THE ROLE OF PERIOSTEUM IN BONE REGENERATION

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PREFACE

Standard oral surgery teaching has long stressed the importance of incorporating the periosteum in mucosal flaps raised during dento-alveolar procedures. In this study, justification for such emphasis has been sought. Although it is widely believed that the osteogenic potential of periosteum is crucial in the repair of defects in alveolar bone, the literature contains surprisingly little direct evidence in support of this concept.

This work has two main components : a review of the literature and an account of animal experiments carried out using the rat.

The literature review has been compiled to give an overview of what is a vast subject. It is intended to convey current thinking, and how these thoughts evolved.

In the main part of the experimental work, a circumscribed bone defect was created in the mandible of the rat using a dental bur, via an intra-oral approach. The overlying periosteum was either excised or retained, and the healing of the defect was studied.

Sacrificing the mental nerve was unavoidable in creating the defect in the only accessible position of the mandible: near the mental foramen. Hence a second study was made in order to evaluate the possible effect of neural damage on the findings of the main experiment.

Finally, conclusions have been presented, drawn from the results of both parts of the research. The techniques used in all experimental procedures simulated as closely as possible those used in clinical oral surgery.

DEDICATION

To the many people who helped to shape my thinking during the preparation of this work. On countless occasions, comments were passed during isolated discussions which made more impact than their originators probably realised.

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CHAPTER I

REVIEW OF THE LITERATURE

1.1 PERIOSTEUM: STRUCTURE AND FUNCTION

The periosteum forms the immediate covering of bone, and consists of two fundamental layers: the outer fibrous layer and the inner osteogenic or cambial layer. In young animals, the cambial layer consists of three cellular sub-compartments. These are first, the pre-osteoblasts, second, a layer of differentiating progeny and third, the functional osteoblasts. The pre-osteoblasts comprise a layer of osteogenic cells and are the precursor cells of the osteoblasts. The layer of differentiating progeny mature to form the functional osteoblasts (Tonna 1974).

In the adult the periosteum is in a quiescent state, and serves for the attachment of tendons. The periosteum also carries blood vessels, lymphatics and nerves; its inner layer retains its osteogenic potency and in fractures is activated to form osteoblasts and new bone (McLean and Urist 1968).

In a study of the morphology and maturation of rat mandibular periosteum, Chong *et al*, (1982) found that the periosteum consisted of a cellular layer and a fibrous layer composed of collagen and reticular fibres. Neither oxytalan nor elastic fibres were detected in the periosteum of these animals up to the age of 56 days.

In each age group studied, which ranged from five to 56 days, periosteal morphology varied at different anatomic sites of the mandible, the variation in patterns being most marked in 25 day old animals. After the soft parts had been removed from the mandible, photomicrographic examination allowed each of these patterns to be correlated with characteristic markings on the bony surface. At 25 days, four morphological patterns were identified according to cellular and fibrous organisation. Each was associated with specific regions of local growth. These patterns reflected the cellular activity or functional state of the periosteum. Chong *et al.* also found that with maturation, the periosteum showed a decrease in the size, number and variety of the cells, and an increase in the size and thickness of the collagen fibres.

In an electron microscope study of periosteal cells in the femora of mice, Tonna (1974) noted intracellular changes, loss of functional activity, loss of various organelles, pinocytosis, progressive cellular degeneration and the presence of lipofuscin in aging fibrocytes, osteogenic cells and osteocytes.

Scott-Savage and Hall (1980) investigated the differentiative ability of the tibial periosteum in the embryonic chick. Their work included study of transverse sections from diaphyses and epiphyses of seven and nine day old chick embryos in close association with the fibrous periosteum of intact host tibiae, at either the

diaphyseal or epiphyseal level. The experimental tissues were placed on culture media for seven days. They found that with the diaphyseal grafts, the fibrous layer of the periosteum gave rise to fibroblasts. However, no osteoprogenitor cells were demonstrated.

Owen (1970), reviewing the origin of bone cells, stated that there were two types of cells in the body capable of induced osteogenesis: the first, the "undifferentiated mesenchyme cells with widespread distribution", and second, those "cells found in marrow tissues which are already predetermined in an osteogenic direction". Owen believed that the exact origin of the cells that contribute to the different stages of fracture repair was not known for certain. She reported that after callus formation in the healing of bone fractures, increased activity, mainly of the osteoprogenitor cells of the periosteum, had been observed, leading to new bone formation on the surfaces of the callus.

Brown and Mayor (1976) considered that fracture healing may occur by the formation of a subperiosteal callus, and that new bone was deposited on the periosteal surface of the callus. They performed an ultrasonic assessment of early callus formation in an animal model and identified two waveform components which correlated with the healing process: first, the "callus wave", which was indicative of early periosteal callus formation and second, the "bone wave", which was indicative of endosteal callus

formation and the strength of the cortical union.

Sebek *et al*. (1972) recognised three layers in periosteum: an outer adventitia, a middle fibroelastic layer and an inner cambial layer. They conceded that some authors regarded the adventitia as part of the loose connective tissue, and thus acknowledged the view that just two periosteal layers existed. However it is interesting to note that whereas Sebek *et al*. described a fibroelastic layer which they believed to possess the greatest mechanical strength, Chong *et al*. (1982) were unable to demonstrate any elastic fibres at all in periosteum.

In an extensive study of the reaction of bone to mechanical stimuli,Sebek *et al*. (1972) included an examination of focal differences in the structure and strength of periosteum. Samples of the membrane were taken from the tibia of 16 calves aged between six and 24 months, and the force required to rupture the periosteum in a longitudinal and transverse direction was measured. This force, the "periosteal strength", was smallest in the middle of the diaphysis and increased rapidly towards the metaphyses. Transverse strength was approximately half that of longitudinal strength. The inner periosteal layer was found to be exceptionally strong at the epiphyseal plate level.

Among the conclustions drawn by Sebek and his co-workers were,first, that the joint ligament attachments, rather than the muscle insertions, were the primary

influence on the thickness and structure of the fibroelastic periosteal layer; second, that the differentiation and activity of the epiphyseal plate may be influenced by longitudinal periosteal fibres, especially those of the deep layer overlying the plate.

Theunissen *et al.* (1974) studied the interrelations among the changes in fibrous periosteum, the growth at the epiphyseal discs and the remodelling at the bony surfaces,in rat tibia. The animals examined were aged from two to twelve weeks. Radiographic, histological and autoradiographic techniques were used, and from their results they hypothesised that the change in shape of the fibrous periosteal envelope, resulting from the growth at the epiphyseal discs, played a major role in the determination of bone remodelling, associated with its increase in length.

Keim (1967) compared periosteal attachment in human long bones of normal adults and adult paraplegics. Whereas periosteum attached smoothly to bone in normal individuals, there was evidence of marked osteoclastic erosion at the periosteum - bone interface in paraplegics; this was attributed to disuse.

Alterations in calcium homeostasis are seen in space flight: Morey and Baylink (1978) reported that rats formed significantly less periosteal bone during 19.5 days on a Russian satellite than did control animals on the ground. This defect had been corrected 26 days after the flight.

Investigating the response to disuse, Uhthoff (1982) studied the activity of the three membranes where remodelling of bone occurs: the periosteal, endosteal and Haversian envelopes. One extremity of each of 60 Beagle dogs was immobilised for periods up to 40 weeks. Twenty four of these dogs were remobilised, prior to changes in bone mass being measured. Immobilisation was achieved in each animal by using a shoulderspica (a type of bandage). In a further 28 dogs, alterations in bone mass were similarly recorded following femoral osteotomies and plating with stainless steel or titanium alloy implants.

Uhthoff found that the three envelopes did not contribute to an equal extent to any change in bone mass. He believed that their contribution was induced by mechanical factors and was dependent on the age of animal and time. Further, the duration of activity of each envelope varied: the contribution of the Haversian envelope to bone loss was found to be temporary. In young adult Beagles, endosteal bone loss was reversed early in the course of continued immobilisation. However, in older dogs, the resorptive activity of the periosteal envelope ceased early, and the permanent loss was due to the activity of the endosteal envelope. Remobilisation led to accretion of bone at the sites where it had been lost.

The transmission and scanning electron microscope (TEM and SEM) studies of Ornoy *et al*. (1980) revealed that periosteal osteoblasts in the tibiae of young rats have alongated processed attached to which were globular structures, known as matrix vesicles. These organelles were also observed on the cell surface and are thought to act as the initial focus of calcification in bone.

In a TEM study, Taylor and Yeager (1975) observed cytoplasmic inclusions in both normal and "stimulated" rat periosteal cells which were bounded by a pentilaminar membrane similar to a tight junction and contained a core of granular material. They could ascribe neither origin nor function to these inclusions, and pertinently pointed out that it was difficult to view them as invaginated tight junctions since insufficient cell to cell contact had been observed to account for the numerous inclusions seen in some cells. Nevertheless the authors speculated that their report may be the first to show morphological evidence that a mechanism of intercellular communication, such as described by Reith (1970) for ameloblasts, might exist in a connective tissue where the individual cells were widely scattered.

A tissue culture system for the formation of bone from isolated rat bone cells has been devised (Binderman *et al*. 1974). The bone cells were obtained either from embryonic rat calvarium and periosteum or from periosteum of young rats. The isolated bone cells were cultured for periods of up to eight weeks, and it was shown that they had the ability to form bone.

Tissue culture studies on bone formation have been performed recently: Heersche (1982), studying bone formation and resorption, cultured periosteum from the calvaria of 17 day-old foetal chick embryos on a medium containing "embryonic extract" and fowl plasma. He found that mineralisation of new bone occurred consistently when a source of organic phosphate, beta-glycerophosphate, was

added to the culture medium. Heersche interpreted the need to add organic phosphate to the culture medium as being an indication of the necessity of having an adequate level of these ions for mineralization to occur.

Tenenbaum and Heersche (1982), using the same model as in the previous work, studied the differentiation of osteoblasts as well as bone formation. Having removed the periosteum from the chick calvaria and left the osteoblasts behind on bone, the membranes were folded with the potentially osteogenic cells (i.e. the cambial layer) in apposition and cultured for up to six days on plasma clots. Osteodifferentiation was observed, followed by the formation of osteoid tissue between the two layers. As in Heersche's study mentioned above, addition of beta-glycerophosphate consistently led to mineralization of the osteoid.

Rosin *et al.* (1963) placed periosteum taken from rat femur in diffusion chambers in the upper abdomen of rats. After periods of up to six weeks, bone formation was found in marginally over one third of these explants.

Diffusion chambers were also used in a study to ascertain the periosteal control of long bone growth in the rat (Houghton and Dekel 1979). The periosteum of right femora of immature rats was circumferentially divided and the middle one third was stripped. The contralateral femora acted as controls. Within 14 days, longitudinal overgrowth of the periosteally divided

femora had occurred. Houghton and Dekel concluded that intact periosteum mechanically checked the growth plate, and that circumferential periosteal division removed this restraint to increase longitudinal growth.

Tonna and Cronkite (1961a) were among the first to use autoradiography to study periosteum. In 1963, they quoted Lacroix (1949) who wrote: "For anyone seeking information on the properties of periosteum, the literature on the subject is almost as disappointing as it is abundant. In it, from time to time, are to be found discussions in which ideas are questioned which one would have thought well established, with the result that at the present day it is hard to find even elementary points on which opinion is unanimous ... the discussion, on which an understanding of so many problems of physiology and pathology of bone depends, seems to have gotten into a rut, out of which its sole chance of escape lies in the acquisition of fresh data".

Fortunately some of this data has been provided by the introduction of autoradiography. This technique is able to detect radioactive material in cells and tissues by placing them in contact with photographic film. As a result, a deeper insight into a number of periosteal activities has been obtained which include cellular migration, origin, transformation, aging, response to external stimuli, proliferative rates and the intracellular turnover of important metabolites. A "flash-labelling" technique has been developed whereby an

animal is injected with tritiated thymidine and later killed. Those cells which were synthesising DNA at the time of death become labelled, and the intensity of the label may allow study of proliferative capacity and total life cycle of the cell. More fully described in an excellent article (1963), Tonna and Cronkite have used this technique to broaden understanding of periosteum.

Tonna (1961) investigated the cellular component of the skeletal system in mouse femora from the time of birth to one year of age. To calculate the labelling index, which is a measure of the intensity of the uptake of tritiated thymidine into the DNA, the number of labelled cells was divided by the total cell population. In periosteum, this index was found to be highest at birth. At the age of eight weeks, the index plummetted to just under one twelfth of its neonatal value and then remained low throughout the period of study.

In this work, Tonna also reported, most interestingly, that the cells of the articulating surface of the epiphysis and disc were derived, at least in part, from migrating chondro-osteogenic cells of the periosteum residing at the perichondrial region. These chondro-osteogenic cells were noted to serve as the progenitor part necessary for both circumferential growth and expansion of the epiphyseal disc.

Tonna and Cronkite (.1961b) examined the repair of femoral fractures in female mice over a period of one hour to 14 days after injury, using tritiated thymidine as a radioactive marker. The results showed that the initial proliferative response in the periosteum and adjacent soft tissues occurred 16 hours after fracture, with the maximum response being detected after 32 hours. The reaction was not limited to the fracture site but extended along the entire femoral diaphysis. Fourteen days post-injury, the labelled population of periosteal cells at the fracture site was still above that of the mice used as controls.

Tonna and Cronkite (1961a) reported that osteogenic cells were a relatively quiescent cell population awaiting a signal for proliferation and transformation, as in fracture repair. This study, also in mice using the "flash-labelling technique", showed that osteogenic cells constituted a self-sustaining cell population which diminished in size with increasing age. Tonna and Cronkite noted that osteoblasts were, in part, self-reproducing and, in part, produced by transformation of pre-osteoblasts; these same authors (1962) stated that "osteoblasts are considered outside of the periosteal mineral compartment when they become surrounded by bone matrix and mineral salts.

More recently, Rodionova (1980) used tritiated thymidine autography in rats and rabbits to prove that the population of osteoblasts in the periosteum of

growing bone was replenished due to the reproduction and differentiation of perivascular cells and pre-osteoblasts.

The foregoing summarises and illustrates the properties of periosteum relevant to this work. In the next chapter, both the early research, and the evolution of ideas concerning periosteum and its role in bone regeneration, are discussed. Finally, the concepts resulting from recent research are presented.

1.2 EARLY RESEARCH

The role of periosteum in bone regeneration has been an issue of controversy for more than 200 years. The man who seems to be credited with making the first scientific observations of bone growth was a young surgeon named John Belchier who, in 1736, was invited to dinner by a "Calico Printer". Belchier noticed that the bone in the cut of roast pork they shared was red. Inquiries revealed that the animal had been regularly fed the residue from madder roots after the alizarin had been extracted from them (Keith 1919). This observation inspired Belchier to feed madder to a cockerel. He reported (1739) that the bone formed over a 16-day course was coloured red.

Henri-Louis Duhamel (1739), a French squire, repeated the work of Belchier on fowls, turkeys, pigeons, and pigs. Similar results were achieved. He reported that only certain parts of the bones were stained, and that the dye was taken up more readily and deeply in young animals than old.

In 1741, Duhamel created bone fractures in experimental animals, and he recorded that the periosteum was primarily responsible for callus formation, assisted by the endosteum. Later (1742, 1743), he noted that madder stained the newly deposited bone only while it constituted part of the diet of the animal. Sectioning bones transversely after intermittent feeding with

madder revealed alternate red and white rings.

Duhamel concluded that bone grew in the same way as wood: by superimposition of layer upon layer. The source of the new layers was the periosteum. Also, the deep layer of the periosteum appeared to serve the same function as did cambium for growing wood; this deep layer is also known today as the cambial layer. However Duhamel observed that as a bone grew, the medullary cavity enlarged. In an attempt to explain this, he encircled the shafts of growing bones with silver wire which eventually cut its way into the medullary cavity. Duhamel believed this had occurred due to expansion of the shaft.

In stark contrast to the beliefs of Duhamel, a Swiss born Anatomy Professor, Albrecht von Haller (1763), argued that arteries were the depositors and builders of bone. Von Haller repeated many of the madder-feeding experiments performed by Duhamel. He concluded that the periosteum was essential for neither callus formation in fracture repair nor bone growth.

Another eminent figure who subscribed to the view of Von Haller was the British surgeon, John Hunter. He noted that after teeth were extracted, their sockets disappeared; that when deciduous teeth were shed, their roots were eroded and their sockets were diminishing in size; that, in a child, the second deciduous molar lay

at the base of the ascending ramus of the mandible, but by adulthood, room had been found for the three permanent molars. From these, and other observations of growing long bones, Hunter correctly deduced that bone grew by a process of continuous remodelling: by deposition and resorption. This explained the silver wire experiments of Duhamel, referred to above - the rings entered the medullary cavity since deposition had occurred on the exterior of the shaft and resorption on the interior. Hunter (1764) confirmed these findings by intermittently feeding madder to growing pigs. After sacrifice, he noticed, as had Duhamel before him, that the dye had been taken up by the newly deposited bone only whilst it was included in the diet. In further experiments, Hunter went on to show that the epiphyseal plate was the major site of growth in long bones. (Bassett 1962).

During the nineteenth century, more men devoted years of study trying to clarify the role of periosteum in the regeneration of bone. One was James Syme, Professor of Clinical Surgery at Edinburgh. In 1835, he was compelled to amputate the leg of a young girl suffering from acute osteomyelitis of the tibia. Later, while dissecting the dead part, he found that the shaft of the affected bone was encased by a stratum of new osseous tissue adherent to, and apparently formed by, periosteum. Following some animal experiments inspired by this observation, Syme became convinced that periosteum had osteogenic potential. However, his colleague from Edinburgh, John Goodsir, was

equally convinced that periosteum was merely a "limiting membrane", and that any neo-osteogenesis observed actually arose from attached particles of bone. Goodsir, initially Curator of the Museum of the College of Surgeons of Edinburgh and later Anatomy Professor in the same city, was among the first to extensively use the compound achromatic microscope. His bone research was all performed during the three years of his curatorship (1841 - 1844), and he appears to have been credited with great advances during this remarkably short period.

Across the English Channel, the Parisian Marie Jean-Pierre Flourens had repeated and extended the work of Duhamel (1842). He was convinced that periosteum had osteogenic potential. He also believed that Hunter was correct in concluding that bone was being constantly remodelled. His compatriot from Lyons, Louis Ollier, conducted a protracted series of animal experiments (1857-1868). At the beginning of this time, Ollier believed that periosteum had no osteogenic capacity. However, by the end of his mammoth research, he had completely changed his mind. His critics claimed that bone particles were attached to the periosteum he had used, yet Ollier, whom Keith (1919) described as a proficient microscopist, used this relatively new instrument to preclude such a possibility; the argument was that the new osseous tissue had arisen from the fragments of bone and not the periosteum.

Ollier concluded that the deepest layer of periosteum was cellular and that it could produce bone. However, according to Burman and Umansky (1930), Ollier thought that ossification varied according to such factors as type and age of animal, operative technique, size and adherence of the transplant, vascularity of the recipient site, functional stimulation given to the transplant, whether the transplant was autoplastic, homoplastic, or heteroplastic, the donor site (i.e. if the bone was long or flat), the integrity of the cambial layer, and the presence or absence of infection.

Spanning the turn of the century was the career of Sir William MacEwen, then at its zenith. Elected to the Chair of Surgery in the University of Glasgow in 1892, MacEwen believed that the periosteum was a major limiting factor in preventing the osteoblasts from being scattered into the soft tissues during their development (1912). This was the same view as that held by Goodsir: that the periosteum was a "limiting membrane". Keith (1919) indicated that the two reached their conclusions independently. Further, MacEwen considered that osteogenesis resulted from the activity of the osteoblasts of bone, as opposed to those found in periosteum. However the work of MacEwen was criticised for two reasons. First, that as there was an almost complete absence of microscopic study of his grafts, direct evidence that bone cells were alive and had formed osseous tissue was lacking. Second, his conclusion that the vitality of the bone could be assessed radiographically had been disproved by Kuttner

(Berg and Thalhimer 1918). Keith (1919) believed that MacEwen had been "less than just" to periosteum, and considered that from anatomical and surgical viewpoints the osteogenic power of the deepest stratum of the periosteum could not be denied.

Nevertheless MacEwen found some support from Murphy (1913), although the latter considered that transplantation of periosteum with bone was advantageous. He found that by raising a strip of periosteum from bone and embedding one end of it into the soft tissues while leaving it attached at the other end, bone was usually formed on the under surface of the periosteum at the osteo-periosteal angle, provided there were osteoblasts attached to it.

Murphy also believed that transplanted bone acted as a scaffold for new bone production and that the transplant itself was always ultimately absorbed. That there was no osteogenesis from any portion of a transplant (including periosteum if present) had been advocated nearly 20 years earlier (Barth 1894). MacEwen's concept of periosteum having the sole role of a limiting membrane received some support (Cohn and Mann 1914, Davis and Hunnicutt 1915). Indeed, Cohn and Mann were unable to demonstrate any osteogenic function in periosteum following an histological study of bone transplants and periosteum in dogs, cats and rabbits.

Bancroft (1921) took an even more extreme view and believed that bone repair was more likely to occur by a chemical deposition of calcium salts in connective tissue, and suggested that the roles of periosteum, endosteum and bone reticulum had over-complicated the issue.

McWilliams' fascinating paper (1914), based on four human bone transplants and a number of animal experiments, indicated an important role for periosteum in the regeneration of bone. He found that by removing an entire cross-section of a bone, regeneration would occur if the periosteum bridging the defect were entirely or partially preserved. This was not the case if all the periosteum were excised.

In the same paper, McWilliams also strongly emphasised that the life of a living bony autograft depended upon its blood supply, irrespective of the presence of periosteum or contact with other osseous tissue. Here it is interesting to recall Von Haller's view of the mid-eighteenth century that bone was formed by blood vessels (Ham and Harris 1971). The importance of the blood supply in maintaining the viability of the cells of a transplant was stressed by Phemister (1914), and was considered essential for the normal union of fractures, (Kolodny 1923). However Davis and Hunnicutt (1915) found that the removal of periosteum had little effect on the nutrition of a bone, unless the nutrient artery was interfered with. When this vessel was cut, radiographic

changes were observed although the bone appeared normal macroscopically.

The production of bone from free periosteal transplants was achieved by some (McWilliams 1914, Berg and Thalhimer 1918, Mock 1928, Fang *et al.* 1934) but not by others (Brown and Brown 1913, Cohn and Mann 1914, Phemister 1914, Davis and Hunnicutt 1915). However Davis and Hunnicutt found that when bone shavings were left attached to free periosteal transplants or pedunculated periosteal flaps, then new bone was formed. In a limited series of experiments, McWilliams (1914) reported that incorporation of periosteum with bone transplants achieved a success rate of 93 per cent in comparison with 48 per cent when periosteum was absent.

As mentioned previously, Cohn and Mann (1914) supported MacEwen's belief that the periosteum was simply a limiting membrane. MacEwen described periosteum as being a fibrous membrane composed of two layers, the inner of which contained many elastic fibres and blood vessels; in addition he recognised an amount of loose areolar tissue between the inner periosteal layer and the bone which allowed "penetration of the osteoblasts emanating from the underlying tissue". MacEwen's view, that "periosteum acts as a limiting membrane to the osteoblasts issuing from the interior of the bone", was opposed by Phemister (1914) and Berg and Thalhimer (1918) who all believed that the inner layer of periosteum had the ability to form bone.

In this era of classic experiments, no agreement was reached as to whether or not it was essential, advisable or unnecessary to leave periosteum attached to bone transplants.

Some considered it more prudent to leave periosteum intact when bone was being transplanted (McWilliams 1914, Davis and Hunnicutt 1915). Phemister found that when a piece of tibial cortex with periosteal and endosteal surfaces removed was transplanted into a bone defect, "creeping substitution"* of the cortex occurred. However, when the periosteum was left intact, osteogenesis producing union and"creeping substitution" were more rapid. As mentioned previously, Barth (1894) and Murphy (1913) considered that osteogenesis did not arise from any portion of a transplant and that its substitution occurred entirely by an ingrowth of new bone from the original osseous margins. Brown and Brown (1913) tried unsuccessfully to produce bone by placing a transplant into subcutaneous tissue or muscle; the presence or absence of periosteum made no difference. Alternatively,by placing a transplant in contact with living bone where it had a function to perform,osseous tissue was deposited in nearly every instance.

The Law of Functional Adaptation was proposed by Wilhelm Roux (Phemister 1914). It stated that there was

*"Creeping substitution" or "creeping replacement" is an old concept which suggested that during the remodelling of bone, the newly formed osseous tissue was responsible in some way for the removal of the old. References to these terms are copious in the literature e.g. Phemister (1914) Weinmann and Sicher (1947), De Bruyn and Kabisch (1955)

a distinct relationship between the form, size and structure of a tissue or organ and the function it had to perform. A change in any one of these factors resulted in corresponding changes to the others. Belief that functional demand stimulated the surviving cells of the transplant was shared by others (e.g. Phemister 1914). If the recipient site demanded no functional activity from the transplant, it tended to be resorbed; this fate uniformly awaited those transplants placed in subcutaneous tissue and muscle (Brown and Brown 1913).

Berg and Thalhimer (1918) performed a series of tibial transplants in cats where the bone was placed either on or within the spleen, subcutaneously, or on the costal cartilages. They concluded that some bone cells (i.e. those cells of bone which are enclosed in completely osseous lacunae and intercommunicate via canaliculae) in the transplants were able to persist for almost a year, but that most of the bone was ultimately resorbed. They differentiated between the very young lacunar cell and the adult bone cell. The young lacunar cell was described histologically as having a large oval vesicular nucleus, was believed to be the active bone producing cell in "creeping substitution" (here, Berg and Thalhimer claim support from Mayer and Wehner 1914), and was an "osteoblast within a lacuna surrounded by uncalcified matrix". The adult bone cell, however, was described as having a smaller, more elongated and more darkly staining nucleus, and was not observed to have the ability to produce bone.

It was generally considered at this time that bone resorption was performed by osteoclasts, the multinucleate giant cells found in Howship's lacunae. However, in the same article, Berg and Thalhimer speculated that some form of biochemical action also contributed to removal of the transplants, which were then replaced either by a "creeping forward" or "gradual infiltration" of the new bone into the transplant.

Klinkerfuss (1924) agreed that solid bone grafts were resorbed and replaced by new bone which he considered was deposited following proliferation of osteoblasts in periosteum, endosteum, and Haversian canals.

Transplanting bone with attached periosteum into various soft tissue sites in dogs, Davis and Hunnicutt (1915) suggested that the periosteum may have a certain protective action on the transplant. For instance, they found that after 128 days and under similar conditions, sections of fibula without periosteum had markedly softened and reduced in size, whereas control sections of fibula covered with intact periosteum were practically intact.

Another highly respected view held in the early years of the twentieth century was that of the German researcher Georg Axhausen. He considered that the bone in a graft always died and was resorbed, but that regeneration occurred from the periosteum which was the only surviving part of the transplant. An intimate vascular connection between the transplant and its bed was formed by periosteum.

(Axhausen 1907, 1908,1909, 1911).

In an apparent attempt to solve the mystery of osteogenesis once and for all, Rohde (1925) published a paper entitled: "Does bone form from osteoblasts or from metaplasia of surrounding connective tissue?" This researcher was adamant that osteoblasts were responsible for bone formation, and that metaplastic bone formation did not take place from ordinary connective tissue. Rohde claimed to have been able to substantiate the powerful regenerative action of the periosteum and that it was destroyed only by either loss of blood supply or through early overgrowth of "non-specific" connective tissue. He firmly believed that the callus formed in a bone defect was of periosteal origin, supporting the view of Kolodny (1923) that the periosteal callus played a far greater role in the union of fractures than did the endosteal callus. Rohde finally stated that *"Fundamental prerequisites for bone formation are living osteoblasts or unused remaining mesenchyme* cells which can develop into osteoblasts".

In an excellent publication reporting animal experiments and clinical experience, Mock (1928) reported that closure of a bony defect with a periosteal transplant had failed due to the shredding of the membrane. Further, denuding the bone of periosteum on either side of a defect led to non-union, and in three out of six of his cases the bone regeneration that had occurred emanated from the periosteum rather than the ends of the fragments. He concluded that

periosteum was necessary for the regeneration of bone, and that in cases of delayed union, un-united fractures and loss of bone substance, periosteal transplants properly positioned around the site of the damaged bone would result in healing and reconstruction of the defect. Also, Mock pointed out that the majority of investigators regarded periosteum as the one layer most indispensable to osteogenesis.

According to Mock, Leriche and Policard (1926) stated that the periosteum had no true osteogenic action, and that it merely checked "osseous infiltration, which spread widely", thus reviving MacEwen's concept of periosteum being a "limiting membrane". Indeed, Burman and Umansky (1930) reported Leriche's belief that successful results in transplantation were due to bone particles attached to periosteum.

In an experimental study of free periosteal auto-transplants being wrapped around the tendons of young rabbits, Burman and Umansky (1930) found that osteogenesis occurred in the majority of their experiments where the cambial layer of the periosteum was present, and in almost none where it was absent. By incorporating bone chips with the transplants, they noted no increased bone formation, contradicting the views of Leriche. In studying bone formation in an embryo, Burman and Umansky believed that it was impossible not to observe periosteal osteogenesis. They pointed out that Leriche mentioned no such studies in his work.

In a tissue culture study of fowl embryos and young chicks investigating the osteogenic capacity of periosteum and endosteum, Fell (1932) resected periosteum from the middle third of long bones or from bone rudiments. These samples were grown in a mixture of equal parts of plasma and embryo extract and were usually transferred to fresh medium every 48 hours. She found that periosteum would form bone in six and ten day-old limb bone rudiments. These results are particularly interesting, since, as Fell point out, the periosteum was deprived of its normal association with cartilage, and of its blood and nerve supply.

In a limited series of experiments in rabbits and dogs, Fang *et al.* (1934) not only believed in the osteogenic potential of periosteum but differentiated between the source of free grafts on the tibial shaft, pointing out that the nearer the source of the graft was to the epiphysis,the greater the osteogenic power. This would seem to correlate well with John Hunter's finding in the eighteenth century that the epiphyseal plate was the major site of growth in long bones (Bassett 1962).

Following a study of autografts in 22 rabbits, Haldemann (1933) concluded that the periosteum was the most important part of a bone graft, both as regards union of fractured bones and survival of the grafts. He noted that if the periosteum was absent from the graft, the fracture failed to unite or union was delayed, and the graft was resorbed. He also believed that the bone cells

of a graft died within a few days and "creeping substitution" occurred.

The enormous contribution made by the early researchers in this field can not be overestimated, particularly bearing in mind the conditions under which they worked. Everything was done in the pre-antibiotic era, and although references are made to aseptic techniques being employed, by the standards of today they can only be described as primitive. This is no reflection either on the ability or tenacity of these workers but simply that many discoveries have been made as the years have passed, bringing to light important facts hitherto unknown. Nowadays, the literature is read with a more critical eye and standardisation of technique and selection of the experimental model are factors which must be carefully examined. For instance, it is probably not unreasonable to suggest that in describing an animal as a "small old black and white mongrel" or a "small spotted white and black female fox terrier 9 months old" (experiments 22 and 14, Brown and Brown 1913), there is insufficient standardisation. Similar examples may be found in the work of others (McWilliams 1914, Phemister 1914, Davis and Hunnicutt 1915).

From the weight of evidence, it must be concluded that the periosteum does have some role in the regeneration of bone but the overriding question is to what extent. While it is hard to understand how there are so many diametrically opposing views, the opinions of all must be respected and

those in the field today are in the debt of the pioneers for their painstaking efforts in laying the foundation for current understanding of this complex subject.

1.3 THE ROLE OF PERIOSTEUM IN BONE REGENERATION

1.3.1 INTRODUCTION

In the sections that follow, an attempt has been made to outline recent research on this vast subject. It has been written with a bias towards clinical application in oral surgery although the references cited come from a wide range of publications, many of which are not dental. The aim has been to cover the scope of the osteogenic potential of periosteum, but it is emphasised that coverage of the section sub-headings is not exhaustive. However, prime consideration has been given to selecting references which best illustrate current ideas of the role of periosteum in bone regeneration.

1.3.2. THE POTENTIAL OF PERIOSTEUM TO FORM BONE BY INDUCTION.

The concept of induction was well described by Slavkin (1974). When embryonic cells, normally associated with a specific path of development, are placed into a dissimilar part of the embryo, and are specifically instructed to take on a different path, induction is said to have occurred.

Urist and Mclean (1952) reported the results of a large number of experiments on induction which included autogenous transplantations of periosteum in rats. These transplants were placed in the anterior chamber of the eye, and new bone formation followed. However, the evidence from this work suggested that the bone was produced directly by the graft rather than resulting from induction due to contact with the transplanted tissues. Nevertheless Urist and Mclean believed that bone formation following transplantation of some other tissues, notably cartilage, was due to induction.

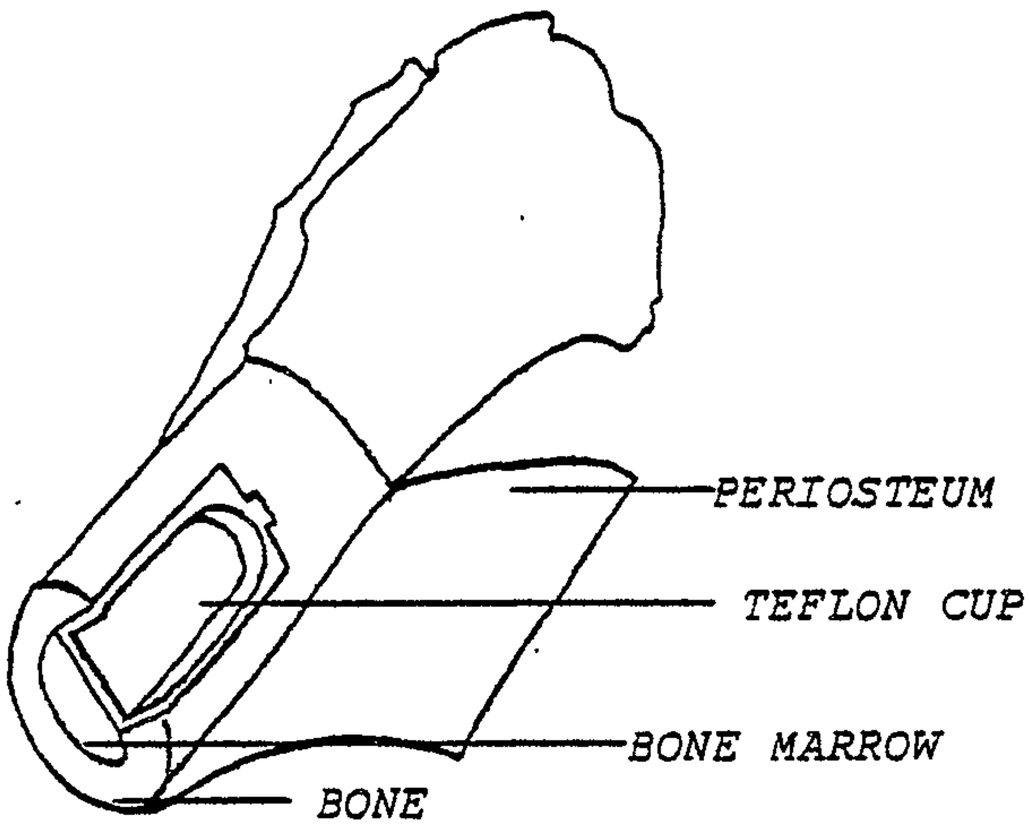
Further, the age of the rat where autogenous periosteal transplantation was being performed appeared to be important: in three week old animals, new bone was deposited, but none was observed in animals of approximately one year. When the periosteum was taken from the region of a fracture and placed in the anterior chamber of the eye, new bone was formed as early as 24 hours after the bone damage had been sustained. Also, they found that the periosteal

transplants produced only compact bone without cartilage or bone marrow. Finally, they proposed that the long established concept of "creeping substitution" (see page 21) was essentially a process of new bone formation by induction.

1.3.3. THE OSTEOGENIC CAPACITY OF PERIOSTEUM ACCORDING TO ANATOMICAL LOCATION.

It is now evident that the osteogenic capacity of periosteum varies from site to site. Periosteum from long bones, mandible and calvaria as well as the dura mater seems to lay down varying amounts of bone in different circumstances. This will be illustrated in the following paragraphs.

The osteogenic capacity of tubular and membranous bone periosteum was qualitatively and quantitatively studied in growing rabbits (Uddstromer 1978). The selected sites were the tibia and the skull. Uddstromer used a Teflon (TM) polytetra-fluoroethylene cup model (Fig. 1); after creation of a bone defect, which extended through the cortex to the medullary cavity, a close fitting rectangular Teflon cup was inserted. Each short side of the cup had a small lip to prevent it sinking into the marrow cavity. The cup was filled with a coagulum of peripheral blood.



(AFTER UDDSTROMER)

Fig. 1

Cross section through rabbit tibia to show placement of Teflon cup.

By suturing the periosteum back over the top of the cup, periosteal osteogenesis could be studied *in situ*; the animals were killed at various post-operative periods from two to 15 weeks. In his quantitative analysis, Uddstromer reported that, after approximately seven weeks, tibial periosteum had produced normal quantities of cortical bone and seven times more than had the same area of skull periosteum. Calvarial periosteum was observed to be incapable of restoring a skull defect completely.

In six cases, Surgicel (TM)* had been placed in the Teflon cup. Examination of the animals six weeks after surgery revealed that considerable diminution of neo-

* *Oxidized cellulose;* *see page 65*

osteogenesis had occurred.

Among the first to use Surgicel in the repair of congenital clefts with periosteum was Skoog (1965). Its functions were to keep inner and outer periosteal layers apart and to obtain a medium for good bone formation, (see page 65).

Using the Teflon cup model in rabbits described above, Uddstromer and Ritsila (1978) studied the osteogenic capacity of periosteal grafts. Tibial periosteum was grafted to the skull, and *vice versa* Investigations also included a study of the formation of periosteal bone when other osteogenic factors were not excluded; the dura mater, for instance, was one such factor, the osteogenic potential of which was described by Sirola (1960).

Results from the qualitative studies of Uddstromer and Ritsila (1978) indicated that in growing rabbits, tibial periosteum grafted to a skull defect restored the latter with compact cortical bone and bone marrow, preceded by a very small amount of woven bone. Where no Teflon cups were used (thus permitting osteogenic factors other than the periosteum to operate), calvarial-like bone resulted.

Quantitatively, tibial periosteum on skull defects was found to have the capacity of complete repair.

Calvarial periosteal grafts applied to the skull resulted in deposition of only small amounts of new bone, whereas, and most interestingly, when transplanted to long bone defects, the osteogenic capacity of these transplants was increased fivefold.

In contrast to tibial periosteum the calvarial periosteal grafts led to incomplete healing of both tibial and skull defects; after initially depositing woven bone (in greater amounts than did tibial periosteum when grafted to the skull), the final structure was that of tubular bone.

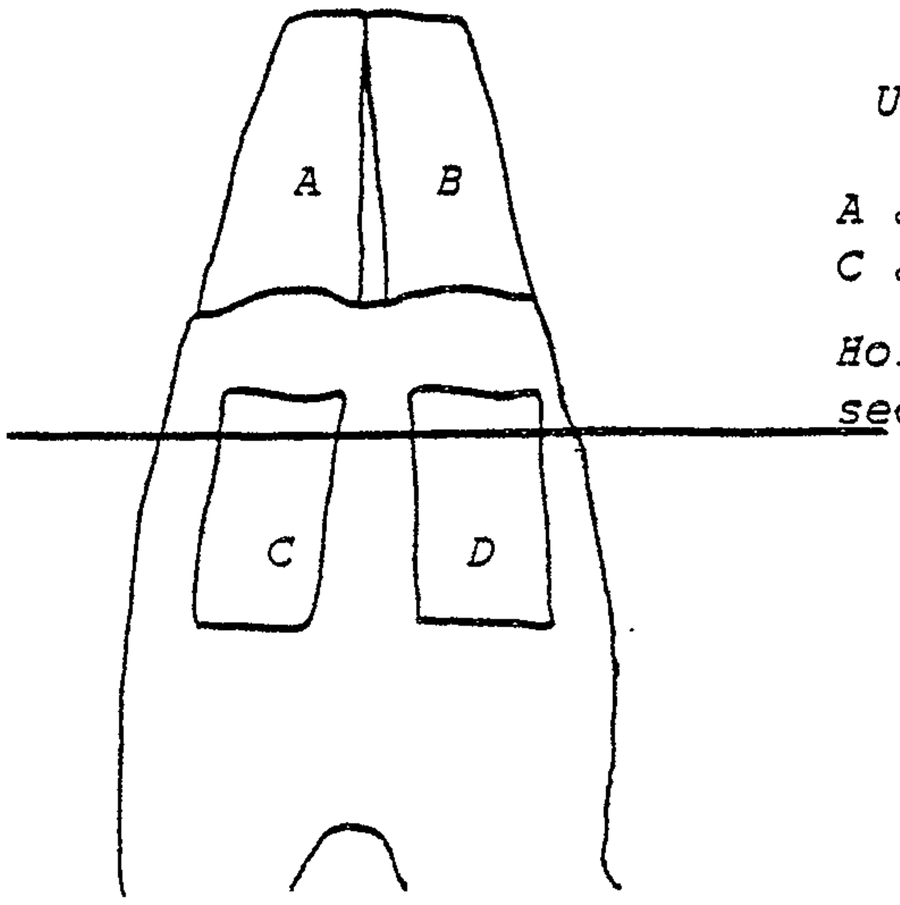
Uddstromer and Ritsila believed that environmental functional demands seemed to influence the type of bone formation and the final structure of the new bone, and that differences existed between the amounts of bone formed by long and membranous bone periosteum. These findings imply a difference in periosteum covering the two types of bone, but no other reference in the literature was found.

Uddstromer and Ritsila (1979) again used the Teflon cup model in growing rabbits to determine, first, the extent of influence exerted by periosteum, cortical bone, endosteum and bone marrow on the healing of long bone defects,and second, the extent to which the dura mater participated in the repair of calvarial defects. They concluded that the periosteum had the most potent healing capacity in the healing of tibial defects whilst the

the cortex, endosteum and bone marrow made minor contributions. However the osteogenic potential of calvarial periosteum proved less than both tibial periosteum and dura mater. For a complete return to normal structure of a calvarial defect, combined periosteal and dural osteogenesis was required.

That tibial periosteum had greater osteogenic potential than mandibular periosteum was reported by Ranta *et al.* (1981). They transplanted free tibial periosteal grafts on to alveolar bone defects in eight adult rabbits; the root surface of the continously erupting mandibular incisors was exposed, the test side being covered with a tibial periosteal graft and the control side being covered with a local periosteal flap (Fig. 2). The defect healed on both sides and the type of bone formed on both control and test sides was identical. However, in seven of the eight animals, two or three times the amount of bone was formed on the test side as on the control side. Eruption of the incisors was symmetrical. These results confirmed the earlier results of the same group (Altonen *et al.* 1977), who found, first, that it was possible to induce bone formation around an erupting rabbit tooth, and second, that a free periosteal transplant from the tibia had a better osteogenic capacity around the tooth than a local periosteal flap.

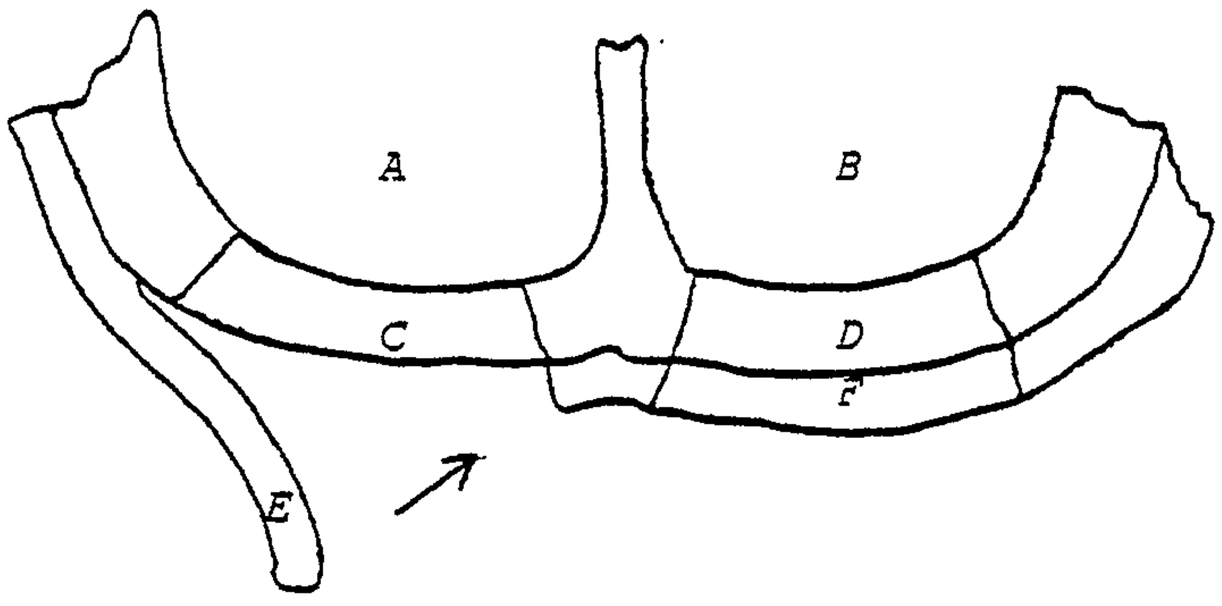
Mattout and Rachlin (1978) performed an *in vitro* study of the role of periosteum and the periodontal ligament in the bone repair process in adult rats. Periosteum was removed from fragments of palatal mucosa, and from pieces of maxillary and mandibular bone which included the margins of tooth sockets. All were placed in tissue culture. The specimens survived longer than 26 days. The ligament side of the bone showed signs of osteogenesis by the twelfth day whereas on the other side; which was devoid of periosteal osteoblasts, no signs of bone deposition were observed.



Undersurface of Rabbit Mandible

A and B ::Continuously erupting incisors
C and D : Test areas

Horizontal line represents plane of section of diagram below.



E : Local periosteal flap on control side
F : Periosteal transplant from tibia.

Fig. 2

(AFTER RANTA)

1.3.4 GRAFTS

1.3.4.1 PERIOSTEAL GRAFTS

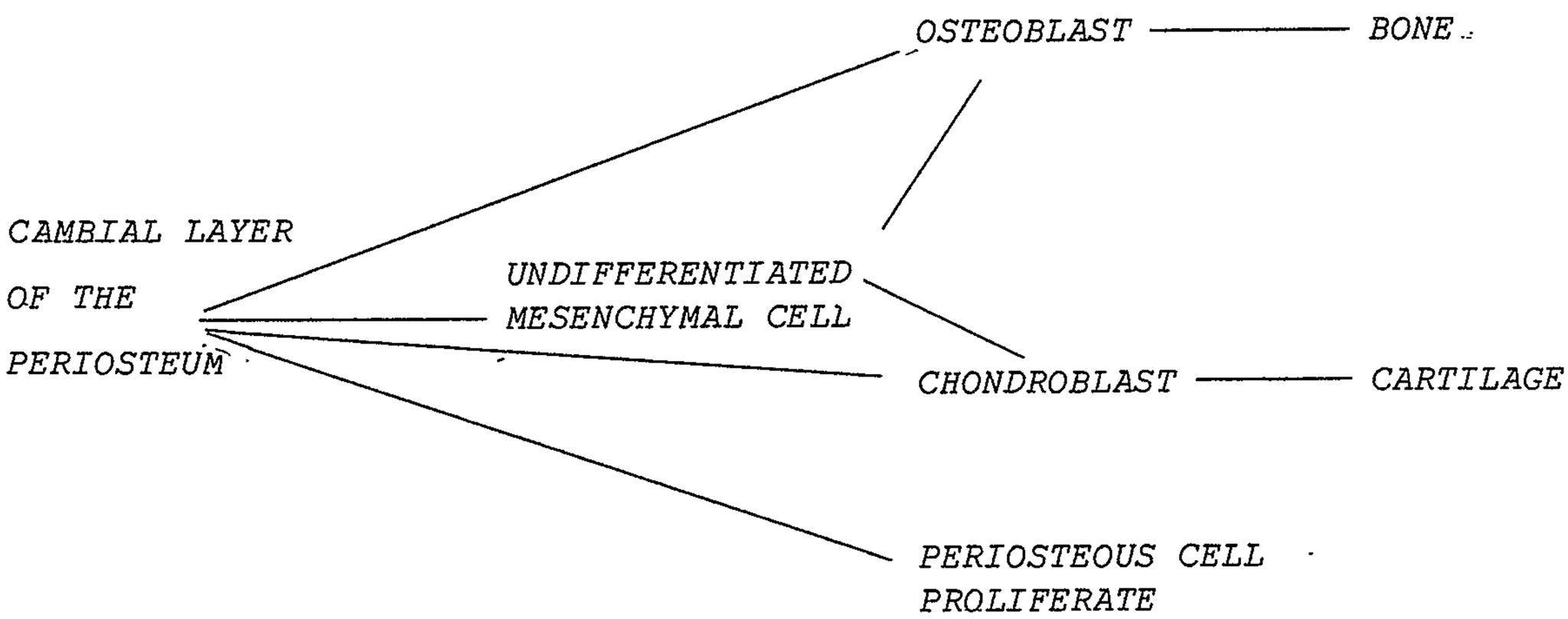
Lacroix (1946) reported on an extensive series of experiments on periosteal osteogenesis in rabbits. These included taking periosteum from different parts of the tibial diaphysis and placing the transplant beneath the renal capsule. He compared the differences in periosteal osteogenesis between young and adult animals. Among his findings were that the graft survived transplantation and then started to form an osseous layer on the fourth day, which reached uniform thickness on the 15th. However, resorption of this layer commenced after approximately six months.

Cohen and Lacroix (1955) attempted to devise a standardised method for assaying the osteogenic ability of a tissue to be used for grafting. They studied autogenous periosteal grafts in rabbits, and found considerable variation in the potency of the tissue to form bone: it depended on the age of the animal and the "character" of the recipient bed used. In contrast to Urist and Mclean (1952)*, grafts into the anterior chamber of the eye showed the least amount of osteogenesis, whereas grafts to the tibia were the most successful in forming new bone. In addition, cartilage formation was often observed. In grading tissue response, Cohen and

* *see page 30*

Lacroix included the amount and type of intercellular matrix (i.e. bone and cartilage) elaborated in response to stimuli which, from analysis of their results, were thought to be primarily chemical in nature, rather than mechanical or circulatory. Cohen and Lacroix considered that alkaline phosphatase was undoubtedly active in the elaboration of bone and that other local substances, undefined in 1955, must also contribute.

Morito (1980) investigated bone and cartilage formation in autogenous periosteal transplantation in rabbits. The animals were divided into two groups: in the first, free periosteal autografts from the tibia were placed under the renal capsule, and in the second, similar transplants were inserted into a diffusion chamber and placed subcutaneously in the abdomen. From the results, it was concluded that the periosteum had osteogenic powers, especially the cambial layer. This stratum was also thought to be able to differentiate into other cell types, either directly or indirectly.



Morito also reported that, in the differentiation of periosteal cells into bone and cartilage, such factors as low oxygen content or contact with other cells seemed important.

In contrast to the work of Morito (1980), cartilage formation was not observed at any stage of the healing process by Najjar and Kahn (1971) who developed an experimental model for the study of osteogenesis and remodelling. They excised a segment of tibial cortex from each of 70 rabbits. Radiographs were taken two weeks and one month postoperatively. Tetracycline was administered to some animals as a bone label, and the animals were sacrificed at intervals up to four months postoperatively. Histological examination revealed that the osteoblastic "blastema" - presumably osteoblasts and their precursors - arose mainly from the periosteum.

In discussing the presence of cartilage during bone formation, Uddstromer (1978) suggested that the variance in experimental findings of a number of workers might be due to the different ages of the animals used.

In recent years a major contribution to the role of periosteum in bone regeneration and growth has come from Scandinavia. Ritsila *et al*. (1972a) published a preliminary report on the experimental transplantation of free periosteum. One hundred and fifty two rabbits were used, their ages ranging between eleven and 149 days at the time of operation. In the majority of the 28

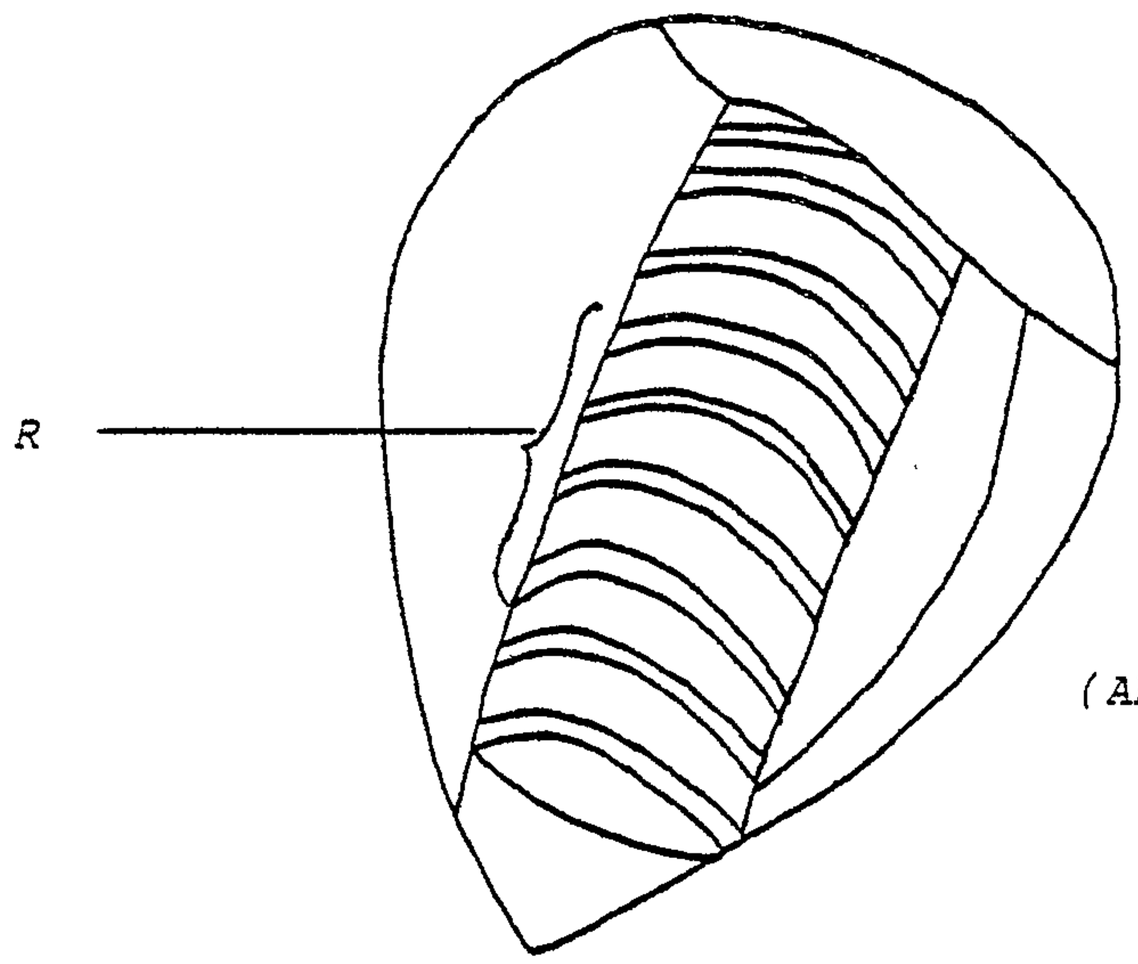
series studied, tibial periosteum was excised and transplanted into various soft tissue sites. These included muscle and subcutaneous tissue. Calvarial periosteum was also transplanted. The recipient locations were mostly autogenous but some were homogenous. Radiographic, histological and autofluorescent techniques were employed to study neo-osteogenesis which was observed in all series.

Ritsila and Alhopuro (1973a) studied the repair of bone defects by free grafting of periosteum in growing rabbits. The defects were created in the calvarium and the ulnar shaft. In the calvarium, autogenous periosteal transplantation led to healing of the wound within two months. In the ulna, an interesting experimental method was used: following resection of part of the shaft, a free fat transplant was interposed to prevent healing of the defect. After two months the fat was removed and periosteum placed over the wound site. Strong bony union resulted, although the authors did not specify how long this took.

Further, Ritsila and Alhopuro reported that transplantation had been performed in the physiological maxillary defect of the growing rabbit. Again, new bone formation was noted, but there was simultaneous growth disturbance of the upper jaw, This proved to be due to surgical stapling of the premaxillo-maxillary suture, incidental to the experimental procedure.

1.3.4.2 PERIOSTEAL GRAFTS AND THE REPAIR OF TRACHEAL DEFECTS

Ritsila and Alhopuro (1973b) published the results of their experiments to reconstruct tracheal defects in growing rabbits, using free periosteal grafts. These autografts, obtained from the tibia, were placed over an artificial cartilage defect created by resecting the anterior parts of the fourth to eighth tracheal rings (Fig. 3). Neo-osteogenesis was constant, and a supporting ring of bone was deposited. Where separate periosteal grafts were used, multiple bony rings were obtained.



(AFTER RITSILA & ALHOPURO)

Diagrammatic representation of operation site to show tracheal rings resected (R).

Fig. 3

The principle has been used for tracheal reconstruction in human patients (Kufass and Pasila 1974). They used free periosteal transplants taken from the tibia in three patients and placed them over the tracheal defect. They reported that neo-osteogenesis occurred in the trachea of one patient (aged two years) within seven weeks and in the second (aged one) within three months. In the third patient (aged twelve), the trachea was described as being stable and showing no sign of collapse after seven months. On bronchoscopy, the only abnormality observed was granulation tissue on the posterior tracheal wall. "Plain x-rays", wrote the authors, "showed probable ossification in the transplant". However, in this patient, a cannula had been necessary following trauma and its removal was prevented by a cricoid stricture. As the third patient was older than the others, it was suggested that age may have been partly responsible for the slower repair. Nevertheless, Kufass and Pasila suggested that clinical application of this procedure should be continued in infants and children.

1.3.4.3 PERIOSTEAL GRAFTS AND SPINAL FUSION

Further work demonstrating the apparent osteogenic power of periosteum included studies of spinal fusion with free grafts. Ritsila and Alhopuro (1975) produced sound bony fusion in both the thoracic and lumbar spines of young rabbits, resulting in local narrowing and "wedging" of the intervertebral spaces, followed by retardation of growth and "wedging" of the vertebrae.

Ritsila, Alhopuro and Snellman (1977) applied the findings of this work to treating experimental progressive scoliosis in growing rabbits. By producing spinal fusion with free periosteal grafts, they found that deterioration of the deformity was prevented. If performed early, the authors concluded that gradual straightening of a slight or moderate scoliosis was possible.

Further, Snellman, Osterman and Ritsila (1977) reported the use of free periosteal grafts in spinal fusion for the treatment of scoliosis and spondylolisthesis* in 55 patients of mean age of 14.5 years.

Biopsies were taken in a second operation, and bone formation was found to be"stable and compact" after six months. The bone formed in the fusion area was found to have occurred by endochondral ossification.

* *A forward displacement of one vertebra upon the vertebra below usually occurring between the fifth lumbar vertebra and the sacrum. The condition is usually due to injury or to a congenital defect of the vertebra.*

However, the results of Friedlander *et al.* (1979) were not so convincing. They aimed to determine the role of free periosteal grafts in arthrodesis* by attempting to produce spinal fusion in rats. This was performed by transplanting autogenous periosteum which was taken from the femur and grafted to either the lower thoracic or upper lumbar vertebrae. Also, Friedlander and his co-workers wished to see if the transplants remained viable and retained their osteogenic potential.

Histological and microradiographic examination revealed that neo-osteogenesis did not occur until twelve weeks postoperatively. A solid arthrodesis was not achieved, and the small amounts of bone detected after that time were described as being "invariably heterotopic". In one third of the animals, radiographs demonstrated that a solid bone bridge had formed at the site of periosteal transplantation. From this observation it would appear that the grafts retained a degree of osteogenic potential in a proportion of the animals.

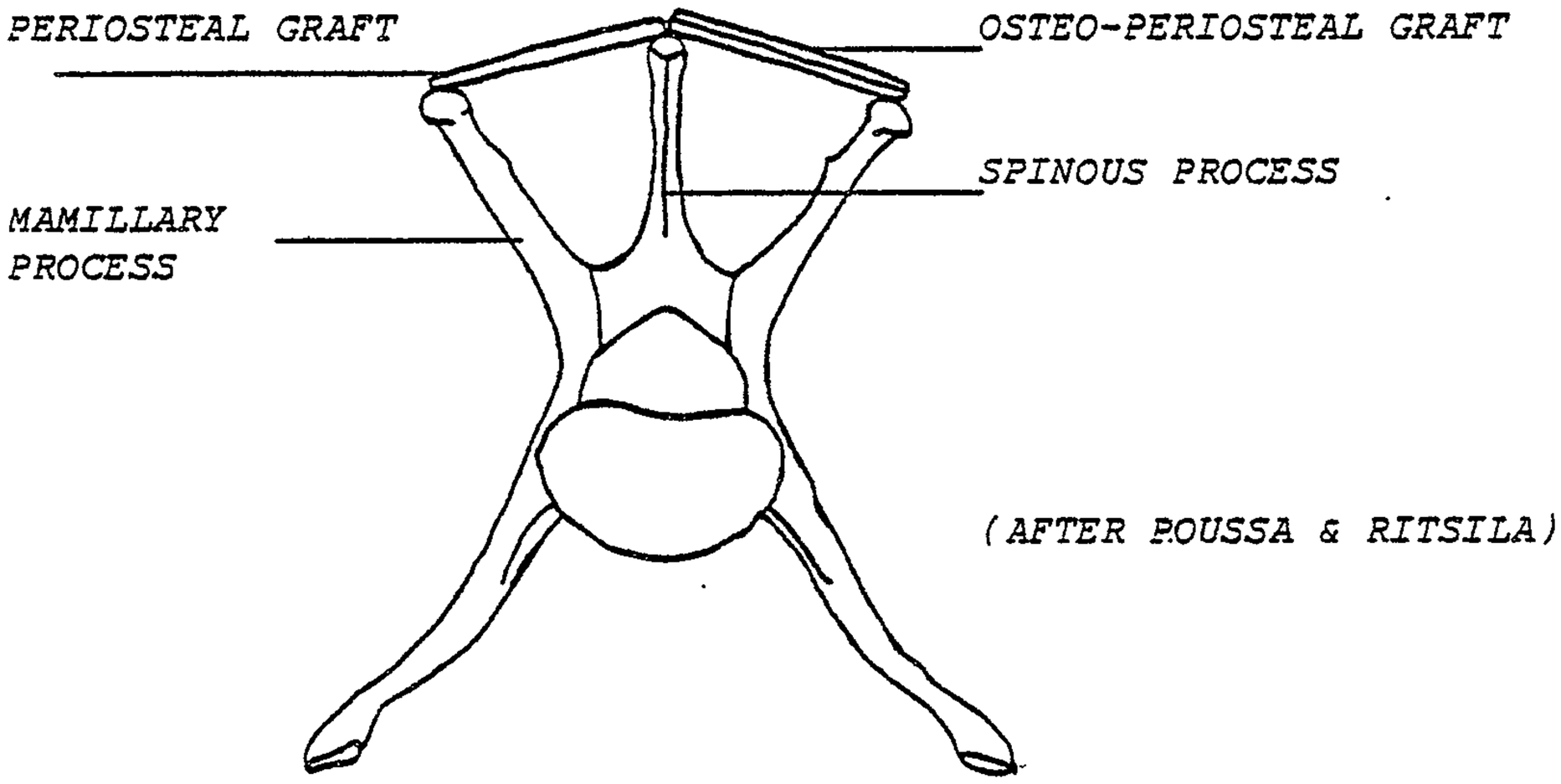
In comparing the results of Snellman, Osterman and Ritsila (1977) to those of Friedlander's group, it should, of course be remembered that species differences may account, at least in part, for the conflicting findings.

* *Surgical fusion of a joint.*

1.3.4.4 OSTEOPERIOSTEAL GRAFTS

Repair of skull defects using outer table osteo-periosteal grafts was reported by Santoni - Rugiu (1969). Twelve patients aged from 23-41 years had acquired the defects through various forms of trauma which included was wounds, road traffic accidents, and necrosis of bone flaps after neuro-surgery. The progress of ten of the twelve was monitored for one year post-operatively, and complete uneventful recovery recorded. It is interesting to note that the author emphasised the importance of ensuring the dura mater was not damaged during surgery (cf Uddstromer and Ritsila 1979, see page 35).

Poussa and Ritsila (1977) compared the osteogenic capacity of periosteal and osteoperiosteal grafts in six weeks old rabbits (Fig. 4). Each osteoperiosteal graft included a layer of bone 200 microns thick. Both kinds of transplant were taken from the tibia and placed on either side of the same lumbar vertebrae between the spinous and mamillary processes. The animals were examined at varying periods up to 28 days post-operatively. Results showed that the periosteal grafts formed a greater amount of bone and, more quickly, than did the osteoperio-steal grafts. However, the bone formed by both types of transplant was observed to originate in the cambial layer of the periosteum.



Lumbar vertebra to show the periosteal and osteo-periosteal grafts sutured between spinous and mamillary processes.

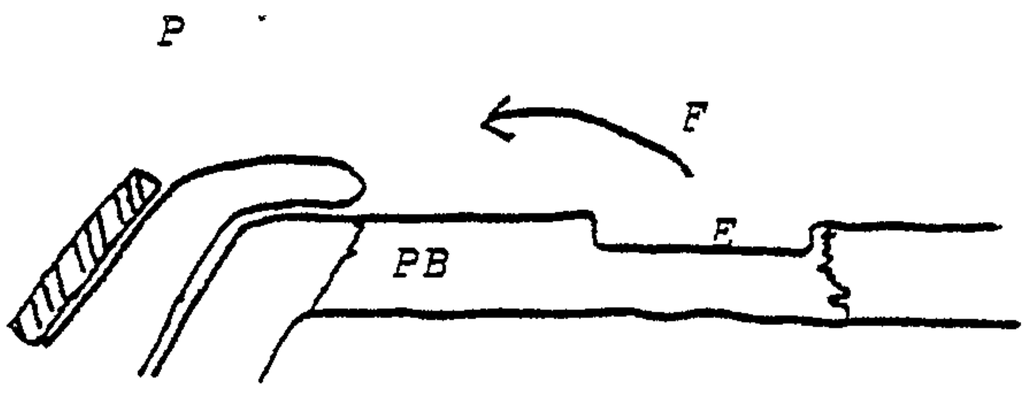
Fig. 4

Further, Poussa and Ritsila (1979) reported that after longer periods (42 and 84 days), no difference could be observed in the progress of osteogenesis. The main feature differentiating events at these longer times was the remodelling of woven bone to form compact bone seen at 84 days.

As a result of the above findings, Poussa, Rubak and Ritsila (1980) investigated the possibility of a thinner bone layer being more conducive to bone formation. Using the same technique as before, they reported that the osteogenic capacity of free periosteum and osteo-periosteum with a layer of bone 100 microns thick was about the same. However, in the same experiment, they also transplanted periosteum with full thickness cortex which was found to have an inferior osteogenic capacity to the other two types of graft examined.

The osteogenic capacity of combined osteoperiosteal-periosteal flaps in the parietal bones of twelve adult rats were studied (Melcher and Accursi 1971). The paired combination flaps, swung posteriorly, were buried in separate pockets of trapezius muscle (Fig. 5). The animals were sacrificed either two or four weeks later. Histological study under the light microscope showed that new bone was deposited subperiosteally in the osteoperiosteal portion of all the flaps. The quantity of new bone deposited was not significantly influenced by the thickness of the bony component of the osteo-periosteal flap. Melcher and Accursi suggested that these observations could have implications in the planning of oral and periodontal procedures in adults.

POSTERIOR *ANTERIOR*



O *(AFTER MELCHER & ACCURSI)*

O = Osteoperiosteal flap
P = Periosteal flap
F = Flap turned back
PB = Parietal bone
E = Cavity created when flap turned back

Fig. 5

1.3.4.5 GRAFTS AND CALCITONIN

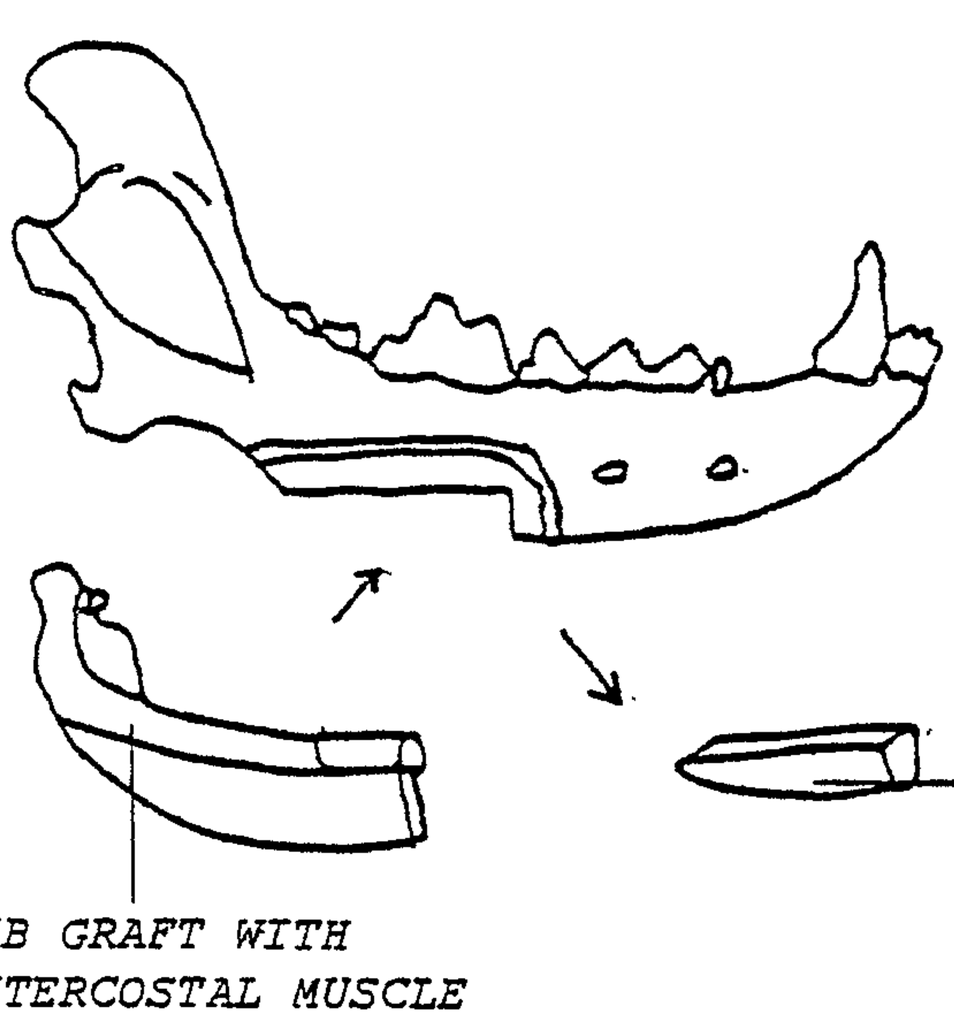
A radiographic study on the influence of periosteum and calcitonin on onlay bone graft survival was reported (Knize 1974). Iliac bone autografts were placed on the nasal bone complex in rabbits, some with periosteum preserved and some with periosteum excised. Where periosteum was retained, the grafts tended to resorb more slowly. The administration of calcitonin appeared to enhance the prospects of the graft both surviving and not being resorbed, whether or not periosteum was present.

Using young rabbits, Golan *et al.* (1976) autotransplanted free tibial periosteal grafts into muscles of the leg and the floor of mouth. Serial radiographs of the experimental sites were taken, followed by histological examination after sacrifice.. Neo-osteogenesis was observed in all animals. The influence of calcitonin on the bone mass was also investigated, but, in contrast to the results of Knize (1974), the hormone could not be shown to have any effect.

1.3.4.6 FREE VASCULARIZED BONE GRAFTS

In the replacement of bone lost from trauma, tumour, infection or certain congenital abnormalities, conventional bone grafting is not always successful. In recent years, other techniques have been tried which have revolutionized bone grafting.

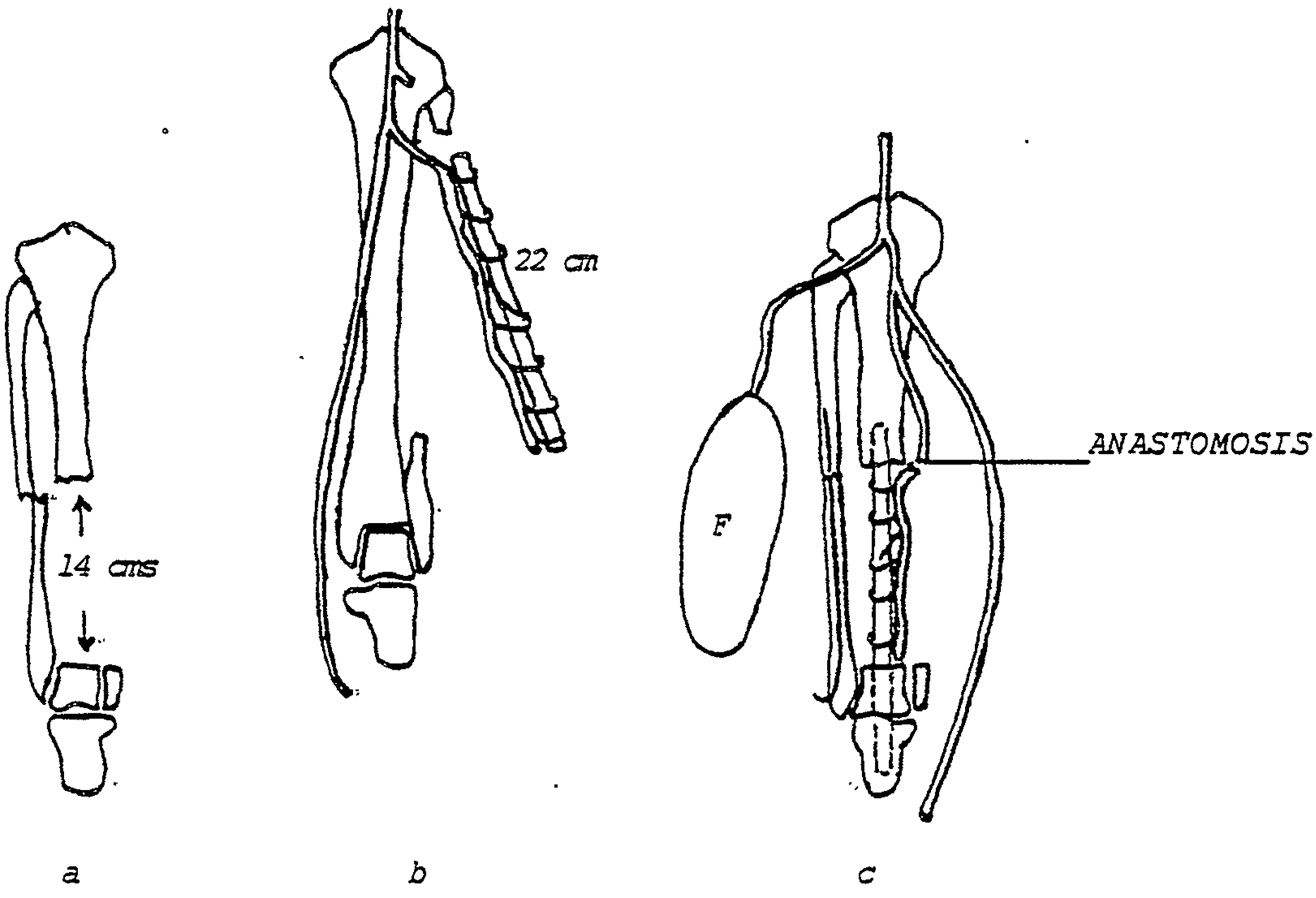
Ostrup and Fredrickson (1974) autotransplanted a portion of rib with adjacent intercostal muscle and pleura to restore a mandibular defect in each of ten dogs, (Fig. 6). The blood supply of this composite graft was reconstituted by microvascular anastomoses of the intercostal vessels to the lingual vessels. The same operation was performed on a further six control animals without the microvascular anastomoses. In eight of the ten experimental animals, the graft was successful. However, only one of the six grafts survived where anastomoses were not performed.



The defect in the mandible of the dog with rib graft about to be positioned. The mandibular periosteum around the defect was excised to eliminate any possible neo-osteogenesis from that source.

Fig. 6

A report extending the use of microvascular techniques in a free vascularised bone graft to help two patients faced with amputation of a limb appeared the following year (Taylor, Miller and Ham 1975). The graft was taken from the contralateral fibula in each. In one of the patients, no patent



(AFTER TAYLOR, MILLER & HAM)

CASE 1. Diagram of the Free Vascularised Bone Graft Transfer.

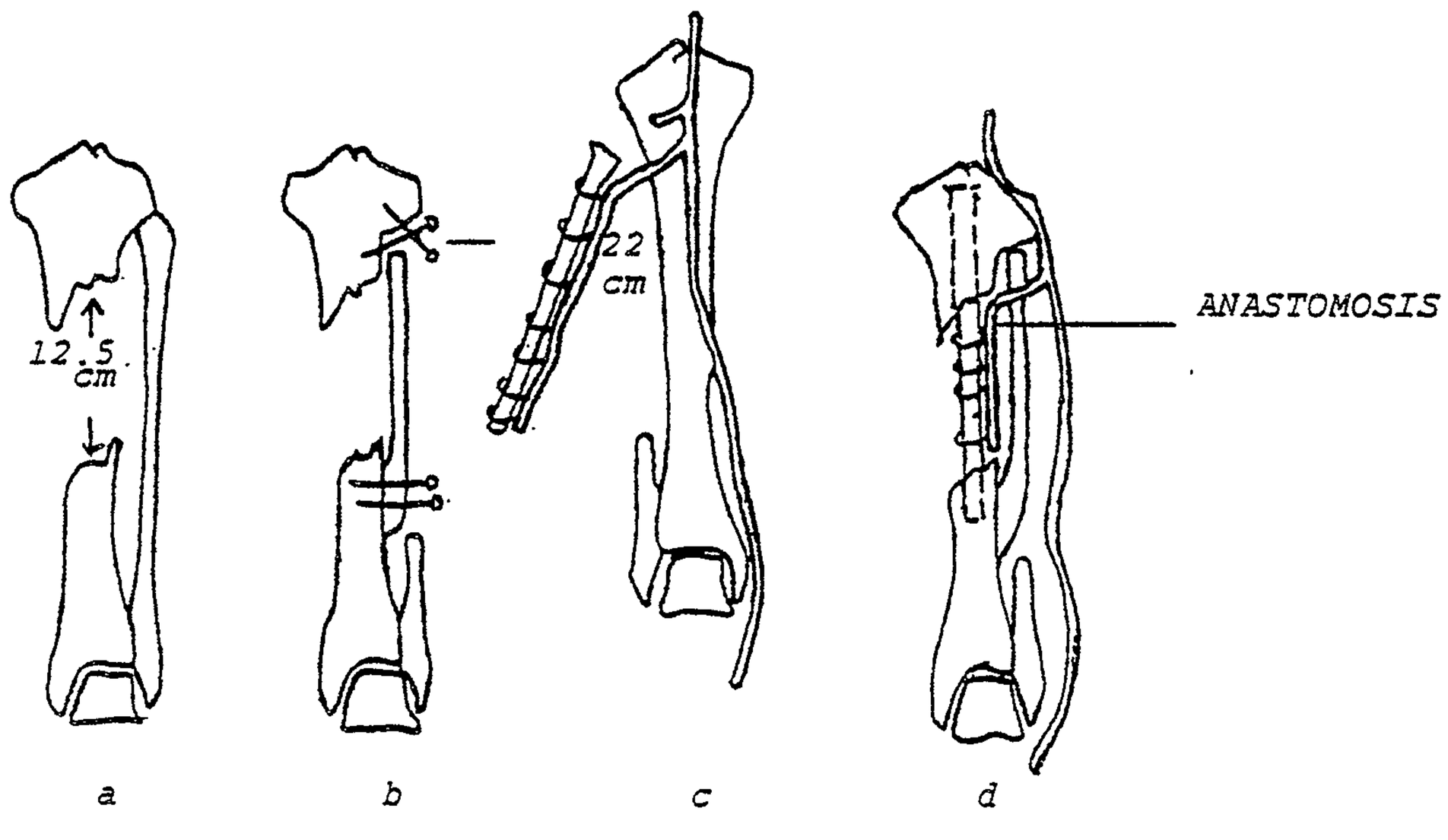
a. The bone defect.

b. Isolation of the fibula on its pedicle in the donor leg.

c. The anastomosis of the composite fibular graft. The free soft tissue flap (F) was necessary to restore a large skin defect follcwing injury and was successfully performed in a separate operation prior to transfer of the free vascularized bone graft. However no patent anastomosis could be demonstrated 14 weeks post-operatively.

Fig. 7

anastomosis was demonstrable after 14 weeks but in the other, an angiogram demonstrated success of the surgery six weeks post-operatively (Figs. 7 and 8).



(AFTER TAYLOR, MILLER & HAM)

Case 2

a. The bone defect.

b. Initially, repair was attempted by transposition of the ipsilateral fibula, but this was unsuccessful.

Union at the upper end of the fibula failed to occur and a pseudarthrosis (arrow) developed.

c. The fibula was taken from the opposite leg, and transplanted to the site of the defect. The fibula is shown isolated prior to grafting.

d. The free bone graft in position. The anastomosis is arrowed, and was shown to be patent six weeks after surgery.

Fig. 8

Finley, Acland and Wood (1978), referring to the previous studies, stated that there were limitations on the size and configuration of such free vascularised grafts. Thus they proposed the use of revascularised periosteal grafts, by means of which neo-osteogenesis may occur. In nine dogs, they transplanted rib periosteum to the groin, and each specimen was divided in half, making a total of 18 grafts. In nine of these tranplants, no micro-vascular anastomoses were created but in the remainder, the intercostal vessels were anastomosed to the local muscular vessels. Examination at six weeks revealed that where no anastomoses had been performed, the grafts had been resorbed. However in those animals where vascular patency had been confirmed, substantial new bone had formed.

In the second part of the experiment, Finley, Acland and Wood created full thickness defects in the tibiae (including periosteum) of ten dogs. In five experimental animals, a periosteal autograft from the rib was sutured in place around the defect and vascular anastomoses created between tibial and intercostal vessels. In the remaining five animals, which acted as controls, the wound was closed and fixation was provided. The animals were sacrificed at varying periods post-operatively. Results showed that, in the experimental animals, the tibiae had regenerated within six weeks and were weight bearing: the dogs were ambulant. However, in the control animals, healing had not occurred within two months.

In a further communication, Acland (1978) suggested that, using their techniques, mechanical stress may be a necessary adjunct to neo-osteogenesis. Further, it had been found in similar canine studies that new bone had been formed in the ulna but not in the cranium, and until results of this subsequent work had been fully evaluated, caution was advised prior to clinical application of their methods.

Puckett *et al.* (1979) attempted to compare the above results with those achieved in traditional bone grafts in unstressed bone. Using three groups of dogs, these researchers found that revascularised periosteum did not produce a significant amount of bone. At four months, revascularised rib grafts had a slightly greater tolerance to stress at the callus site than traditional bone grafts, but the incidence of non-union was the same. The authors concluded that the use of revascularised bone grafts should be reserved for situations in which traditional bone grafting techniques were unlikely to be successful.

Van den Wildenberg *et al.* (1983) compared the osteogenic capacity of revascularised periosteal grafts from different sites in 56 African pygmy goats. After securing external fixation to provide satisfactory immobolization, a length of tibial midshaft was excised. Radiological examination revealed that revascularised grafts of tibial periosteum firmly bridged the defect within eight weeks but

revascularised grafts of costal periosteum did not.
In a letter to the British Journal of Plastic Surgery concerning the clinical use of revascularized periosteal grafts, Goris and Van den Wildenberg (1983) expressed their belief that, although the behaviour of such transplants was not fully understood, the grafts had great clinical significance and there should be no discouragement where procedures, which had been done for good clinical and surgical reasons, had failed.

1.3.4.7 COMBINATION GRAFTS

In this section, miscellaneous grafting experiments which illustrate periosteal behaviour are described. These have been collectively referred to as combination grafts.

De Bruyn and Kabisch (1955) investigated bone formation by fresh or frozen and autogenous or homogenous transplants of three types: bone, bone marrow and periosteum. New Zealand white rabbits were used.

Of the three fresh autogenous grafts, they found that periosteum resulted in osteogenesis in the smallest percentage (58) of samples. However, the differences in the incidence of bone formation in the various experimental animals could not be shown to be statistically significant, and thus it was provisionally concluded that fresh autogenous transplants of bone, bone marrow and periosteum had osteogenic potential.

In contrast, the incidence of bone formation by frozen autogenous transplants, fresh homogenous transplants and frozen homogenous transplants was much lower, varying from zero to eight per cent.

In fresh autogenous periosteal transplants, histological examination provided no clue as to the origin of the osteogenic cells. However, osteogenesis by similar autogenous transplants of bone was thought to originate from surrounding connective tissue. This suggested to the authors that the transplant released an osteogenic inductor causing the transformation of mesenchymal cells into osteogenic elements.

De Bruyn and Kabisch (1955) found that by freezing autogenous periosteal transplants, the bone forming activity of the grafts was destroyed, possibly by killing osteogenic cells. This received some support from Wolfgang Axhausen (1956) who histologically examined autogenous frozen bone grafts covered with periosteum in the dog. Axhausen observed necrosis of the periosteum, and he regarded this as proof of the fact that the osteogenic tissue was completely killed by freezing.

However, in the same publication, Axhausen reported that new bone formation took place as long as 53 days after the grafting was performed. His conclusion was that transformation of "non-specific connective tissue" of the host into osteogenic tissue must have taken place.

"Consequently", he wrote, "the osteoblast and induction theories are combined into a unified concept".

Axhausen summarised his thoughts by stating that bone regeneration occurred in two phases: the first and physiologically more important phase originated in the pre-existing specific cells and began after several days; the second, originating in the non-specific connective tissue, commenced after several weeks. However, he considered that both phases were stimulated by the activating influence of bone necrosis.

With this concept of two-phase theory in mind, Gray and Elves (1982) investigated the relative osteogenic contributions of different cells (bone marrow, endosteum, periosteum and osteocytes) during the first 16 days after implantation of cancellous autografts. Using a radioactive strontium labelling technique to measure neo-osteogenesis in rats, grafts including the various cell types from the ilium * were placed subcutaneously. The results showed that the endosteal lining cells together with narrow stroma contributed 60 per cent of the new bone formed, and the periosteal cells 30 per cent. Neither the free haemopoietic marrow cells nor the osteocytes were found to have made any significant contribution.

The role of the periosteum on the fate of pedicle osteocutaneous grafts has been investigated by Canalis,

* *the lateral flaring portion of the hip bone.*

Hemenway and Ward (1979). Using dogs, these researchers attempted to clarify the role of periosteum in the survival of pedicle assisted bone grafts. Three types of procedures were performed.

First, a pedicle osteocutaneous graft was developed by raising a forehead flap on one side and suturing its distal end to parietal muscle fascia. The bony element consisted of the anterior table of the frontal sinus.

Second, and on the other side, a pedicle periosteal graft developed in an identical manner to the first procedure, except that only periosteum was sutured to the fascia.

Third, the remaining anterior table of the frontal sinus was autografted to under the skin of the calvarium. This was a control.

The specimens were evaluated at regular intervals over a 40 week period and the findings were as follows. The free bone grafts (i.e. the anterior table of the frontal sinus) were resorbed, and no osteogenesis from the pedicle periosteal grafts was observed. In contrast, the pedicle osteocutaneous grafts maintained their viability and progressive, vigorous neo-osteogenesis was observed. Eventually partial substitution of the original graft by new bone of periosteal origin was noted, and it was stressed that the periosteum had been shown to have the leading role in the restructuring process of these grafts.

The ultimate purpose of animal studies is, of course, to find techniques which can be used for the treatment of human patients in conditions for which there is no treatment or the available treatment is unsatisfactory. In addition, deeper understanding of how established techniques work may be obtained. Such an example is the clinical problem of congenital tibial pseudarthrosis. King (1976) performed some experiments on an unspecified number of dogs to try to find a solution to the management of this condition. Having accepted that periosteum had osteogenic properties, King raised pairs of pedicled periosteal flaps from the subcutaneous tissue of the tibia. These flaps were tubed and anastomosed. After developing the surgical technique, King reported that in the last 24 animals operated upon, one in three of the tubes had ossified completely.

Later in the experiment, pseudarthroses were induced in the middle third of the tibiae of a further ten animals in order to see if hypertrophy would occur under the stress of weight bearing. It was found that in each of 24 specimens examined, some hypertrophy of the bone had occurred. In three of the specimens where the graft had been placed in parallel with the pseudarthrosis, this hypertrophy was associated with complete ossification of the transplant, by-passing the pseudarthrosis.

Erol and Spira (1980) provided support for King's work following the publication of the results of a study

on complete periosteal bone regeneration after subtotal ostectomy in animals. A segment of whole tibia was excised from each of six female pigs. The metaphyses and the periosteum were both kept intact, the latter being sutured back into position. In a seventh animal the periosteum was removed, otherwise being treated in the same manner as the first six. Examination using radiographic, microangiographic and histological techniques was carried out. Within three months, bone regeneration was found to be complete and uniform in the experimental group, but disorganised and incomplete in the control.

Thompson and Lewis (1976) assessed the role of free periosteal grafts in improving the survival of autogenous onlay bone grafts. They used two groups of rabbits. In the first, they placed iliac bone autografts with attached periosteum on to the nasal bones. In the second, they applied iliac bone autografts denuded of periosteum to the same site, but this time placed overlay grafts of free nasal bone periosteum on the transplants. Serial radiographs over a twelve month period, plus histological examination at the end of that time, suggested that the grafts including free periosteum survived better. This was attributed to increased rapidity of vascularisation and osteogenic activity in the periosteal osteoblasts of the second group.

Thompson and Lewis suggested that clinical application of these findings might allow accurate contour sculpturing of onlay bone grafts, while in no way impairing graft survival.

1.3.5 REPAIR OF THE CLEFT PALATE : THE ROLE OF PERIOSTEUM

Free periosteal grafts have been used in the primary repair of congenital maxillary clefts. Ritsila *et al.* (1972b) reported on a series of eleven patients who were all operated upon between the ages of eight and ten weeks. The periosteum was taken from the tibia. They wrote: "The bone forming capacity of free tibial periosteum when transplanted to the maxillary cleft is undoubted. After two weeks there is callus in the area of transplantation, and after six weeks definite bone can be observed in the area." Ritsila *et al.* pointed out that the advantage of a free periosteal transplant over a bone graft was that it did not enlarge the operation significantly; also, the periosteal grafts were easily available and, most important, the baby was caused "no trauma worth mentioning". They also stated that "it was unnecessary to detach maxillary periosteum which, as well as being technically difficult, may cause disturbances to normal periosteal bone growth".

Further, Ritsila, Alhopuro and Rintala (1976) reported on the use of free tibial periosteal grafts being used in the repair of 26 congenital maxillary clefts. In 23 of these, rapid bone formation occurred at the transplantation site overbridging the bone defect. No conclusion on the permanent value of these bridges was presented, but the authors pointed out that the strong osteogenic capacity of the free periosteal grafts

had been well demonstrated. It is particularly interesting that teeth were observed to have erupted through the bridges of bone in some patients.

An earlier researcher in the use of periosteal flaps in the repair of clefts was the late Tord Skoog. He reported (1965) an operative technique for reconstruction of both complete and incomplete clefts to the primary palate (Fig. 9). Periosteal flaps were used to create bony continuity between the premaxilla and the lateral maxillary segments. The technique also aimed to prevent maxillary collapse and contribute to the restoration of the symmetry of the alveolar arch. In the eight cases treated, the results after six months were described as very promising. However, Skoog stated that it was not possible to assess the long term advantages of the procedure.

Rosselli (1982) advocated closure of bilateral cleft lip and palate in one stage using a free periosteal graft from the tibia. Pointing out that two operations were usually performed a few months apart (first one side, then the other), he considered that there were a number of disadvantages which included the following.

First, there was a greater chance of deviation of the nasal septum - premaxilla.

Second, there were greater dangers in repeated periosteal grafting in the same place after so short a time.

Third, there was an increased chance of finding unequal results in the osteogenesis and healing of the two sides.

Fourth, when the free periosteal grafts were taken from opposite tibiae, two scars were produced instead of one.

By carrying out the repair in one procedure, Rosselli held that bone was deposited more evenly on the two sides and better stability of the segments was obtained. Further, he hoped it would avoid any possible complications arising from enforced prolongation of the interval between operations.

In conclusion, although Rosselli stated that he had sometimes been dissatisfied with the results of the two stage procedure, he conceded that the issue was unresolved and that it was wise to wait before passing unconditional judgement.

Hrivnakova *et al*. (1983) published a comparison of maxillary growth in clefts after primary bone implantation and after bridging a gap with a periosteal flap. The bone used for transplantation was rib. The periosteal flap technique was similar to that employed by Skoog. Two hundred and forty five patients received bone grafts compared to the 183 in whom the periosteal flap technique was performed. The authors concluded that on the basis of clinical tests, radiographs and examination of study models, distinctly better overall results were obtained

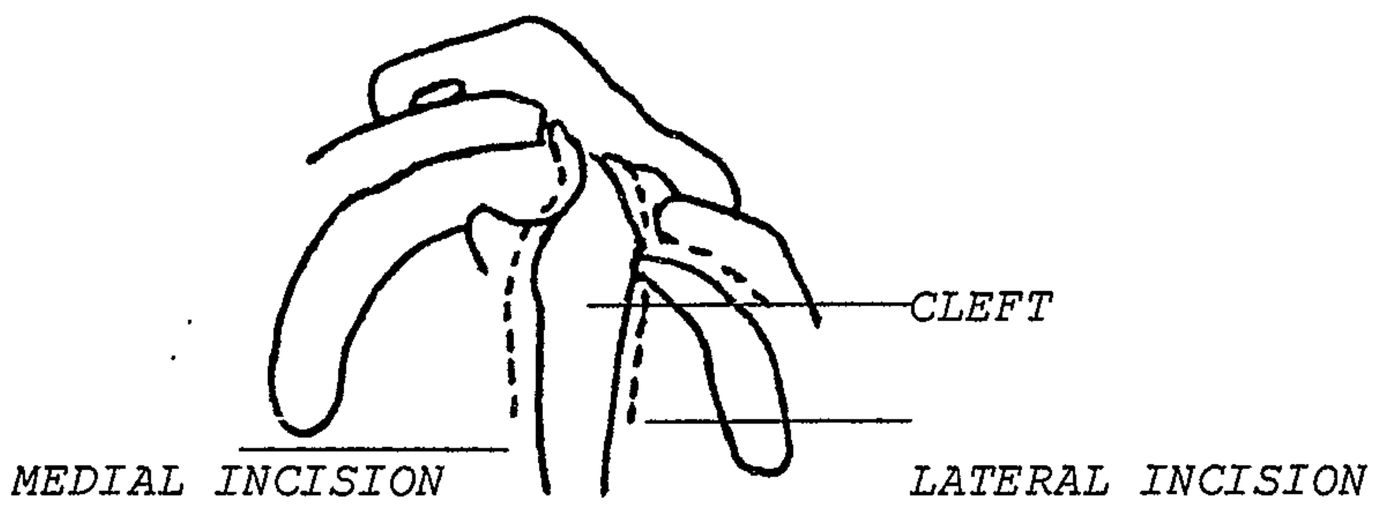
with the latter. They also stated that, in their experience, not only was the periosteal flap technique better than primary bone implantation, but it was also better where no bone graft had been used at all.

1.3.5.1 OXIDISED CELLULOSE AS AN ADJUNCT TO CLEFT PALATE REPAIR

An extensive paper was published reporting on the use of periosteum and oxidized cellulose (Surgicel TM) for bone restoration in congenital clefts of the maxilla (Skoog 1967). The technique involved the placement of periosteum to periosteum all round the cleft (Fig. 9) The osteogenic capacity of the periosteum was stated to be indisputable, and in the 83 clefts operated upon, significant maxillary collapse never occurred. Furthermore, it was found that the stability of the maxillary segments induced by the periosteal repair was such that appliances to prevent medial collapse of the lateral segment were not required postoperatively.

Where greater amounts of periosteal bone were needed, it proved necessary to keep the flaps suitably separated by using Surgicel as a scaffold. The oxidised cellulose proved to be satisfactory in all respects and clinically improved cosmetic results were claimed.

Engdahl (1972) studied bone regeneration in maxillary defects in 320 rabbits. At the age of three weeks, these animals were subjected to a standardized unilateral maxillary resection including the premaxilla-maxillary suture. The material was divided into groups according to the periosteal lining of the resection cavity: the membrane was either left intact, partially excised or



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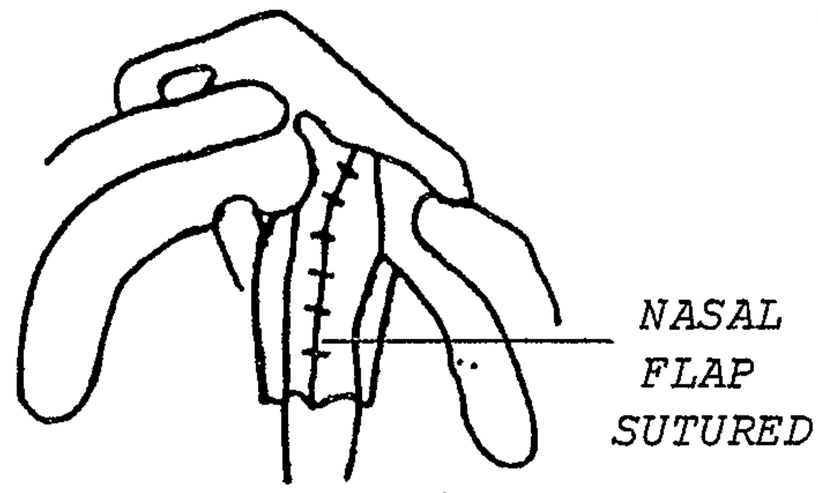

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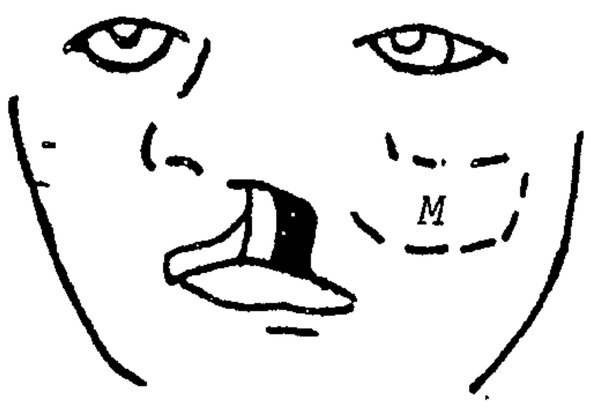

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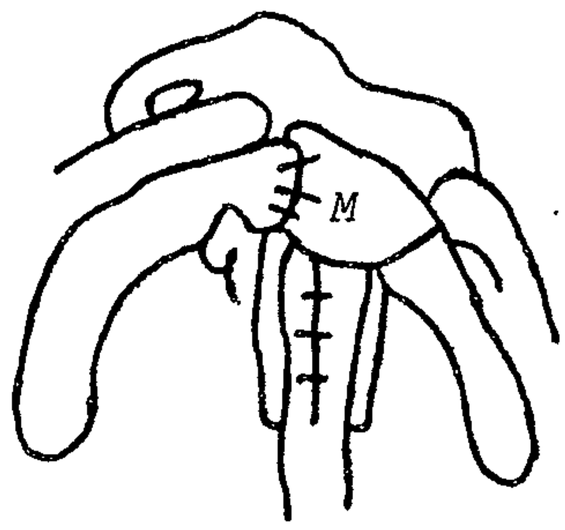


d

The operative procedure designed to create periosteal continuity across the maxillary cleft, used by Skoog.

a. *Lateral aspect of the cleft. The incision is made along the border of the defect from between upper and lower nasal cartilages anteriorly to a point approximately two centimetres behind the alveolus.*

   The lateral border of the piriform aperture and the maxillary cleft are exposed. A mucoperiosteal flap is raised from the inner aspect of the bone which is advanced mesially.

   Medial aspect of the cleft. The incision is made from the vomer, over the anterolateral aspect of the premaxilla and towards the anterior edge of the septum. The periosteum is carefully elevated and the mucoperiosteal - perichondrial flap mobilized.

b. *The medial and lateral flaps are sutured with 3.0 Chromic catgut and tied on the nasal side.*

c. *Periosteum covering the anterolateral aspect of the maxilla is mobilized.*

   This flap is based medially along the infra-orbital margin and on the lateral aspect of the nasal pyramid.

   A periosteal flap is raised by incising laterally below the infra-orbital foramen. The original excision for exposure in the vestibulum is extended laterally to meet this upper border.

d. *The anterolateral maxillary flap (M) is rotated almost 180° to its position across the cleft and sutured to the periosteal edge on the anterior aspect of the premaxilla. Thus periosteal continuity is created on both aspects of the maxillary cleft. The use of double periosteal flaps aimed to maximise the prospects of subperiosteal bone deposition. The principle of the technique has been applied to both complete and incomplete clefts.*

Fig. 9

completely excised. The bone defect was filled with a variety of media: blood, Surgicel, bone marrow aspirate and rib bone grafts.

The results demonstrated the great importance of the periosteum in bone regeneration. When the resection cavity was completely lined with periosteum, reconstitution of virtually normal anatomy occurred "to an amazing degree". Partial periosteal lining produced inferior results. With total periosteal excision, hardly any bone regeneration was noted. Where the bone defect was filled with either blood or bone marrow aspirate, rapid satisfactory bone formation was found to have occurred. Surgicel delayed bone formation and seldom was the resection defect repaired fully. Bone grafting of the wound rapidly produced a large amount of new bone, mainly independent of the periosteum.

Rintala *et al.* (1974) reported the results of the operation performed on the alveolar clefts of 63 children. Fifty four had defects in both the primary and secondary palate, and the remaining nine in the primary palate only. The procedure used was the maxillary periosteal flap technique introduced by Skoog (Fig. 9).* Surgicel was applied in 40 of the cases. The results are shown in Table 1.

* *See page 66*

	Overall	Surgicel Used	No Surgicel Used
Number of patients	63	40	23
	%	%	%
Distinct bone bridge	54	53	56
Diffuse bone bridge	22	22	22
No Bone	24	25	22

Table 1. Results are expressed as percentages of the number of patients.

The presence of Surgicel did not appear to influence the deposition of bone.

In a study which included evaluating the effect of the periosteal flap technique (Skoog 1965) on cleft width, Ranta *et al.* (1974) found that patients with a well formed bridge of bone between the alveolar segments displayed at least the same degree of narrowing of the cleft as those in which no neo-osteogenesis had been established radiographically. These observations were based on following 36 patients with unilateral complete cleft lip and palate. In 24 of these cases, Surgicel was applied, and the cleft narrowed by an average of 3.0 millimetres. In the remaining twelve cases, cleft narrowing averaged 2.6 millimetres.

Closure of primary palatal clefts in twelve infants using Skoog's technique was reported by O'Brien (1970). There were ten unilateral clefts and two bilateral. In some cases, Surgicel was used. He found that bone formed spontaneously in the primary cleft, was evident within six months and its quantity increased with time. Moreover, satisfactory alignment of the alveolar arch was achieved. Further bone deposition followed

subperiosteal implantation of Surgicel at the age of one year in four cases, with lessening of the asymmetry. No interference with maxillary growth was observed.

O'Brien concluded that full evaluation of his results would take a considerable period of time, but that the preliminary findings were promising.

1.3.5.2 INHIBITION OF MAXILLARY GROWTH BY SURGICAL INTERVENTION

The frequent clinical observation that maxillary growth may arrest following various forms of cleft palate surgery prompted Freng (1979a) to investigate the restorative potential of mucoperiosteum when made to cover both nasal and oral sides of a midpalatal defect.

The growing domestic cat was selected as a model since it had several points of similarity to human anatomy: a proper hard palate, continuous dental arches, a deciduous and permanent dentition and a "nearly equal skeletal construction". However, one experimental disadvantage was considered to be the relatively long period from birth to maturity. Rodents, which do not have this drawback, were considered, but rejected on anatomical grounds. A total of 45 cats were used, divided into three groups of 15.

The first group served as unoperated controls. All were observed to have open midpalatal sutures.

In the second group, a midpalatal cleft was created in each animal and the defect was covered by mucoperiosteum on both nasal and oral aspects. At maturity, complete osseous regeneration was obeerved in all animals, but only five were reported to have regained a normal looking midpalatal suture. The palatal halves in the remaining ten were observed to be united by bone bridges.

In each of the third group of cats, the oral mucoperiosteum was simply raised and replaced without resection of bone - this was an attempt to test the soft tissue reaction of the surgical trauma. In three of the animals, bone bridges across the suture were reported to have formed, similar to those described above.

The presence of bone bridges suggested to Freng that normal palatal growth might be retarded. He concluded that, since this experiment was a kind of periosteoplasty (as performed by Skoog 1965), there may be some support for the fact that maxillary growth may arrest following cleft palate surgery.

Several years prior to Freng's work, Kremenak *et al.* (1970) investigated whether or not mucoperiosteal denudation of the palatal shelf bone adjacent to the deciduous molars was the single surgical variable responsible for the inhibition of maxillary growth. Beagle dogs were used. In one group, a strip of mucoperiosteum approximately halfway between the midpalatal suture and the deciduous teeth was excised. In the other, as well as the mucoperiosteal strip being excised, the remainder of the membrane was elevated to the midline and the major palatine neurovascular bundle cut. The variables studied in the two groups had been selected due to the frequency of their being components of palatal cleft repair procedures in humans.

Analysis of the ensuing facial growth of the animals, especially the palatal shelf breadth, revealed that mucoperiosteal denudation of the palatal shelf bone adjacent to the deciduous molars did indeed appear to be the single surgical variable responsible for inhibition of maxillary growth.

In a study on the growth effects of different oronasal flaps, (Stauffer, Latham and Curry 1981), an experimental model was designed to test the hypothesis that the periosteum of the nasal cavity may have an inherent tendency for bone resorption, whereas oropalatal periosteum may have an inherent osteogenic character. Using mongrel puppies, the model was designed so that one of three different types of flap could be placed against a length of autogenous whole rib in a "standard alveolar environment". These flaps incorporated either palatal mucoperiosteum, nasal mucoperiosteum or buccal mucosa. Neo-osteogenesis predominated in the bone covered by palatal mucoperiosteum. However, resorption resulted under the other two types of flap, although this was initially slower under buccal mucosa.

It was concluded that the hypothesis under scrutiny was valid, and these findings may have important surgical implications in man.

1.3.5.3 ORTHODONTICS AND THE CLEFT PALATE

The relevance of orthodontics to cleft palate repair procedures was recognized by both the Finns (Ritsila *et al*. 1972b) and the Swedes (Skoog 1967). Indeed, Skoog reported that the deformity of his congenital cleft palate patients was recorded by both pre- and post-operative plaster models as well as cephalometric drawings made from radiographs whilst treatment progressed (1967).

The orthodontist working with Skoog was Hellquist who published extensive work on facial skeleton growth after periosteal resection in rabbits and guinea pigs (1972). One hundred and two animals (87 rabbits, 15 guinea pigs) were divided into four groups. Periosteum was removed from nasal and frontal bones, maxilla and premaxilla. Osteometric, radiographic and histological studies were performed, and it was found that periosteal resection always affected the subsequent growth of the underlying bone.

However, from his analysis, Hellquist concluded that removal of the periosteum in young growing rabbits had only slight influence upon growth of the facial skeleton, unless the maxilla was involved. In the latter case, growth changes ranging from slight to severe were observed since the blood supply to the periosteum was

unavoidably damaged on account of the specific anatomy of the rabbit. In the guinea pigs, facial skeleton growth was barely affected. Histological examination of resected periosteum from the left nasal bone and premaxilla - maxilla of 17 rabbits revealed that new periosteal connective tissue with osteogenic capacity was formed within ten days of surgery. Interestingly, after four months, the new periosteum was still thicker than that of the control side. Only after six months did its thickness tend to return to normal.

Further observations were made. The regenerated periosteum appeared to adhere more firmly to the underlying bone compared with the control side. Increased subperiosteal bone formation was observed after periosteal resection, new woven bone becoming histologically apparent within ten days of surgery.

The author considered that adjacent periosteal tissue with osteogenic potential regenerated within ten days, as mentioned above. In rabbits, Hellquist proposed that the rapidity of new bone formation may have been due to a long standing, increased vascularisation of the regenerated thicker periosteum. A marked increase in bone thickness was found on the operated side of the nasal bone and premaxilla-maxilla region from 18 days after the procedure.

In addition, Hellquist reported that in those rabbits where periosteal resection was confined to the premaxilla and anterior part of the nasal bone, increased appositional bone growth was seen on the anterior part of the maxilla from which the periosteum had not been excised. He pointed out that these findings agreed with the observations by other authors that the periosteal proliferative response to trauma extended to involve the adjacent intact periosteum.

Finally, Hellquist noted that changes in the facial skeleton of the rabbits and guinea pigs after periosteal resection demonstrated that decreased growth in one area could be spontaneously compensated for by overgrowth in other areas.

The influence of primary periosteoplasty on maxillary growth and, consequently, the occlusion of the deciduous dentition in cases of complete unilateral cleft lip and palate was investigated in children from infancy to the age of five years (Hellquist and Skoog 1976). Sixty-six patients with total unilateral clefts of the primary and secondary palate were operated upon, 36 of whom had periosteoplasty in conjunction with their repair procedure, and 30 of whom did not and served as controls.

New bone formation was reported to have occurred within the alveolar cleft of all patients in whom periosteoplasty had been performed. In about half, neo-osteogenesis was marked. With repeated periosteoplasty, bone formation was increased and new

bone bridging the cleft was found consistently.

Hellquist and Skoog concluded from this study that infant periosteoplasty was effective in restoring the bony framework across a cleft and that the procedure did not retard or impair growth of the maxilla up to five years post-operatively.

Subsequently, Hellquist *et al.* (1983) modified this opinion in the light of their findings of a longitudinal study of delayed periosteoplasty* to the cleft alveolus. The ages of the 35 patients (24 boys and eleven girls) all with clefts involving the maxillary alveolar process ranged from five to 17 years. The mean age at operation was 6.4 years. It was reported that, in patients younger than seven years at the time of surgery, good bone formation was found in 80 per cent after delayed periosteoplasty compared to 47 per cent after infant periosteoplasty. Further, the formation of new bone after delayed periosteoplasty continued for several years. No resorption of new bone was observed. When compared with Scandinavian cleft lip and palate patients who had been subjected to neither periosteoplasty nor primary bone grafting, no negative

* *Hellquist (1982) stated that primary periosteoplasty was carried out up to the age of two years in connection with primary surgery, whereas delayed periosteoplasty was performed at any time during the juvenile period.*

effect on occlusion or craniofacial growth was found after delayed periosteoplasty in patients up to the age of 17 years.

It was concluded that, in young individuals between the ages of four and seven years, delayed periosteoplasty might be the superior method for maxillary alveolar arch reconstruction.

1.3.6 PERIOSTEUM AND FRACTURE HEALING

The biology of fracture healing has been recently discussed in an excellent article (McKibbin 1978). Among the many relevant factors in this remarkable process, he quoted the work of Mulholland and Pritchard (1959) who stated that the absolute width of the bony gap and the tissues occupying this gap were important in the course and outcome of fracture repair. They created 174 rib and fibular fractures of known initial width in rats, and carried out an histological and radiographic examination of the results.

It was shown that the widest gap which would be bridged with new bone unaided was one millimetre in the fibula and two millimetres in the rib. However, leaving a periosteal bridge in rib fractures enabled gaps of eight millimetres to be united by bone. It was also found that a polythene tube, crossing the gap and ensheathing the fractured ends of the fibula, allowed gaps of seven millimetres to be spanned.

In a similar study, the influence of periosteal continuity on the mode and speed of repair of a bone gap was investigated (McClements, Templeton and Pritchard 1961). They found that in the presence of an intact periosteal bridge, gaps of any width in a rib were repaired inside three weeks. However, localized cautery damage in the periosteum inhibited neo-osteogenesis

indefinitely in the immediate vicinity of the injury. If a periosteal bridge were absent, it was found that a bony gap in the third metatarsal, initially two millimetres wide, was incompletely bridged with new bone in 20 weeks. Although the repair process was observed to be different in the rib and the third metatarsal, it was concluded that the presence or absence of a periosteal bridge was a decisive factor in determining the speed and mode of fracture repair.

Experimental osteogenesis at fracture sites and gaps was investigated in the fibulae of 120 rats (Narang and Laskin 1976). The aims were to investigate the repair of bone defects without grafting, and to determine the role of the periosteum in the repair. A variety of techniques was used to create the defects. The animals were sacrificed at varying intervals up to twelve weeks post-operatively. Clinical, radiological and histological studies revealed that, when either periosteum or bone contact or both of these factors were present following fibular fracture or creation of a fibular gap, neo-osteogenesis and remodelling of bone occurred. However, small and large fibular fracture gaps without periosteum led to non-union, interposition of fibrous tissue and lack of new bone.

Narang and Laskin concluded from their experiments that bone contact was more important than periosteum for normal healing. Nevertheless, where bone contact was not possible, the presence of periosteum assumed a more crucial role.

1.3.6.1 BLOOD SUPPLY OF BONE AND PERIOSTEUM IN RELATION TO FRACTURE HEALING

In man, the three systems of blood vessels (nutrient, metaphyseal-epiphyseal and periosteal) are present in all tubular bone (Trueta 1974). In man, and in the dog, the inner two thirds of the cortex of a long bone is supplied by branches of the nutrient artery. The periosteal circulation can maintain viability in long bones when the medullary circulation has been interrupted by displacement of fracture fragments or by surgery. (Rhinelander and Baragry 1962).

However, Macnab and de Haas (1974) reported that in the dog, at least 90 per cent of the cortex is supplied by the endosteal circulation. These researchers studied the role of periosteal blood supply in the healing of tibial fractures. They stated that in fractures, the longitudinal blood supply from endosteal vessels was interrupted, whilst the transverse blood supply from periosteal vessels sustained viability on both sides of the fracture site. It was found that the intact periosteum sealed the fracture gap and the periosteal vessels revascularized the distal fragment.

Macnab and de Haas concluded that the integrity of the periosteum was of vital importance in determining the rate of healing of a fractured tibia.

Further, using microangiographic and histological

techniques, Hulth and Olerud (1964) had noted hyperplasia of pre-existing periosteal vessels during a study of early fracture callus in normal and cortisone-treated rats. Similar observations, regarding an increased number of periosteal blood vessels, were made in a microangiographic and histological study of the healing of undisplaced closed fractures in adult mongrel dogs, although the medullary vessels were thought to be the dominant supply to the cortex in both the normal circulation and that supplying a healing fracture (Rhinelander and Baragry 1962).

1.3.6.2 GRAFTS OF BONE AND PERIOSTEUM IN FRACTURE REPAIR

That osteogenesis and vascularity were closely related was reported by Zucman, Maurer and Berbesson (1968) as a result of their study on the effect of autografts of bone and periosteum in recent diaphyseal fractures. Zucman *et al.* (1968) created single and triple level fractures in individual rabbit tibiae and either excised the periosteum or left it *in situ*. The types of autograft used were cortical onlay bone, cancellous bone or free periosteum. The periosteum was found to enhance union of the fractures, but the bone grafts led to no demonstrable osteogenesis. It would seem that this work was highly traumatic to the rabbits since over half of those operated upon died prematurely.

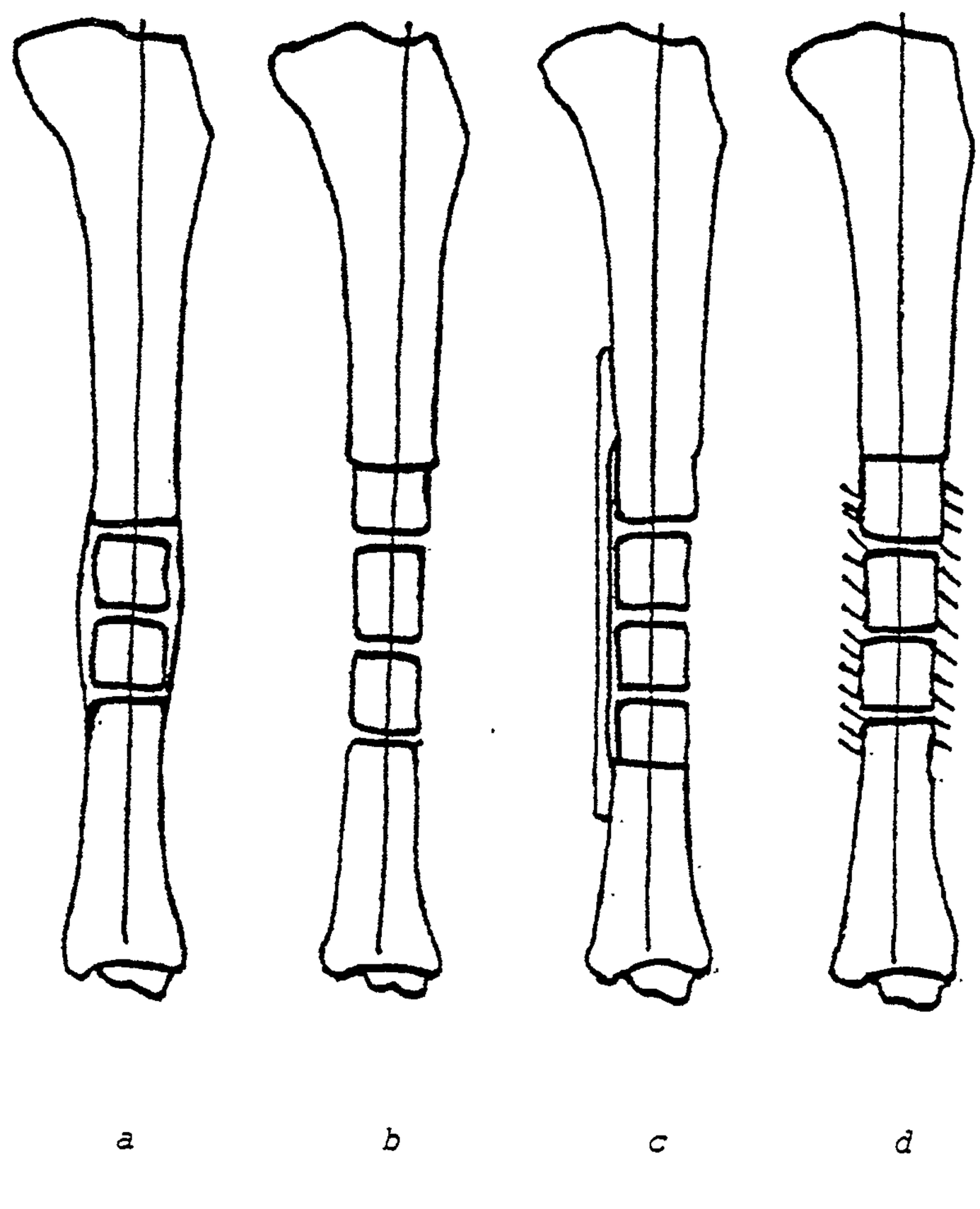
Following upon this work, Zucman and Piatier-Picketty (1970) studied the importance of bone marrow, periosteum and bone grafts in recent severe diaphyseal fractures in rabbits.

They found, first, that triple level fractures (Fig. 10) with excision of periosteum did not unite after three months.

Second, the addition of a cortical bone autograft bridging these fractures did not increase the rate of bone union.

Third, autografted cancellous bone chips did not lead to more callus formation, although bone marrow autografts did improve callus formation and increased the rate of union.

Fourth, the addition of free periosteal grafts led to complete bone union in more than 50 per cent of cases, but when an osteoperiosteal graft was placed, it seemed inactive.



(AFTER ZUCMAN & PIATIER-PICKETTY)

a. *Subperiosteal triple level fracture*
b. *Triple level fracture with wide excision of periosteum*
c. *Triple level fracture with cortical bone autograft*
d. *Triple level fracture with autograft of cancellous bone chips (shaded area)*

Fig. 10

1.3.6.3 MEDULLARY DESTRUCTION

Destruction of the medullary cavity of the tibia in rabbits and the femur in dogs by either reaming, brushing or suction was carried out (Danckwardt-Lilliestrom 1969). Examining the effect on the diaphysis, a number of conclusions pertaining to the periosteum were drawn.

First, the periosteal blood vessels were able to maintain circulation in practically the entire cortical vascular bed when the medullary vessels were destroyed.

Second, the periosteal vessels reacted with increased filling when injected with contrast medium. They also became tortuous.

Third, the periosteum reacted with increased bone formation; this occurred more rapidly in growing rabbits than adult rabbits. The periosteum formed either mature lamellar bone or woven bone. While deposition of the former was believed to be an acceleration or revival of normal osteogenesis, the immature bone was thought to result from callus formation promoted, at least in part, by bone marrow forced out of the medulla by the reaming.

Fourth, the medullary cavity was revascularised mainly from vessels which, in the diaphysis, penetrated the cortex from the periosteum.

Fifth, the periosteum was observed to react more intensively in the dog, and always formed some woven bone. This was not a regular finding in the adult rabbit.

The abundant blood supply from the periosteal vascular system appeared to be of enormous value. This seemed to confirm some of the findings of Zucman (1960) who studied the vascular connections between periosteum, bone and muscle in rabbits. Zucman reported that the periosteal vessels were in communication with vessels in the tibial cortex, and also had rich anastomoses between their segmental components.

However, a finding of much greater clinical significance was that a marked dilatation of the periosteal vessels was observed to occur if the periosteum was stripped from its attachment to bone.

In an attempt to attain a greater understanding of the treatment of fractures by intramedullary nailing after reaming, Danckwardt-Lilliestrom *et al.*(1970) conducted further investigations. They found that a nail placed in the medullary cavity, fully fitting the fracture region, did not in any way impair osteogenesis at the site of healing, since a good vascular supply was provided in the early stages by the periosteal vessels.

The formation of periosteal bone on medullary evacuation was regarded as being so consistent as to

warrant an investigation to find whether this phenomenon could be used as an experimental bone formation model (Danckwardt-Lilliestrom *et al*. 1972). Rabbit tibiae were used, one side being operated upon, the other serving as the (unoperated) control. Oxytetracycline was used as a bone label. Statistical analysis of the results indicated that the bone formation on the treated side was significantly increased compared to the untreated side, and that the model showed a good reproducibility.

1.3.6.4 PERIOSTEAL PROLIFERATIVE RESPONSE TO INJURY

In an autoradiographic study, using tritiated thymidine, on cellular proliferation in the formation of fracture callus in rat tibiae (Kernek and Wray 1973), the cambial layer of the periosteum was shown to be the dominant site of new cell formation. The results also suggested that these cells were the major source of fracture callus. Interestingly, Trueta (1974) reported that suppression of the peripheral callus by surgical introduction of plates and screws caused a severe interference with the normal process of bone union.

In addition, Kernek and Wray (1973) believed that the periosteal cell had the potential to become an osteoblast or a chondroblast, and that the chondroblast of early callus tissue may become osteoblastic. However, these researchers raised a particularly valid point concerning interpretation of autoradiographic studies: some cell labelling may have been due to reutilization of tritiated thymidine from dead cells and extruded nuclei in reticulocyte formation, rather than genetic transference during cell replication.

The cellular response to tibial fracture in five week old albino mice was investigated (Hyldebrandt *et al*. 1974). Using autoradiographic techniques, the periosteal proliferative response was observed to commence between eight and sixteen hours after fracture, reaching a maximum in 20-28 hours. The response

was most marked at the fracture site, but was also demonstrable on the tibial diaphysis and, most interestingly, on the contralateral non-fractured tibia.

Tibial and fibular fractures were created on rats in a study to investigate osteolysis after rigid fixation (Aro *et al*. 1982). Rigid intramedullary fixation of a tibial fracture was reported to cause the development of non-union in the associated fibular fragments. In contrast, with flexible fixation of the tibial fracture, the associated fibular fracture healed normally. The tibial fractures healed uneventfully in both cases.

Histological examination revealed that a plexus of encapsulated nerve endings (Pacinian corpuscles) existed in the periosteum of the fibula at the distal tibio-fibula junction. It was postulated that these neural elements could detect the stress protection provided by the rigid intramedullary nail in the tibia, with the consequence that the normal remodelling process failed and non-union resulted. However, with flexible fixation, the periosteal neural mechano-receptors were thought to sense the loading of the fibula when the tibia was bent during weight bearing, and thus played an important part in the healing process.

1.3.6.5 THE ROLE OF ELASTIC FIBRES IN FRACTURE HEALING

In contrast to the work of Chong *et al*. (1982), Murakami and Emery (1966) stated that the presence of elastic fibres in the periosteum had been well-known for many years. However, the latter reported that very little work had been carried out concerning the role of these fibres in neo-osteogenesis and fracture healing. Thus, paying special attention to elastic fibres in periosteum, embryonal subperiosteal bone formation in chick wings and healing fractures of guinea pig humeri were examined histologically.

It was found that, in subperiosteal bone formation in both models, elastic fibre proliferation occurred at the outer edge of the proliferating osteogenic cell in association with neo-osteogenesis. A "very close relationship" was noted between the cells among the elastic fibres and the osteogenic cells.

Further, Murakami and Emery (1967) found that these cells within the elastic fibre layers appeared to function as "elastoblasts" during the early stages of subperiosteal bone formation in guinea pigs. The cells within the innermost part of elastic fibre layers appeared to be undifferentiated cells which could be transformed into either elastoblasts or osteoblasts.

When the periosteum was torn, no elastic fibre formation was observed, fibrous proliferation and cartilage formation being the dominant features of the healing process. In this situation, the cells between the elastic fibre layers appeared to differentiate into fibroblasts and chondroblasts.

1.3.6.6 THE ROLE OF MAST CELLS

The mast cell is prevalent in the dermis of man. It is generally found in the loose connective tissue along small blood vessels and near fat cells. It originates from mesenchymal cells in the foetus, but its precursors in adult tissue are unknown. The mast cell is round or oval, averages between twelve and fifteen microns in width and has a small round nucleus. Within its cytoplasm there are numerous granules containing secretory material. The functions of the mast cell relate to the action of the substances contained within the secretory granules. These include heparin (an anti-coagulant), serotonin (a vaso-constrictor) and histamine (increases the permeability of the post-capillary venules leading to their greater permeability to plasma proteins, and stimulates leucocytic emigration). Further, hyaluronidase may be synthesised by the mast cell. This enzyme reduces the viscosity of the ground substance, thus increasing connective tissue permeability. It has been suggested that the mast cell participates in maintaining a normal functional relationship between blood vessels and the connective tissue compartment (Rhodin 1977).

Mast cells are postulated to have a role in fracture healing. Lindholm, Lindholm and Liukko (1967) created experimental fractures in rats and found aggregations of mast cells in the mesenchymal part of the periosteal callus. These increased in number from the third to the thirteenth day of repair. They concluded that the mast cells were intimately involved in the process of bone repair in rats,mainly in the region where pre-formation of cartilage takes place. They considered it likely that the mast cells "furnished substances" essential for the process of endochondral ossification.

In a quantitative study on mast cells in the callus tissue of experimental fractures in rabbits, Lindholm and Lindholm (1970) found mast cell aggregations in both periosteal and endosteal parts of the callus "cuff", but counts were considerably higher in the former on the fifth to seventh days after fracture. They speculated that the differing counts might have been due to biomechanical factors, and might have correlated with the excess of cartilage formation where movements at the fracture site occurred.