The Regeneration of bone

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INTRODUCTION

Definition of Bone Regeneration.

Bone repair in its broadest sense includes the healing of either a general or a local impairment of the skeleton regardless of the causative agent. While the subject of general repair is one of great importance in the wider aspects of medicine and biology it is the narrower implications of local repair that are of special interest to the surgeon. Local damage of bone may vary much in cause and severity and the reparative response may vary accordingly. Thus, accidental trauma, operation, inflammation and neoplasm all produce their own types of lesions, and the healing reaction of each shows the special imprints of the causative factor.

Bone repair is seen in its simplest form as the reaction to uncomplicated injury and it is this phase to which this discussion will be limited.

There are so many articles on the experimental studies of bone regeneration that the student is soon lost in the maze of conflicting statements. It is just as the question, "How Does Bone Grow?" -- a controversy almost two centuries old in the age of modern surgery and undoubtedly a favorite of contention among the ancients. The French and German journals are replete with similar (regeneration) articles.

The problem herein discussed is not a new one. In an endeavor to find out from the literature of the past what theories have been held regarding regeneration of bone, I naturally turned to the question of repair of fractures, as it seemed to me the only phase of the subject which would lead to a comprehensive understanding of the problems involved. Even after an extensive review of the literature, I find that
I have not been able to include the newer biochemical developments in this field of bone regeneration, as that phase of research represents a definite problem in itself.

The literature on growth, regeneration and repair of bone has been surveyed often and although these controversial matters have been frequently studied, it is an aid to assemble in outline all the available bibliographic material related to these subjects. In presenting this summary of the literature, the classification of Bull has been followed.

"Quid dican de ossibus? quae subjecta corpori, mirabiles commissuras habent, et ad stabilitatem aptas, et ad artus finiendos accommodatas, et ad motum, et ad omnem corporis actionem."
---Cicero, De Natura Deorum

"Scrape a bone and its vessels bleed; cut or bore bone, and its granulations sprout up; break a bone, and it will heal; or cut a piece away, and more bone will readily be produced; burn it, and it dies."
---Charles Bell, Anatomy

From H. A. Harris
In order to emphasize the complex system of bone formation and development, a few important anatomical and embryological facts must be mentioned. Even in the normal development of bone we find a considerable dispute as to the exact origin of its various elements. It is not surprising, therefore, that controversy should arise as to the method of regeneration of bone.

All the connective or supporting tissues of the body, except neuroglia and the reticulum of the thymus, are derived from the mesoderm. This does not imply, however, that all the mesoderm is transformed into connective tissues. Bone develops relatively late in embryonic life, after the muscles, nerves, vessels and many of the organs have been formed.

Toward the middle of the nineteenth century, an idea was advanced by the histologist Reichert (1). In a broad conception of the tissues of the organism, this author demonstrated that bone and cartilagenous tissue should be considered as derivatives by adaptation from connective tissue. Since his time, in bone as well as in cartilage, we have been able to establish the essential feature of connective tissue: the collagen material, a substance which by boiling or hydrolysis, produces gelatine or "glue." Bone and cartilage, tendon, or adipose tissue, are derivatives of this connective tissue. They all belong to the family of connective (supporting) substances; all are derived from the primitive mesenchyme, and, from the standpoint of their evolution, they have equal importance.
Tissues of the connective tissue group undergo ready and frequent transformations. Connective tissue may present a series of evolutionary types which are variable in their degree of complexity and adaptation. Embryonic connective tissue is formed of numerous cells and a fundamental substance which is more or less abundant and contains very fine fibrillae. The first step in the development of this true fibrillar form of connective tissue from mesenchyme is the formation of fibrils and fibers. While it has been held by many investigators that the fibrils arise in and from the homogeneous intercellular substance, the best substantiated view is that they arise within and from the cytoplasm of the mesenchymal cells. In the first instance they become separated from the cytoplasm and lie free in the "ground" substance in bundles (fibers). In the second instance they arise in the ground substance of the mesenchyme apart from the cells. This first step in development gives rise to a loose, delicate tissue in the embryo, known as embryonal connective tissue, from which all the adult forms, except reticular tissue, develops.

In any fibrous tissue, such as areolar, or the denser forms (fascia, tendons, ligaments), the structure depends upon the secondary arrangements of the fibers and not upon any peculiarity of origin. Under the influence of functional adaptations of its fundamental substance, adaptations which are peculiar and of a mechanism as yet little known, undifferentiated connective tissue may become specialized in some degree and assume various structural types; it may become loose connective tissue, a structure for filling spaces and to secure sliding movements; fibrous tissue with solid bands, powerful agents of resistance and
traction; aponeurotic tissue, tendinous tissue, elastic tissue, etc.
The less differentiated the connective tissue matrix, the better the
bone is physiologically. The more delicate the connective tissue frame-
work, the greater becomes its mechanical value. As Leriche and Policard
(2) stated: ".....The physiological worth is inversely proportional to
the histological differentiation....." Bone is a metamorphosis of
connective tissue.

Thus we see that the basis for development of osseous tissue is
embryonic connective tissue, although in one type of development carti-
lage precedes bone. Two types of ossification are recognized -- intra-
membranous and intracartilaginous or endochondral. In intramembranous
ossification calcium salts are deposited in ordinary embryonic connect-
tive tissue. In intracartilaginous ossification hyalin cartilage first
develops in the same general shape as the future bone and the calcium
salts are afterward deposited within the mass of cartilage. According
to Kolliker (3), Robert Nesbitt was the first to point out that bones
are not indurated or transmuted cartilages, but are new formations,
produced around the cartilages which are later destroyed. Moreover,
Nesbitt showed that certain bones develop directly from connective
tissue without having been preformed in cartilage.

Harris says (4): "Since Nesbitt in 1736 described this occurrence
of ossification in membrane without the intervening stage of cartilage
formation and endochondral calcification therein, much time has been
wasted in didactic exercises in relation to bone formation. Briefly we
may say that membrane bone is essentially characterized by two-dimensiona
growth, particularly in those areas where its protective function is marked." The bones of the cranial vault, of the face, and the clavicles appear by a process of ossification in primitive mesenchyme from the 39th to the 55th day of foetal life. Such ossification, involving bones which are essentially flat, does not require that orderly progression from mesenchyme to cartilage with subsequent calcification of the cartilages before it is replaced by bone.

As said previously, the membrane bones are those of the face and the flat bones of the skull. They include the interparietal or upper part of the occipital, the squamous and tympanic parts of the temporal, the medial pterygoid plate of the sphenoid, the parietal, frontal, nasal, lachrymal, zyomatic (malar) and palate bones, together with the vomer, maxilla and almost the entire mandible. Nesbitt correctly concluded that there is but one method of bone formation, whether or not it takes place in relation with cartilage, but he was unaware of the existence of cells, and believed that bones were produced from an ossifying juice derived from the blood.

Intramembranous Ossification.—

This is the type of ossification by which many of the flat bones of the skull and face are formed. The region in which these bones are to develop consists of embryonic connective tissue. Bone formation begins with the production of a layer or spicule of matrix which stains red with eosin. As to the origin of this matrix there is the same difference of opinion which obtains in regard to other intercellular products. It has been asserted that it proceedes from osteogenic fibers, which are modified
white fibers of the connective tissue. Between the osteogenic fibers, calcareous granules are deposited until the fibers are lost in a homogeneous calcified matrix. According to this opinion the matrix is essentially an intercellular formation. Others consider that the matrix is produced by a transformation of the exoplasm of bone-forming cells, or osteoblasts.

As soon as the connective tissue fibers become impregnated with calcium salts the areas become known as calcification centers. In each of these areas the cells increase in number, the tissue becomes very vascular and some of the cells, becoming more or less round or oval, with distinct nuclei and a considerable amount of cytoplasm arrange themselves in single, fairly regular rows along the bundles of calcified fibers.

These differentiated cells or osteoblasts are derived from mesenchymal or young connective tissue cells. Two characteristic differences in the behaviour of the original mesenchymal cells lead to the formation of cartilage on the one hand and bone on the other. In cartilage the cells lose their original protoplasmic processes, and matrix is formed on all sides of them. In bone the mesenchymal network is retained, and the cells deposit the matrix only from one surface, that which rests on an already formed spicule.

After the differentiation of the osteoblasts, the whole tissue is called osteogenic tissue. Under the influence of the osteoblasts a thin layer of calcium salts is deposited between the osteoblasts and the calcified fibers. In this way the first true bone is formed, and the calcification center becomes an ossification center.
The young connective tissue cells, which seem to be attracted by the osteogenic fibers or the bone spicules, around which they become arranged in an epithelioid layer of osteoblasts, are thus still connected by fine protoplasmic processes both to each other and to the neighboring unaltered fibrocytes. A variable number of white fibers, already present in the intercellular spaces, become incorporated in the bone matrix.

There is great variation in the shape of the osteoblasts. Often they are pyramidal, but they may rest upon the bone either by a broad base or a pointed extremity. Their round nuclei may be in the part of the protoplasm next to the bone, or away from it as far as possible. Active osteoblasts tend to be cuboidal or columnar, but as bone production ceases they may become quite flat. Their nuclei show changes similar to those seen in gland cells, changing from clear and vesicular when the cells are most active to dark and cloudy when activity has passed its peak, and pycnotic when the cells become inactive and flat. The cells form bone only along that surface which is applied to the matrix, and only in the intercellular spaces. New matrix is thus formed around any cell or protoplasmic process which lies at the edge of the matrix, and osteoblasts which have ceased to produce matrix are buried by their neighbors. Since the inactive, flat osteoblasts are those most often thus buried, the lacunae formed are flattened and parallel to the surface; and since the protoplasmic processes still connect the buried cells with active ones, the lacunae are also connected to the surface or to each other by canaliculi, the little canals formed by the deposition of matrix around the processes. Buried osteoblasts are called bone cells. They
differ from cartilage cells in having access through the canaliculi to the tissue fluids. Therefore they do not develop fat droplets and rarely divide or die, like cartilage cells. If they continue to produce matrix, thus becoming more widely separated, it is only to a slight extent and in young bones; they are therefore quite inactive. Harris (4) states that the bone corpuscles and osteoblasts control the absorption and deposition of calcareous matter; the marrow controls in large measure the supply of the formed elements of the blood. Leriche and Poli-card (5) regard osteoblasts as fibroblasts with only a low osteolytic capacity, whose function it is to oppose and restrict osseous extension. According to their view the osteoblasts are "useless parasites of osseous tissue."

It may be well to advance at this point a few of the theories regarding the origin of the osteoblast.

The origin of the osteoblast has been a subject of dispute from the earliest days of histology. On the one hand, Ranvier (6) has stated that "the matrix of the calcified cartilage liquefies; the cartilage cells proliferate, become free, and give birth to an embryonic tissue, the cells of which, surrounding themselves with a new kind of matrix, become the bone corpuscles." This was one of the first descriptions of that physiological heteroplasia which has attracted the attention of so many modern morbid anatomists. This view has been supported in turn by Retterer (7) and Macwen (8) and Van der Stricht (9). On the other hand, the majority of observers such as Brachet (10) and Carey (11) state that the cartilage cells which are liberated from the spaces in the calcified cartilage give
rise either to the reticulum of the bone marrow or to osteoblasts. Widely conflicting views have been put forward. Wingate Todd (12) maintains that osteoblasts do not enter skeletal tissue along blood vessel tracks, but are fibroblasts or connective tissue cells which have undergone certain characteristic modifications, and may or may not have passed through a "chondroblast" stage. H. B. Fell (13), working the laboratory of the late Dr. Strangeways, maintains that in the developing bone of the embryonic fowl the chondroblasts degenerate and disappear, and the perichondrium differentiates into two layers of the future periosteum, a superficial fibroblastic layer and a deep osteoblastic layer. Stump (14), on the other hand, is a protagonist of the extreme heteroplastic hypothesis and states that the primitive connective tissue of the osteogenetic area in mammalian embryos gives rise to the chondroblasts of cartilage, the fibroblasts of the perichondrium or periosteum, the osteoblasts and osteoclasts of osseous tissue, and the hemoblasts and reticular cells of the marrow.

Some observers have tried to distinguish between the bone corpuscles included between the lamellae of the bone and have reserved the term osteoblast for those cells which lie on the free surface of the lamellae in proximity to the marrow cavity or Haversian canals.

Harris (15) in a study of developing bone suggests that the senescent cartilage cells in the zone of calcification may undergo one of two fates: "Either on the one hand, as is true for the vast majority of chondroblasts, senescence is followed by death, or on the other hand, certain favourably situated senescent cells are saved from death by a process of rejuvenescence. Wherever the advancing capillary loops succeed in removing the calcified trabeculae, there is always the opportunity for certain
nutritive substances to reach the senescent cell, and produce that rejuvenescence and metaplasia which lead either to the chondroblast persisting as a bone corpuscle or to the chondroblast undergoing rapid division so as to produce two, four, or eight daughter-cells which are veritable osteoblasts.... This mechanism whereby the senescent chondroblast is saved from complete decay has much in common with the process of spore formation in certain bacteria; and the process of rejuvenescence and subsequent cell division of the senescent chondroblast may be as rapid and as evasive as the actual process of sporulation of the anthrax bacillus.... The chondroblasts may give rise to a bone corpuscle under the influence of a smaller supply of blood-borne substances than is necessary to form active osteoblasts. On the other hand, as in rickets, the chondroblasts may give rise to a low-grade osteoid bone corpuscle in the poorly calcified trabeculae, whenever the supply of blood-borne substances is inadequate to produce rapid differentiation of osteoblasts. This is illustrated in almost any section of rachitic bone where the diaphysis presents cartilage islands in which the chondroblasts are becoming converted into low-grade bone corpuscles, but the active liberation of high-grade osteoblasts on the surface of the poorly calcified trabeculae is absent. Such cells might with advantage be termed "osteoidoblasts." 

The spicules of bone, containing bone cells and beset with osteoblasts, increase in size and unite with one another, so as to form a spongy network enclosing areas of vascular connective tissue. These areas are not entirely surrounded by bone, but retain connections with the exterior, through which the vessels may enter and leave. These spaces among the trabeculae are known as primary marrow spaces and contain osteo-
genetic tissue. It is evident that if the spicules continue to thicken, while new ones were added at the periphery, the bone would soon become quite solid and heavy. This is prevented by the destruction or resorption of certain spicules, which begins at a very early stage.

In sections of bone, the places where absorption is going on may be recognized by the presence of large multinucleate cells, which Kolliker (16) in 1873 named bone destroyers or osteoclasts. They are shapeless masses of protoplasm without any limiting membrane, containing usually from one to twenty nuclei. In the largest of them, Kolliker counted from fifty to sixty nuclei. Since the early description of the osteoblast by Gegenbaur (17) and the osteoclast by Kolliker, the genesis and function of the latter, like that of all giant cells, has been the subject of dispute. Kolliker believed that they arose from osteoblasts through repeated cell division. Notwithstanding that the formation of giant cells by fusion of pre-existing cells is the prevailing view in pathology, the evidence upon which it is based is singularly lacking. The view that giant cells arise from the rapid nuclear division of a single cell at least has in its favour that such a process is a normal occurrence of extremely wide distribution in the animal and plant world. The osteoclast may be regarded, according to Harris (18), as a special type of response to an urgent demand for remodeling of bone. Osteoclasts are found along the surface of the bone, sometimes forming rounded elevations or caps at the extremities of spicules, and sometimes imbedded in shallow excavations known as Howship's lacunae.

The spongy bone formed is covered on its outer side by a layer of
of connective tissue which from its position is called the periosteum, and which represents a part of the original embryonic connective tissue membrane in which the bone was laid down. During its development the periosteum becomes an exceedingly dense fibrous membrane which is closely applied to the surface of the bone.

In a growing embryo, provision must be made for increase in the size of the cranial cavity to accommodate the growing brain. This is accomplished in the following manner. On the inner surface of the newly formed bone, the osteoclasts appear. Whether they are the specific agents in dissolution of bone has been questioned by Arey (19). On the contrary they appear to be degeneration cells, produced by those conditions which lead to the dissolution of bone. While the destruction of bone is going on on the inner surface, new bone is being formed on the outer surface, especially under the periosteum where the osteoblasts are most numerous. Thus the layer of bone gradually comes to lie farther and farther out and the cranial cavity is enlarged. So long as the cranial cavity continues to enlarge the new bone is of the spongy variety, but toward the end of development the trabeculae become thicker and finally come together to form the compact bone characteristic of the roof of the skull.

The processes of bone formation just described take place both in membrane and in cartilage bones. As the membrane bone enlarge, the central portion, through resorption, becomes loose, spongy bone (substantia spongiosa), which is enclosed on all sides by an outer layer of compact bone (substantia compacta). In the flat bones of the skull the compact substance forms the outer and inner "tables," which have the spongy diploe
between them. The cartilage bones likewise consist of spongy and compact portions.

Intracartilaginous Ossification.--

In this type of ossification hyaline cartilage is first formed in a shape which corresponds very closely to the shape of the future bone. For example, the femur is first represented by a piece of hyaline cartilage which develops from the original embryonic connective tissue. On the surface of the cartilage a membrane of dense fibrous connective tissue, known as the perichondrium, develops. In most cases, ossification begins about the middle of the piece of cartilage, corresponding to the middle of the shaft of a long bone. The cell spaces enlarge and in some cases the septa of matrix between the enlarged spaces break down, so that several cells may lie in one space. The cell spaces radiate from a common center, but a little later they come to lie in rows parallel with the long axis of the mass of cartilage. During these early changes lime salts are deposited in the matrix of the cartilage in this region, and the portion so involved is known as a calcification center.

So far the process is preparatory to actual bone formation. Then small blood vessels from the perichondrium (periosteum) grow into the cartilage, carrying with them some of the embryonic connective tissue. These little growths of connective tissue and blood vessels are known as periosteal buds. The septa between the enlarged cartilage cell spaces break down still further, forming still larger spaces into which the periosteal buds grow. Many of the connective tissue cells are transformed into osteoblasts -- oval or round cells with distinct nuclei and a considerable amount of cytoplasm -- and with the fibers and blood vessels constitute osteogenetic tissue. The cartilage cells in this region disinte-
grate and disappear, and the cavity formed by the coalescence of the cell spaces constitutes the primary marrow cavity. From the primary marrow cavity osteogenetic tissue pushed in both directions toward the ends of the cartilage. The transverse septa between the enlarged cartilage cell spaces break down, leaving a few longitudinal septa which form the walls of long anastomosing channels which are continuous with the primary marrow cavity. The osteoblasts arrange themselves in rows along the septa of calcified cartilage and a thin layer or lamella of calcium salts is deposited between them and the cartilage. Successive lamellae are deposited in the same manner and some of the osteoblasts become enclosed to form bone cells. The cartilage in the center gradually disappears. This region where bone formation is going on is known as an ossification center and the irregular anastomosing trabeculae of bone with the enclosed marrow spaces constitute primary spongy bone.

From this time on, ossification gradually progresses toward each end of the cartilage, and at the same time a special modification of the cartilage precedes it. Nearest the ossification center the cartilage cell spaces become enlarged arranged in rows and contain cartilage cells in various stages of disintegration. Some of the septa break down, leaving larger, irregular spaces; the remaining septa become calcified. Passing away from the center of ossification, there is less enlargement of the cell spaces and they have a tendency to be arranged in rows transverse to the long axis of the cartilage; there is also a lesser degree of calcification. The region of modified cartilage at each end of the ossification center passes over gradually into ordinary hyaline cartilage and is known as the calcification zone. It always precedes the formation of
bone as the latter process moves toward the end of the cartilage.

Along with this type of ossification just described subperiosteal ossification also occurs. Beneath the periosteum (perichondrium) is a layer of connective tissue the cells of which are transformed into osteoblasts. They deposit layers of calcium salts on the surface of the cartilage in the same manner as around the trabeculae inside the cartilage.

The transformation of the spongy bone into compact bone is peculiar in that the former is dissolved and then replaced by new bone. Whether this dissolution occurs through the agency of the large multinucleated cells known as osteoclasts is not certain. By the process of dissolution the marrow spaces are increased in size and are known as Haversian spaces. Within these spaces new bone is then deposited layer upon layer under the influence of the osteoblasts, until the Haversian spaces are reduced to narrow channels, the Haversian canals. The layers of bone are the Haversian lamellae. The interstitial lamellae in compact bone have two possible origins. They may be the remnants of certain lamellae of the original spongy bone which were not removed in the enlargement of the primary marrow spaces, or they may be parts of early formed Haversian lamellae which were later more or less replaced by other Haversian lamellae.

In the development of bone mechanical factors play an essential part not only in the formation of the bone itself but also in the establishment of its form and internal structure. Carey (20), in his studies on certain mechanical phases of development, concludes "that cartilage and bone are not self-differentiated, nor are they self-crystallized products," but represent "cellular responses to the varying intensity of the stresses
and strains produced by resistance (pressure) counteracting the growth of the skeleton in its blastemal state, that is, while the cells are closely compacted prior to the appearance of the specific tissue. Koch (21) has concluded that the "normal external form and internal architecture of the human femur results from an adaptation of form to the normal static demands, or normal function of the bone."
Hippocrates held that callus was formed from the marrow, as the marrow was thought to be the nutritious juice of the bone. Galen held that callus was due to excessive nutritious juice brought to the bone by the blood. In 1609, Jacque de Marque (22) demonstrated that marrow could not of itself furnish the materials for callus. In 1694, Dellamotte (23) resected subperiosteally six inches of a tibial shaft in a case of compound fracture and found that eight months later a new shaft had formed.

Antivivisection sentiment was so strong prior to this time that a surgeon, Jobi Merken (24), had been threatened, in 1862, with excommunication because he had placed a piece of dog's skull in a defect in the skull of a soldier. This being the case, we can understand that knowledge of the function of the periosteum and bone fragments marked time.

Two hundred years have elapsed then since the beginning of the debate over the importance of the periosteum in bone growth, regeneration and repair; and in spite of the vast amount of experimental and clinical evidence submitted by the adherents of the two views, namely, the one that the periosteum is an osteogenic membrane, and the other that it is merely an inert limiting membrane, there exits today the same two factions, as in the early part of eighteenth century.

The theories of growth and regeneration of bone can be classified in five groups, each view being exemplified by the statement of an outstanding exponent:

I. Ollier in 1867 maintained that growth and replacement of bone are due to specific osteogenic activity of the osteo-
blasts of the periosteum.

II. Barth in 1893 said that all transplanted bone dies and is replaced by proliferation of new bone from the surrounding host bone into the dead transplant.

III. Axhausen brought forth in 1908 the view that repair of bony defects and the replacement of bone grafts are effected by deposition of bone by periosteum and the endosteum.

IV. Macewen in 1912 claimed that bone grows and repairs itself by proliferation of the bone cells. The periosteum serves no osteogenic function and acts merely as a limiting membrane to the growing bone.

V. In the same year (1912), Baschkirzew and Petrow wrote that all new bone is formed by metaplasia of the pre-existing connective tissue in the region where it is to be laid down.

I. GROWTH AND REPLACEMENT OF BONE ARE BROUGHT ABOUT BY SPECIFIC OSTEOGENIC ACTIVITY OF THE PERIOSTEUM.

As we have seen previously in this paper, Dellamotte (23) in 1694 obtained a new shaft after subperiosteal resection.

In a most interesting review of this subject, Keith (24) presents many important facts connected with the early study of bone growth. He shows how Belchier (25), in 1736, after investigating the chance observation of a calico printer who had found that the bones of his madder-fed pigs were stained red, discovered that only new bone took the red stain.
Dulamel (26), in 1739, utilizing the important discovery of Belchier, came to the conclusion, from experimental work, that the new bone was formed by the periosteum, assisted by the endosteum. He determined that bone was laid down by the deep layers of the periosteum (comparable to the cambium layer of growing wood) and that madder was deposited only in newly formed bone. He found that growth in the length of bones took place at the ends, but he did not study the epiphysis. Periosteum produced bone "as an exogenous stem grows from the inner layer of the bark."

Goodwin (27), in 1800, ascribed osteogenesis to periosteum.

The death of Hunter occurred in 1793. Early in the nineteenth century the noted surgeon, James Syme (28), carried on his experimental investigations on the repair of bone. In 1835, he removed one and three-quarters inches from the radius of both the right and the left legs of young dogs. On the right, he removed the periosteum and the left, this membrane was preserved in place. When the dog was killed at the end of six weeks, the missing portion of the left radius was regenerated; whereas, on the right, where the periosteum had not been preserved, a gap still remained. In another experiment, he placed tin-foil beneath the periosteum and found that the periosteum produced a layer of bone on the tin-foil. Thus, the pendulum swings once again in favor of the osteogenic power of the periosteum.

Contemporaneous with Goodsir was Marie Jean Pierre Flourens (29), who repeated Dulamel's experiments. In his works, published in 1842, he concluded that Dulamel was right, and that new bone was deposited on the
surface of old bone, under and by the periosteum. He also agreed with Hunter that, coincident with bone building, there was bone absorption.

In 1859, Malgaigne (30), in his surgery, stated: "To sum up then, callus is formed by an effusion of plastic lymph, probably secreted from the periosteum and the medullary tissue, perhaps also by the surface of the fracture."

In the next period, we find the work of Ollier (31), published in 1867, in which he touches on practically all the phases of bone growth and repair. He performed various experiments, to find out whether the periosteum was osteogenic. He turned out flaps of periosteum from the bone, about the neighboring muscles, and found that new bone was formed on the under surface of the periosteum. He found that periosteum transplanted to subcutaneous tissue formed bone. The opponents of the osteogenic function of the periosteum claimed, as had Goodsir (32), that the new bone was due to growth of adhering bone particles that remained attached in the process of removal from the cortex. Ollier attempted to meet this objection by examining the detached pieces of periosteum with a hand lens; and he stated that he could not find any osseous plaques on his detached pieces of periosteum. It is interesting to note that Ollier found that osteogenesis differed in different animals, and even in different bones of the same or other animals. He was well aware of the fact that the periosteal graft from the rabbit had greater powers of reproduction than those from the cat; also, that a periosteal graft from the nasal bone of the cat would hold, while that from a similar bone of the rabbit would fail to do so. He found that the transplanted dura mater was more efficient in osteogenesis than the pericranium. He discovered
that the osteoblasts were fewer in the deeper layers of the periosteum of old animals than in that of young animals, but that they could be increased by the stimulation of injury to the bone. An explanation of the discrepancies in the findings of early and recent investigators may be due in part to the differences in osteogenesis of different animals and bones as observed by Ollier. Ollier concluded from his studies on fractures that the periosteum formed the greatest amount of callus; the endosteal surface, a lesser amount; and the bone itself, the least. From his studies on the behaviour of bone grafts, he determined that the most suitable material was a piece of living bone with both the periosteum and the marrow surface intact.

Cushing and Marpurgo (34), also in 1899, found that after several hours' preservation outside the body, transplanted periosteum retained its vitality and osteogenic power.

Colvin (35), in 1907, performed subperiosteal resection of entire bones for osteomyelitis in several cases and later found that complete regeneration of their shafts had taken place.

Tomita (36) (1908) stated that new growth is from the inner layer of the periosteum and from the marrow cells. The cells of the bone itself have not power to form new bone.

Nakahara and Dilger (37) (1909) found that free transplants of periosteum caused regeneration of bone in a fair proportion of cases.

Zondek (38) (1910) after a study of experimental fractures in mice, attributed an osteogenic function to the periosteum in the formation of callus.

In 1910, Janeway (39) successfully transplanted fresh bone with
adherent periosteum. The radiographs of the case demonstrate a progressive increase in the size of the transplant. Janeway states: "We must assume as a result of the conclusions of research work (not his own) upon implanted bone and periosteum that the implanted bone itself had died, but that periosteum and marrow had lived and replaced the old bone with new." Cohn (40) did not think that Janeway's X-ray pictures bear out the assumption, as in none of them was there evidence of a rarefying process which is characteristic of bone death. Janeway further states that transplants are osteo-conductive, and that all regenerative changes are solely due to the living and regenerative power of periosteum and marrow. He overlooked Sir William Macewen entirely, although the later had published in 1909 cases of successful bone transplantation, done in one case thirty years before; and in some of his cases Macewen had not transplanted periosteum with the bone.

In 1910, Lobenhoffer (41) concluded from his work that transplanted bone dies off but the periosteum remains alive.

Other investigators have made use of very fine pieces, or of an emulsion of periosteum for transplantation. Pochhammer in 1911 stated that the periosteum produces cartilage and bone after fracture. He (42) scraped off the "cambium" layer of the periosteum and transplanted it into muscle with negative results; but when he transplanted teased pieces of the entire periosteum, bone was formed in fifteen per cent of the cases. In another series of experiments he first scraped off the "cambium" layer of the periosteum and then the outer surface of the bone; a mixture of the two was transplanted into muscle and small nodules of bone were formed in ten to fourteen days. He refers to the experiments of
Berthier's in which were found bone formation following transplantation of pieces of periosteum into muscle.

Again in 1912, Pochhammer (43) published an article. Many authors had used pedicled flaps instead of free transplantation in order to obtain additional evidence of the power of the periosteum to form new bone. At this time Pochhammer described how he stripped almost the entire periosteum from the humerus of rabbits, allowing it to remain attached to the bone only at the lower end of the shaft. He then arranged the periosteum into the form of a tube which he filled with muscle. He found a small amount of new bone only at the lower end of the tube where the periosteum remained attached to the shaft of the humerus. He then filled the periosteal tube with blood clot instead of muscle and found a considerable increase in the amount of bone formation. These experiments give no absolute proof as regards the regenerative property of periosteum, but they show the marked stimulating effect which blood clot exerts on the repair of bone.

Murphy (44) in 1912 mentions in his article that periosteum from a young individual, when transplanted into a fat, or muscle tissue bed in the same individual, may produce a lasting bone deposit. No experimental proof was given. He also believed that there is always complete absorption of periosteum-free bone when it is transplanted into tissue in which there is no bony surrounding. Murphy sites cases in which he raised a strip of periosteum from the shaft of a bone, leaving it attached to the epiphysis in one case, and to the shaft in the other. He carried these strips between the fasciculi of the adjacent muscle and reattached the free end to the cut edge of the periosteum on the shaft. He found new
formation of bone on the under surface of the periosteum.

Trinci (45), in 1912, stated that transplanted periosteum is capable of causing early regeneration of bone. He referred to similar results obtained by Bonomme. Trinci in his experiments excised one-half to one centimeter of the shaft of the fibula of dogs. He bridged this gap with periosteal flaps which were turned down from the remaining bone stumps. In the microscopical preparations of the early cases he found new formation of bone in the center of the gap, where its only possible source was periosteum; and in the late cases there was evidence of union between this central new formed bone and that which developed at the ends of the shaft. He also emphasized the importance of blood clot and bone particles as a stimulating material for bone formation from the lower layers of the periosteum, and stated that there is less active growth of bone if these substances are lacking.

Attention must be called to the work of T. Jokoi (46) who in 1912 used an emulsion of periosteum, which he injected either subcutaneously or intramuscularly. He found that six out of ten experiments on rabbits showed active bone formation following autoplastic transplantation, and that even after seventy days there was a tendency to proliferation. In homoplastic transplantation there was active growth while in heteroplastic cases negative findings were the rule. If fresh blood was injected with the emulsion there was no increase in bone formation but if fibrin was used active increase of bone development resulted. If heminjected the cambium layer of the periosteum alone there was no bone formation, and even if there were also small particles of bone these underwent resorption. He referred to a similar and previous work by Nakahara and Dilger
In 1912, Carrel (47) cultivated periosteum on his special media and found that when this growing periosteum is transplanted into subcutaneous tissue, it leads to bone formation.

Haas (48), in 1913, almost simultaneously with the appearance of Macene's work, published an article entitled, "Regeneration of Bone from Periosteum," in which a number of experiments were reported showing that the periosteum had the power to regenerate bone. In later years he felt that this article did not give due attention to the other important osteogenic areas. He concluded that periosteum, especially in the presence of blood-clot, had the power to regenerate bone; that regeneration of bone is not solely dependent upon the presence of pre-existing bone; and that regeneration of bone was never found excepting when periosteum was present.

Schepelmann (49) (1913) said that periosteum when transplanted into the omentum, mesentery and liver and other organs caused growth of persistent new bone. It is important, he stated, to preserve vascularity and the integrity of the cell and to use the entire periosteum.

Oeschner (50) (1914) stated that after the placing of bone grafts in defects the periosteum causes regeneration of complete shafts of bone.

Mayer and Wehner (51) (1914), in experiments on animals, found that transplanted periosteum produced new bone. Adult bone cells demonstrated no proliferative ability. "Most of the bone cells of the transplant eventually die," they wrote, "though frequently the grafts is revascularized sufficiently early to preserve some of the bone cells. New bone growth is dependent upon activity of the periosteum and the endosteum."
Again in 1914, Haas (52) wrote: "It is apparent that the periosteum is very actively concerned in the regeneration of bone."

In 1914 McWilliams (53) said: "The life of living graft depends entirely on its receiving sufficient blood supply to keep it alive, and nothing else.... Without periosteum there is always uncertainty as to whether grafts, even though they be small, will acquire enough blood to keep them alive. With the periosteum on the grafts one is sure of their living as a result of one or two things happening: either a sufficient blood-supply, or a resupply to the grafts of living cells, derived from the periosteum, which reproduce the bone." He did not know exactly what influence the periosteum had. He believed that (1) its presence affords a better blood supply to the grafts, or (2) the periosteum, in the event that the bone cells in grafts die, supplies fresh cells to the grafts and from these regeneration takes place.

Todig (54), in 1917, in experiments on dogs, found that many of the components of the graft survived, particularly the perioskeletal and subperiosteaal tissues. "The periosteum will actively form new bone," he wrote.

Berg and Thalhimer (55), writing in 1918, stated: "Fully developed bone cells, in the accepted sense of the term, that is, cells within well calcified lacunae, have never been shown by microscopic observations to have divided and formed new bone." Other views of these investigators were as follows: The periosteum of a living autogenous graft remains alive after transplantation, and thereby the life of the transplanted bone is maintained. Periosteum devoid of adherent bone cells, when transplanted into foreign tissue, produces bone. Endosteoan and osteoblasts lining the Haversian canals in bone transplants produce bone very actively.
The cambium layer, when adherent to transplanted cortex, produces bone.

Mayer (56) (1919) wrote: "The fully developed bone cell has no power of division and bone growth results from the activity of cells lying between the bone and outer layers of the periosteum."

From a report of Delangeniers and Lewin (57) (1920) the following statement is quoted: "One can be certain that a layer of bone, with its periosteum, produces new bone and that this bone gradually grows and replaces lost bone."

Todd (58) (1920) expressed the following belief: "Cancellous tissue (endosteum) is one of the chief agents in regeneration of bone, and, like the cambium layer of the periosteum, should be treated conservatively at operation. Compact bone plays a very minor part in regeneration."

Simon (59) (1922) stated that living autologous bone with periosteum is the ideal graft, since the periosteum aids in proliferation and revascularization of new bone.

Kolodny (60) (1923) wrote: "An adequate blood supply of the periosteum is essential for normal union of fractures. The periosteal callus plays a far greater role in union of fractures than the endosteal callus."

Haas' (61) investigation in 1924 was concerned with determining the role played by the periosteum and endosteum in the healing of fractures in transplanted bone. He felt that his findings would have an important bearing on the question of the behaviour of the periosteum and endosteum in the growth and regeneration of bone, under usual conditions, as well as in transplants. The basic principle of his work, as he states it, is dependent on the fact that a fractured transplanted bone with both the
periosteum and endosteum intact will heal even when placed in a muscle bed remote from any other osseous tissue. A fractured bone when placed in muscle beds is in a most unfavorable environment because of the complete destruction of the original blood supply, the loss of osseous contact with normal bone and failure of functional stimulation. He states: "In other words it is working a very low threshold of physiologic stimulation, and therefore necessarily would be quite sensitive to any further injury, such as removal of the periosteum or the endosteum, or both.... In order to attain a maximum of reparative power for any bone lesion, it is deemed essential to preserve the integrity of both the periosteum and the endosteum."

Subperiosteal resection of a portion of the entire shaft of a bone the seat of osteomyelitis, has been advocated by a few authors since Ollier first described this procedure in 1857. Baumann and Campbell (62) (1926) give an excellent report of this procedure in a number of cases, and conclude that regeneration of the shaft takes place from the retained periosteum; that the role of the endosteum in the regeneration is slight and that success can be obtained in all but about ten per cent of cases other than tuberculosis.

Mock (63) in a resume of the literature covering this subject up to 1928 says: "The outstanding fact in this resume is that to periosteum alone, of all the bone layers, is ascribed a definite role in bone regeneration." In addition to this uniformity of opinion concerning some power in the periosteum for bone regeneration Mock repeatedly observed the following facts which to him gave additional evidence for his argument in favor of periosteal osteogenesis:
(1) When the bone is exposed at operation for old ununited fractures, the cortex is roughened and completely denuded of periosteum for a distance on either side of the site of the fracture. The ends of the fragment may be osteoporotic or osteosclerotic but, in either event, periosteum is absent for a variable distance from the ends of the fragments.

(2) In cases of delayed union of fractures, following severe trauma or trauma from repeated efforts at reduction, which are finally operated, the periosteum is very thin, almost impossible to raise without tearing and shredding, and oftentimes has completely disappeared from the ends of the fragments.

(3) In the application of a Lane plate in a recent fracture, say within the first two weeks, the results are far better than when a Lane plate is used in a case of delayed union or an ununited fracture. In the latter the plate is usually applied to a bone with very poor periosteum or one completely denuded of it.

(4) Subperiosteal resection of ribs, phalanges, metacarpal, and metatarsal bones for osteomyelitis, other than tuberculosis, almost uniformly results in regeneration of the bone, evidently from the retained periosteum. When the periosteum is completely removed with the bone proper, this regeneration does not occur or is greatly delayed.

Mock concluded by stating that periosteum is necessary for the repair of bone and therefore in cases of delayed union, ununited fractures, and loss of bone substance, periosteal transplants, when properly fitted about the site of the damaged bone, will result in healing and reconstruction of the defect.

D. B. Phemister (64) in an interesting article written for publication in 1935, feels that there can be little doubt from clinical and experimental evidence that the less the periosteum is disturbed the more quickly and surely the fracture heals. Experimentally he found that excision of the periosteum from the fragment ends delays callus formation and ossification. He goes on to state that these facts indicate that
the connective tissue outside the periosteum does not function as efficiently as the periosteum in the process of healing of a fracture.

To close this section of this paper, it may be said that there are numerous articles, dealing with the regenerative power of the periosteum; in its normal position, as a free transplant, or in relation to a transplanted piece of bone. Even with the better facilities for working, convincing proof, for or against the osteogenesis of the periosteum, is still lacking. The question naturally arises as to what explanation can be made for differences in the conclusions of the various investigators. The observations of Ollier, on the difference of osteogenesis of different animals, and even of different bones in the same animal, explains some of the discrepancies in the results. Furthermore, there is a greater activity of the osteogenic cells in the young animals than in the older animals, and this fact may lead to error in interpretation. A considerable amount of confusion has arisen over the exact definition of the periosteum, the addition of new terms and the arbitrary statements that neither can be proved or disproved. In the first place, the definition of the periosteum must be based, according to many authors, on the histologic structure of that membrane. If one set of observers claim that the inner cambium (cellular) layer of this membrane does not belong to the periosteum while another group maintain that it does, there will be no uniformity of opinion until that point is settled. Haas (65) states that the removal of the periosteum is a surgical procedure, and one cannot submit the specimen to a histologic examination and at the same time to a physiologic test. It is his further opinion that, by the careful removal of the periosteum without the use of very sharp instruments, one can uniformly obtain
specimens of periosteum free from osseous elements. To quote him: "In old animals, the periosteum is more firmly adherent, but there is a natural cleavage plane between it and the cortex. The cohesive force that exists between the structural components of the cortex is stronger than that between periosteum and cortex; so that there is not much chance of removing bone particles unless the bone is scraped with a sharp instrument. From the practical standpoint and in the routine practice of the surgeon, the periosteum is considered as that membrane which he strips away from the cortex. If this tissue is osteogenic, it is of prime importance to him, and he will use especial care to preserve it. The teaching, on the basis of unaccepted hair-splitting histologic investigations, that the periosteum is not osteogenic is wrong, and may be a source of harm. Fortunately, most of the surgeons who hold that the periosteum is not osteogenic advise that it be preserved for some other reason, such as its serving as a path for ingrowth of blood vessels."
II. ALL OF THE TRANSPLANTED BONE DIES AND IS REPLACED BY PROLIFERATION OF NEW BONE FROM THE SURROUNDING HOST TISSUE

Barth (66) in 1893, in transplanting bone to trephine holes in the skulls of dogs, found that all the elements of the graft died and were absorbed and that new bone proliferated from the surrounding living bone. The final success in grafting depended largely on intimate contact between the graft and the living vascular bone. The periosteum, cortex, and marrow died, and all the bone acted at first like a foreign body. The graft was gradually replaced by new-formed bone from the adjacent bone-producing tissue. This is the process called "creeping replacement." (In 1908, Barth repudiated this idea and came to believe that the periosteum was the necessary element in the life of a graft).

Marchand (67) (1901) stated that bone is replaced by proliferation in the necrotic substance of young bone cells from the surrounding cortex.

Grekooff and Frangenheim (68) (1901) maintained that the source of newly formed bone is in the osseous matrix of the bed into which the bone is transplanted.

The view of Murphy (69) (1913) was that the transplanted bone forms a scaffold for the Haversian vessels from both ends of the living bone to pass through, forming a callus. Transplanted bone is only osteoconductive. It must be in contact with fresh living bone if proliferation is desired and complete resorption is to be prevented. In 1913, Murphy (69) seemed to have changed his views, as follows: "Normal periosteum completely detached from bone and transplanted into a muscle tissue bed in
the same individual, if he be young, may produce a permanent bone deposit, but only if osteoblasts remain attached to the lower layer of the periosteum. The periosteum of itself is not osteogenic; it is rather a limiting membrane." It will be noted that in 1912, Murphy (44) supported the periosteal theory of bone regeneration.

Gallie (70) (1914) transplanted bone without periosteum in dogs. The bone grafts died and were revascularized. Dead bone was resorbed and new bone produced by the bone cells, which invaded the grafts along the route of the new vessels.

Brown and Brown (71) (1915) stated that all transplants are ultimately absorbed. The periosteum seems to have little influence on the early establishment of a blood supply.

Ely (72) (1919) wrote: "Three things are necessary for bone formation: (1) Blood vessels. (2) Loose meshed fibrous tissue, a homogenous matrix of granular or necrotic material. (3) A stimulus, physiological or pathological, as the case may be....It is seen therefore that neither periosteum nor marrow is necessary for bone formation and that neither of them forms bone, in the proper meaning of the word."

Gallie and Robertson (73) (1919) said that when bone is transplanted, the cells on the surface or in the Haversian canals may live and proliferate; the remainder die and are absorbed. New bone proliferates on the periosteal and endosteal surfaces of the graft.

Ely (74) (1924) stated that when a piece of bone is transplanted to the soft tissues of an animal, the bone and its marrow die; some vascularization of the marrow begins, and shortly thereafter, formation of bone.
C. Ray Murray in 1934 states: "The primary healing following a fracture is always by a tissue indistinguishable from granulation tissue; this tissue can be, and often is, derived in large part from tissue entirely outside of the bony structures; in fact, following tissue necrosis in the soft parts quite distant from bone, the formation of bone occurs in numerous sites in the body following disease and injury and can be produced experimentally in granulation tissue without the introduction of any osseous elements."
III. REPLACEMENT OF BONE GRAFTS AND REPAIR OF BONE DEFECTS ARE
BY PROLIFERATION OF BONE FROM THE PERIOSTEUM
AND THE ENDOSTEUM

Radzimowsky (76) (1891) said that when living, periosteum-covered
bone is transplanted, the tissue proper dies, but the periosteum lives and
produces new bone, which is deposited not only on the surface of the trans-
planted dead bone but in its lacunae and in the enlarged Haversian canals.

Benonme (77) (1885), in studying fractures in rats, found that bone
in the immediate vicinity of the fracture died. His belief was that it
was resorbed and replaced by new bone which was formed from the osteo-
genic layer of the periosteum.

In 1904, Nichols (78) stated that repair of a bony defect occurs by
proliferation of epithelioid cells from the periosteum, accompanied by
blood vessels, and by proliferation from the layers of cells lining the
inner surface of the cortex (endosteum). The cortical bone seems to have
a very limited or no power of proliferation.

Axhansen (79) (1908) published the results of his extensive experi-
mental work. He came to the conclusion that the graft did live, and the
chief source of the living osteogenic cells was the periosteum and, to
a lesser degree, the lining of the medullary cavity. He found that bone
grafts without periosteum were much less viable than those with periosteum,
although he admitted that some results may be obtained with bare grafts.
He believed that when bone is transplanted into a bony bed it makes no
difference whether the transplanted bone be living or dead, or whether
it be covered with periosteum, but that there is greater probability of
successful regeneration if it is alive and covered with periosteum. He
also mentions the value of making longitudinal incisions in the periosteum. A more detailed study of Axhausen's work reveals: He performed and reported in detail 146 experiments on animals with many different types of grafts. He wrote: "There is marked cellular proliferation under the periosteum, also the marrow showed proliferation, absorption and production of new bone under the periosteum, by proliferation from the endosteum, and around the new vessels which penetrate the dead bone. The chief source of the young bone which replaces the necrotic bone of the transplant is the periosteum, next in order comes the marrow and endosteum."

Law (80) in 1908 for removal of a malignant growth resected the upper end of the humerus of a boy, aged 8 years, and replaced it with a graft from the tibia. The arm was amputated eleven weeks later owing to recurrence of the growth. The graft was richly vascular, and bone cells were resorbed. There was marked periosteal and endosteal proliferation, and new bone was forming around the new vessels.

 Lexer (81) (1908) stated that the bony tissue of a transplant is gradually absorbed and is replaced by bone which is formed from the periosteum chiefly and from the medulla in part, and that the periosteum also aids in cementing the graft to the wound and in stimulating capillary invasion and early nutrition.

 Lobenhoffer (82) (1908) expressed the belief that in a transplant with periosteum the cortical bone dies and is absorbed and the periosteum remains viable and produces new bone.

 Albee (83) (1913) wrote: "It is believed that periosteum and marrow substance on the bone graft serve an important role in aiding to establish an early and more abundant blood supply from the recipient bone to the
transplant....It seems very probable that the amount of Haversian blood supply is in a very large degree, if not wholly responsible in determining whether the bone graft lives as such or acts as an osteo-conductive scaffold." And then again in the same year he found that periosteum when transplanted into muscle did not regenerate new bone. He thought that the outer layer of the cortex is necessary and agreed with Macewen in most respects.

McWilliams (84) in 1914, upon reviewing his results of both animal and human transplantations, first thought many of them seemed contradictory. On further analysis it appeared to him that these contradictions could be readily explained if looked at in this light: "The life of a living graft, whether transplanted in the human being or in animals, whether transplanted with or without its periosteum, is probably entirely dependent on its being supplied with sufficient blood to keep it alive." He felt that this would be the solution of the many vexed, theoretical considerations of bone grafting, and would explain the apparent contradictions and varying results obtained in bone transplantations. He stated that practically every bone graft made with its covering periosteum would live and grow if asepsis were attained. His question, "What will happen to grafts without their periosteum?" is answered by his statement: "The life of a graft without periosteum will depend entirely on its blood supply. If it obtains sufficient blood supply, then it will live and grow; if not, then it will die. A small graft without periosteum will have a better chance of getting sufficient blood to its cells than a large one without its periosteum; but in either case there is always some doubt as to whether the graft will live, if it be without its
periosteum. The periosteum on a graft does one of two things, or possibly both, namely: (1) By its presence it so favourably influences the nutrition of the graft, that is, increases the blood supply to its cells, as to keep it alive, or (2) In case the bone cells in the graft die from insufficient nourishment, the periosteum supplies living cells to the graft, by means of which the bone is regenerated.

Phemister (85) (1914) wrote: "Osteogenesis in bone repair occurs from the inner layer of the periosteum, from the endosteum, and to a much less extent from bone cells and fibrous contents of the Haversian canals." He stated, furthermore, that the viability of a transplant depends on the ability to secure nourishment; therefore, the outer cells survive and proliferate.

From Albee (86) in 1914: "The endosteum, marrow, and periosteum should be included in the graft, as they play a most important role in aiding to establish an early and sufficient blood supply from the recipient tissues to the cortical part of the graft. The endosteum is also actively osteogenetic as well as the inner layer of the true periosteum."

Haas (87) in 1914 expressed the following view: "The periosteum is directly and actively concerned in the regeneration of bone. The regeneration of bone also takes place from the marrow but to a more limited degree."

Gill (88) (1915) transplanted entire metatarsal bones in dogs and noted good healing in most cases, with little necrosis of bone. He stated: "Revascularization of a graft takes place early and first reaches the exterior -- the most vital portion of the graft."

Brooks (89) (1917) wrote: The living bone transplanted with the
periosteum and endosteum is the only type of implant which has osteo-
genetic properties. Osteogenesis is from the junction of the peri-
osteum and cortical bone rather than from either alone."

Berg and Thalheimer (90) in 1918 reported: "The few transplants
which include endosteum, though not enough to allow any definite con-
clusions to be formed, showed an even greater growth from endosteum than
any other transplants, even including periosteum."

Nathan (91) in 1921 expressed his belief as follows: "Bone is
produced solely by osteoblasts. The osteoblasts are always confined to
the cambium layer of the periosteum or the endosteum. The osteoblasts
are also found in the bone marrow." Bone grafts, he said, should include
the periosteum and endosteum.

Albee (92) in 1923 said that most of the blood supply to a graft
is derived from the marrow and that an early blood supply is essential
to the life of a graft.

Klinkerfuss (93) in 1924 wrote: "Solid bone grafts in the main die,
are absorbed and replaced by new bone tissue resulting from the proliferating
osteoblasts of the periosteum, endosteum and Haversian canals."

Haas (94) from his experiments in 1924 found that "(1) A fracture
in a transplanted bone from which the periosteum has been removed may
unite, either when transplanted to the muscles of the back or reimplanted
in its normal position. (2) A fracture in a transplanted bone from which
the endosteum has been curetted may unite, either when transplanted to
the muscles of the back or reimplanted in its normal position. (3) A
fracture in a transplanted bone from which both periosteum and endosteum
have been removed did not unite, either when transplanted to the muscles
of the back or when reimplanted in its normal position. (4) The chief
source of osteogenic cells, for the repair of a fracture in a transplanted bone, is from the osteoblasts of the periosteum and the endosteum. The presence of either is sufficient for union. The periosteum plays a relatively more active part than the endosteum. (5) The explanation of cases of non-union not due to mechanical tissue interference or gross malpositions must be sought for in the factors that inhibit osteogenesis simultaneously in both the periosteum and the endosteum. (6) In order to attain a maximum of reparative power for any bone lesion, it is deemed essential to preserve the integrity of both the periosteum and the endosteum."

Carl Rohde (95) in his article published in 1925 gives a very interesting discussion of the problems involved in the regeneration of bone. His thoughts are as follows: "As a result of injury, the blood-vessel system of the bone involved reacts by filling the blood vessels and new blood vessels are formed. As a result of hyperemia all the functions of the involved bone are increased, and with this, the regeneration changes begin. The causes of regeneration in chronological order are; trauma, hyperemia, and products of tissue destruction. Their results are: hyperplasia, proliferation, and hypertrophy of the specific and nonspecific tissue elements, which under normal conditions, develop into fibrous scar tissue or a pseudo-arthritis....Periosteum is composed of two layers — an outer layer, the adventitia; and an inner, the fibro-elastoc or cambium layer. For bone regeneration, both layers, and in proper relationship, are necessary." In his discussion of the role of the periosteum in bone regeneration he showed that the periosteum plays
a most important role in the regeneration of bone. He concluded that the normal union of the different layers of the periosteum (cambium layer and adventitia) is necessary for bone regeneration. With bones, it is the same as with all other tissue and organs: life, function, and regeneration are possible only so long as the circulation leading to the tissue in question is intact. He explains the variations in experimental results in this manner: "The main cause for bone regeneration is to be sought in cases in which the periosteum and bone are united, for between the periosteum and the compact bone, where the cambium layer of cells are retained, bone regeneration takes place. From these facts it develops that under the usual experimental conditions the periosteum of old animals does not form bone." He found that when only marrow and endosteum were used, a pseudo-arthrosis constantly resulted. His opinion of the role of compact bone in bone regeneration is that the cortex denuded of periosteum and marrow and endosteum does not take part in bone formation. But as the denuded compact bone again becomes nourished, periosteal regeneration and bone formation take place from the osteoblasts of the Haversian canals. And upon the problem of metaplasia of the surrounding tissues he states: "The connective tissue elements of the periosteum, the marrow and endosteum, as well as the non-specific connective tissue of the vicinity never develop through metaplasia into bone." He gives as fundamental prerequisites for bone formation living osteoblasts or unused remaining mesenchyme cells which can develop into osteoblasts. These bone-building cells, without any spontaneously developed deposit of calcium salts and without artificially
brought bone-building substances can take the organic and inorganic substances from the living organism which they need for the building of bone. He concluded that bone-building power is found only in specific bone-building tissues (osteoblasts of the periosteum and marrow endosteum). Metaplastic bone building from the usual connective tissue does not take place. Heterotopic bone formation in soft tissue is from the unused remaining mesenchymal cells, which through traumatism, infection, toxic stimule, or disturbances of metabolism, may abandon their indifferent state at any time and commence to build bone.

Johnson (96) (1927), after proving that the intact circulation of nutritive arteries, which supply the bone marrow and inner half of the cortex, allows most rapid bone repair, in his ingenious study of the blood supply of the diaphysis, states: "The medullary callus was the earliest and most active factor in the repair of cortical defects." He also shows that the periosteal blood supply, which nourished the outer half of the cortex only, stimulates bone growth to a lesser degree and is unable to afford collateral circulation to the medulla in less than four weeks.

Kartaschew (97) (1930) stated that the periosteum and the endosteum are important in transplanting bone. Small chips of bone permit circulation to reach the Haversian canals earlier and aid the endosteal proliferation.

Haldeman (98) in 1932 said that the periosteum plays the chief part in the healing of fractures. Endosteal (medullary) callus aids in the healing of fractures but in the absence of periosteum is often unable to complete the union.
In 1934, McGaw and Harbin (99) stated: "Histologically, the richest supply of osteoblasts is found in the bone marrow. No one, however, has suggested that marrow tissue alone can be used as a free graft to stimulate or to hasten osteogenesis. These experiments seemed to indicate that bone marrow and endosteum, which are easily obtained, may be of clinical use for this purpose." In their series of experiments they attempted to determine the use of bone marrow and endosteum as a free graft to stimulate or hasten osteogenesis. A small mass of marrow and endosteum removed from the tibia in dogs was substituted for a three-eights inch resected segment of fibula in the same subject. The opposite fibula was similarly resected for control. They concluded that bone marrow and endosteum play a very active role in the formation of callus and new bone in the dog.

Prewitt and Easton (100) found that the laying down of fibroblasts and endothelial tissue for sustentacular and circulatory functions, respectively, in soft tissue, does not result in mineral deposition as in the case of osteogenesis. The presence of these tissues, however, is universal in successful bone repair. To the question, "Wherein then does bone repair differ from ordinary soft tissue repair?" they say: "The cells of bone marrow and endosteum play a very active role in new bone formation. It seems logical therefore, to suppose that locally a system of fibroblasts is formed in the meshes of which the periosteal, endosteal and marrow cells are supported. The rapidly growing endothelial cells develop into a sustaining circulatory network which permits of the elaboration of such enzymes by the bone cells as are necessary for the production of new bone."
Here again we find that opinions vary as to the role played by the different tissues of bone in its regeneration. One investigator may regard the periosteum, another the endosteum, and another the cells of bone itself, as the important factor in the union of fractures, in the correction of defects, and in the obtaining of satisfactory results in transplanting bone. It should be mentioned that there are investigators who regard the periosteum, endosteum, or bone cells themselves as capable of producing bone and that the other tissues of the bone have no function whatsoever. And there are some men who believe that the formation of bone after trauma, etc., and in transplants, is dependent largely and sometimes entirely on a metaplasia of the surrounding connective tissue.
IV. THE PERIOSTEUM HAS NO OSTEOGENETIC FUNCTION. BONE GROWS AND REPAIRS ITSELF BY PROLIFERATION OF THE BONE CHIPS

Havers (101) (1692) studied the microscopic structure of bone and determined that the periosteum is merely a connective tissue, a limiting membrane which vascularizes the bone.

With advent of the microscope, it became possible to study the minute histologic structure of the various constituents of bone, and to trace the different stages in its growth and development. John Good sir (102), working about 1841, had the opportunity of utilizing an improved microscope for the study of his specimens on bone growth. In spite of the fact that he worked with Syme, he came to diametrically opposite views regarding the function of the periosteum, concluding that it was not osteogenic, but that in its removal small pieces of bone were carried with it and these grafts served as centers of reproduction for new bone. Although he was a follower of John Hunter, he found that it was not the arteries that formed the bone but specialized cells, the bone corpuscles, which also had the power to carry away bone. He conceived that, under the stimulation of inflammation, the canalicular cells became active, and further, from his study on the necrosis of shafts of long bones, he concluded that the periosteum had no bone forming power but was simply, as he termed it, "a limiting membrane," and the regeneration which took place depended wholly on the proper substance of the shaft that became adherent to it. It is interesting to note how closely the ideas of the present day proponents of the non-osteogenic power of the periosteum
correspond with those advocated by John Goodsir.

Macewen (103) in 1912 recalled Goodsir's statement that the periosteum was merely a limiting membrane. He believed that any signs of osteogenesis were due to the presence of osseous tissue detached from the underlying cortex; and he presented additional experimental work and clinical facts to substantiate his contention. He said that the bone cells of the cortex, occupying the lacunae of the bony substance itself, are the active agents in the life and regeneration of a transplant. The periosteum is only a limiting membrane and takes no part in osteogenesis. The periosteum acts merely as a "limiting membrane to the osteoblasts issuing from the interior of the bone....The vegetative capacity of the bone cell is fully as great as that of the epithelial cell." The periosteum is the medium through which the bone gains some of its blood supply, but it is not indispensable. Many cases have been reported in which subperiosteal resections of bone were made and in which there was no replacement by bone tissue. Diaphyseal bone is reproduced by the proliferation of osteoblasts derived from pre-existing osseous tissue. Regeneration takes place independently. "The periosteum plays no part in the reproduction of bone after transplantation."

It is believed that Macewen has minimized the importance of the periosteum in just as great a degree as the proponents of the view that the periosteum is osteogenic have exaggerated that idea. He found that pieces of bone without periosteum, when transplanted into muscle, showed definite signs of proliferation.

Geddes (104) in 1912 wrote that bone derives from the ectoderm and not from mesoderm. Osteoblasts arise from the cells of the ectoderm,
correspond with those advocated by John Goodsir.
and migrate as individuals to the sites of bone formation, passing through the periosteum enroute. Periosteum, far from being an osteogenetic membrane, is a limiter of bone formation. Cartilage, a mesodermal tissue, has, when it precedes bone, the function of providing a scaffolding upon which the osteoblasts can move. Osteoclasts are composed of the fused bodies of one or more cartilage cells, with numerous osteoblasts living within the protoplasmic mass cells, with numerous osteoblasts living within the protoplasmic mass cell inclusions (multiple nuclei).

McWilliams (105) (1912) in using ribs in experimental transplants, found that the periosteum seemed to hinder the early development of a blood supply. It served no osteogenic function.

Vogt (106) (1913), after subperiosteal resection of the shaft of a humerus, found no evidence of regeneration of bone.

Gallie and Robertson (107) (1914) studied the healing of defects produced in bones of young and old animals. The periosteum, they thought, is merely a fibrous membrane without osteogenic function and osteogenesis appears to be solely a property of the endosteum and to be as energetic in the absence as in the presence of the periosteum.

Moore and Corbett (109) (1914), in experimental transplantation of bone in animals, found that fascia is a suitable substitute for periosteum. "Periosteum is not an essential element in the healing of bone," they stated.

Cohn (110) (1914) believed in Macawen's theory and attempted to find a suitable explanation of the regeneration of bone through the agency of the osteoblast, the embryonic bone cell:
(1) Primary ossification proceeds through cartilage; in fact, the osteoblast is the result of division and liberation of the nuclei of cartilage cells.

(2) Primary periosteum is a connective tissue tube in which the centers of ossification are slid down. Without the deposition of such centers the bone is not formed and there is then any one of the possible congenital anomalies due to the absence of a part (acheiria).

(3) Bone is living tissue, and as such must undergo a constant process of renewal and repair. Such changes can only occur, according to Macewen, as follows:

(4) Following stimuli to bone, the cells on the interior proliferate, and escape through the Haversian canals into the subperiosteal space; there they find room for proliferation and may ultimately contribute to the breadth of the shaft.

It is apparent that Cohn believed with Macewen in the inherent osteogenic function of bone transplants. He concluded: "We believe that small bone transplants are osteogenetic and not essentially osteo-conductive. Periosteum has no osteogenic function, but is rather a limiting membrane. Periosteum is not essential to the repair of defects in bone."

Lewis (111) (1914), after clinical observations, concluded that gaps in bone are filled by growth from the ends of the divided shaft under periosteal limitation and control.

Groves (112) (1914 and 1917) expressed the belief that the periosteum is the product and not the mother of bone. All the osteogenic properties of the periosteum are due to the more or less accidental presence of the
outer layer of bone cells adherent to its deep surface. Living bone is
the chief source and origin of callus, which grows mainly from its outer
or periosteal surface. The periosteum is chiefly a limiting membrane of
bone. The dense bone can live, grow, undergo repair and produce fresh
periosteum after the periosteum has been removed.

Davis and Hunnicutt (113) (1915), after experiments on dogs and
rabbits, concluded that periosteum alone (even when osteoblasts are de-
monstrated) does not produce bone when transplanted. Periosteum with
bone shavings attached produces new bone, they said. Particles of bone
and accompanying osteoblasts are necessary for the production of bone.
The nourishment of bone is in no way affected by the stripping off of
the periosteum. The periosteum acts as a limiting membrane. Growth of
bone into defects is from the shaft itself.

Cohn and Mann (114) (1916) stated that the periosteum is a source of
added blood supply and a limiting membrane. The endosteum and the cortical
osteoblasts lying under the periosteum are the sources of formation of
callus. Transplants are usually absorbed.

The results of Dobrowolskaja's (115) (1916) experiments, especially
those with compact bone, seemed to explain in some measure the process
which takes place in the organism in bone-grafting operations and in the
healing of splintered bone, and to throw some light on its mode of action.
As a matter of fact, these two processes are quite analogous, because
every piece of bone which has been entirely separated off by fracture
may be regarded as a free-bone "transplant! He states that a detached
piece of bone must of necessity play some active part in the process of
bone regeneration. Islets of osteogenetic tissue have been noticed round
a piece of bone transplanted without its periosteum. Probably this osteogenetic formation arises from the growing cells of the transplanted piece; but the newly-formed bone only acquires permanent vitality when connected with the bone matrix, by means of which it is brought into relation with the central nervous system and the other normal conditions of life. From his experimental findings after culture, incubation and study of small pieces of bone of small animals, he concluded: "Bone tissue is capable of producing a luxuriant growth in vitro. The living elements of compact bone tissue are also capable of developing new cells. The islets of osteogenetic tissue round a piece of bone deprived of its periosteum, and transplanted in the soft-tissues, probably arise from the growing cells of the transplanted compact bone. When the bone is transplanted with its periosteum, the growth is evidently more active, and this would explain the more favourable clinical results of such transplantations. In order to obtain due strength, it is necessary that the bone should be connected with the bone-matrix, through which it enters into normal conditions. Blood coagulum aids the growth of osteogenetic cells by means of its fibrinous network." He also mentioned the fact that the organism finds in the piece of transplanted bone a source of organic salts, which are necessary for bone regeneration.
V. NEW BONE IS FORMED BY METAPLASIA OF THE PREEXISTING CONNECTIVE TISSUE IN THE REGION WHERE IT IS TO BE LAID DOWN.

No sooner had Duhamel's doctrine that the periosteum was osteogenic been announced than a faction arose under the leadership of Haller (115), who in 1766, maintained that the periosteum was not osteogenic. He wrote: "We cannot yet distinguish the bones themselves of which the first appearance is mucus.....Thus the muscles, by their action, draw out the processes from the bone and dilate the small cavities into large cells; and likewise incurvate the bones and variously modify their shape.....The bones at first are soft and of a mucus nature; then they acquire the consistence of jelly; and this afterwards becomes a cartilage, without any change of parts, as far as can be observed Cartilage however is not so imperceptibly converted into bone. It never happens without the red blood making a passage for itself into the vessels of the bones. Round these vessels are formed cellular texture and laminae, which the vessels themselves seem to compress into a medullary tube.....But even a bony callus never becomes sound till newly formed red vessels have penetrated its substance.....The periosteum covers the bones as membranes cover the viscera.....nor does the periosteum at all adhere to the bone, except in the epiphysis." Haller maintained that the periosteum was merely a vascular covering that served for the nourishment of the bone, and that it took no part in the formation of repairing callus; or in the growth of bone. He believed that the callus was formed by the broken bones; that the arteries were the depositors and builders of bone, and that they could form bone anywhere within the limits of the periosteum.
The next important observer was John Hunter (117), who began his epoch-making work about 1750. He was a firm believer in the teachings of Haller and did a considerable amount of investigation to confirm his opinions. He wrote in 1786 as follows: "Bones receive most of their nourishment from the surrounding parts, as from the periosteum.... Bones grow by two processes going on at the same time and assisting each other; the arteries bring the supplies to the bone for its increase; the absorbents at the same time are employed in removing portions of the old bone, so as to give to the new proper form.", Thus Haller he made the additional and important discovery that living tissue had the power to absorb bone. He upheld the belief that the arteries were the depositors of bone and that, in fractures, the deposition of bone usually commenced at the broken ends of the bone.

Blessig (118) (1859) observed calcification of the kidneys of rabbits killed from four to six days after ligation of the left renal artery.

Paul (119) (1886) stated that senile degeneration of arteries presents three states: (1) Calcareous degeneration; (2) Irritation about these plates from fracture or other injury, leading to inflammatory proliferation; (3) Ossification in this young proliferation tissue.

Barth (120) (1895) placed a piece of incinerated bone in the peritoneal cavity of a cat. Six weeks later he found it penetrated by connective tissue and in several places by true bone lined by osteoblasts.

Pollack (121) (1901), in examining lungs obtained at one hundred postmortem examinations, was able to demonstrate the presence of osseous
nODULES IN SIXTEEN CASES. HE EXPRESSED THE BELIEF THAT OLD SCAR TISSUE FORMS OSTEOID TISSUE AND BONE BY METAPLASIA.

SACERDOTTI AND FRATTIN (122) (1902) FOUND BONE PLAQUES IN THE KIDNEYS OF THREE OF FOUR RABBITS OF WHICH THE RENAL VESSELS WERE Ligated. IN TWO CASES THE URETERS WERE TIED. TRU CORTEX, PERIOSTEUM AND ENDOSTEUM WERE PRESENT.

PASCHARISSKY (123) (1905) PRODUCED BONE IN A RABBIT'S KIDNEY IN FROM THREE TO FOUR MONTHS BY LIGATING THE VESSELS.

MAXIMOW (124) (1906) FOUND BONE AS EARLY AS FIVE WEEKS AFTER LIGATION OF THE RENAL PEDIClE.


BUNTING (126) (1906), AFTER STUDYING SClerotic AORTAS, WROTE: "THE FACTORS IN METAPLASIA BONE FORMATION IN VESSELS ARE EXTENSIVE SCLEROSIS WITH THE PRESENCE OF CALCIUM DEPOSITS, TRAUMATIC OR INFLAMMATORY DISTURBANCE IN THE CALCIFIED AREA, WITH PEnETRATION BY GRANULATION TISSUE AND THE FORMATION OF BONE."

HARVEY (127) (1907) APPLIED IRRITANTS TO THE WALL OF THE AORTAS OF RABBITS TO INDUCE DEGENERATION. BONE, HaverSian CANALS, AND MARROW WERE FORMED, AND HARVEY STATED: "NEW BONE FORMATION COMMENCES IN AREAS PREVIOUSLY NECROTIC. THIS PROCESS OF NEW BONE FORMATION IS ONE OF METAPLASIA."

BUERGER AND OPPENHEIMER (128) (1908) EXPRESSED THEIR VIEWS AS FOLLOWS: "IT IS GENERALLY CONCEDED THAT THE PRESENCE OF LIME AND YOUNG CONNECTIVE TISSUE IS ESSENTIAL TO HETEROPLASTIC BONE FORMATION. DUE TO SOME STIMULUS, VESSELS..."
vessels penetrate the diseased media, young connective tissue proliferates and comes in contact with lime deposits. True bone is formed."

Pearce (129) (1909) wrote: "True bone was found in the scar tissue of the kidney in six animals. Bone formation occurred as thin lamellae in the scar tissue immediately beneath the mucosa of the pelvis."

Baschkirzew and Petrow (130) (1912), on the basis of experiments on both animals and of clinical observation, elaborated the theory that the regeneration of bone takes place by metaplasia from the surrounding connective tissue cells. They present an interesting explanation regarding the regeneration of bone when transplanted into muscle. They believe that the majority of bone corpuscles die, and that only those which receive better nutrition and possess especial vitality remain alive. The presence of periosteum or marrow is not considered necessary for regeneration, even though large pieces of bone be transplanted. They believe the chief source of regeneration to be a proliferation of the surrounding young connective tissue elements of the muscle, which penetrate into the vascular spaces and canals of the bone, where, through a process of metaplasia, they acquire the properties of osteoblasts and regenerate the new bone. They called attention to the fact that Ollier had also suggested such a possibility in his earlier work. In clinical application the periosteum is admitted to be of value, not for its property of regeneration of bone, but because of its aid in directing bone growth, and serves as a protecting membrane for the new bone. The periosteum is also said to produce new bone, which, however, is soon absorbed.

Todd (131) in 1912 wrote: "Osteoblasts do not enter skeletal tissue along the blood vessel tracks, but are fibroblasts or connective tissue cells which have undergone certain characteristic modifications and may
or may not have passed through a chondroblast stage."

Strauss (132) (1914) made ureters experimentally from the abdominal wall. Bone formed in the layer of the fascia transversalis. No degenerative changes had taken place where bone was formed. The bone that had formed closely resembled normal bone.

McWilliams (133) (1914) stated: "Connective tissue seems to be essential in the formation of bone. Osteoblasts are indistinguishable from fibroblasts. The first occurrence in bone formation is the arranging of fibroblasts (osteoblasts) around a blood vessel. In this new fibrous tissue calcium is deposited by some unknown influence, which goes on to the formation of bone.....There is some other factor for making for the life of grafts than the periosteum or contact with living bone; and this I take to be a sufficient blood supply."

Moschowitz (134) (1916) reported several cases of calcification or ossification in the ovary. "Blood vessels, osteoblasts, bone cells, and marrow (in large part at least) are merely differentiations of the mesenchymal cell unit," he wrote.

Neuhof (1917) (135) wrote: "In fascial transplants into experimental defects in the urinary bladder, macroscopic plaques of true bone appeared.....That previous bone or periosteum is not necessary for the formation of new osseous tissue was, of course, demonstrated by finding such tissue developed in situations far removed from the skeletal system.....The periosteum-like layer ensheathing the bone plaques can be accounted for satisfactorily on the theory of metaplasia of adjoining connective tissue and similarly, metaplastic changes in connective tissue
included between the bone trabeculae will explain the development of bone marrow."

Asami and Dock (136) (1920) ligated renal vessels and ureters of rabbits, and bone plaques formed later. Young fibroblasts accumulated to form a membrane-lined structure. Formation of bone began in the loose vascular connective tissue, close under the transitional epithelium of the calices.

Phemister (137) (1923) found that in fascial transplants to the bladders of dogs (the urine of which is always acid) bone always formed. In similar transplants in rabbits and sheep (the urine of which is alkaline) there was no calcification or ossification. Lime slats were deposited from the lymph or blood in the portion of the transplant bordering on the lumen, where nutritional conditions were poorest, necrosis was greatest and the acidity was increased by contact with the acid urine of the bladder.

Leriche and Plicard (138) (1926) stated: "(1) The function of bone is the result of a metaplastic change in the connective fundamental substance. This metaplasia takes place in three stages: (a) transformation of the connective tissue by an edematous infiltration with a multiplication of connective fibrils; (b) infiltration by a special substance, chemically undefined -- the pre-osseous substance; (c) deposits in that substance of a calcareous mixture of calcium phosphates and carbonates. (2) Osseous metaplasia can occur in all types of connective tissue; embryonal type, fibrous type, etc. The numerous forms of osseous tissue, as found among men and animals, are the result of that process. (3) In osseous meta-
metaplasia, the cells do not play the part classically attributed to them, that is to say the osteoblasts do not secrete directly osseous substance between the cells. Such a conception is erroneous. The osseous transformation of connective tissue as a phenomenon independent of all cellular action. It is an interstitial and humoral process....The perios-teum is modified (to an embryonal state) by a change of circulation or by edema; it becomes then a ground for ossification. It is passively ossified -- it does not make bone in an active manner."

They further state that osseous metaplasia of connective tissue is a reversible process. Bone appears and disappears with great facility. There is a continual state of unfixed equilibrium. In transplantation of bone the formation of new bone depends on the resorption of the transplant. It seems that as a result of rarefaction of bone there is produced a localized oversupply of calcium, which provokes an osseous metaplasia in the surrounding connective tissue. "The resorption of bone is specially directed by humoral phenomena, dependent on the circulatory activity in the bone."

To sum up the views of Policard and Leriche it may be said that they maintain that bone formation is essentially a metaplasia of fibrous connective tissue involving the deposition of calcium phosphates and carbonates in the edematous connective tissue. Osteoblasts are regarded as fibroblasts with only a low osteolytic capacity, whose function is to oppose and restrict osseous tissue extension. According to their view the osteoblasts are 'useless parasites of osseous tissue.' The osseous metaplasia of connective tissue is regarded as a reversible process, and
bone absorption follows any local increase of vascularity in response to vasomotor control.

Keith (139) (1927) stated that many cases have been reported in which bone formed in the scars left by laparotomy. Osteoblasts, which are directly concerned in the formation of bone, are probably not transported by the bloodstream. Fibrous tissue anywhere can produce bone if it reverts to embryologic fibroblasts (embryonal state) or edematous fibrils and if calcium is present. As the arteries proliferate, lamellae of bone appear around them. The cells which assume a bone forming role are derived from the endothelium of the capillary system or from the reticulo-endothelium. Perhaps the action of an enzyme is necessary to stimulate this proliferation.

Huggins (140) (1931) found that when the urine has been diverted from the bladder of the dog, bone still forms in a fascial transplant to the wall of the bladder. The bone forms only in the transplant, and the newly formed epithelium of the transplant is the essential factor in the osteogenesis.

Abbott and Goodwin (141) (1932) stated that the mucous membrane of a dog's bladder when transplanted into muscle forms an epithelium-lined cyst wherein new bone is deposited after twenty days.

Ghormley and Stuck (142) (1936) stated: "It is our feeling, and this is corroborated by many writers, that the periosteum of a young person under certain conditions may stimulate formation of new bone but that the periosteum of older persons, at least on the shafts of the long bones where no muscular attachments are found, is a thin, almost non-functioning, ligamentous substance... The union of grafts and probably any new
formation of bone are largely due to transformation of local cells into a matrix, or basic substance, which under certain stimuli adds to itself the calcium and other salts necessary for its transformation into bone. We are convinced that the process of reducing the calcium content of the bone may hasten its union in a transplanted position. This may be due, again, to the fact that a decalcified graft is more readily permeable by this matrix, but we feel that an added stimulus is brought about by the chemical change of decalcification.

In meditation over this large amount of literature I find that with the development of bone surgery there arose the question of the importance of the periosteum in bone regeneration and repair. Clinical and experimental observation has failed to show whether the regenerative processes have their origin in the periosteum or in the cells of the bone itself. Much has been learned from the careful study of the changes which occur in transplantation of bone and periosteum, but wide differences of opinion have arisen as to the relative importance of the bone and the periosteum and the surrounding connective tissue elements in initiating the regenerative processes.
Bone cannot be considered as an inert support as is apparent from much evidence, both cellular and chemical. All fractures heal as do wounds elsewhere, unless there is a mechanical, chemical or anatomic bar to the healing. This healing in common with that of wounds of the soft parts, takes place through the medium of new connective tissue known as granulation tissue. Growth and repair are properties of living matter that have a great deal in common, and a study of one may yield results of importance in the understanding of the other. In certain lower forms of animal life and in the least specialized tissues of higher forms, repair may be almost identical with growth except in the matters of time, velocity and the nature of the stimulus bringing it about. Thus, repair following amputation of the leg of a salamander or the tail of a tadpole may proceed to the point of complete restitution of the missing part with its various types of tissue, and repair following injury of certain epithelial and connective tissues in man may approach the same order of perfection. Bone is one of the tissues in which repair simulates growth both in its physiologic and morphologic processes to a very considerable degree.

The capacity of fractured bones to solidify in a few weeks and to regain their functional activity may be regarded as one of the manifestations of nature's remarkable healing power.

The initial quick uprising of forces which form the basis of fracture healing is stimulated and controlled by nervous factors. Acute pain, generally experienced at the site of the fracture, is the signal
which calls attention to the injury. According to Turner (143), it is
the response to the painful stimuli which results in the initiation of
the steps which constitute the local pathology of a fracture. In the
repair of a fracture, nature's problem is to restore bone structure in
such a way that normal function may be resumed.

The trauma fractures the bone, tears or strips periosteum, produces
a hematoma and damages ends of fragments sufficient to kill osteocytes
for a variable distance back from the fracture line. Function is inter-
rupted because of pain and loss of mechanical support.

The blood free in the tissues and the dead tissue resulting from
the injury constitute tissue irritants. There is in effect an aseptic
inflammation; the parts surrounding the fracture are rapidly infiltrated
by hemorrhage, edema and inflammatory exudate.

Following this pathologic change there is clotting, i. e., laying
down of a fibrin network, from the hemorrhage and exudate in the tissues.
Organization of the hematoma as the first step in the healing of broken
bones has been known since fractures were first studied histologically.
Bier (144), emphasized the importance of the hematoma not only as a sub-
stratum but also as a stimulant for the growth of new bone. Haas (145)
found that the most striking features of his experiments was the in-
fluence of blood-clot in stimulating the formation of new bone. There
can be little doubt that the blood-clot exerts some specific influence,
since the presence of various foreign substances has failed to stimulate
bone regeneration. Pochhammer (146) found that the addition of agar-
agar, gelatine and living muscle tissue did not cause any increase in
bone regeneration. Kugelmass and Berg (147) by the injection of five
cubic centimeters of a one per cent solution of trypsin in the fracture site, digested away the blood clot and produced delayed healing, while by the injection of fibrinogen into the fracture site more than the average amount of callus was produced. Potts (148) believed the hematoma about a fracture to be suitable medium for the deposition of calcium salts and the formation of bony callus. Fibrin he found to be less effective than blood as a medium about a bone injury into which osteoid tissue may grow.

The fibrin network joins the bone ends and adjacent soft parts in a web of interlacing fibrils along which tissue repair is to take place. Thus it is seen that the blood is one of the important factors in the healing process. It clots about the bone ends, and in so doing forms a framework for the ingrowth of new tissue.

As a result of the death of tissue, hemorrhage and progressive circulatory stagnation (lymph and blood vascular) following rapidly after fracture, the pH of the local tissue fluid becomes acid. With this acidity there occurs over the next week or ten days a decalcification of the fracture site and the adjacent dead bone is held locally in the soft parts probably by chemical affinity for fibrin and collagen. This is Murray's (149) conception of this phase in the repair of fractures.

Stasis hyperemia has been used clinically since the time of Ambroise Paré to promote the formation of callus in fractures. An artificially produced and permanently maintained hyperemia will exercise a powerful stimulus on the tissues and tissue elements which participate in callus formation. Many observers have noted the correlation between venous stasis and the overgrowth of bone in pathologic states. Pearse and Morton (150) concluded that venous stasis caused stimulation of bone growth. They
observed that accelerated healing due to venous stasis was manifested by earlier formation of callus and earlier union.

Coincident with the change in pH at the fracture site, the healing process proceeds, as with any other wound, by the growth of new fibroblastic cells along the fibrin network, from all available connective tissue sources -- marrow cavity with its so-called endosteum, periosteum and fascial planes and muscular stroma, if they are opened into by the fracture.

This healing process is what one would expect in any wound and is explicable, if at all, on the same basis as all wound healing. At some stage during the process calcium is deposited in the healing tissue to surround the cells, and the tissue then is called callus instead of granulation tissue. Whether or not osteoid tissue (early callus) contains calcium or merely a pre-osseous substance in which calcium is deposited, as claimed by Leriche and Policard (151), whether or not specific bone-forming cells are involved in the process, either by direct growth and migration of osteoblasts from adjacent bone and periosteum and endosteum or by metamorphosis of connective tissue cells in reply to physiologic demand, whether or not ferment activity (phosphatase is an integral part of the story) and, if so, whence the ferment is derived are a much debated problem and cannot be considered here because of the extensiveness of the literature on the subject. That, in itself, is a problem in itself. The consensus of opinion is that the calcium deposited in the newly formed connective tissue is for the major part derived from local decalcification at the fracture site and that its deposition
occurs coincidentally with alteration of the pH of the tissue fluids locally toward the alkaline side as a result of the removal of tissue death products and the correction of stagnation of tissue fluids by a restoration toward normal of the lymphatic and blood vascular circulatory efficiency of the part (152).
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