AN

EXPERIMENTAL STUDY

OF

BONE TRANSPLANTATION

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FROM

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VOLUME I

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PREFACE

The experimental work embodied in this thesis was carried out in the Anatomy Department of the University of Glasgow.

I am particularly indebted to Professor Wyburn for the constant encouragement and advice he has given me and for the benefits of his kindly criticism.

I also wish to express my thanks to the technical staff for the considerable volume of detailed work so willingly and ably carried out by them.

Part of this work - Observations on Bone Transplants in the Anterior Chamber of the Eye was published in the Glasgow Medical Journal in October, 1949, and a contribution based on the work described in this thesis was delivered at the International Congress of Anatomists, Oxford, July, 1950.

September, 1951.

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INTRODUCTION

It is a century and a half since the first successful bone transplantation was carried out in animals and almost a century since the first authentic case of bone grafting in man. During this period, many investigators have carried out experiments and performed operations involving bone transplantation and there has been much discussion as to the choice of materials and methods which should be employed. The object of the present work is to attempt to find an answer to some of the outstanding problems.

When bone is removed from one individual and transferred to an individual of the same species, the transplant is said to be autogenous. Bone tissue removed from one individual and transplanted to another individual of the same species is described as a homogenous transplant and when the two individuals are closely related, it is called a syngenesious transplant. Finally, bone tissue removed from an individual of one species and transplanted to an individual of another species is described as a heterogenous transplant. It is now generally accepted that autogenous transplantation is, in all circumstances, the most favourable, but the

use of the other forms of bone tissue remains a subject of controversy which provides many of the unanswered problems. In view of the serious disability which results from the failure of union following the loss of continuity, it is natural that bone was the first type of connective tissue to be used as a graft. It is fortunate, therefore, that it is one of the most successful of the connective tissues in grafting procedures.

In spite of the work - clinical and experimental - on bone grafting, the following questions remain unanswered:-

A. The fate of different types of bone transplants - autogenous, homogenous, heterogenous.

B. What happens to the various tissue elements of bone and what part do they play in the event of recovery or replacement of a transplant?

C. The significance of the host tissue reaction and the extent to which it influences the final histological picture.

D. The optimum size and form of transplants.

E. The influence of the age factor in relation to the donor and the host.

F. The nature and source of the stimulus to osteogenesis.

The scope of the present work was defined by an attempt to answer these questions. A study of the literature on experimental bone transplantation indicates that the majority of the investigations have been of a short term nature. It was therefore decided that the necessary information could best be obtained by carrying out a considerable number and variety of experiments and by examining specimens removed at stated intervals, from ten days up to six months. Furthermore, it was thought that by implanting bone tissue not only into bone but also into muscle and into the anterior chamber of the eye, the fate of the transplant and the details of the histological processes involved could be more accurately assessed.

HISTORY

The history of bone transplantation is closely linked with the history of experimental work on bone growth and repair. Hippocrates (460 B.C.) believed that following injury to bone or cartilage, repair was effected by some other form of connective tissue. According to Galen (130 A.D.), the fractured ends of bone were united and solidified by an ossifying fluid supplied from the blood stream. The Galenic theory was not disputed until the 17th century when Havers carried out fundamental work on bone structure. He gave the first accurate description of bone structure including the vascular canals which have immortalised his name. In the 19th century, Von Volkmann completed the study of the blood supply of bone. Sir Arthur Keith (1918) relates how John Belchier, a London surgeon, when dining with a friend - a calico printer - in 1736, remarked that the bone in a leg of pork was red instead of white and found that his host fed his pigs on madder which he used in calico dyeing. Belchier then carried out experimental work on osteogenesis - work which was extended by Duhamel (1741) who observed that madder only stains growing bone. He described the two layers of the periosteum and named the inner layer "cambium" recognising it as the osteogenic layer. John Hunter(1746)

used madder fed animals to investigate bone growth and found that bones grow in length at their extremities. In this way, the experimental study of bone growth and bone repair was started and further knowledge of the processes involved was increased by the work of Syme and Goodsir in Edinburgh, Macewen in Glasgow, and Ollier in France whose work in particular influenced later researchers.

Any history of bone grafting must repeat the oft told story of the Russian nobleman who, in 1670, received a blow in the head from a sabre and subsequently had an extensive bony defect. The surgeon is reputed to have filled the gap with a piece of bone from the skull of a dog. Sir William Macewen (1881) refers to it as "the fabulous 17th century case". Buscarlet (1881) also describes it in some detail and remarks on the frequent allusion to it in the literature.

There are four epochs in the history of bone transplantation and each had the contribution of at least one outstanding experimentalist. In the first half of the 19th century, experimental work on animals was carried out by Merrem, Walther and others - work based on the 18th century investigations into growth and repair of bone. During the second half of the 19th century and including the early part of the 20th century, many names appear in the literature on bone grafting but probably the most

significant work was that of Macewen in Britain, Ollier in France and Nussbaum and Lexer in Germany. They experimented on the use of bone grafts in both animals and humans. The third historical period had as its outstanding personality Albee who was primarily responsible for the great advances in the clinical aspects of bone grafting. The fourth and final phase which includes the last two decades has been concerned with the factors responsible for new bone formation and particularly during the last ten years, with the study of homogenous bone and its preservation.

Modern work on bone transplantation stems from the experiments of Ollier (1825 - 1900). He is credited with the first successful free bone transplantation in the human. In 1859, he described experiments carried out in rabbits where he exchanged segments of the radius from one foreleg to the other and from the foreleg of one animal to that of another. In his conclusions, he noted that autogenous grafts were more successful than homogenous grafts. Nevertheless, he reported successful hetero transplants between animals in phylogenetic ascent, for example, from chickens to rabbits or from rabbits to man. In his book on Resection of Bones and Joints, written in 1885, he reviewed the whole field of bone grafting and many of the modern concepts regarding growth and repair of bone initiate from his work. He carried out many experiments on the heterotopic transplantation of bone,

e.g. under the skin and in other situations where bone is not normally present and satisfied himself of the success of these procedures. His criterion of success was the degree of vascularity and formation of new peripheral bone. In common with many other early workers, he attempted to transplant periosteum but reports only one success. His conclusions may be summarised as follows. Fresh bone covered by periosteum remains alive when transplanted but autogenous bone survives better than homogenous or heterogenous bone. Homogenous or heterogenous bone may be implanted in the animal body without being extruded. There is a fundamental difference between bone tissue from the same species and bone tissue from different species. Only autogenous bone increases in thickness by the deposition of new bone under the periosteum and this increment is the only reliable criterion that the graft continues to live. Other types of bone graft die and become sequestra with a foreign body reaction. When a permanent replacement of lost bone tissue is desired, autogenous bone covered with periosteum should be used.

The teachings of Ollier remained undisputed until the late 19th century during which many others worked on aspects of bone growth and grafting, including Percy, Paterson, Macewen, Nussbaum, Lexer, Poncet, Marshall, White, Shermann, Jaksh, Ricard, Adamkiewicz, Middledorpf,Flourens, Wolff, Ferrari, Durante, Moose and others. Barth (1893)

claimed that transplanted bone tissue dies and merely provides mechanical fixation until such time as it is replaced by new bone developed from the host tissue and not from the periosteum or any other part of the graft. He records this as his result, irrespective of the type of bone graft - autogenous, homogenous or heterogenous, dead or alive, with or without periosteum, untreated or boiled. It is interesting to note that as early as 1886, Sir. William Heath of Philadelphia in his Dictionary of Practical Surgery, suggested that human bone grafts might be obtained from amputated limbs and thus early envisaged the use of homogenous bone.

The use of foreign substitutes for bone was investigated by Bircher of Berne (1886) when he advocated intra-medullary ivory pegs and by Howard Marsh of London (1887) who used bone knitting needles to pin the tibia after resection.

Macewen of Glasgow (1874) recorded a case where he transplanted the parietal bone from a dog into a defect in the human skull with apparent success. Nevertheless, he states that in view of the findings of Ollier and others, he is not prepared to urge this as a surviving bone graft. He affirms, however, that the vegetative capacity of the bone cell is commensurate with that of the epithelial cell and is capable not only of survival but

of proliferation when grafted. He believed, however, that the smaller the fragments of bone, the greater the proliferation.

Curtis (1892) succeeded in healing an un-united fracture of the tibia by separating a segment of the fibula and pushing it across sub-cutaneously into a tibial defect. The following year (1893) he condemned the use of large free grafts, pointing out that they are difficult to insert and often undergo necrosis.

Macewen (1881) described the famous case of the boy in whom he built up, by stages, the shaft of the humerus, using fragments of homoplastic bone removed at osteotomies. Sometimes the transplanted bone was extruded but the majority of fragments remained in situ and at succeeding operations were seen to bleed freely. He was able eventually to complete the humeral shaft, proving beyond doubt that homogenous bone may be transplanted and satisfactorily repair bone defects. In the same year he wrote a brief historical resume of bone transplantation and recorded the following results of his own work to support the vitality and viability of transplanted bone tissue:-

Detached portions of bone with periosteal covering were washed with carbolised solutions and replaced. They increased in thickness, were surrounded by callus and united firmly to neighbouring bone.

A portion of bone devoid of periosteum and completely separated was detached and placed at a short distance from its original site. It became firmly attached and increased in bulk. A cylindrical portion of fractured humerus was re-attached and joined up satisfactorily. Portions of toes and fingers containing bone re-united after complete separation.
Phalanges of the finger when denuded glove-like will survive when the glove is replaced.

Referring to the homogenous bone fragments which he used to rebuild the shaft of the humerus, Macewen suggested that the vitality and viability of the transplant was indicated by the following facts:-
Only a few grafts were extruded. The others increased in size. Callus was formed from the earlier transplants when their ends were refreshed to insert the new grafts. The bones were vascular when refreshed at subsequent operations. The surrounding membrane had the appearance of normal periosteum.

The literature on bone transplantation up to the beginning of the 20th century was reviewed by Axhausen (1908) and from the examination of many human and animal transplants, he concluded that Barth's statement regarding the failure of survival of any type of bone transplant is incorrect. He agreed with Ollier's findings regarding the relative

incompatability of the hetero graft and considered that autogenous bone is always preferable. Unlike Ollier, he believed that the essential requirement was the presence of living periosteum, that the transplanted bone itself dies, irrespective of the structure of the bone tissue transplant or the relationship between donor and recipient and that new bone from the surviving periosteum replaces the dead bone.

Lexer commenced his publications in 1907 and continued them throughout the first part of the present century. His extensive experimental work coincided with the technical advances made by Albee in America and they both showed particular interest in the transplantation of entire joints but record no successes in man.

Macewen (1912) writing on the importance of the periosteum, stated that it was a limiting membrane only and that regeneration took place from the osteoblasts in the graft itself. Brown (1913) suggested that living bone, if transplanted into bone, survives, provided there is functional demand, whereas bone transplanted into soft tissue only survives temporarily. Phemister (1914) showed that in the case of fragmented grafts, though some pieces die, osteogenesis occurs around most of them. Dobrowolskaja (1916) reported on the cultivation of bone tissue in vitro and was able to produce active cell growth from cancellous

tissue with or without periosteal covering. Hey Groves (1917) recalled attention to the claim that a bone transplant is osteogenic and not merely osteoconductive, indicating that a certain number of the cells survive and proliferate.

Albee did not concern himself much with animal experiments or with the controversy as to which of the constituents of bone were responsible for the healing of the graft but rather he proceeded on the basis of the well established fact that autografts were successful. He believed that all the elements of bone should be used. He proved in his practice that perfect technique, combined with strict asepsis and adequate fixation of the graft would lead to success in practically all cases. Indeed, from the technical point of view, there have been few material advances since Albee established his methods and it was he who improved and perfected the work of earlier investigators and whose methods established the bone graft as a routine orthopaedic procedure. Names associated with that of Albee during the third epoch are - Phemister, Brown, Hey Groves, McWilliams, Winnett Orr, Arbuthnot Lane and Jones.

The first world war (1914-18) brought a large number of serious bone injuries and provided an opportunity for the study of technical improvements. The bone graft emerged from this testing period showing a preference for

the autogenous graft, that the homogenous graft was less satisfactory and the heterogenous graft rarely successful. The massive onlay or inlay graft was preferred and it was concluded that the grafts should be at least three times the length of the defect with a period of fixation of three to six months.

During the past 30 years, experimental work on the bone graft has been concerned with:-

Improved technique in the use of autogenous grafts.

The relative value of cortical and cancellous bone grafts and of the large single grafts as compared with multiple small grafts.

The use of substitutes for autogenous bone.

Experimental production of bone and cartilage and investigation into the factors responsible for bone formation.

The preservation of homogenous bone and its storage in the form of a bone bank.

Barney Brooks (1920) introduced his method of delayed autogenous bone grafting, with the object of ensuring a quicker and more effective re-vascularisation of the transplant. He experimented on dogs and the graft was separated from its bed but not removed. It was transplanted 10 days later by which time it had acquired a covering of osteoblastic tissue and he claimed that by this means osteogenesis was more active. The delayed autogenous graft was also thought to be of greater value because a certain amount of decalcification increased permeability of the graft. Orell (1937) introduced the bone grafts described by him as Os Purum and Os Novum. Os Purum consists of heterogenous or homogenous bone treated by physical and chemical

means so that a large proportion of its organic content is removed - the object being to reduce the reaction of the host to the transplant and to render the transplant more permeable. Os Novum is prepared by the implantation in the tissues of splinters of Os Purum. These become surrounded by new bone formation and it is considered that when the graft is removed and applied to its new site, this young osseous tissue will actively promote osteogenesis.

In the last epoch investigation has centred on osteogenesis normal and abnormal, and the histological processes involved in bone transplantation. In this respect the contributions of Leriche and Policard, Wells, Shands, Robison, Heinan, Annersten, Lacroix, Blum, Pollock, Ghormley, Urist, in particular have added to our knowledge. Whereas Macewen, Goodsir and others considered that osteogenesis was a specific function of the bone cell, Wells (1911) claimed that osteoblasts differentiated from non-specific C.T. cells in response to variations in the local calcium concentration. Leriche and Policard (1928) held similar views and sought experimental proof therefore.

Robison (1923) succeeded in isolating an enzyme which he called phosphatase, and considered was the controlling factor in osteogenesis. Pollock (1922) and Levander (1938) observed new bone formation following the intra-muscular injection of an extract of bone. Annersten (1940), Bull (1944) and several other investigators have carried out experiments along these lines, and Lacroix (1947) from the results of his work has consistently urged the existence of a specific osteogenic substance.

DESCRIPTION OF EXPERIMENTS, METHODS AND MATERIALS.

The results described are based on a series of 214 animal experiments, consisting of bone transplantations in rabbits. The animals used were bred from a mixed stock obtained from various sources. Extremes of age were avoided but younger animals and closely related animals were specially noted. Throughout the series, an autogenous transplant and a homogenous transplant were performed at one session with bone taken from the same source, so that there are available for examination 107 specimens of autogenous transplants and a like number of homogenous transplants. The bone tissue was left in situ for varying periods of time, specimens being removed for histological examination at intervals of 10 days, 21 days, 42 days, 80-120 days and 180 days after operation.

Several groups of experiments were performed. In the first group, bone tissue was transplanted into the depth of the gluteal muscles. In the second group, bone tissue was transplanted into a freshly prepared site in the ilium. In the third group, bone tissue was implanted into the anterior chamber of the eye. In the fourth group, bone tissue was preserved by refrigeration and later implanted into a prepared site in the gluteal muscles. In the fifth group, heterogenous bone was implanted into the gluteal muscles.

TABLE SHOWING THE NUMBER OF EXPERIMENTS IN EACH GROUP.

	Duration of Implantation in Days.				
	10	21	42	80-120	120-180
GROUP A					
Into Muscle					
Autogenous	8	8	6	6	6
Homogenous	8	8	6	6	6
GROUP B					
Into Bone					
Autogenous	6	6	6	6	6
Homogenous	6	6	6	6	6
GROUP C					
Into Anterior Chamber of Eye					
Autogenous	7	7	6	4	4
Homogenous	7	7	6	4	4
GROUP D					
Refrigerated Bone					
Autogenous	6	-	6	-	-
Homogenous	6	-	6	-	-
GROUP E					
Heterogenous Bone	-	-	6	-	-

ANAESTHESIA.

In all experiments, general anaesthesia was used, consisting of pre-medication with nembutal, followed by the administration of open ether. During the preliminary operations, two deaths occurred under anaesthesia but thereafter there were no deaths throughout the series.

TRANSPLANTATIONS INTO MUSCLE.

The field of operation was carefully prepared and with aseptic technique the crest of the ilium was exposed. The abdominal muscles were separated from it and a segment of bone 15 mms. x 5 mms. was removed. Adherent muscle tissue was excised but the periosteum was left intact. In 6 experiments a segment of cortical bone 15 mms. in length and consisting of one third of the substance of the bone was removed from the diaphysis of the femur. The excised bone was divided into four equal parts and placed in normal saline at body temperature. The gluteal fascia was incised and two fragments were inserted into the depth of the muscles - autogenous transplant. The incision in the gluteal fascia was closed and the muscles re-apposed to cover the site of removal from the iliac crest. The other two fragments were then implanted in the depth of the gluteal muscles of another animal - homogenous transplant. 68 transplantations into muscle were performed

and the bone tissue left in situ for varying periods as shown in the table on page 16 . Each animal was x-rayed a few days after operation in order to confirm the presence and position of the bone fragments. Further x-rays were taken at intervals of a week for the first month and thereafter at increasing intervals so that in each case a series of x-rays is available to show the position of the transplant and the radiological changes which it undergoes. At the times stated, the animals were killed and the transplants removed, together with the surrounding soft tissue. Each specimen was x-rayed on removal. Serial sections of 10 μ in thickness were cut, stained with Hematoxylin and Eosin and prepared for microscopic examination.

TRANSPLANTATIONS INTO BONE

The crest of the ilium was exposed and the gluteal muscles raised to display the outer aspect of the ilium. Using a trephine, a full thickness disc of bone, 7 mms. in diameter was removed from the ilium on the right side. A second disc, 6 mms. in diameter, was removed from the ilium of the opposite side and into this prepared site the first disc was fitted so that exact apposition was achieved - autogenous transplant. The second disc was then fitted

into a site prepared in the ilium of another rabbit by the removal of a disc 5 mms. in diameter - homogenous transplant. The soft tissues were accurately re-apposed to minimise bleeding. 60 transplantations into bone were carried out and the transplants left in situ for varying periods, as shown in the table on page 16 .Each animal was x-rayed a few days after operation in order to confirm the presence and position of the bone fragments. Further x-rays were taken at intervals of a week for the first month and thereafter at increasing intervals. At the arranged intervals, the animals were killed. The whole ilium was removed, the adherent soft tissue excised and the specimen x-rayed. After decalcification, the bulk of the specimen was reduced, leaving only the transplanted disc and the iliac bone immediately surrounding it. Sections 10 μ in thickness were cut, stained with Hematoxylin and Eosin and prepared for microscopic examination.

TRANSPLANTATION INTO ANTERIOR CHAMBER OF EYE.

The technique of intra ocular implantation was described in detail by Schochet in 1920 but several articles describing the use of the method appear much earlier. As long ago as 1877, Cohnheim appears to have originated the method and it was subsequently used and described by Salomonsen (1879), Baumgarten (1880) and Klebs (1883). The method was used by H.S.N. Greene (1940) who described its advantages as a means of studying tumour cell growth.

The anterior chamber of the eye is, for several reasons, a suitable site for the growth of transplanted tissue. The implantation can be effected with ease. There is little or no disturbance to the animal and the tissue which is visible through the cornea grows under optimum conditions of body temperature and nutrition. According to Greene, the aqueous humor is not an isolated fluid. There is no selective barrier between it and the circulating blood and it participates in all the general body reactions. Greene found that following the growth of tumour cells in the anterior chamber of one eye, the anterior chamber of the opposite eye was resistant to re-inoculation, thus indicating that it participated in immune reactions. Bone tissue transplanted into the anterior chamber is free from the local influence

of other bony tissue and therefore living bone cells found in such a transplant are either survivors of the original transplant or are host tissue cells which have differentiated in response to some osteogenetic stimulus. The bony implants remain clearly visible within the anterior chamber of the eye.

The iliac crest was exposed and a segment of bone removed by cutting forceps. This was sub-divided into smaller fragments about 2 mms. in diameter which were placed in sterile normal saline. The conjunctiva was grasped with forceps and the eye ball dislocated, a simple procedure in the rabbit. The cornea was punctured obliquely by a small sclerotome, just in front of the limbus and fragments of bone were inserted through this small aperture into the anterior chamber. An iris repositor was used to push the fragments into the anterior chamber and if necessary to replace any herniation of the iris. Although the aqueous humor escaped freely through the incision, the normal tension was restored within 24 hours. Following the operation, albucid drops were instilled into the conjunctival sac but in no case was there any ocular infection and the animals seemed quite undisturbed by the operation. 56 transplantations into the anterior chamber were performed - the transplants being left in situ for varying periods as shown in the table on page 16.

The transplants were examined in the anterior chamber by means of slit lamp illumination but the observations were of little value in assessing the condition of the bone tissue. X-ray examination of the specimens in situ was not possible. At the arranged intervals, the animals were killed and the specimens consisting of the small transplants, together with surrounding connective tissue and a varying amount of ocular tissue were removed and x-rayed. Sections 10 μ in thickness were cut, stained with Hematoxylin and Eosin, and prepared for microscopic examination.

PRESERVED BONE TRANSPLANTS

The crest of the ilium was exposed and a segment of bone 15 mms. x 5 mms. was removed; adherent muscle tissue was excised but the periosteum was left intact. This fragment of bone was divided into four equal parts, wrapped in sterile gauze, moistened with normal saline solution and stored in a refrigerator at +1°C to +5°C for 72 hours. Thereafter two fragments were implanted in the gluteal muscles of the animal from which the bone was taken - autogenous transplant - and the other two fragments were implanted into the gluteal muscles of another animal - homogenous transplant. 24 transplantations of preserved bone into muscle were carried out, of which

12 remained in situ for 10 days and 12 for 42 days. Each animal was x-rayed a few days after operation and at weekly intervals thereafter. At the times stated, the animals were killed and the transplant removed. Each specimen was x-rayed on removal. Serial sections 10 μ thick were cut, stained with Hematoxylin and Eosin and prepared for microscopic examination.

HETEROGENOUS BONE TRANSPLANTS INTO MUSCLE

Two fragments of human cancellous bone 6 to 8 mms. in diameter were implanted into a prepared site in the gluteal muscles of the rabbit - heterogenous transplant. 6 experiments of this kind were performed and the bone was left in situ for 42 days. Each animal was x-rayed a few days after operation, at weekly intervals thereafter and each specimen was x-rayed on removal. Serial sections 10 μ thick were cut, stained with Hematoxylin and Eosin and prepared for microscopic examination.

DESCRIPTION OF OBSERVATIONS.

The same procedure was adopted throughout the description of the transplants. An autogenous and a homogenous transplant of similar age group is described alternately in each set of experiments as follows:-

(1) Description of x-ray appearance of transplants in situ and after removal.

(2) Histological examination of the sections of the transplants.

(a) General picture - viability of transplant, host tissue reaction etc.

(b) The matrix of the transplant and its cellular content.

(c) Included cartilage tissue.

(d) The soft tissue occupying and lining the endosteal spaces, the haversian canals and any transplanted periosteum - that is the tissue sometimes referred to as skeletal connective tissue.

(e) Marrow tissue.

(f) The host tissue reaction and its relation to the transplant.

BONE TRANSPLANTS INTO MUSCLE.

10 DAYS AUTOGENOUS TRANSPLANTS.

X-Ray examination of the transplant in situ confirmed the presence and position of the bone fragments. Examination of the specimen on removal shows that the fragments are separate and have undergone no radiological change.

The general histological picture (Fig. 1) is that of trabeculae of cancellous bone with marrow filled spaces. Surrounding the bone tissue of the transplant and separating it from the muscle tissue is a capsule of host connective tissue. New bone formation with trabecular formation is already taking place in the zone between this connective tissue reaction and the transplant.

Although the bony matrix of the transplant is well stained, the majority of the lacunae are empty with no evidence of nuclear staining except in the peripheral parts of the trabeculae (Fig. 2). Here the well stained nuclei indicate the survival of the less mature cells. Surrounding the transplant and lining its endosteal spaces is a layer of large cells in close contact with the bone. At some places these cells form a regular layer like the endosteum of mature bone. Elsewhere there are multiple layers of proliferating cells loosely arranged which have assumed the appearance of

osteoblasts and migrate as if to invade the matrix. The marrow tissue is of normal appearance.

The surrounding host connective tissue reaction (Fig. 3) is vigorous, and forms a capsule separating the transplant from the muscle tissue. Peripherally it consists of smaller spindle shaped cells, but they become larger and more ovoid until they assume the form of osteoblasts close to the transplant (Fig. 4). Young bone tissue in trabecular formation is being laid down in this region, in apposition to the transplant. Some of the osteoblasts particularly large, appear to invade the transplant.

10 DAYS HOMOGENOUS TRANSPLANTS.

X-Ray examination of the transplants in situ and of the specimens after removal shows that the bone fragments are separate. There is no radiological evidence of structural change.

Histological examination shows that the bone is dead (Fig. 5). The matrix is well stained but has a somewhat opaque quality. In the majority of the specimens the lacunae are all empty and there is no nuclear staining. In two specimens (Expts. 23, 120) the mature osteocytes have disappeared but some of the younger cells at the periphery of the trabeculae have survived. Islets of cartilage (Fig. 8) frequently seen in sections of bone from the rabbit iliac crest are alive, showing well stained

nuclei and the marrow tissue is of normal appearance. The transplant is demarcated by a dense outline (Figs. 5, 6) which is due to commencing lysis at the periphery of the matrix. In this zone (Fig. 6) but outwith the dense line there are large nucleated cells or osteoblasts which are laying down pre-osseous tissue. This represents the earliest stage of what has been described as " creeping substitution ".

The host connective tissue reaction has similar features to those seen in the corresponding auto transplant but it is more intense. Its cells are spindle shaped peripherally but more rounded and larger close to the transplant, and a new feature is the presence of a round cell " lymphocytic " infiltration with some plasma cells (Fig. 7). Where cartilage tissue of the transplant is in immediate contact with the host tissue the reaction of the latter is vigorous (Expt. 121) and differentiation into osteoblasts with the deposition of new bone in trabecular formation is more advanced than elsewhere.

21 DAYS AUTOGENOUS TRANSPLANTS.

X-Ray examination of the transplants in situ reveals no radiological change. Examination of the specimens on removal shows that the outline is smooth and some rarefaction has taken place.

On histological examination the matrix is well stained. Considerable areas, particularly the smaller trabeculae and the periphery of the larger trabeculae contain the large and well stained nuclei of young bone cells but there are many devitalised regions with empty lacunae (Fig. 9). The large cells, potential osteoblasts, surrounding the transplant and lining the endosteal spaces form a regular single layer in some parts but elsewhere they are actively multiplying and migrating to repopulate the matrix of the transplant. Cartilage survives and its ground substance stains more deeply than that of the transplanted bone tissue (Fig. 10). Where the transplant consists of dense cortical bone (Fig. 11) there is an entirely different picture. The cells which are nearly all mature osteocytes degenerate leaving vacant lacunae except in the immediate vicinity of the endosteum and the haversian canals. The connective tissue cells lining these spaces proliferate and commence to repopulate the transplanted matrix, but the process is slower than when the transplant is of cancellous bone.

The host tissue reaction is similar to that of the 10 day specimens, consisting of spindle shaped cells differentiating into osteoblasts and laying down young osseous tissue in contact with the transplant. When the transplant includes cartilage (Fig. 10) the host tissue reaction is more intense and its cells differentiate into osteoblasts and chondroblasts laying down young chondro-osseous tissue. This young tissue appears to invade the bony matrix of the transplant and to replace it.

21 DAYS HOMOGENOUS TRANSPLANTS.

X-Ray examination of the transplants in situ reveals no radiological change. Examination of the specimens on removal shows that there is no alteration in the density of the bone but the outline is irregular.

The transplant is quite devitalised (Fig. 12), the matrix has an opaque appearance and none of the transplanted osteocytes have survived. The sharp outline is again apparent and in some specimens this denser zone is being invaded by large cells resembling osteoclasts (Figs. 12, 15) but in others (Fig. 13) the a-cellular matrix has no histological contact with the surrounding tissues. In striking contrast to the dead bone, the incorporated cartilage has survived (Fig. 14). The soft tissue of the endosteal spaces (Fig. 12) consists of spindle shaped cells loosely arranged. These cells do not differentiate into osteoblasts

and there is no peripheral layer of large cells surrounding the bone trabeculae as seen in the autogenous transplants. Marrow tissue is present but is being replaced in many specimens by loose connective tissue (Figs. 13, 14).

The host tissue reaction consists of proliferating connective tissue with the typical spindle cells, some lymphocytes and plasma cells. Close to the transplant, the host connective tissue cells have differentiated into osteoblasts which are laying down bone trabeculae adjacent to the graft. This more deeply staining bone is replacing parts of the periphery of the transplant (Fig. 16).

42 DAYS AUTOGENOUS TRANSPLANTS.

X-Ray examination of the transplants in situ and of the specimens on removal shows that the two implanted fragments have united. The texture of the cancellous bone is apparent but there is marked rarefaction. The transplants have a smooth clearly defined outline due to a surrounding layer of denser (cortical) bone.

At 42 days the transplant consists of living bone, the matrix is well stained and cellular except in the middle of the larger trabeculae (Fig. 17) where the lacunae are empty. The well stained nuclei of the osteocytes are larger than those of mature rabbit bone but their numbers and distribution are similar (Figs. 18, 19). Islets of cartilage tissue survive but in specimen No. 103 (Fig. 21)

the bone cells are few in number even adjacent to cartilage. The endosteal spaces (Figs. 17, 18) are occupied by normal surviving marrow and loose connective tissue but a well defined layer of larger cells forms an endosteal lining in close contact with the trabeculae. A study of the section with higher magnification (Fig. 19) discloses that cells from this layer penetrate, in some way or other, the bone matrix and replace the degenerated osteocytes. Since, however, the cell population of the transplant now approximates to the normal, there is no longer the active proliferation and migration seen in earlier specimens. Where there is a young donor and a young host there is a comparatively greater proliferation of the cells of the transplant (Figs. 17, 20).

The host connective tissue reaction is now less active-looking. The cells (Figs. 17, 20) are predominantly fibrocytes and there is little evidence of the differentiation into osteoblasts described in the younger transplants. These host connective tissue cells form a capsule of fibrous tissue around the bone - a fibrous tissue periosteum - the innermost layer of which persists as the osteogenic (cambium) layer clothing the new bone formed from the host tissue.

42 DAYS HOMOGENOUS TRANSPLANTS.

X-Ray examination in situ and after removal shows that the grafted segments of bone have become fragmented. The outline is irregular and there is no evidence of the formation of a cortical layer.

The transplanted bone is dead (Figs. 22, 23, 24). The matrix has a dense outline, is less well stained and its lacunae are all empty. There is now little evidence of the invasion of this dense peripheral zone by the large cells seen at earlier stages; there is in fact no histological contact between the calcified matrix and the soft tissue occupying the endosteal spaces. This endosteal tissue consists of loosely arranged elongated cells. There is no differentiation into osteoblasts, and no normal marrow tissue. Cartilage when present, has a devitalised appearance with but a few surviving cells.

The host connective tissue (Fig. 25) resembles young granulation tissue, consisting of proliferating cells with large nuclei and numerous capillaries. In some zones (Fig. 26) lymphocytic infiltration is pronounced. Young connective tissue cells are differentiating into osteoblasts and laying down new bone (Figs. 25, 27), characterised by its deeply staining matrix and large osteoblasts. In some regions, as the process of substitution advances (Figs. 27, 28), the matrix of the dead transplant undergoes lysis and

the dissolving periphery recedes, wave-like. This receding edge stains more deeply than the rest of the matrix thus accounting for the dense outline so characteristic of the homo transplant. The new formed bone which also has a well stained ground substance with numerous large osteoblasts, follows closely the retreating edge of the dissolving matrix but there is always an interval between them.

80-120 DAYS AUTOGENOUS TRANSPLANTS.

X-Ray examination of the transplants in situ and of the specimens on removal shows that the bone fragments have usually united. They have trabecular structure with an outer layer of dense bone. In one specimen (Expt. 22), where the fragments remain widely separated, each shows differentiation into an outer cortical layer and inner trabeculated cancellous tissue.

The majority of the transplants have now the histological features of normal rabbit bone (Fig. 29). The matrix is well stained and populated by mature-looking osteocytes (Fig. 31), though in the larger trabeculae (Fig. 30) osteoblasts are still invading the a-cellular regions of the matrix. Where the autogenous transplant consists of dense bone (Fig. 33) recovery is delayed and, while there are osteoblasts at the periphery of the bone and the regions surrounding the endosteal spaces and haversian canals, elsewhere the matrix is devoid of cells.

The endosteal spaces contain marrow and loose connective tissue (Fig. 29) and are lined with a single layer of larger cells immediately adjacent to the bone tissue, except where the process of repopulation still continues, and masses of large proliferating cells (Fig. 30) adjoin the bone and are seen to migrate into the matrix.

The host connective tissue (Fig. 32) forms a fibrous capsule separating the transplant from the muscle but the transition from spindle shaped connective tissue cells to rounded osteoblasts with the deposition of young bone trabeculae continues in some places. This new bone has endosteal spaces lined by large cells and encloses marrow tissue. Where the transplanted tissue includes cartilage (Fig. 34) the host tissue reaction is vigorous and its cells differentiate into both osteoblasts and chondroblasts resulting in the formation of young chondro-osseous tissue.

80-120 DAYS HOMOGENOUS TRANSPLANTS.

X-Ray examination of the transplants in situ and of the specimens on removal shows that not only do the segments fail to unite but that fragmentation occurs. There is no cortical layer and the outline is ragged. In two specimens (Expts. 27, 126), the transplants are considerably reduced in size but in none is there any apparent loss of density.

The general picture is that of a sequestrum surrounded by mature connective tissue and with no evidence of new bone formation (Figs. 35, 36, 37, 38). The bone matrix is sharply outlined and a detailed survey of this dense peripheral zone reveals that in all but a few areas (Figs. 35, 38) there is no cellular invasion and the sequestrum like transplant is isolated from the surrounding tissue. Cartilage tissue has survived in some specimens (Figs. 36, 37) although it is in immediate contact with dead bone. The endosteal tissue (Figs. 37, 38) consists of loosely arranged elongated cells, and there is an absence of the layer of large cells - the differentiated osteoblasts - which line the endosteal spaces of the autogenous transplant. Marrow tissue is no longer present. In the sections from one specimen (Expt. 123), (Fig. 39) where the donor and host are closely related - syngenesious transplantation - bone tissue and marrow have survived.

The host connective tissue consists of mature fibrous tissue and the lymphocytic infiltration present at earlier stages has almost disappeared. There is no cell differentiation and osteogenesis; on the contrary, young bone tissue laid down in apposition to the transplant is quite devitalised (Fig. 40), its deeply staining matrix being honeycombed by the empty lacunae previously occupied by large osteoblasts.

180 DAYS AUTOGENOUS TRANSPLANTS.

X-Ray examination in situ and of the specimens on removal shows the transplant as a small irregular bone, consisting of cancellous tissue surrounded by a cortex of dense bone with a smooth outline. The fragments have united into one bony mass and the transplant is of normal density and texture. There is one exception (Expt. 119), in which the fragments have not united, the periphery is irregular and there is no evidence of a cortex.

Histologically, the general picture is that of normal, mature bone (Figs. 41, 43) surrounded by a capsule of fibrous tissue which separates it from the muscle. The ground substance stains well and is populated throughout by osteocytes, but at the periphery of the trabeculae and the regions surrounding haversian canals the cells are larger and osteoblastic in type. The endosteal tissues comprise spindle shaped cells which give place to a uniform layer of

larger cells lining the trabeculae. In some places these potential osteoblasts proliferate and cells can be seen to invade the matrix (Fig. 43). The marrow tissue is abundant (Fig. 42), and healthy in appearance, and in several specimens the transplant has formed a bony ring enclosing it. In the specimen already mentioned (Expt. 119), the transplant is devitalised, bony lacunae are empty and the clear-cut outline of the trabeculae has no connection with the soft tissue in the endosteal spaces. Cartilage tissue is also dead and shows the large vacant lacunae of the cartilage cells.

The host reaction now consists of fibrous connective tissue forming a capsule around the transplant (Figs. 41,43), with typical fibrocytes, except those in close contact with the periphery of the bone. Adjoining the bone the cells are larger and more ovoid and can be seen invading the bone matrix like the osteoblasts of the endosteum. These cells constitute the osteogenetic layer of the periosteum.

180 DAYS HOMOGENOUS TRANSPLANTS.

X-Ray examination of the transplants in situ and of the specimens on removal shows that in most cases the grafts are reduced to fragmentary remnants with an irregular outline and no evidence of differentiation into cortical and cancellous bone. In one specimen (Expt. 143), the bone shadow is just visible and in another (Expt. 144), it has entirely disappeared.

The region of the transplant now consists of a space enclosed by a capsule of mature fibrous tissue infiltrated in places by lymphocytes. In some specimens (Expts. 24, 25, 165), the transplant is present as a small sequestrum (Fig. 45) with a disintegrating matrix, empty lacunae, and its outline frayed and separated from the surrounding tissue. In other specimens (Expts. 143, 144), no bone or cartilage tissue can be seen and the transplantation site consists of a ring of fibrous tissue enclosing some large crystals (Fig. 46) the remains of the inorganic elements of bone. In one specimen (Expt. 166), healthy cartilage tissue is present alongside healthy bone with young, well staining bone cells. This chondro-osseous tissue forms a ring enclosing marrow tissue and has an appearance similar to that of the autogenous transplant of the same age.

When the transplant is reduced to a sequestrum or has

entirely disappeared there is no evidence of living endosteal tissue and no marrow tissue is seen (Figs. 45, 46).

The host connective tissue forms the fibrous tissue capsule enclosing the site of the transplant, but it does not differentiate into the osteogenic layer characteristic of earlier stages. In the specimen (Expt. 166) in which the cartilage and bone have survived, the surrounding host connective tissue (Fig. 47) provides the osteoblasts which form the layer of large cells closely apposed to the transplant and invading its periphery.

SUMMARY OF OBSERVATIONS.

There is no radiological change in the autogenous transplant at 10 days, but at 21 days some decalcification is apparent. At 42 days and later the two fragments have united, the outline becomes smooth and the transplant has the appearance of a small bone with cancellous tissue surrounded by a cortex of dense bone. The homogenous transplant does not undergo decalcification, but remains a dense shadow with an irregular outline, like a sequestrum. The fragments do not unite and there is no cortical formation. Later, at 80-120 days fragmentation occurs and the specimen is gradually reduced until in some experiments it disappears entirely.

During the first 21 days the autogenous and the homogenous transplants are histologically similar, the mature osteocytes die so that the transplanted matrix becomes devoid of cells except at the periphery of the trabeculae where the young bone cells survive. Even at this early stage, however, cellular degeneration is greater in the homogenous transplants than in the autogenous transplants. The transplanted soft tissue survives and in the autogenous specimens at 10 days it is actively proliferating and already showing differentiation into osteoblasts. These cells are capable of migration and make their way into the substance of the matrix to repopulate it. Where this function is proceeding vigorously the endosteal osteoblasts are arranged

in clusters and merge into the transplanted matrix blurring the bony outline. The repopulation of the autogenous transplant by cell migration from the endosteum proceeds until the trabeculae have the normal complement of cells. The endosteal cell proliferation then ceases and osteoblasts form a uniform layer of cells lining the endosteal spaces. At 80 days the osteocytes of the transplant are mature cells and by the end of six months the transplant has the appearance of healthy adult rabbit bone.

In the homogenous transplants there is no endosteal reaction and formation of migrating osteoblasts. By 42 days the bone is sequestrum like, all the osteocytes are dead, and the endosteal tissue consists of undifferentiated spindle shaped cells. The outline of the matrix is definite. At the end of six months the transplant is either completely absorbed or is a sequestrum.

Cartilage which is frequently present is transplants removed from the crest of the ilium, part of the epiphysis, survives in both autogenous and homogenous specimens up to 42 days. Thereafter, although it remains healthy in the autogenous transplants it becomes progressively devitalised in the homogenous transplants until in the later specimens it is quite dead. This cartilage tissue in the autogenous transplant together with the immediately surrounding young bone tissue survives much better than the mature bone tissue. In syngenesious transplants the cartilage survives and behaves in a fashion similar to that in the autogenous transplants.

Marrow tissue remains healthy in all the autogenous transplants, it withstands transplantation well and new marrow appears in the endosteal spaces where osteogenesis has occurred. In the homogenous transplants, by the end of 21 days, the marrow is being replaced by undifferentiated connective tissue, and after 42 days there is little evidence of it in any of the sections.

When bone tissue is implanted in a muscle bed, the host reacts to its presence by forming around it a capsule of young fibrous tissue. In the early stages there is little difference in the reactions evoked by autogenous and homogenous transplants except that in the latter lymphocytes and plasma cells infiltrate the surrounding capsule and in some places form dense aggregations. Both autogenous and homogenous transplants induce active osteogenesis in the host tissue, the young cells close to the transplant proliferate and differentiate into osteoblasts which lay down young bone tissue characterised by its trabeculae of deeply staining matrix and large cells. Where the transplant is autogenous these osteoblasts form a periosteal layer, similar to the endosteal layers already described, and from this osteogenetic layer cells migrate into the matrix to aid in its repopulation. Where the transplant is homogenous, the periphery is clearly outlined by its more deeply staining dissolving edge and as the matrix gradually recedes it is replaced by the young bone tissue laid down by the host. The presence of cartilage in the transplant, whether autogenous

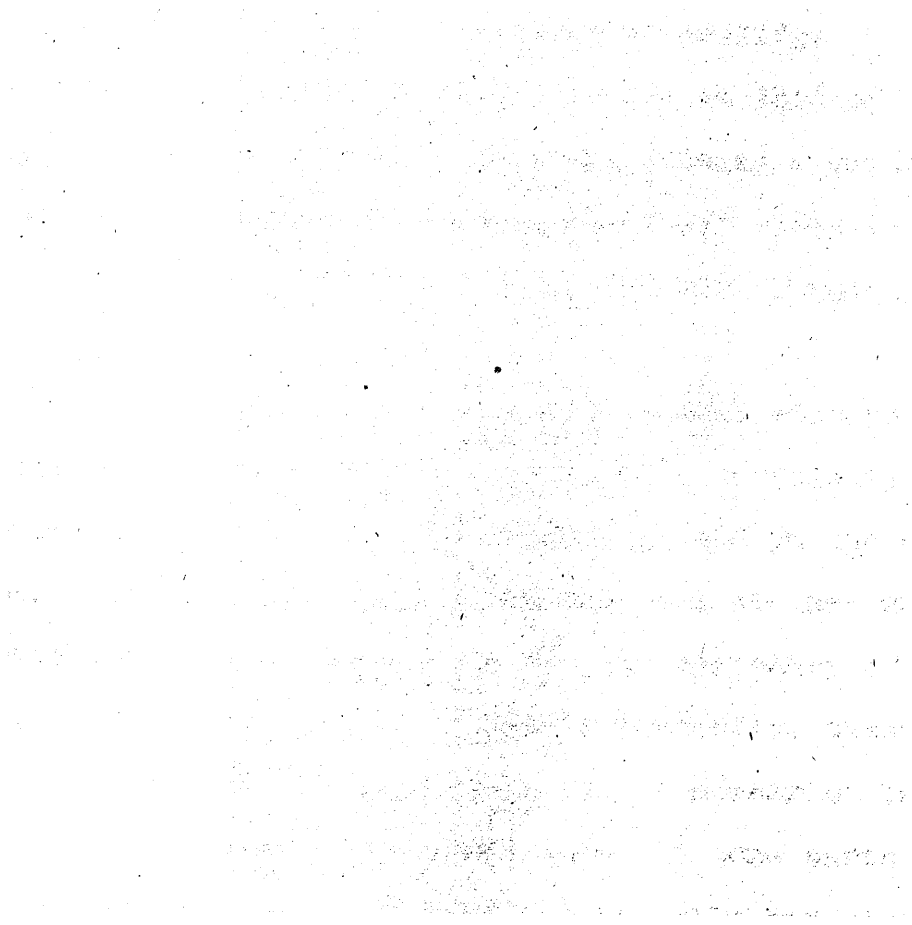
or homogenous, seems to provoke greater activity in the various reparative processes; osteogenesis in the host tissues is vigorous and the new formed tissue is a mixture of young bone and cartilage.

By the end of 42 days, at which time the autogenous transplants are considerably revitalised, the host reaction subsides, the fibrous tissue matures and the osteogenetic layers of cells in contact with the bone come to form a uniform layer; proliferation, migration and invasion has ceased and the outline of the transplant has become more definite. At later stages the maturing host tissue capsule becomes a fibrous periosteum of which the innermost layer remains as the osteogenic or "cambium" layer.

With the homogenous transplant, the sequence of events is different. At 42 days the new bone formation and substitution still continues, but it later subsides and the maturing host tissue forms a fibrous capsule with no osteogenic layer. The transplant is now a sequestrum and even the young bone tissue laid down by the host tissue to substitute it undergoes necrosis. Finally the transplant remains as an encapsulated sequestrum or it undergoes lysis and is absorbed.

The living bone which results from the implantation of autogenous transplants is thus not only derived by the repopulation of the transplanted matrix from its own skeletal connective tissue but includes new bone formed by the host tissues. Where the transplant consists of dense bone with a higher proportion of mature osteocytes, the cell degeneration

is greater and repopulation is delayed since it is difficult for the migrating osteoblasts to permeate the denser matrix. The syngenesious transplant behaves in a manner similar to the autogenous transplant especially if it consists of young tissue.



BONE TRANSPLANTS INTO BONE.

10 DAYS AUTOGENOUS TRANSPLANTS.

On x-ray examination of the transplants in situ, and of the specimens on removal, the disc of bone is clearly visible. There is no change in the density of the transplanted bone as compared to that of the surrounding ilium and no radiological evidence of healing.

The general histological picture is that of a partly devitalised disc of bone accurately fitting a circular defect in the ilium and surrounded on all sides by a layer of organising blood clot in which new bone formation is taking place.

The matrix of the transplant is well stained but many of its cells are degenerated (Fig. 48), especially in the central areas of the larger trabeculae and in the denser bone. Young bone cells with large well stained nuclei occupy the smaller trabeculae and the periphery of the larger trabeculae. The endosteal connective tissue has proliferated and differentiated into a number of large cells. These form a uniform endosteal layer in some parts but elsewhere they multiply into clusters of cells and migrate to repopulate the bony matrix of the transplant.

The host tissue forms a capsule surrounding the

transplant on all sides and consists of young connective tissue with spindle shaped cells, some of which are differentiating into large round osteoblasts (Fig. 49). These cells become enveloped in a ground substance arranged in the form of ill defined trabeculae. This young bone tissue is closely applied to the transplant and even at this early stage it is difficult to determine a line of demarcation between transplant and host (Fig. 48).

10 DAYS HOMOGENOUS TRANSPLANTS.

X-ray examination of the transplants in situ and of the specimens on removal shows the same features as in the corresponding autogenous transplants.

The general picture on histological examination closely resembles that of the autogenous transplant. The matrix is well stained and osteocytes are present (Fig. 50), but closer inspection shows that many of the nuclei are small and devitalised. Connective tissue cells occupy some of the endosteal spaces but they are less abundant and do not exhibit the proliferation seen in the autogenous transplant. Marrow tissue survives and has a healthy appearance.

The connective tissue reaction of the host is similar to that in the corresponding autogenous transplant. There is some round cell infiltration but it is not a marked feature. Large spindle or oval shaped connective tissue cells are differentiating into osteoblasts which form young osseous tissue in close apposition to the transplant.

21 DAYS AUTOGENOUS TRANSPLANTS.

X-Ray examination of the transplants in situ and of the specimens on removal shows little radiological evidence of change. The disc is clearly visible and its density is unaltered.

The matrix of the transplant is well stained and young bone cells with large nuclei occupy the smaller trabeculae and the periphery of the larger trabeculae (Fig. 53). In the centre of the large trabeculae, most of the cells have died and the lacunae are either empty or contain the shrunken remains of osteocytes. The endosteal spaces have marrow of normal appearance and loose connective tissue (Fig. 53). A uniform layer of larger cells lines the trabeculae and from this layer osteoblasts migrate into and repopulate the matrix. The denser bone (Fig. 51) does not appear to be invaded by these young bone cells, but osteogenesis proceeds around it and results in the formation of a closely apposed layer of bone. This new bone can be distinguished from the transplant by its more deeply staining matrix and large cells, and it is in actual contact with the transplant (cf. homo-transplant where there is no contact between the graft and the new bone) (Fig. 27).

The host connective tissue reaction (not so extensive as in the bone transplants into muscle) forms a capsule immediately surrounding the transplant (Figs. 51, 52).

Large spindle shaped cells differentiate into osteoblasts which lay down young osseous tissue. In the outer zones this young bone tissue is deeply staining and contains many osteoblasts with large round nuclei, but close to the transplant it has a more mature appearance and consists of well defined trabeculae.

21 DAYS HOMOGENOUS TRANSPLANTS.

X-Ray examination of the transplants in situ and of the specimens on removal shows the disc clearly; there is no alteration in density and no evidence of healing.

In the majority of the specimens at this stage the transplants are either dead or considerably devitalised (Figs. 54, 55, 56). The matrix has an opaque quality and the lacunae are empty. The transplants have a dense outline and appear to have no histological contact with the surrounding tissue (Fig. 56). In one specimen (Expt. 154), osteocytes at the periphery of the trabeculae have survived (Fig. 54) but mostly the lacunae are empty or house shrunken, degenerated cells. The endosteal spaces are occupied by loose connective tissue and although marrow is present in some sections, it has degenerated. There is little evidence of osteoblastic reaction and consequently no new bone formation from the endosteal cells of the transplant, except in the specimen (Expt. 154) quoted above, where some endosteal cells are proliferating and differentiating.

There is a capsule of host connective tissue with large spindle or oval shaped cells (Fig. 56). These cells differentiate into osteoblasts and have laid down young bone tissue close to the transplant. In one specimen (Expt. 170) where the bone disc was implanted in a young animal, the host tissue reaction is particularly vigorous. The young connective tissue cells have differentiated into both osteoblasts and chondroblasts (Fig. 55) and formed a chondro-osseous tissue. The dead transplant is being absorbed and replaced by this young tissue.

42 DAYS AUTOGENOUS TRANSPLANTS.

X-Ray examination of the transplants in situ and of the specimens on removal shows that healing proceeds until at this stage, the disc outline is difficult to recognise. There is no radiological evidence of rarefaction.

The bony matrix of the transplant is well stained and contains many osteocytes. The smaller trabeculae (Fig. 57) are entirely repopulated but the central areas of the large trabeculae still show empty lacunae. Where the transplant is more dense (Fig. 58) the greater part of the matrix is devoid of cells although they are present at the periphery of the transplant and around endosteal spaces and haversian canals. The transplanted endosteal tissue (Fig. 57) has proliferated and formed large osteoblasts which are arranged in multiple layers or groups, closely

applied to the bone trabeculae. Some of these large osteoblasts migrate, invade and repopulate the matrix. Similarly the dense bone (Fig. 58) has a layer of large cells around it, but here invasion is slower.

The host tissue forms a capsule surrounding the transplant and from this new bone, trabeculae have been formed. At this stage, it has become difficult to see any line of demarcation between the revitalised transplant and the new bone trabeculae laid down by the host, except where the transplant consists of dense bone which is comparatively a-cellular.

42 DAYS HOMOGENOUS TRANSPLANTS.

X-Ray examination of the transplants in situ and of the specimens on removal still shows the outline of the disc clearly.

The appearances vary in different specimens but the general histological picture is of a devitalised disc of bone surrounded by a host tissue in which new bone is being formed to replace the transplant. A number of the transplants are sequestra - e.g. Expt. 156 - and the matrix is a-cellular but the empty lacunae are still visible. There is no evidence of the invasion and repopulation of this matrix by young bone cells, but rather it is being replaced by new formed bone - "creeping substitution".

The host connective tissue has formed trabeculae of young bone surrounding the transplants. (Fig. 60). These also invade the spaces between the trabeculae and are slowly replacing the transplant.

80-120 DAYS AUTOGENOUS TRANSPLANTS.

X-Ray examination of the transplants in situ and of the specimens on removal shows that the process of recovery and healing proceeds until, at this stage, the outline of the disc cannot be defined.

The transplant cannot be distinguished from the host tissue on histological examination. The process of healing is complete and there is no evidence of dead or devitalised bone. The host connective tissue reaction has subsided and the bone tissue has a mature appearance.

80-120 DAYS HOMOGENOUS TRANSPLANTS.

X-Ray examination of the transplants in situ and of the specimens on removal shows that the bone discs cannot be identified from the surrounding bone tissue, except for some rarefaction at the site of implantation.

Histologically the transplant cannot be distinguished from the host tissue. There is no evidence of dead or devitalised bone, or any indication of the host tissue reaction characteristic of the earlier transplants.

SUMMARY OF OBSERVATIONS.

There is no radiological change in either autogenous or homogenous transplant during the first two weeks. Thereafter the autogenous disc is less well defined and by the end of six weeks it is difficult to distinguish its outline. The homogenous disc can still be recognised at six weeks but it gradually loses definition and at 80 days it is no longer apparent.

Both autogenous and homogenous transplants are considerably devitalised at 10 days, the mature cells are dead and only the young cells at the periphery of the trabeculae and in the endosteal spaces have survived. Endosteal proliferation and cell migration occurs in the autogenous specimens, but in the homogenous transplants the spaces are occupied by loose connective tissue showing no evidence of differentiation. There is cytological evidence of recovery in the autogenous transplant at 21 days and by the end of six weeks cancellous bone is normal. Where the transplant consists of dense bone the recovery is much slower. The homogenous transplant at six weeks is like a sequestrum, its cells are necrosed and the periphery of the matrix is undergoing lysis, and there is no evidence of the proliferation and migration of osteoblasts seen in the autogenous transplants. In some of the endosteal spaces new bone is laid down, apparently by the transplanted tissues, to substitute the dissolving edge of the transplant.

Marrow tissue survives transplantation and has a normal

appearance at the early stages, but whereas in the autogenous transplants it remains healthy, it becomes devitalised in the homogenous transplants after 3 weeks and in later specimens it is gradually replaced by undifferentiated loose connective tissue.

The host tissue reaction at 10 days consists of a capsule of young connective tissue in which cell differentiation and osteogenesis is responsible for the deposition of trabeculae of young bone close to the transplant. In the autogenous transplants this new bone matures and by the end of 42 days it is difficult to distinguish it from the transplanted bone, except where the transplant includes dense bone which is comparatively a-cellular. Around the homogenous transplant there is also a capsule of young connective tissue in which osteogenesis proceeds, but here the young bone tissue not only occupies the space between host and transplant, but it also permeates the spaces between the trabeculae using the dead matrix as a scaffold for its extension, and thus gradually replaces the dead transplant.

In the majority of specimens, autogenous and homogenous, it is impossible to distinguish the transplanted disc at 80 days. Where, however, the transplanted bone is dense, a-cellular areas with closely apposed osteoblastic activity can still be seen. The autogenous transplant recovers, it is repopulated by cells from the transplanted endosteal and periosteal tissues, and bony union to the host tissue is effected by osteogenesis in the host tissue reaction.

The homogenous transplant on the other hand dies, all its cells, mature and young, undergo necrosis. It is replaced by new bone formed by the host tissue.

When bone is transplanted into a prepared site in bone, it undergoes the same histological changes as when transplanted into muscle, but in the former case, the osteogenesis in the host tissues obscures the later phases of repopulation in the autogenous transplant, and substitution in the homogenous transplant. In the homogenous transplant into bone the dead matrix is substituted by host osteogenesis, until it is entirely replaced, whereas in the homogenous transplant into muscle limited substitution is effected by new bone tissue formed from non-osseous connective tissue which becomes osteogenic in response to the presence of the implants.

BONE TRANSPLANTS INTO THE ANTERIOR CHAMBER.

10 DAYS AUTOGENOUS TRANSPLANTS.

X-Ray examination of the specimens on removal reveals no radiological change at this stage.

The general picture is that of trabeculae of living bone enclosing healthy marrow tissue. Where the transplant is in contact with ocular structures, there is host tissue reaction in the form of proliferating connective tissue, but where the surface of the transplant lies free in the aqueous humor there is no such reaction.

The matrix is well stained and contains normal osteocytes (Fig. 61), but in the central areas of the largest trabeculae there is cell degeneration. The endosteal spaces contain healthy marrow and young connective tissue cells some of which differentiate to form a uniform layer of large osteoblasts in apposition to the trabeculae. Where cartilage tissue is present it is alive and the osteoblastic proliferation in its vicinity is vigorous. Cell migration into and repopulation of the matrix appears to be taking place as in the transplants into muscle and bone.

The host tissue reaction consists of spindle shaped cells which differentiate into large osteoblasts forming a periosteal layer apposed to the matrix of the transplant. Cells migrate into the matrix from this layer to replace the degenerated osteocytes.

10 DAYS HOMOGENOUS TRANSPLANTS.

X-Ray examination of the specimens on removal shows no radiological change.

The general picture, in the majority of the specimens is that of devitalised bone surrounded by a capsule of host connective tissue. The matrix is well stained and the bone cell lacunae are all empty (Fig. 62). The periphery of the transplant has a dense outline and has no histological contact with the surrounding tissue. The endosteal spaces contain loose connective tissue but there is no indication of differentiation into osteoblasts and therefore no large cell layer surrounding the trabeculae.

The host tissue reaction shows spindle shaped cells which form a capsule of young fibrous tissue around the transplant, and in some areas large cells resembling osteoclasts invade the dense periphery of the transplant where lysis is proceeding. Even in specimens where cartilage is present there is no evidence of osteogenesis.

21 DAYS AUTOGENOUS TRANSPLANTS.

X-Ray examination of the specimens on removal shows that rarefaction has occurred in the transplanted bone.

The matrix is well stained but there is more cell degeneration (Fig. 63), fewer mature osteocytes and only the young osteoblasts in the peripheral parts of the trabeculae have survived. The endosteal spaces are filled

with proliferating connective tissue composed of spindle shaped cells which are differentiating into large osteoblasts and forming an endosteal layer close to the bony matrix. Here and there they gather in clusters of cells and migrate into the substance of the matrix. Where cartilage is present it has survived and the surrounding osteoblastic reaction is more pronounced. Marrow tissue of normal appearance is present in the endosteal spaces. In one specimen (Expt. 112) where the transplant was taken from an older animal the bone is entirely devitalised and the osteocytes have disappeared even from the periphery of the trabeculae.

The host reaction is similar to that seen in the 10 day specimens, but proliferation and differentiation into osteoblasts is more active. The osteoblasts in the proximity of the transplant migrate into it and aid in its cellular repopulation but there is no evidence that they lay down young bone tissue.

21 DAYS HOMOGENOUS TRANSPLANTS.

X-Ray examination of the specimens on removal shows no change in the density of the bone shadow - the homogenous transplant has not undergone rarefaction.

The bone tissue is devitalised (Figs. 64, 65), although the matrix is well stained the lacunae are empty except for a few cells which appear to have invaded it from the endosteum. Where cartilage is present it has survived and marrow tissue

in the endosteal spaces is of healthy appearance. The endosteal tissue consists of young connective tissue cells but it has not formed the uniform lining of osteoblasts so characteristic of the autogenous transplants. Some of the cells, larger than the others, have invaded the matrix of the transplant which is being absorbed.

The host tissue reaction consists of spindle shaped cells differentiating into osteoblasts (Fig. 65). This proliferating tissue surrounds the fragments and some of its cells are invading the matrix of the transplant. In some areas osteogenesis is proceeding and young bone with deeply staining matrix is present.

42 DAYS AUTOGENOUS TRANSPLANTS.

X-Ray examination of the specimens on removal shows marked decalcification.

The matrix of the transplant is well stained and contains many large osteocytes (Figs. 66, 67). Where the bone is denser there are some areas in which the lacunae are still empty. Cartilage has survived and is surrounded by active and healthy bone tissue. The endosteal spaces contain marrow of normal appearance and loose connective tissue which has proliferated to form a layer of large osteoblasts close to the trabeculae. These cells migrate into the matrix to replace the degenerated osteocytes of the graft. In some specimens the surface of the transplant lies free in the

aqueous humor and has a covering layer of large cells or osteoblasts (Fig. 67). These cells are part of the transplanted tissue, they proliferate on the surface and appear to migrate into the matrix.

The host tissue reaction consists of spindle shaped cells forming a capsule which may surround the whole or part of the transplant. The cells of the capsule proliferate and differentiate into osteoblasts which form a layer of large cells some of which migrate and invade the matrix of the transplant.

42 DAYS HOMOGENOUS TRANSPLANTS.

X-Ray examination of the specimens on removal shows a dense shadow, no decalcification has occurred.

The transplant is quite dead (Fig. 68) the matrix contains numerous empty bone lacunae and has a dense, sharply defined outline. Some cartilage cells have survived but there is no evidence of living bone tissue in their vicinity. The endosteal spaces are practically empty, they contain no marrow tissue, and only a few scattered cells are present.

The host connective tissue reaction (Figs. 68, 69) consists of spindle shaped cells permeated by lymphocytes. There is a clear line of demarcation between the sequestrum like transplant and the host tissue with no histological contact. Young bone tissue, which has been laid down by the host connective tissue (Fig. 69) has become completely devitalised.

It is recognised by its more deeply staining matrix and large empty lacunae.

80-120 DAYS AUTOGENOUS TRANSPLANTS.

X-Ray examination of the specimens on removal shows that recalcification has occurred and it is now possible to distinguish cancellous bone surrounded by a cortical layer of dense bone.

The matrix is well stained and although in some specimens (Fig. 70) there are still areas devoid of cells, in older specimens (Fig. 71) the osteocytes are normal in number and appearance. Where the repopulation is still proceeding the cells are large and osteoblastic in type. There is marked endosteal activity (Fig. 70) and differentiation into migrating osteoblasts.

The host reaction of large connective tissue cells surrounds the transplant and its cells form a periosteal layer of osteoblasts. These cells form clusters of osteoblasts at the periphery of the younger transplant and some migrate into the matrix to complete the repopulation. In older specimens (Fig. 71) the migration ceased and a more uniform and usually a single layer of cells surrounds the transplant. The outline of the bone is now well defined and smooth and thus accounts for the x-ray appearance of a cortical zone.

80-120 DAYS HOMOGENOUS TRANSPLANTS.

X-Ray examination of the specimens on removal shows the dense shadow of a sequestrum with irregular outline and no evidence of differentiation into cancellous bone and cortex.

The transplant has the appearance of a sequestrum (Figs. 72, 73). Where the transplanted bone was removed from a mature animal the lacunae are all empty (Fig. 72). The periphery of the transplant and the margins of the endosteal spaces are clearly outlined by a more deeply staining zone. The endosteal spaces are empty and contain neither marrow nor connective tissue. The appearances are somewhat different (Fig. 73) where the transplant was taken from a young animal and contains cartilage surrounded by young bone tissue. The matrix is honeycombed by large empty lacunae and the cartilage is considerably devitalised although some living cells are seen in the vicinity of the endosteal spaces.

The endosteal tissue consists of spindle shaped cells, differentiated osteoblasts which have formed new bone to replace the dead matrix of the transplant. This new bone recognised by its deeper staining matrix is also undergoing necrosis as evidenced by empty lacunae.

The host fibrous tissue reaction surrounds the transplant on all sides except in those specimens where part of the bone lies free in the aqueous humor. The cells close to the transplant have now matured (Fig. 72) and form a capsule of fibrous tissue separating the bone from the ocular structures.

Nevertheless the bony outline is still clearly defined and has no cell continuity with the host tissue. In the specimens taken from younger animals (Fig. 73) the connective tissue cells of the host reaction close to the transplant are differentiating into osteoblasts which lay down young bone to replace the transplant, but the osteogenesis is less active than in the earlier specimens and some of the new formed bone has necrosed.

180 DAYS AUTOGENOUS TRANSPLANTS.

X-Ray examination of the specimens on removal shows that the transplant has now the typical appearance of a small irregular bone; cancellous tissue surrounded by a cortex of dense bone.

The histological appearance is that of mature rabbit bone (Fig. 74). The matrix is well stained and the cells are normal in appearance and numbers. The endosteal spaces contain loose connective tissue composed of spindle shaped cells, while larger and more rounded cells form an endosteal layer lining the trabeculae. In the main this is a uniform single layer of osteoblasts but in some sections proliferation and migration is evident. Marrow tissue where present has a healthy appearance.

The host tissue reaction has subsided and now consists of a capsule of comparatively mature fibrous tissue. The cells in immediate contact with the periphery of the transplant

are larger and form a more regular periosteal layer and there is little evidence of proliferation and migration.

180 DAYS HOMOGENOUS TRANSPLANTS.

X-Ray examination of the specimens on removal shows the dense shadow of a sequestrum with an irregular outline and no differentiation of bone tissue.

The homogenous transplant now consists of dead bone (Fig. 75). The matrix has empty lacunae and its edges which have no cellular contact with the surrounding soft tissue are sharply outlined. The endosteal spaces contain a few scattered connective tissue cells but there is no evidence of proliferation or osteoblastic activity. Where cartilage was included in the transplant (Fig. 76) it is now quite devitalised and the surrounding matrix shows the characteristic large empty lacunae. New bone which has formed close to the cartilage, and may be recognised by its deeply staining matrix is also devitalised.

The host reaction consists of a capsule of mature fibrous tissue, which even in its deeper layers show no differentiation or osteoblastic activity. The new bone laid down by the host tissue, in contact with the transplant is now quite dead and has the same sequestrum like appearance except that it stains more deeply.

SUMMARY OF OBSERVATIONS.

No radiological change is seen in either autogenous or homogenous transplant until after 21 days. The autogenous transplant then undergoes rarefaction whereas the homogenous transplant continues to show the dense shadow of a sequestrum. By the end of 80 days recalcification is appearing in the autogenous transplant, which acquires the x-ray appearances of a small round bone with cancellous tissue enclosed by a cortex of denser bone.

When bone fragments are implanted into the anterior chamber of the eye they obtain varying degrees of contact with the ocular tissues. In some cases the bone lies free in the aqueous humor, in others one surface of the transplant is in contact with host tissue, or it may become enclosed by the ocular tissue.

Both autogenous and homogenous transplants become devitalised during the first 3 weeks, mature osteocytes die, only the young bone cells at the periphery of the trabeculae and the endosteal tissues survive. The degree of cell necrosis is however greater in the homogenous than in the autogenous transplants. Where cartilage tissue is present, either autogenous or homogenous, it survives and many of the young bone cells surrounding it also remain alive. On the other hand transplants consisting of mature bone from older animals show a greater degree of cell necrosis.

After 3 weeks the difference between autogenous and homogenous transplants becomes apparent, in the former the endosteal tissues are undergoing active proliferation and migrating cells invade the matrix of the transplant, whereas in the latter the endosteal spaces are filled with loose connective tissue which has no contact with the deeply staining dissolving edge of the bone matrix.

Where the surface of the autogenous transplant lies free in the aqueous humor it acquires a covering layer of large osteoblasts, and proliferation and migration of these cells into the matrix aids in repopulation of the transplant.

By the end of 3 months the autogenous transplant resembles mature rabbit bone whereas the homogenous transplant is a sequestrum. The endosteal proliferation and cell migration in and around the autogenous transplant continues until the transplant has a normal cell population, thereafter the masses of osteoblasts give place to a uniform layer lining the endosteal spaces. In the homogenous transplants the loose connective tissue gradually disappears until the endosteal spaces are practically empty. Marrow tissue survives throughout in the autogenous transplants, whereas in the homogenous transplants it is gradually replaced by connective tissue.

Cartilage and the young bone tissue in its vicinity survives better than mature bone. In the autogenous transplants containing cartilage there is not only greater cell survival but also more active proliferation in the endosteal tissues

and hence more rapid repopulation of the devitalised matrix. In the homogenous transplants there is again greater cell survival for a time, but ultimately even the cartilage undergoes necrosis. The endosteal reaction is vigorous to begin with and in some specimens new bone is laid down in the endosteal spaces, but by the end of three months this has all subsided and even the new formed bone is dead.

The host reaction is similar to that surrounding transplants into muscle. Except where the bone lies free in the aqueous humor it is surrounded by a capsule of young connective tissue. With the homogenous transplant there is in addition lymphocytic infiltration.

In the autogenous transplants the host tissue differentiation is well marked at 10 days and proliferation and migration continue until the transplanted matrix has a normal complement of cells, a stage reached at 3 - 4 months. Thereafter proliferation subsides and a uniform periosteal layer is formed so that the host tissue ultimately contributes an outer fibrous periosteum and an inner osteogenic layer. In the homogenous transplants there is little or no evidence of cell migration into the matrix, the osteoblasts derived from the host reaction lay down new bone to substitute the transplant but this osteogenesis soon abates and by the end of 3-4 months the new formed bone is devitalised and becomes a part of the sequestrum. The surrounding host tissue reaction ultimately forms a fibrous tissue capsule enclosing the dead transplant.

In general, the series of histological changes which follow bone transplantation into the anterior chamber are very similar to those seen when bone is transplanted into muscle. There is however less host connective tissue reaction and in the autogenous transplants little or no new bone formation so that a better opportunity is provided to study the changes in the transplanted bone itself. The fragments are smaller, and the proportion of surviving bone cells is greater, and especially where the surface of the transplant lies free in the aqueous humor the fate of the transplanted cells and the part played by them in the recovery can be readily followed.

FROZEN BONE TRANSPLANTS INTO MUSCLE.

42 DAYS AUTOGENOUS TRANSPLANTS.

X-Ray examination of the transplants in situ and of the specimens on removal shows that rarefaction has occurred but the outline of the fragments is smooth and in some experiments they have united to form a single bony mass.

The transplant consists of living bone, the matrix is well stained and contains normal osteocytes (Fig. 78). The endosteal spaces are occupied by normal looking marrow and connective tissue. Lining the spaces, and closely apposed to the trabeculae are large osteoblasts which form a uniform single layer in some parts but elsewhere they are arranged in clusters of proliferating cells from which migration into and repopulation of the matrix is proceeding.

The host reaction consists of young fibrous tissue whose cells, close to the transplant have differentiated into osteoblasts forming a periosteal osteogenic layer. From this layer cells migrate into the matrix and aid in its repopulation.

42 DAYS HOMOGENOUS TRANSPLANTS.

X-Ray examination of the transplants in situ and of the specimens on removal shows that the segments remain dense and have an irregular outline, there is no differentiation into cancellous and cortical zones.

At this stage the transplant is either dead or considerably devitalised (Fig. 79). The matrix is well stained but the lacunae are empty or contain the degenerate remains of cell nuclei. The dense peripheral outline, indicating early lysis, is being invaded by large cells from the endosteal spaces. These spaces contain marrow tissue which has survived and loose connective tissue from which are developed the large invading cells.

The host reaction forms a capsule of young connective tissue surrounding the transplant. Besides fibroblasts and capillaries numerous lymphocytes are present. Close to the transplant the connective tissue cells have differentiated into large osteoblasts, which are invading the deeply staining periphery of the transplant.

80 DAYS HOMOGENOUS TRANSPLANTS.

The x-ray appearances of the transplants in situ and of the specimens on removal are similar to those described for the 42 days specimens. The fragments do not unite and there is no differentiation of cancellous and cortical bone.

The general picture is that of a sequestrum like bone surrounded by a capsule of fibrous tissue (Fig. 70). The matrix is well stained but all the lacunae are empty. The periphery of the trabeculae has a dense outline indicating bone lysis, but it has no cell continuity with the surrounding soft tissue. The endosteal spaces contain loosely arranged connective tissue cells but there is an absence of any cellular differentiation.

The host reaction is composed of mature fibrous tissue which encapsulates the transplant. Its deeper cells close to the bone do not differentiate into osteoblasts, there is neither osteogenesis nor invasion of the matrix by host tissue cells.

HETEROGENOUS BONE TRANSPLANTS INTO MUSCLE.

21 DAYS TRANSPLANTS.

X-Ray examination of the transplants in situ and of the specimens on removal shows that necrosis and fragmentation proceeds until it is no longer possible to distinguish the original segments. The bone has become irregular in outline and density.

The general picture is of necrotic bone surrounded by a vigorous host reaction (Fig. 77). The matrix is well stained but the lacunae are all empty; the endosteal margins have a dense outline indicating lysis and the periphery of the transplant is irregular. There is no evidence of endosteal tissue or of osteoblastic differentiation, and all the elements of the transplants have died.

The host reaction forms a capsule of connective tissue around the transplant separating it from the muscle. Its cells are fibroblasts and a marked round cell infiltration consisting mainly of lymphocytes but also some polymorphonuclear leucocytes. There is no evidence of an osteoblastic reaction or of any osteogenesis, although some of the host tissue cells appear to invade the periphery of the matrix.

GENERAL DISCUSSION

The bone graft has many surgical applications, but its immediate purpose is either to provide stability or to promote osteogenesis. Osteogenesis which is the more important may be achieved either by the survival and proliferation of the transplanted cells or by the stimulation of new bone formation in the host tissues. The graft can act as the anlagen for the extension of bone growth from the host tissue.

The points of interest which emerge from the observations are:-

(1) Survival capacity of the transplants.

(2) Cell repopulation of the transplants.

(3) New bone formation.

Survival Capacity of Transplants

Autogenous Bone.

The results described here show that there is impairment of vitality of living autogenous bone transplants during the first 10 days. The mature osteocytes die so that most of the bony matrix has no cells, and for the first two or three weeks the only surviving cell elements are the young bone cells. The soft tissues, endosteum, periosteum and haversian canals survive and at 10 days show active proliferation. The matrix remains healthy and its periphery is in immediate contact with the surrounding tissue. There is a gradual

transition rather than an abrupt demarcation.

Homogenous Bone.

In living homogenous bone transplants the cells degenerate more rapidly and completely, including both mature and young osteocytes, until at 42 days the transplant has no living cells. There is no proliferation of the connective tissue in the endosteal spaces and what remains in the early stage is a loose connective tissue with typical elongated cells, which finally disappears leaving the endosteal spaces empty. The matrix withdraws from the surrounding tissue and lysis of its edges gives a characteristic marginal deep staining, which contrasts with the more opaque appearance of the rest of the matrix. Syngenesious transplants behave like autogenous transplants. The site of implantation, whether muscle, bone or anterior chamber of the eye, has no effect on the survival capacity of either autogenous or homogenous transplants.

It is now generally accepted that homografts, with some exceptions, act as antigens, and promote an active immunity in the host, manifest in the tissue reaction responsible for their ultimate destruction. Until the immunity reaction develops, however, homografts such as skin and nerve, appear to survive, 'take' and be satisfactorily vascularised. The nourishment of a bone graft is a more formidable task than vascularising a soft tissue graft, hence the early onset of osteocyte degeneration common to both autogenous and homogenous transplants.

Thus the bone homograft has never, even in the early stage, the histological appearance of a healthy graft.

The literature on the survival capacity of bone grafts, particularly homografts, is controversial. In 1893 Barth stated that all the elements of a bone transplant die irrespective of the sources of the bone, and quite recently Reynolds and Oliver (1949) after performing experiments in dogs to compare autogenous and homogenous transplants, confirmed this. McWilliams (1916) believed that living bone grafts survived and that it was not essential that they should be in contact with living host bone. Albee repeatedly affirmed as late as 1944, that the autogenous transplant lives and grows in the same way as a grafted twig. Cotton, Phemister (1914), Hey Groves (1914), Ghormley and Stuck (1934) and Ghormley (1942) have each produced evidence to show that all the young cells of autogenous bone survive transplantation. Many attempts have been made to utilise homogenous bone in bone grafting operations from the time of Macewen (1878) to the more recent work of Ghormley (1942), Bush (1947) and Weaver (1949), but as Bush states there is a paucity of reliable information regarding the histological fate of the homograft. Animal experimental work has been performed by many investigators including lexer, Hey Groves, Albee, Phemister and Reynolds and Oliver, but with conflicting results. Many have claimed success in the use of the homogenous transplant but others - for example, Phemister - considered that the whole of the homogenous transplant is devitalised

and ultimately replaced, and Reynolds and Oliver came to the conclusion that none of the elements of bone whether autogenous or homogenous survive transplantation. Medawar (1948) has found that some other homografts survive intra-ocular implantation but the bone homograft into the anterior chamber of the eye has the same fate as homografts elsewhere and becomes a sequestrum.

In this series of experiments, while many elements including young cells survive in the autotransplant the homotransplant, despite some temporary survival of the young cells, finally degenerates completely and becomes a sequestrum.

Heterogenous transplants.

With heterogenous bone transplants degeneration and cell necrosis is very rapid. Not only do the mature osteocytes die but the young bone cells and the connective tissue cells of the endosteum and periosteum become devitalised during the first ten days. In the light of recent and past work it seems there have been no real successes with heterogenous bone although a few claims have been made. Ollier (1859) reported some successes with heterogenous transplants when the recipient was of a higher order phylogenetically than the donor and Macewen (1874) claimed that his well known transplantation of parietal bone from a dog into a human effected a cure. This work agrees with established opinion that heterografts of bone as of other tissues are destroyed by a vigorous host reaction.

Bone Transplants preserved by Freezing.

Autogenous Bone.

The histological appearance 42 days after implantation in muscle is more or less similar to that of the fresh autogenous transplant. The mature osteocytes have died while the peripherally placed young bone cells survive and the connective tissue of the endosteum and periosteum proliferate and differentiate into osteoblasts.

Homogenous Bone.

After 42 days the graft is completely necrotic and there is no intrinsic reparative osteogenesis. The host tissue reaction is less active than in the fresh homogenous transplants and perhaps in consequence of this, there is significantly no evidence of the temporary osteogenic activity of host tissue found in the fresh homogenous transplants. The effect of freezing on tissue vitality has been very thoroughly investigated. Carrel (1912) records the growth of infant skin stored at 3 °C. for 42 days after removal and likewise the growth of embryonic cartilage and bone has been reported after refrigeration for 22 days, Waterman (1944). Tuffier (1911) reported favourably on the results of freezing of bone and cartilage for two months and Keith (1934) confirmed this for bone chips. Similar confirmatory evidence is given by Inclan (1942), Webster (1944), Matthews (1945) and Strumia and Hodge (1945). Bush (1947) examined biopsy specimens of twelve cases of grafting with homogenous bone. He describes a capsule of young connective tissue surrounding the graft,

in which there was active osteogenesis. Weaver (1949) with 49 operations and Wilson (1950) with 307, confirm the clinical success of the frozen homogenous bone grafts. The preservation of bone tissue in merthiolate solution has been shown by Morgan, Jamieson, Powell (1933), O'Connor (1939), Brown and De Mere (1948) and Reynolds and Oliver (1949) to be a safe, simple and inexpensive method of preservation.

It can seldom be necessary to employ preserved autogenous transplants. The preserved homogenous transplant is clinically as efficient as the fresh specimen, and has the advantage that it can be stored and thus made readily available.

Age of the Transplant

When the graft is taken from the region of the iliac epiphysis and consists of cartilage and developing bone, the cell survival is greater in both autogenous and homogenous transplants. In the autogenous transplants all the cartilage cells survive throughout, together with many of the young bone cells, so that in contrast to the older autogenous transplants, the matrix largely retains its cell population. In the corresponding homogenous transplants, on the other hand, the young bone cells ultimately degenerate and even the cartilage tissue dies. Considering the success of homogenous transplants of hyaline cartilage, which have been shown clinically and experimentally to survive, the degeneration of epiphyseal cartilage was unexpected. In this respect, at any rate, epiphyseal cartilage seems to have anticipated the

physiological individuality of its future condition.

Marrow Tissue

Marrow tissue is apparently well able to survive transplantation. There is no evidence of degeneration of marrow in the autogenous transplants at any stage in any of the sites of implantation, and at the end of six months the endosteal spaces contain abundant healthy marrow tissue. In the homogenous transplants, the marrow cells survive longer than the osteocytes, but there is a gradual degeneration and at the end of 6 to 8 weeks the marrow tissue is replaced by loose connective tissue. Marrow survives in the syngenesious transplants.

Influence of type of bone and size of graft on survival capacity

The results obtained with autogenous transplants in this series of experiments show a greater survival capacity of cancellous bone as opposed to cortical bone. There are two factors concerned:- (a) cell population and (b) blood supply.

(a) Cell population.

Cancellous bone has a large surface area and thus contains a higher proportion of young osteocytes than dense bone. In autogenous bone transplants examined after 10 days, the peripheral cells of cancellous trabeculae have survived whereas the matrix of cortical bone is practically a-cellular. The numerous endosteal spaces of cancellous bone are a reservoir of potential osteoblasts, while in cortical bone

the sites of formation of osteoblasts are limited to the periosteum and haversian canals.

(b) Blood supply.

The survival of some of the cells of autogenous transplants depends on the establishment of an adequate blood supply. Abbott et alia (1947) have shown that transplanted tissue dies unless vascularised within 10 days. Cancellous bone with its network of endosteal spaces can be easily penetrated by new capillaries, while in cortical bone, apart from haversian canals, such penetration is difficult. Horowitz (1948) has pointed out that bone with red marrow is more rapidly vascularised than that containing only fatty marrow which inhibits the growth of young capillaries. Preceding vascularisation, there is an early critical phase, during which the transplant depends on tissue fluids for its nourishment. It is obvious that cancellous bone can cope more efficiently with this a-vascular period than the denser cortical bone.

In the very small fragments of cancellous bone implanted into the anterior chamber of the eye there was a higher proportion of surviving cells than when the site of implantation was muscle or bone. This was possibly due to the more favourable conditions during the initial a-vascular period, when the fragments were bathed in nourishing aqueous humor.

Leriche and Policard (1928), Mowlem (1941, '44, '45), Motti (1936) all state that transplants of cortical bone do

not survive. Hellstadius (1944), on the other hand, in a comparative series of experiments concluded that transplants of cortical bone were successful and that there was no evidence of the survival of cancellous transplants. Abbott et alia (1947) examined cancellous and cortical transplants histologically after 10 days and stated that the transplants of cortical bone showed evidence of devitalisation, whereas the cancellous bone transplants survived in a healthy condition. Marrow tissue survives throughout in autogenous transplants without an initial period of diminished vitality.

The size of the transplant

Attempts have been made to increase the survival capacity of bone transplants by subdividing them into multiple fragments, and even by grinding them into a powder and using this in the form of a suspension. The possibility of destroying a large proportion of the cells limits such a procedure. Macewen in 1881, discussing the best method of bone transplantation, suggested subdividing large grafts into many smaller fragments, and this idea was adopted by Heath (1886), Motti (1936), Hallock and Halford (1938), Hellstadius (1944) and Henry (1948).

In the current experiments, transplants of autogenous cancellous bone had the best survival capacity and small grafts survived better than large ones. The type and size of transplant did not of course in any way influence the ultimate death of homogenous transplants.

Cell Repopulation

Throughout the description of the autogenous transplants it has been repeatedly stated that there is proliferation and differentiation of the cellular elements of the osseous connective tissues into osteoblasts which migrate into the transplant to replace the degenerated osteocytes. The histological picture is strongly suggestive of such a process. Nevertheless, it is recognised that there are objections to the facile acceptance of what is a postulate rather than an asseveration. How do these cells penetrate a calcified matrix? Is their progress preceded by a softening process by means of osteoclastic activity? There is no evidence of the presence of large numbers of osteoclasts. A ready alternative explanation is that the osteocytes do not die. They undergo necrobiosis analogous to cromatolysis of damaged nerve cells and their histological reappearance is overt evidence of their recovery. At one stage, however, the lacunae are completely empty with no evidence whatsoever of devitalised cells. Moreover, the autogenous transplants do not regain normal cell population until about 80 days - too long, it is thought, to represent a period of recovery of necrobiotic cells. The marked proliferation and marshalling into clusters of osteoblastic-like cells differentiated from the connective tissue of transplant and host would seem to betoken activity of some sort or other - it might be said - entirely devoted to new bone formation. But in transplants of cancellous bone there is no endosteal

osteogenesis despite the collection of osteoblasts. Further, there is nothing to suggest that the 'normal bone' of the older transplants is a complete replacement of the original transplant. Consequently, in the work it has been assumed that a cellular migration and replacement of effete osteocytes occurs.

Such a cell migration has been described by Fell (1932, 1933) and Gaillard (1942) in their 'in vitro' experiments with bone. They observed cells migrating from haversian canals, inter-trabecular spaces and sub-periosteally and massing into colonies which formed new bone trabeculae.

Intra-ocular transplantation with a minimum host reaction produces conditions approximating to growth in a culture medium. There are sections of such transplants with a regular procession of osteoblasts from an endosteal space through the dense matrix, and it is difficult not to be convinced that this is a cell migration to repopulate the empty lacunae.

A relevant digression here is consideration of the mechanism for dealing with wear and tear of normal bone. In the soft tissue, such as muscle or fibrous tissue, there is always a certain quantity of embryonic connective tissue - a ready source of repair and tissue renewal. It is known that there is a continuous process of absorption, renewal and remodelling of normal adult bone - which would include a replacement of ageing osteocytes. It is unlikely that there is proliferation of osteocytes imprisoned in a hard matrix,

and still less so that the osteocyte enjoys the long lease of life credited to the nerve cell. It may thus well be that cell migration is an essential part of this day-to-day reparative process.

Type of Bone

The majority of the transplants used in these experiments consisted of cancellous bone, and the description applies mainly to that type of bone. Cancellous bone has a large amount of osseous connective tissue which, in this type of transplant, proliferates and supplies large numbers of migrating osteoblasts.

Cortical bone has less osseous connective tissue and here cell replacement is delayed as though production of osteoblasts was slower, coupled perhaps with the mechanical difficulty of penetrating the denser matrix.

Another significant difference already mentioned is the absence of endosteal osteogenesis in cancellous bone, in contrast to the new bone produced here in transplants of cortical bone.

OSTEOGENESIS IN BONE TRANSPLANTATION.

Many theories have been advanced to explain the initiation of osteogenesis and diverse views offered concerning the importance of the various tissue elements. John Hunter (1746), showed that bone is not an inert inorganic substance but is in a constant state of cellular activity. Flourens (1847) repeated and confirmed this work. When Goodsir (1868) and Macewen (1912) reported their results following bone transplantation, they expressed the view that bone is formed by the direct action of specific cells or osteoblasts. Havers (1691) who was perhaps the first investigator to study bone formation in detail, inquired into the source of the calcium deposited in the matrix. The importance of calcium in osteogenesis was emphasised by Wells (1911), and Leriche and Policard (1928) considered that calcium salts exerted a specific influence on the connective tissue cells causing them to grow and differentiate into osteoblasts and marrow cells. Since 1923 when Robison's work on phosphatase afforded a new conception of osteogenesis attempts have been made to explain the mechanism in terms of biochemical stimuli.

INTRINSIC OSTEOGENESIS.

Autogenous Bone.

The transplanted bone was taken from the ilium and thus was mostly cancellous bone with a small amount of cortical bone. New bone formation by the tissue of the transplant was related only to the dense cortical bone and was laid down by the osteoblasts differentiated from the skeletal connective tissue of the transplant. For example there was a layer of new bone in close apposition to the original bone of the transplant - this was an addition and not a substitution process. The superior value of cancellous bone as a transplant which has been established experimentally by Gallie (1931), Ghormley (1942), Higgs (1946), and confirmed clinically by Brown (1946), Lawson Dick (1946), and Gibson and Lowden (1946), is not due to any large scale intrinsic osteogenesis. Indeed some workers, for example, Reynolds and Oliver (1949) claim that there is no intrinsic osteogenesis and the transplant is replaced by an appositional growth of new bone from the host tissue, but this opinion is contrary to the results obtained here which show that intrinsic osteogenesis does occur to a limited extent around dense bone.

The view that bone formation is a cellular activity led to efforts to determine whether the cells concerned

were periosteal, endosteal, marrow cells or mature osteocytes of the matrix. Although Havers (1691) studied the periosteum in detail and recognised its two layers he considered that it functioned only as a limiting and vascularising membrane. Duhammel (1739) credited to periosteum with active participation in the formation of bone, and Ollier (1867) showed that regeneration of bone does take place from the periosteum, according to Macewen from the inner or osteogenic layer. Lexer (1907) with some reluctance concedes a minor osteogenic role to the inner layer of the periosteum. Axhausen (1908) claimed that osteogenesis does not occur in a transplant devoid of periosteum, whereas Weatherill (1913) reported successful transplantation of bone from which the periosteum had been removed. Lobendorffer (1910) was convinced that all transplanted bone tissue dies except the periosteum which survives and forms new bone. Macewen (1912) stated that osteoblasts within the bone tissue itself are mainly responsible for the regeneration of the transplant and that the periosteum is not essential, but Haas (1913) considered the periosteum of prime importance and Oeschner (1914) was non committal. Lewis (1914),McWilliams (1914), and Albee decided that periosteum, endosteum and calcified tissue all participate in the regeneration of bone transplants. Bull (1928) performed experiments using transplants with and without periosteum and was unable to find any real difference in the amount of bone formation in each. More recently Hellstadius (1944) investigated the role of the periosteum

in osteogenesis and concludes that on the whole, new bone formation is slower if the transplant is stripped of periosteum. In the experiments described there was no attempt to remove the soft tissue and all the transplants had periosteum on at least one surface. The transplants consisted mainly of cancellous bone and therefore included a large proportion of endosteal tissue. From the observations made it can be stated that the osteoblasts which repopulate the a-cellular areas of the matrix are derived from the endosteum, periosteum and haversian canals none of which can therefore claim to be the exclusive source of osteogenesis.

Homogenous Bone.

There are very few surviving cells in the homogenous transplants at 10 days and these soon degenerate. The skeletal connective tissue either disappears or atrophies within a few weeks. Marrow tissue survives for a time but ultimately dies and is replaced by non-specified connective tissue, and even the younger transplants including the epiphyseal cartilage ultimately undergo degeneration. There is therefore no intrinsic osteogenesis in the homogenous transplants.

Syngenesious Transplants.

These behave in all respects in a manner similar to autogenous transplants, although in some cases the cell degeneration is greater and the recovery slower.

Preserved Bone Transplants.

Frozen autogenous and frozen homogenous bone transplants both behave in a manner similar to the corresponding fresh bone transplants.

EXTRINSIC OSTEOGENESIS.

In both autogenous and homogenous transplants there is new bone formation by the tissues of the host. In the homogenous transplants this new bone later becomes necrotic.

This production of new bone by the host tissue following bone transplantation has been known for a long time. Barth (1894) regarded all new bone formation as a function of the host tissue. Baschkirzew (1911) and Baschkirzew and Nemilow (1912) recorded the formation of new bone around transplants from which they had removed both periosteum and endosteum, and later Baschkirzew and Petrow (1912) concluded that osteogenesis only occurs in the surrounding host connective tissue.

When autogenous bone is transplanted, the host tissue reacts to its presence by the proliferation of connective tissue cells which form a capsule around the implant. Towards the transplant the tissue cells gradually increase in size and become large round or ovoid. These cells are osteoblasts and where they come into contact with transplanted matrix they form an osteogenic layer from which migration and invasion of the transplant takes place. Here there is also the deposition of young osseous tissue within ten days. This formation of a capsule of osteoblast like cells around the transplants has been observed and recorded by many workers including Axhausen (1909), Baschkirzew and Petrow (1912), Mayer and Wehner (1914), Phemister (1914), Wereschinski (1925),

de Josselin,de Jong and Eykman van der Kemp (1928), Engstrom (1943), Levander (1938), Abbott et alia (1947).

After a period varying from 2-4 months there was no further new bone laid down by the host tissue which settled down to form a fibrous periosteum with an inner quiescent osteogenic layer next to the bone. X-Rays at this stage show the surrounding peripheral layer of new cortical bone.

Extrinsic osteogenesis is particularly well marked where the transplants are fitted into host bone, indeed early and abundant new bone formation obscures the picture and after six weeks it becomes difficult to delineate the graft. Where the transplant consists of young bone tissue, including part of the iliac epiphyseal cartilage host osteogenic reaction is more vigorous and if cartilage is exposed on the surface of the transplant, the differentiated host cells lay down chondro-osseous tissue. Wenger (1945) reported the successful use of epiphyseal cartilage as a transplant and obtained satisfactory growth of bone. Brooks and Hudson (1920) and Keith (1934) also emphasised the importance of the age factor, they found that bone grafts in young animals were 100% successful but in older animals were only 60% successful. This may be due to the fact that they stimulate greater extrinsic osteogenesis.

The occurrence of osteogenesis in the host tissue is part of the general problem of bone formation common alike to bone development, bone growth and heterotopic ossification. In the case of bone transplants it is obvious however that

the evocator is the donor bone tissue, and the issue has been further narrowed down in that bone previously fixed in alcohol is still osteogenic, (Nageotte (1920), Polettini (1922),) therefore living bone cells are not the essential factors.

There has been a spate of theories, and a great diversity of substances given credit from time to time for the osteogenesis succeeding grafting operations and elsewhere. Lexer (1924) advanced the theory that osteoblasts from the transplant sow the young connective tissue of the graft bed and give its cells osteogenic properties. Leriche and Policard (1928) insisted, and their views were accepted for some time, that calcium salts was the potent factor but this was later discredited. For a time phosphatase was regarded as an osteogenic stimulant, Blum (1944). The claim by a number of workers including Lagos and Romero (1946), and Dobbs and Mason (1949), that bone formation could be stimulated by the injection of alcohol and various irritants into the tissues did not help towards a solution of the problem.

As long ago as 1922, Polettini advanced the theory of a specific osteogenin liberated from bone tissue and this has had support from the work of Levander (1938), Annersten (1940), and Bertelsen (1944), who observed new bone formation following the intra muscular injection of alcoholic extracts of bone.

A working hypothesis based on the conception of a substance osteogenin has been provided by the work and well

known writings of Lacroix (1947) and is now widely accepted. This substance while destroyed by boiling appears to survive previous treatment of the bone by fixation in alcohol and it appears to be present in higher concentration in epiphyseal cartilage. The more active and prolific osteogenesis following the transplantation of young bone, and particularly that containing epiphyseal cartilage described here, certainly suggests a more potent quantitative and qualitative osteogenic capacity.

Homogenous Bone

Following the transplantation of homogenous bone into muscle there is a vigorous host connective tissue reaction including a round celled infiltration. This is probably a manifestation of an acquired immunity. As with autogenous transplants connective tissue cells differentiate into osteoblasts which lay down new bone around the transplant. There is however a marked absence of cell migration and the lacunae of the matrix remain empty. Another characteristic of homogenous transplants is what Axhausen (1908) described as "Schleichender Ersatz" and Phemister (1914) as "creeping substitution". The new bone formed by the host follows the retreating edge of the dissolving matrix of the transplant but always separated from it by an interval. When the homogenous bone is taken from a young animal and includes cartilage tissue the host osteogenesis is greater and may include new formed cartilage. By 30 days all new bone or cartilage has become necrotic - an addition to sequestrum

now formed by the dead transplant.

When the transplantation site is bone the changes are not easy to follow after 42 days. There is active new bone formation from the host bone which ultimately replaces the necrotic transplant and remains as living bone. In the intra-ocular transplants there was only a small amount of new bone formation which like the graft itself became necrotic.

If current opinion is accepted then the homogenous transplant acts as an antigen and stimulates the formation of antibodies within the host. The host tissue reaction and subsequent death of the transplant can then be interpreted in terms of immunity concepts. Despite the immunity reaction there is osteogenesis of host tissue. This implies that homogenous bone can act as an osteogenic stimulus and that the stimulant "osteogenin" or otherwise is not individually specific; a fact not only of biological significance but of considerable practical importance. It is also perhaps surprising to find that the serological reaction does not interfere with the response of the host tissue to the osteogenic stimulus. A consideration of the manifold examples of heterotopic ossification - clinical and experimental emphasises our lack of knowledge of the fundamental processes involved in tissue metaplasia and renders further speculation at this stage unprofitable.

While it would appear quite natural that when homogenous bone is grafted into bone, the new bone formed by the host remains alive, it is puzzling to account for the death of the host bone when the transplant is put into muscle or the

anterior chamber of the eye. It is as if the response of the host bone were a normal reparative process, while elsewhere the osteogenic stimulus vanished with the death of the transplant before the new bone had acquired its morphological independence.

Although the results of the use of homogenous bone in animals has been reported by a long list of workers - Belchier (1738), Merrem (1810), Syme (1831), Flourens (1847), Ollier (1867), Goodsir (1868), Wolff (1870), Middledorpf (1891), Axhausen (1908), Phemister (1914) and many others in more recent times, there is no unanimity of opinion but only confusion and contradiction. The surgical use of homogenous bone dates from the famous case of Sir William Macewen who employed bone from several patients to restore a defect of the humerus. Foetal bone and bone from new born infants was used by Poncet (1886) and by Anschutz (1909) the former without success, the latter with complete success. Bone removed from amputated limbs was implanted by Grasse (1899), Hebber (1910), Rovsing (1910), Stukkei (1912) and only Rovsing admitted failure. Sumter (1904) and Kuttner (1913) used homogenous bone removed from cadavers with satisfactory healing. Henry Wade of Edinburgh (1914) carried out homo transplantation and reported successes followed up 6 years later, but McWilliams (1916) and Albee (1915) were both convinced that the homogenous graft is much inferior to the autogenous graft. Ghormley (1942) summarising the results of many operations from 1934 onwards concluded that the homogenous transplant may be successful but was undecided on the relative values

of autogenous and homogenous bone. Smith (1937), Inclan (1942), Armstrong (1945), reported successful cases but considered that there is not yet sufficient evidence to show that they are as reliable as autogenous transplants. Bush (1947) while claiming considerable clinical success stated that there is little information available regarding the histological fate of homogenous grafts, and Henry (1948) concluded that when a sufficient amount of autogenous bone is not available the use of syngenesious or homogenous bone should be considered.

From the information obtained here by histological examination of a comparable series of autogenous and homogenous transplants at regular intervals up to 6 months, the answer to this controversial question is quite simple - the autogenous transplant survives and is incorporated in the host as living healthy bone, the homogenous transplant becomes a sequestrum to be finally absorbed or replaced.

Syngenesious Transplants

Syngenesious transplants behave like autogenous transplants. There is no round celled infiltration and the new bone formed by the host tissue survives to become a part of the transplant. Loeb (1945) conducted experiments in tissue transplantation and considers that syngenesious tissue occupies a position midway between autogenous and homogenous tissue. Henry (1948) used syngenesious bone in grafting operations taking the bone from close male relatives of his patients. He found the method satisfactory and

concluded that when autogenous bone is not available the use of syngenesious bone should be considered. As the host reaction against the graft is due to the formation of antibodies in the blood serum, it was natural to test the fate of homogenous transplants where host and donor were of the same blood group. The results have been disappointing, perhaps not surprising in view of the rapidly increasing knowledge of the specificity of individual blood.

Heterogenous Transplants

There is no new bone formation after transplantation of heterogenous bone into muscle. The host tissue reaction includes an infiltration of lymphocytes and polymorphonuclear leucocytes, and proliferation but no differentiation of connective tissue. Presumably the more potent antigenic effect of the heterogenous tissue evokes a more severe response from the host. This brings about the rapid destruction of the transplant including, of course, any osteogenic factor. Ollier (1859), Macewen (1874), Paterson (1878), Kummel (1891), McGill (1889), Moty (1895), Allison (1910), Kutner (1911), all tried heterogenous bone transplants and some including Macewen reported satisfactory results. According to Orell (1937) heterogenous bone converted into os purum and os novum is successful. There has always been the sporadic claim for the successful transplant of tissue and organs of other species. Admittedly, in the experiments described here there was no heterogenous transplantation into host bone but the results of

transplantation elsewhere support the generally accepted view that host tissue reacts quickly and vigorously to such foreign tissue to overwhelm and destroy it.

CONCLUSIONS.

Autogenous transplants have a considerable survival capacity and although their vitality is impaired during the first two weeks they are later repopulated by osteoblasts. Homogenous transplants despite some temporary survival of young cells finally degenerate and become sequestra. Syngenesious transplants behave like autogenous transplants. Frozen homogenous bone although alive when implanted does not survive. Heterogenous transplants are rapidly destroyed by the host tissue reaction.

In all transplants mature osteocytes imprisoned in calcified matrix die within ten days but the peripherally placed cells of autogenous bone survive. The skeletal connective tissue survives in autogenous transplants and is the source of the osteoblasts which migrate to repopulate the a-cellular areas of the matrix. There is no qualitative difference between endosteum and the osteogenic layer of periosteum, they subserve the same function of farming young osteoblasts. Cartilage tissue survives throughout in the autogenous transplants but is ultimately destroyed in the homogenous transplants. Autogenous and syngenesious marrow tissue is well able to survive transplantation but in homogenous transplants it gradually degenerates.

Cancellous bone has a greater survival capacity than cortical bone and small bone fragments survive better than large fragments. The small cancellous graft is more quickly and fully repopulated than the large cortical graft.

Young autogenous bone and epiphyseal cartilage survives transplantation better than mature bone, repopulation is more rapid and its presence evokes active and prolific extrinsic osteogenesis.

The host tissue reaction to the presence of an autogenous bone transplant is a normal reparative process consisting of the proliferation of connective tissue cells and their differentiation into osteoblasts, some to repopulate the matrix and some to form new bone. The transplantation of homogenous bone is followed by a vigorous host reaction - probably an immunity reaction. There is no repopulation of the transplant and the new bone formation induced in the host tissue soon degenerates.

Bone tissue transplanted into non-osseous tissue stimulates osteogenesis. The existence of a substance, 'osteogenin' liberated by the bone would provide a basis for the explanation of the histological appearances observed. This substance would appear to be more abundant in young bone and in epiphyseal cartilage, to withstand preservation by freezing, and is not individually specific. When

autogenous bone is transplanted into bone the resulting osteogenesis is induced partly by the transplant and partly by the host bone. When homogenous bone is transplanted into bone osteogenesis is a function of the host tissue and the new formed bone remains alive and proceeds to replace the transplant.

The most favourable type of bone graft is autogenous cancellous bone implanted in small fragments. Although the large cortical graft provides stability multiple cancellous grafts should be employed when the object is to promote osteogenesis and healing.

The use of syngenesious bone should be considered when autogenous bone is not available.

Frozen homogenous bone is clinically as efficient as fresh homogenous bone, and has the advantage of being readily available in quantity.

Autogenous transplantation in young individuals is more successful than in adults. Epiphyseal cartilage and young bone tissue as for example from the iliac crest survives well and heals rapidly. The clinical homograft which is substituted by host bone tissue is replaced more rapidly in the young.

SUMMARY.

This thesis includes a short history of experimental and clinical bone transplantation.

Two hundred and fourteen animal experiments are described in which autogenous, homogenous, heterogenous and preserved bone transplantations were made into bone, muscle and the anterior chamber of the eye and the specimens removed at intervals varying from ten days to six months.

X-Rays were taken of the transplants in situ and of the specimens on removal and histological examination of prepared sections was carried out and recorded.

The results are discussed under the headings of survival capacity, repopulation of transplants, and osteogenesis.

Conclusions are stated based on the findings.

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AN

EXPERIMENTAL STUDY

OF

BONE TRANSPLANTATION

BY

JOHN HUTCHISON MB. F.R.F.P.S.

FROM

THE DEPARTMENT OF ANATOMY

UNIVERSITY OF GLASGOW

VOLUME II

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Autogenous Bone transplant into Muscle.

10 Days.

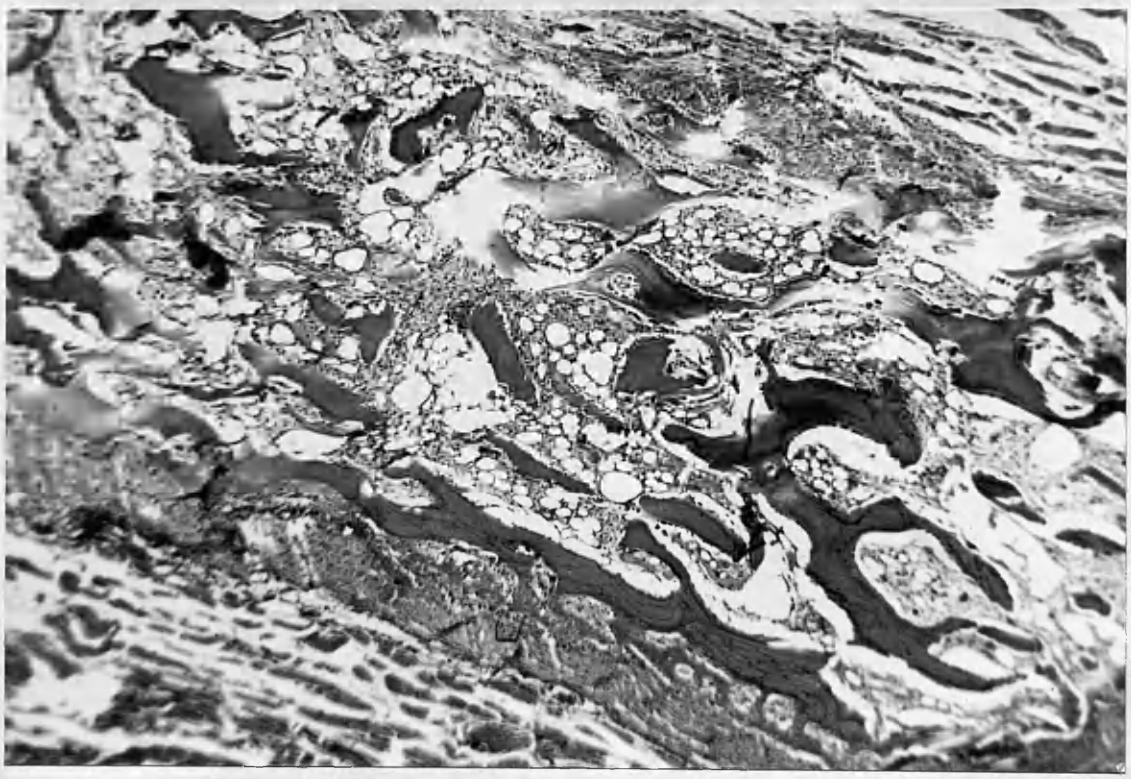

Fig:1 x 50. A general picture of the transplant, with surrounding host connective tissue reaction in which new bone is being formed. The muscle tissue is seen at the periphery.

Autogenous Bone transplant into Muscle.

10 Days.

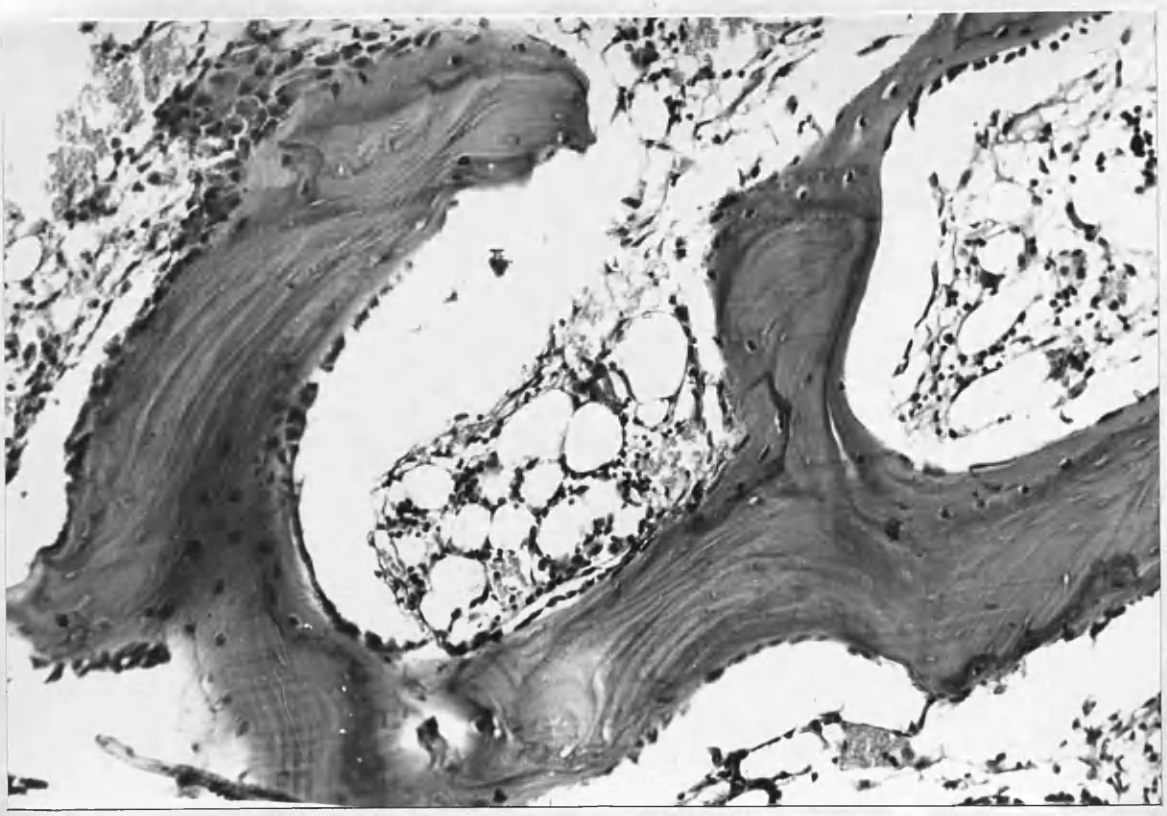

Fig:2 x 200. The majority of the bone cells have disappeared. The surviving endosteal cells are seen with commencing proliferation. The marrow tissue is of normal appearance.

Autogenous Bone transplant into Muscle.

10 Days.

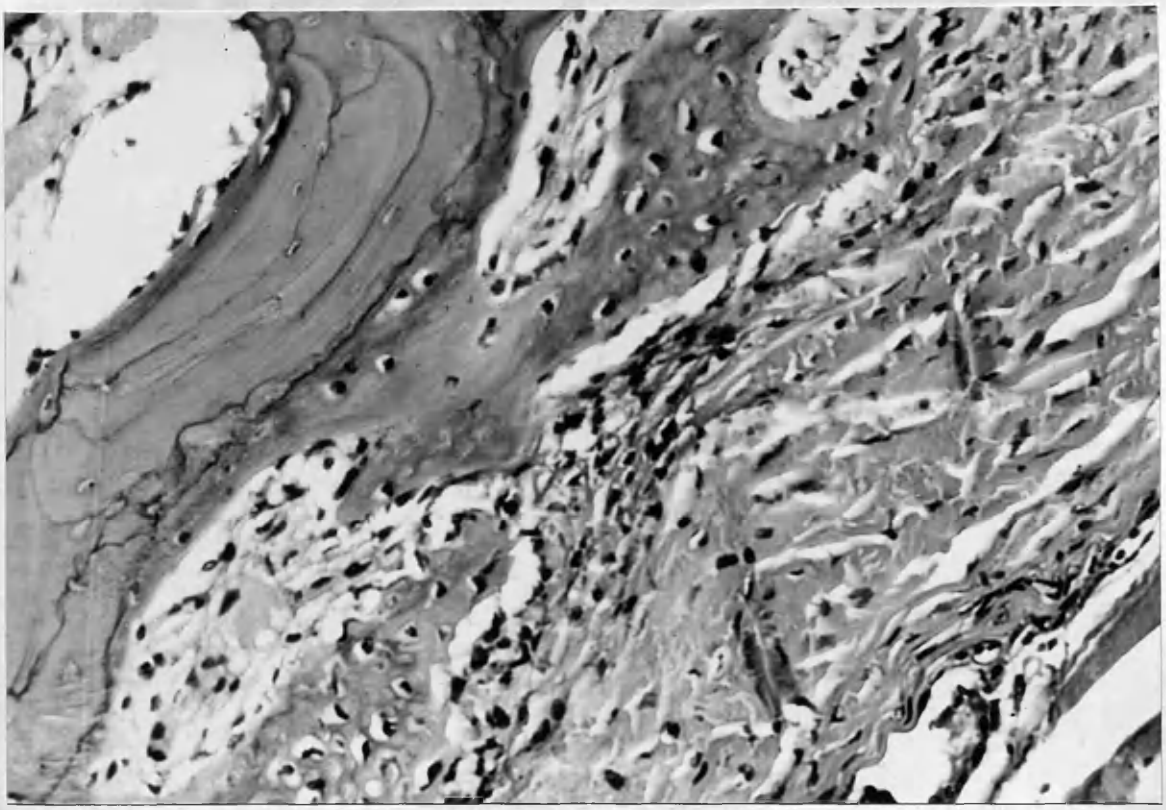

Fig:3 x 250. The host connective tissue reaction consisting of large spindle shaped cells shows differentiation into osteoblasts with new bone formation closely apposed to the transplant.

Autogenous Bone transplant into Muscle.

10 Days.

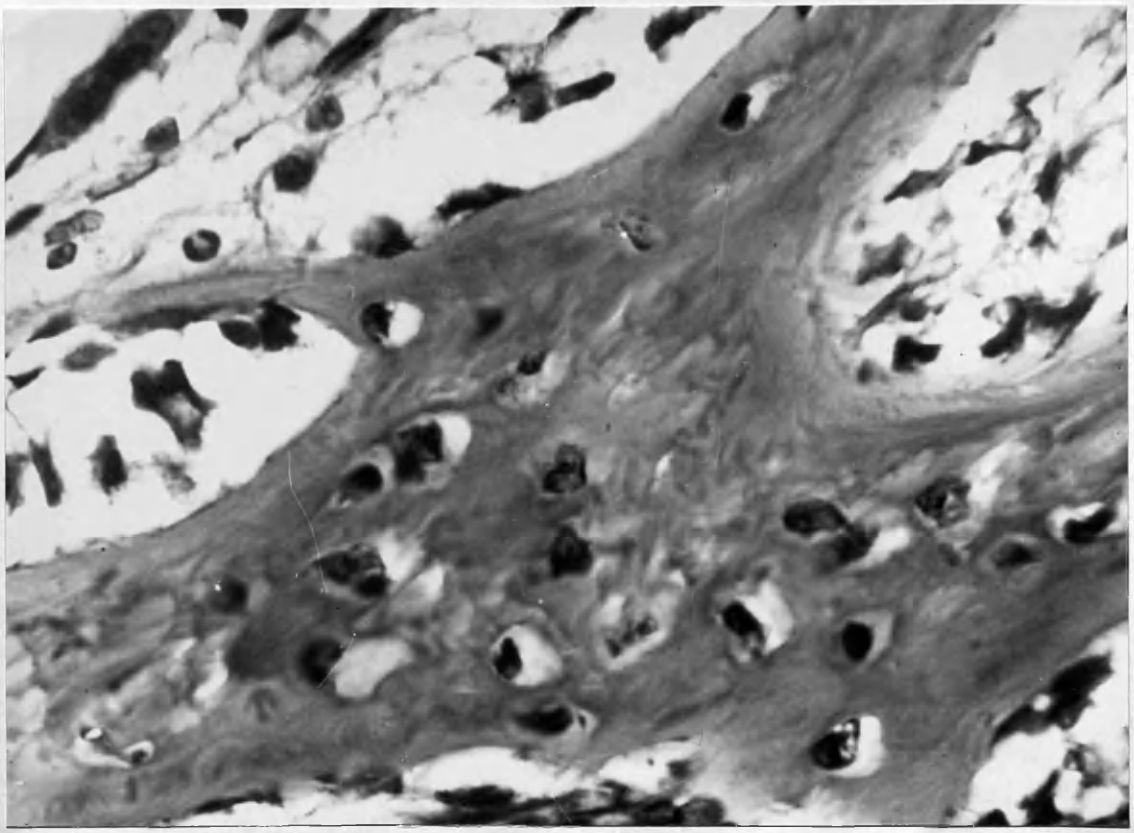

Fig:4 x 750. Large osteoblasts in the new osseous tissue arising from the extra skeletal connective tissue.

Homogenous Bone transplant into Muscle.

10 Days.

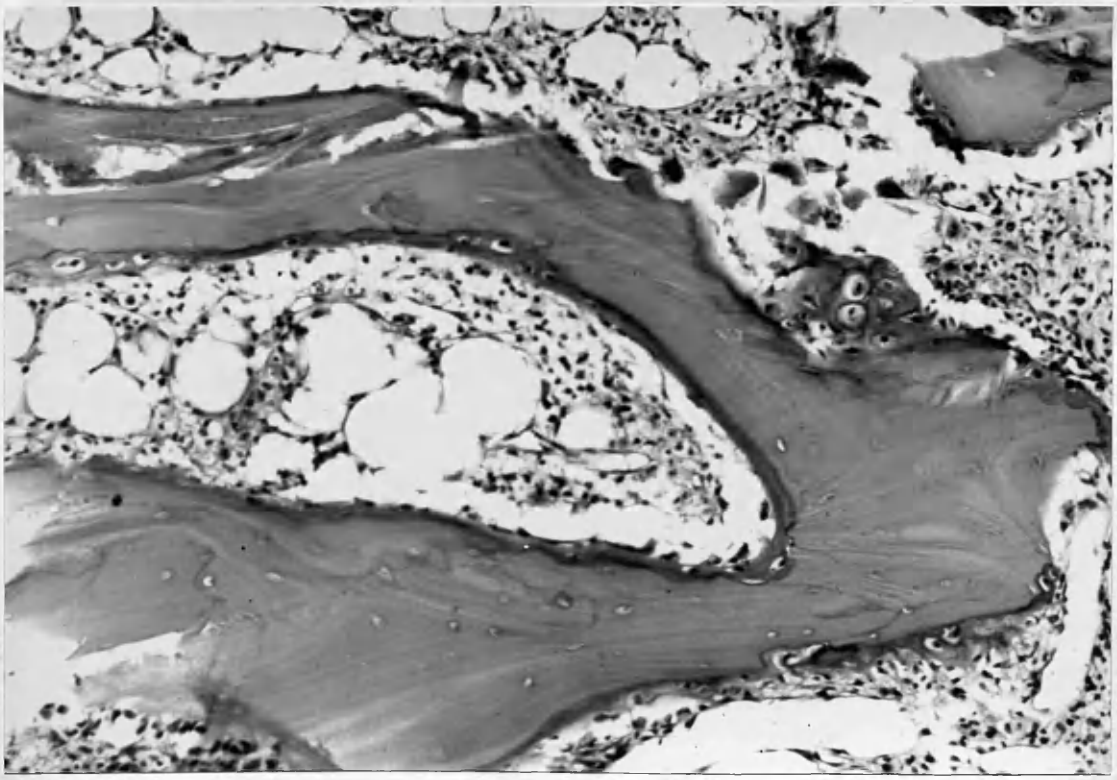

Fig:5 x 200. Dead homogenous bone with its empty lacunae. The edges of the fragments have a dense outline due to bone lysis and osteoclastic activity.

Homogenous Bone transplant into Muscle.

10 Days.

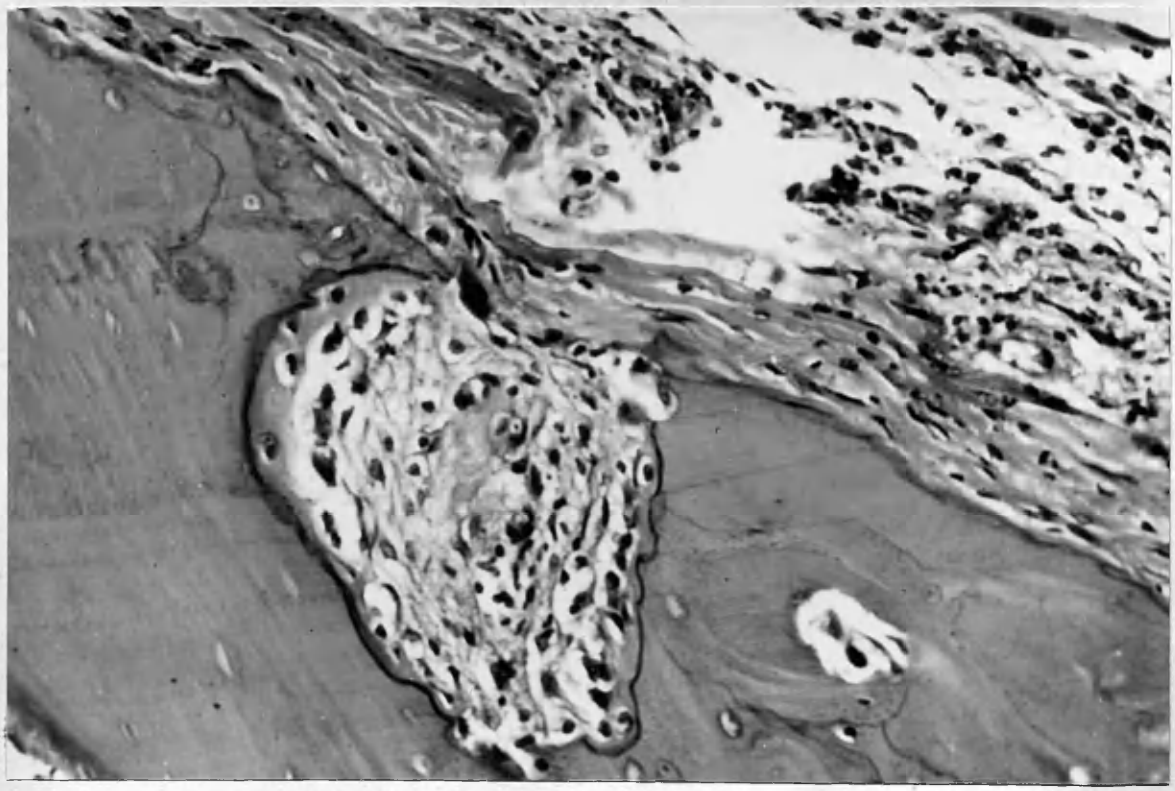

Fig:6 x 250. The dense outline of the homograft indicating bone lysis and osteoclastic activity is clearly demonstrated.

Homogenous Bone transplant into Muscle.

10 Days.

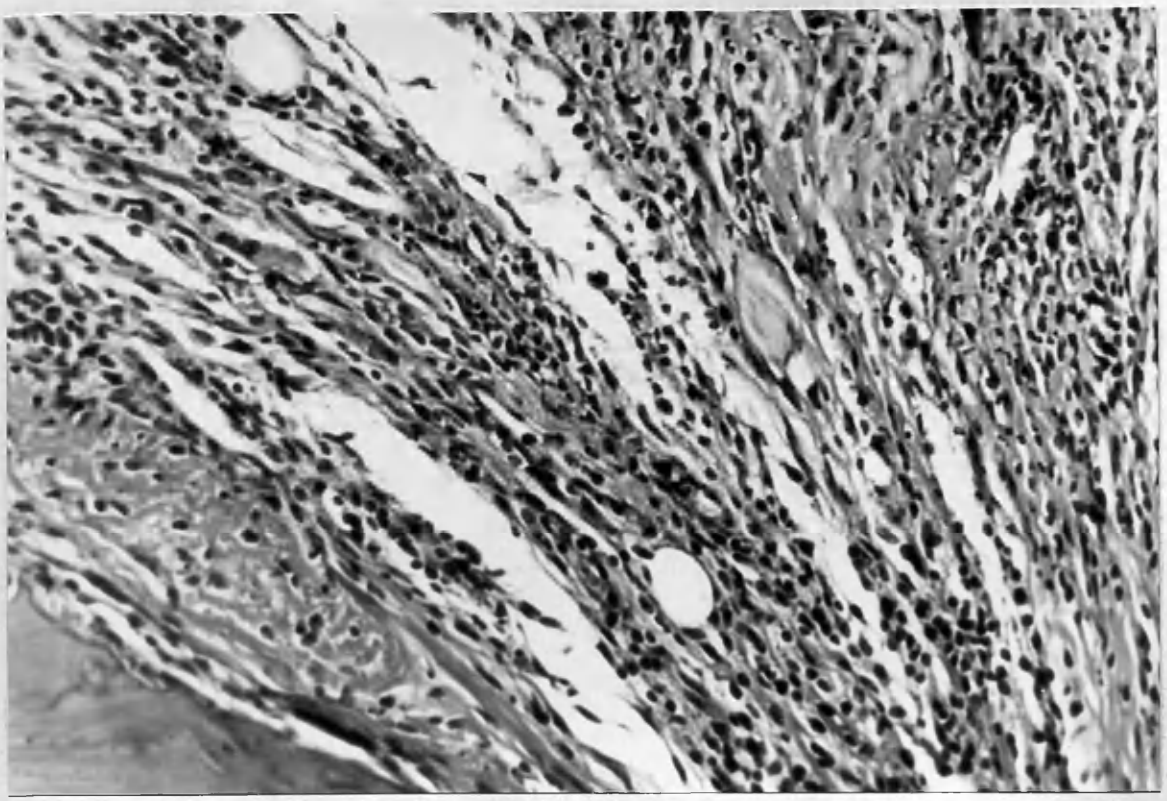

Fig:7 x 250. The host connective tissue reaction surrounding the transplant, characterised by a dense lymphocytic infiltration and large spindle shaped cells.

Homogenous Bone transplant into Muscle.

10 Days.

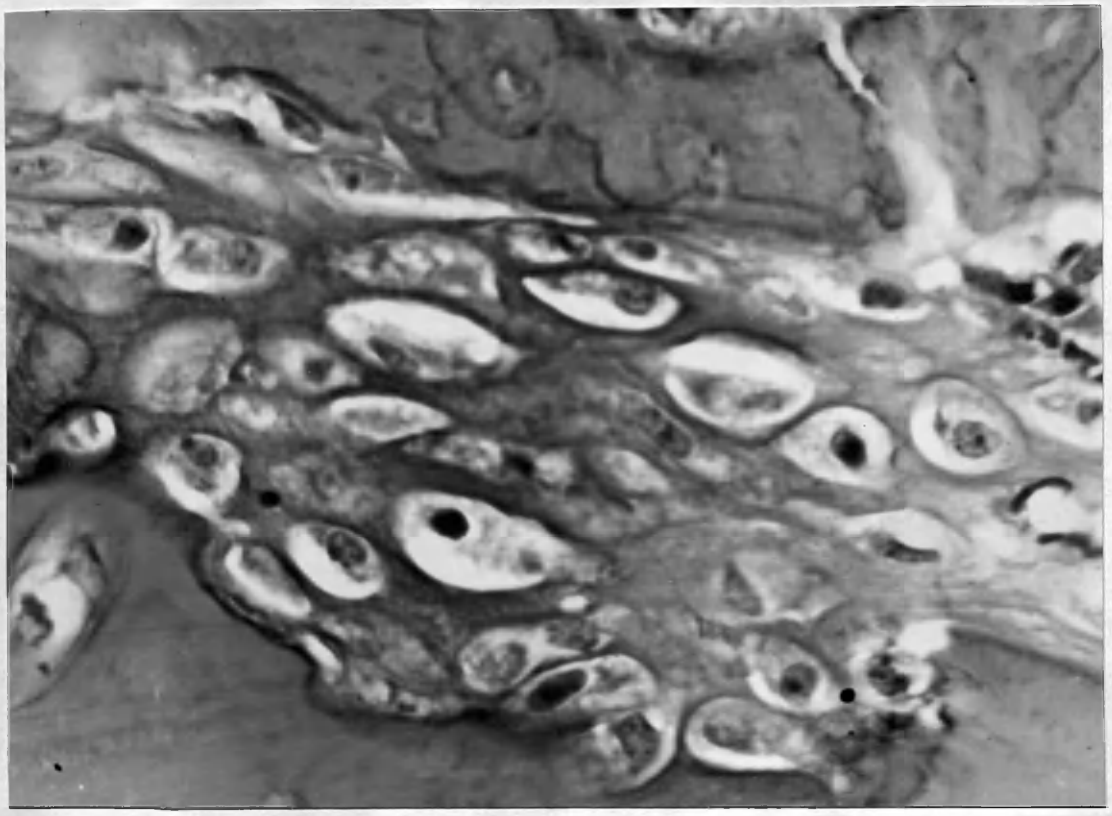

Fig:8 x 750. Large cartilage cells have survived, but the bone tissue is quite dead.

Autogenous Bone transplant into Muscle.

21 Days.

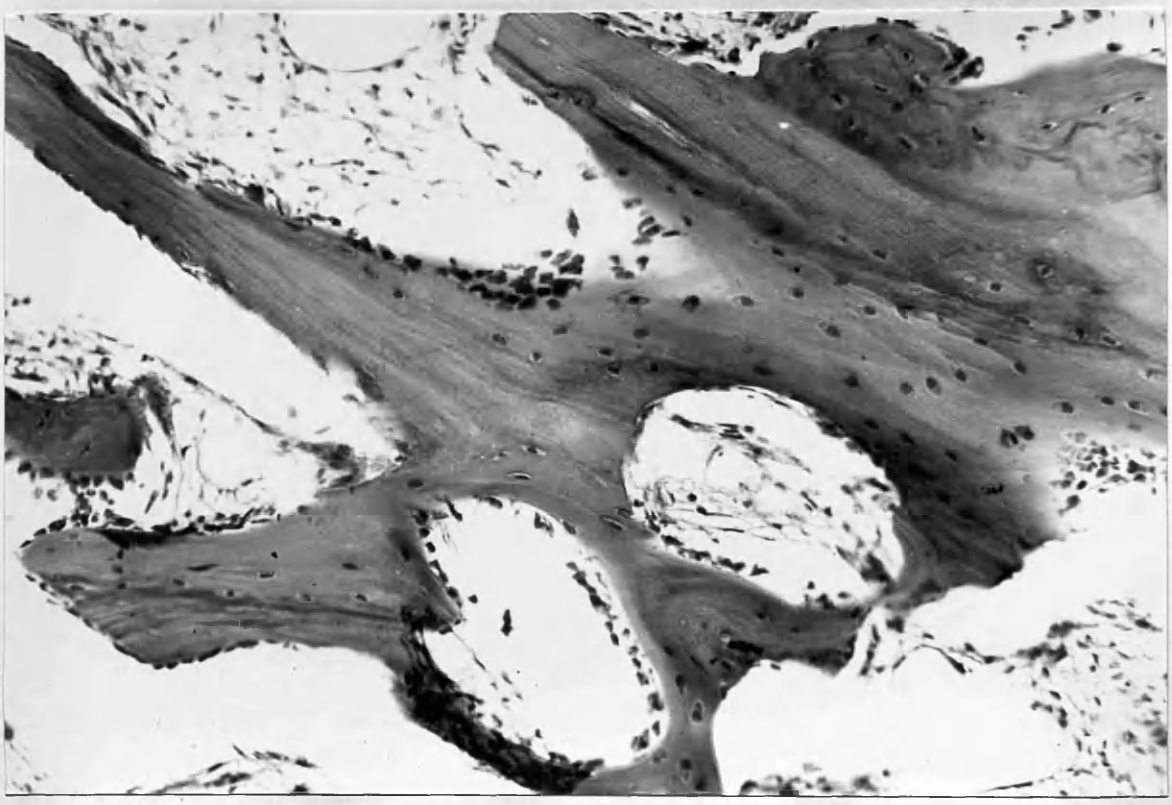

Fig:9 x 200. The phenomenon of migration of osteoblasts from the endosteal proliferation is seen. Many areas are still devoid of nuclei, the mature osteocytes having died.

Autogenous Bone transplant into Muscle.

21 Days.

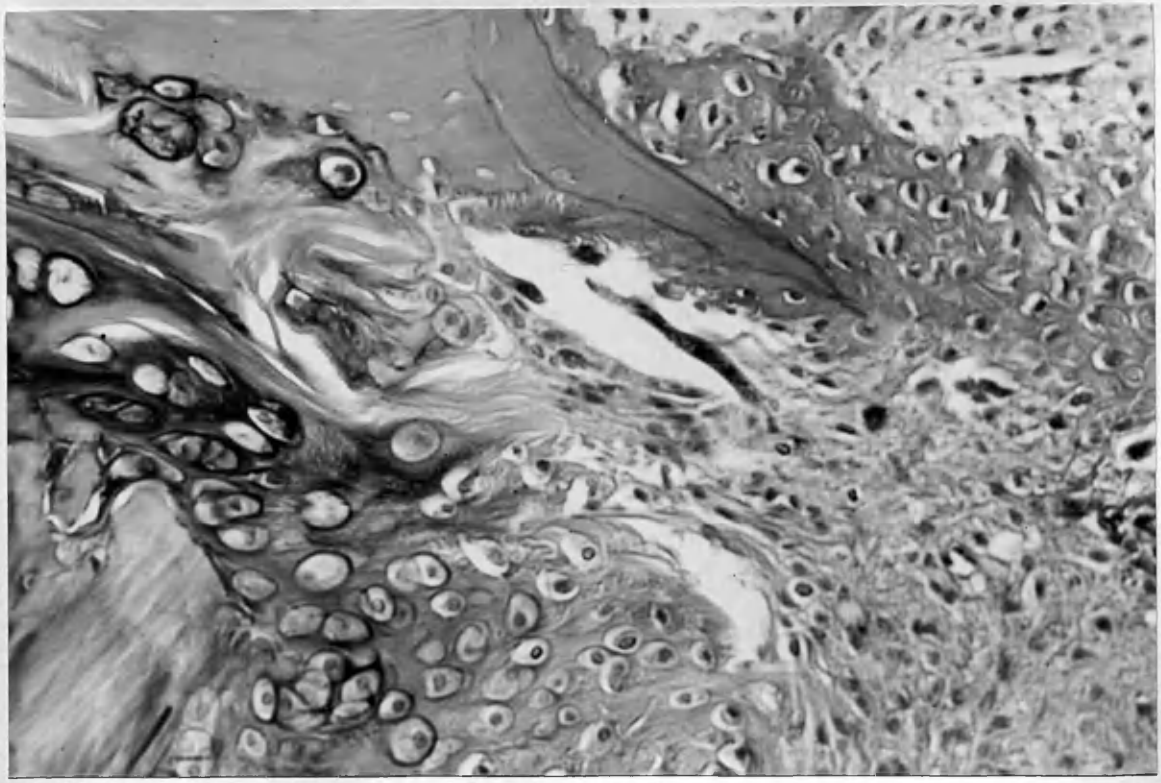

Fig:10 x 250. Cartilage cells have survived with active formation of chondro-osseous tissue in its immediate vicinity. The new osseous tissue being closely apposed to the transplant.

Autogenous Bone transplant into Muscle.

21 Days.

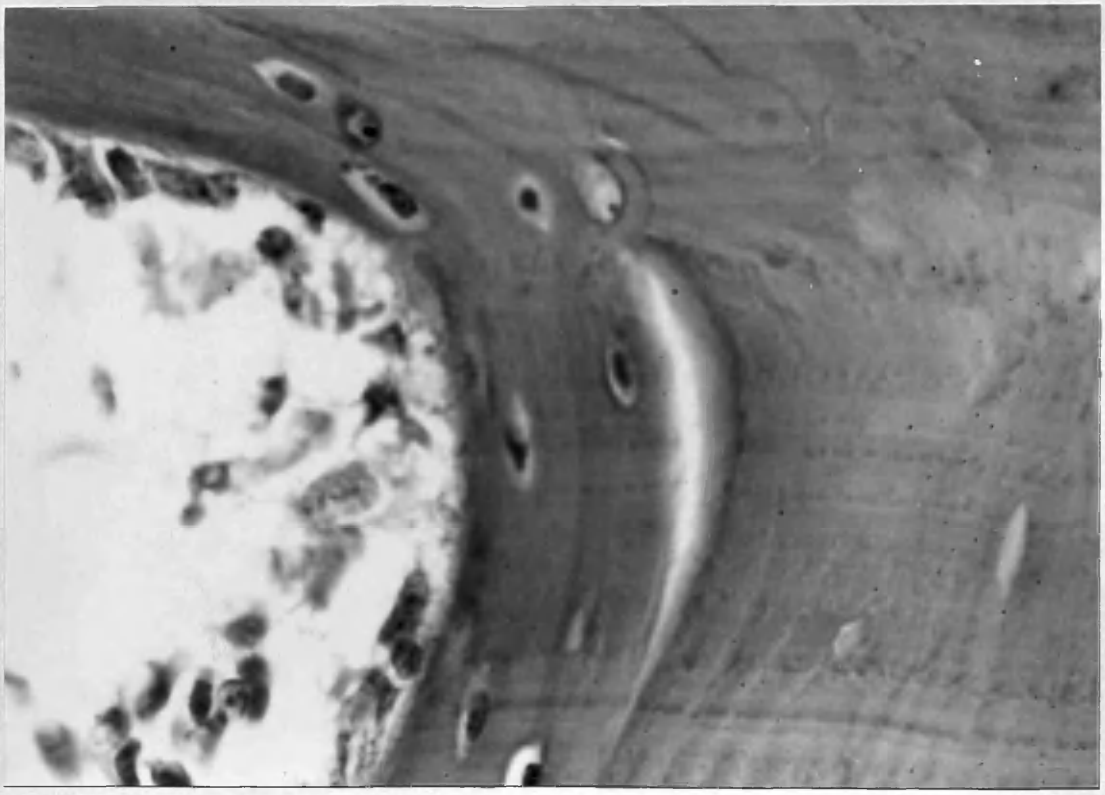

Fig:11 x 700. Autogenous cortical bone transplant which has become devitalised. The lacunae are vacant except at the periphery where invasion is commencing.

Homogenous Bone transplant into Muscle.

21 Days.

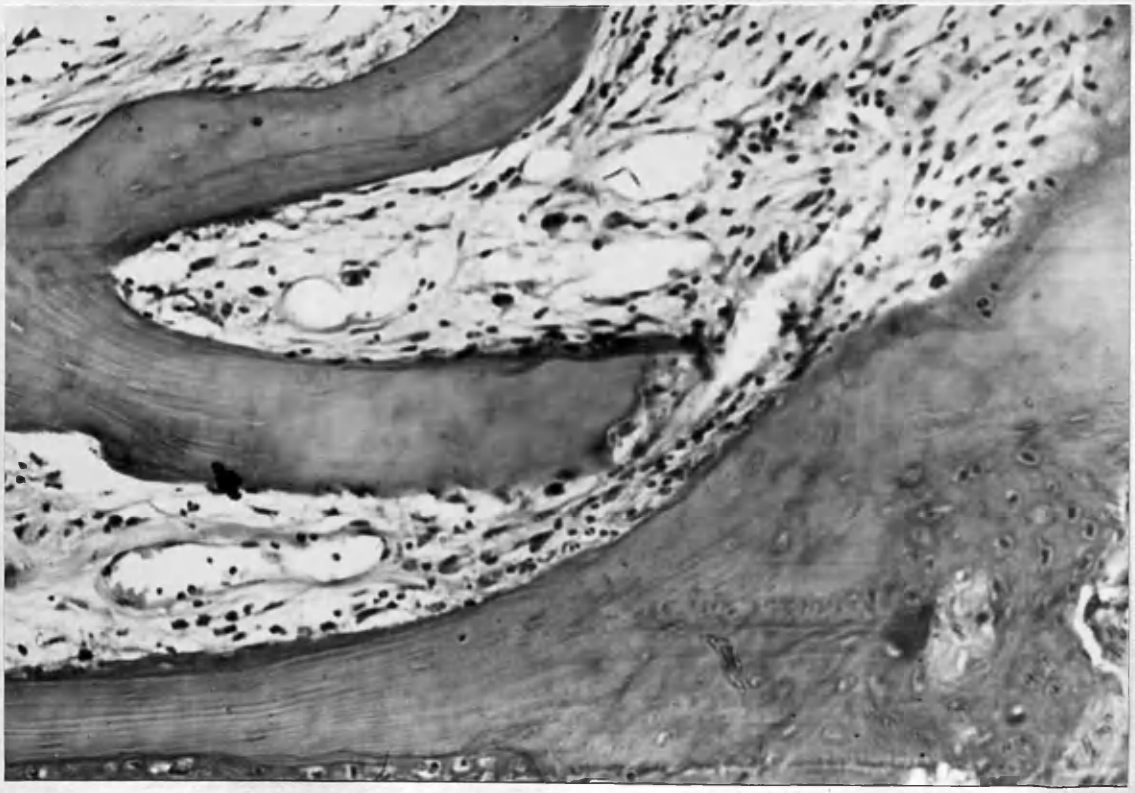

Fig:12 x 200. Devitalised bone tissue, the dense outline of the transplant demarcates it clearly from the surrounding connective tissue.

Homogenous Bone transplant into Muscle.

21 Days.

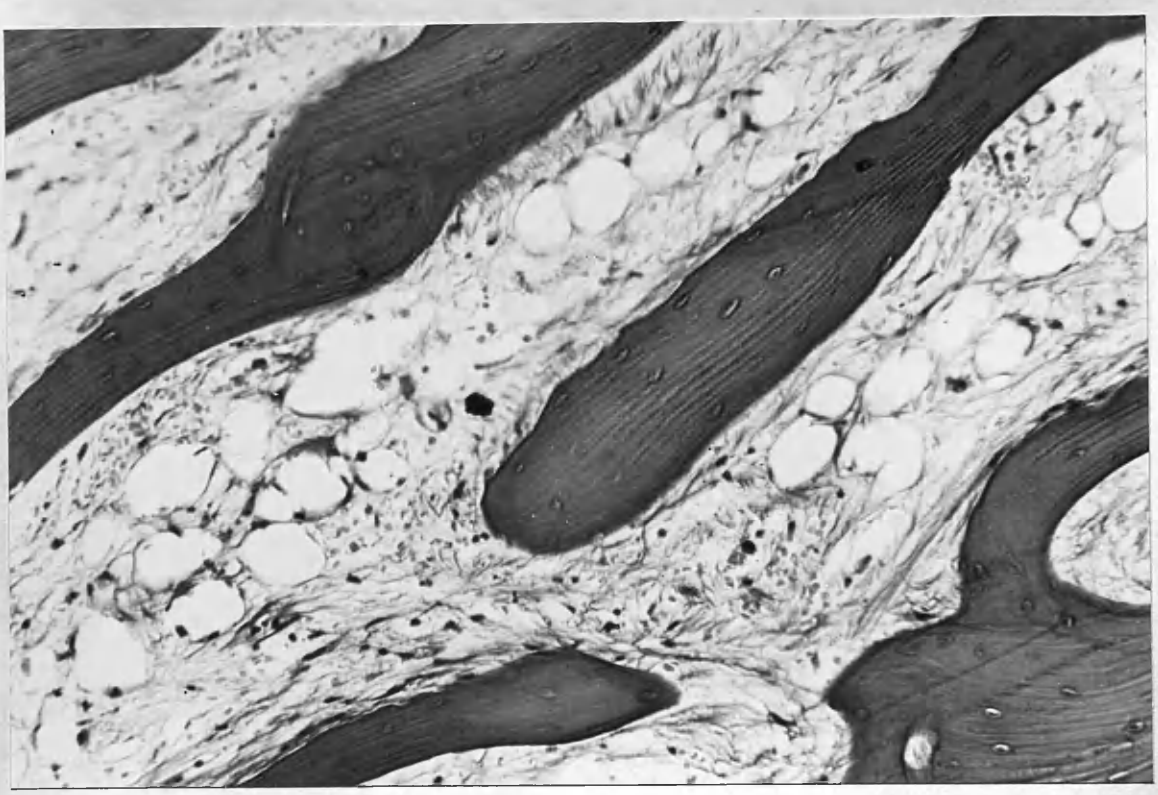

Fig:13 x 200. The sharply defined outline of the dead transplant is evident, there is no endosteal osteoblastic activity.

Homogenous Bone transplant into Muscle.

21 Days.

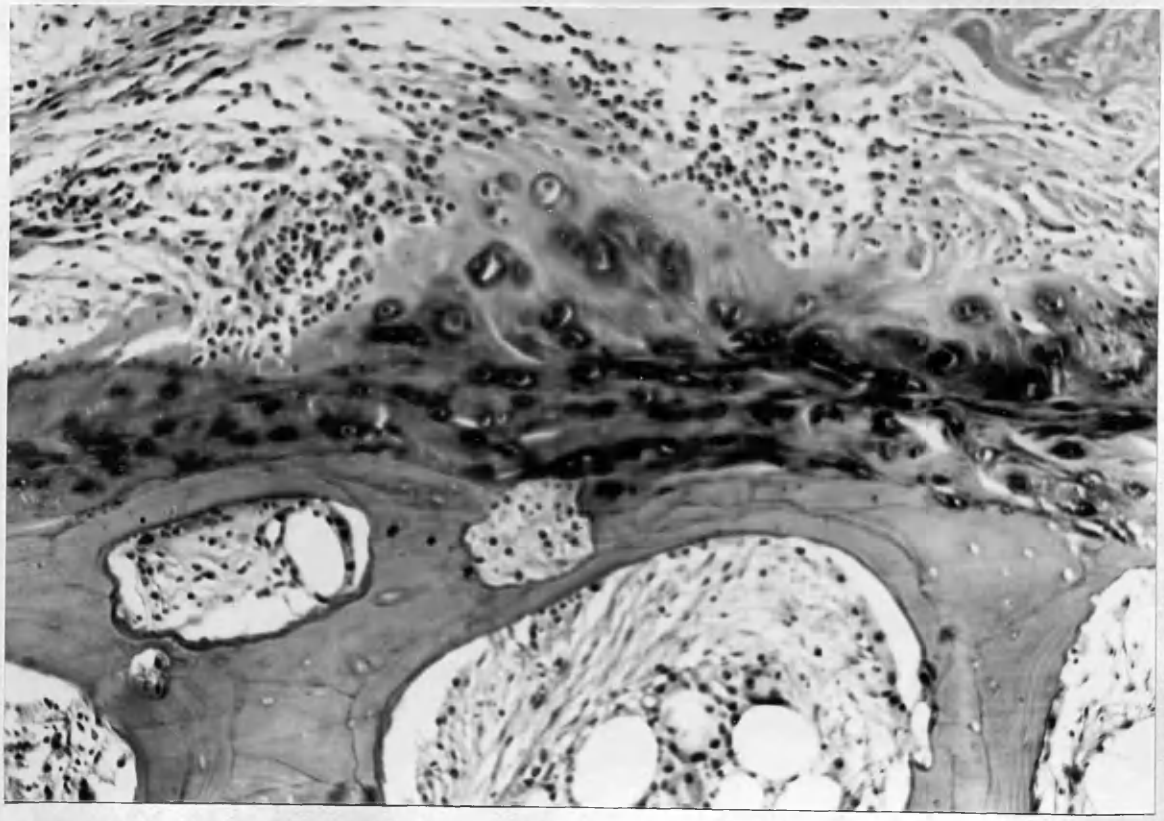

Fig:14 x 200. Though transplanted cartilage tissue has survived, the bone tissue is dead. The round cell reaction of the host tissues is seen.

Homogenous Bone transplant into Muscle.

21 Days.

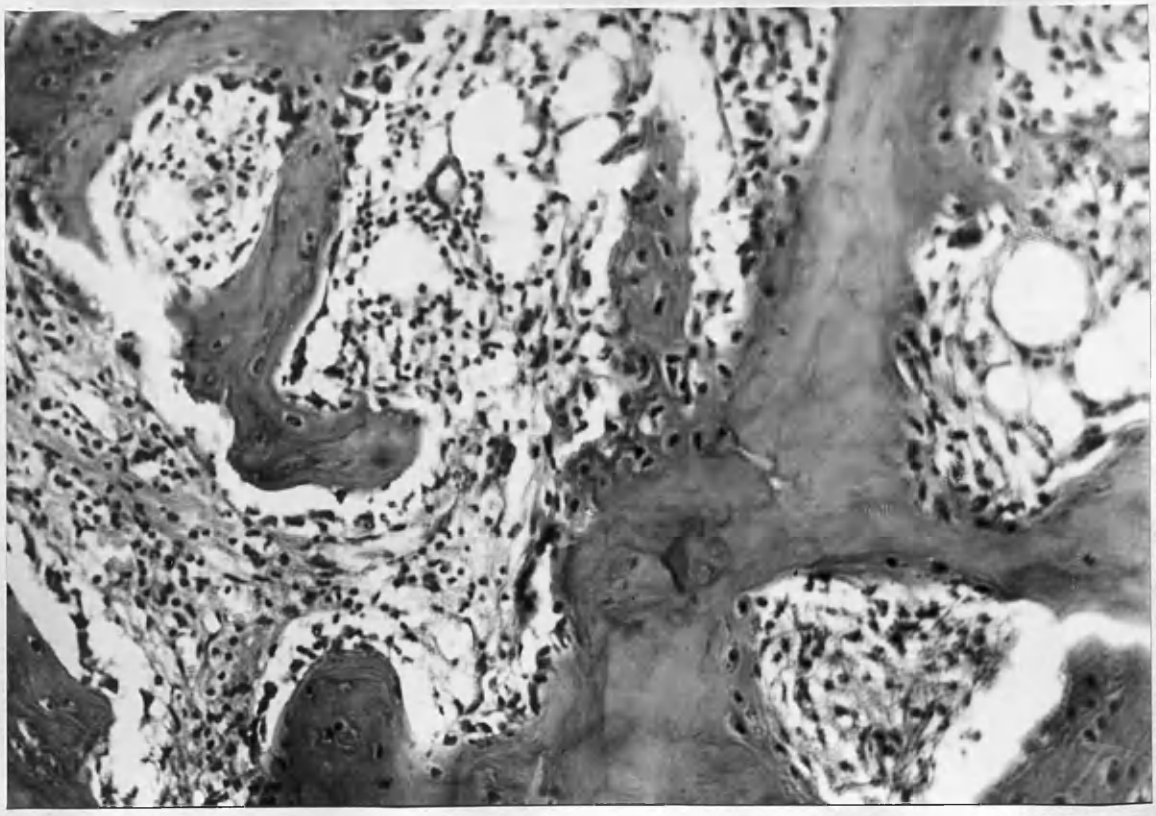

Fig:15 x 200. New bone arising in the connective tissue of the host is closely applied to the dead bone of the transplant and substitution is taking place.

Homogenous Bone transplant into Muscle.

21 Days.

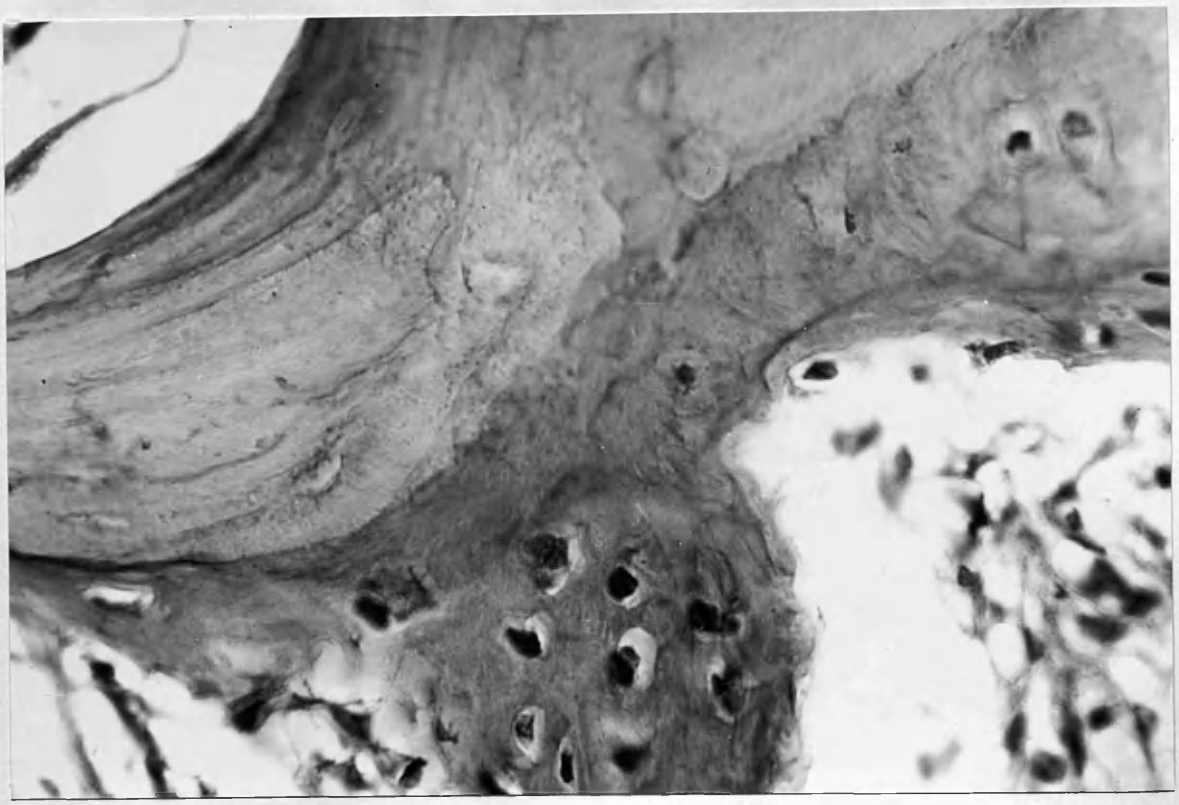

Fig:16 x 750. The new bone is continuous with the dead bone of the transplant. Its osteoblasts have well stained nuclei, and its matrix is more deeply stained than that of the dead transplant.

Autogenous Bone transplant into Muscle.

42 Days.

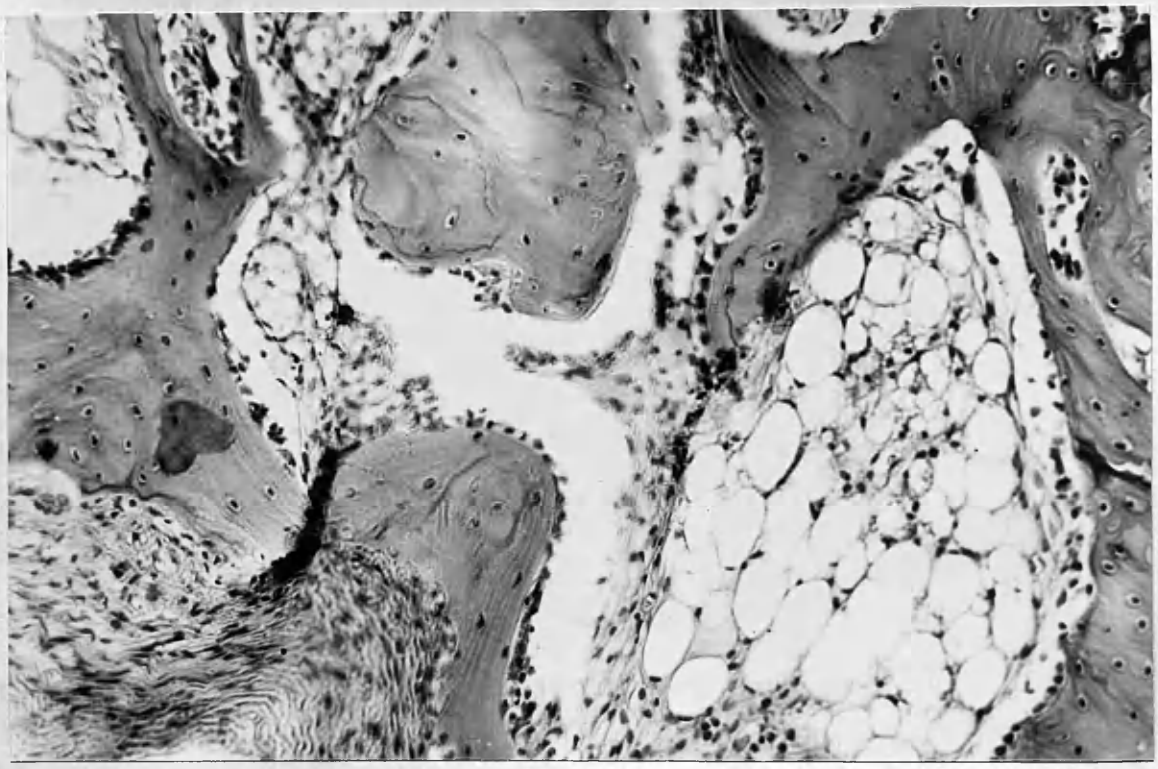

Fig:17 x 200. The matrix is well populated with osteocytes. The endosteal spaces are lined by a regular layer of cells in close contact with the bony matrix. In some areas these cells form multiple layers laying down new bone.

Autogenous Bone transplant into Muscle.

42 Days.

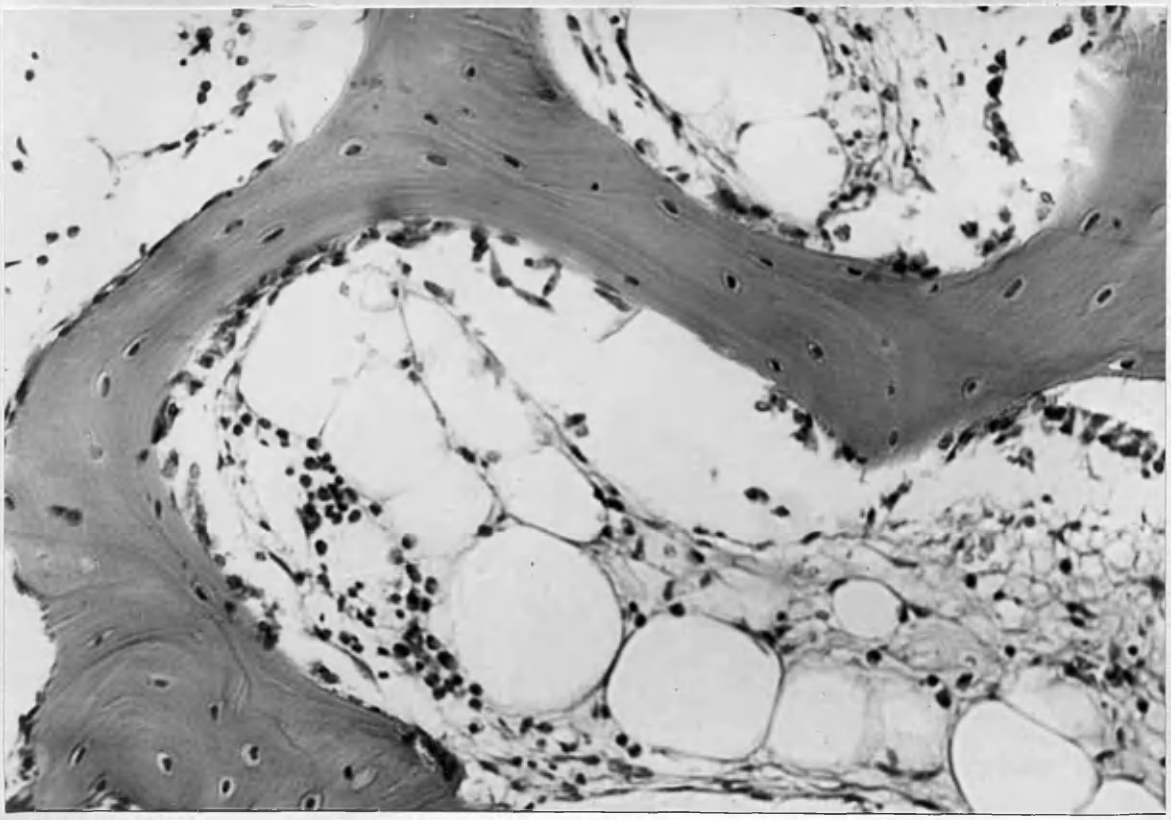

Fig:18 x 250. The smaller bone trabeculae have a comparatively normal appearance at this stage. Osteocytes are present and the surrounding endosteal cells are reduced to a regular layer.

Autogenous Bone transplant into Muscle.

42 Days.

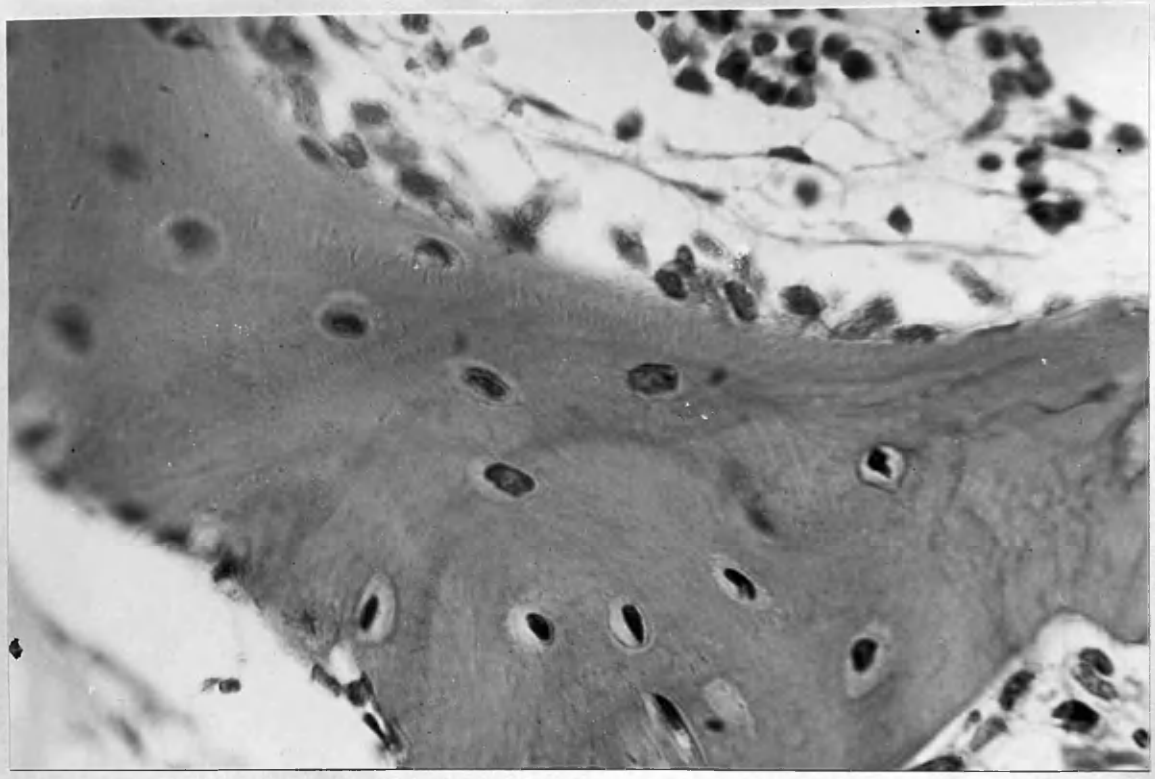

Fig:19 x 700. Osteocytes in the substance of the transplant with osteoblasts at the periphery.

Autogenous Bone transplant into Muscle.

42 Days.

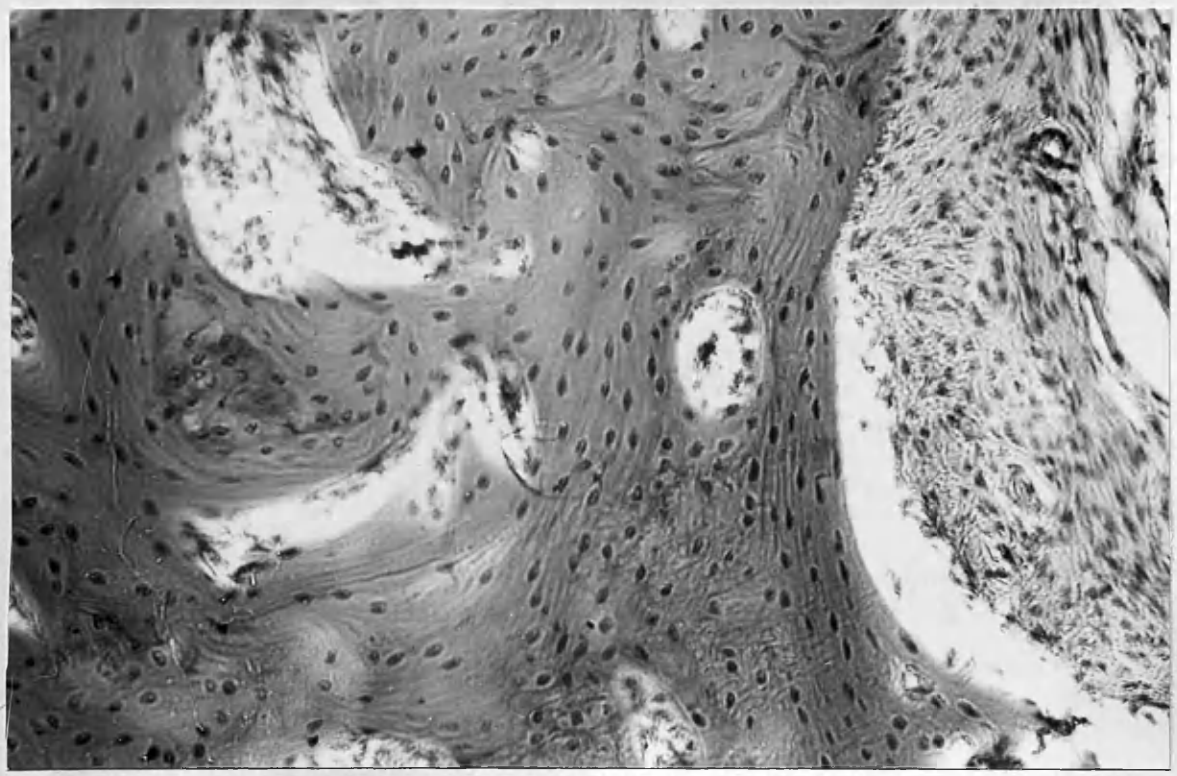

Fig:20 x 200. Very active bone tissue of an autogenous transplant in a young animal.

Autogenous Bone transplant into Muscle.

42 Days.

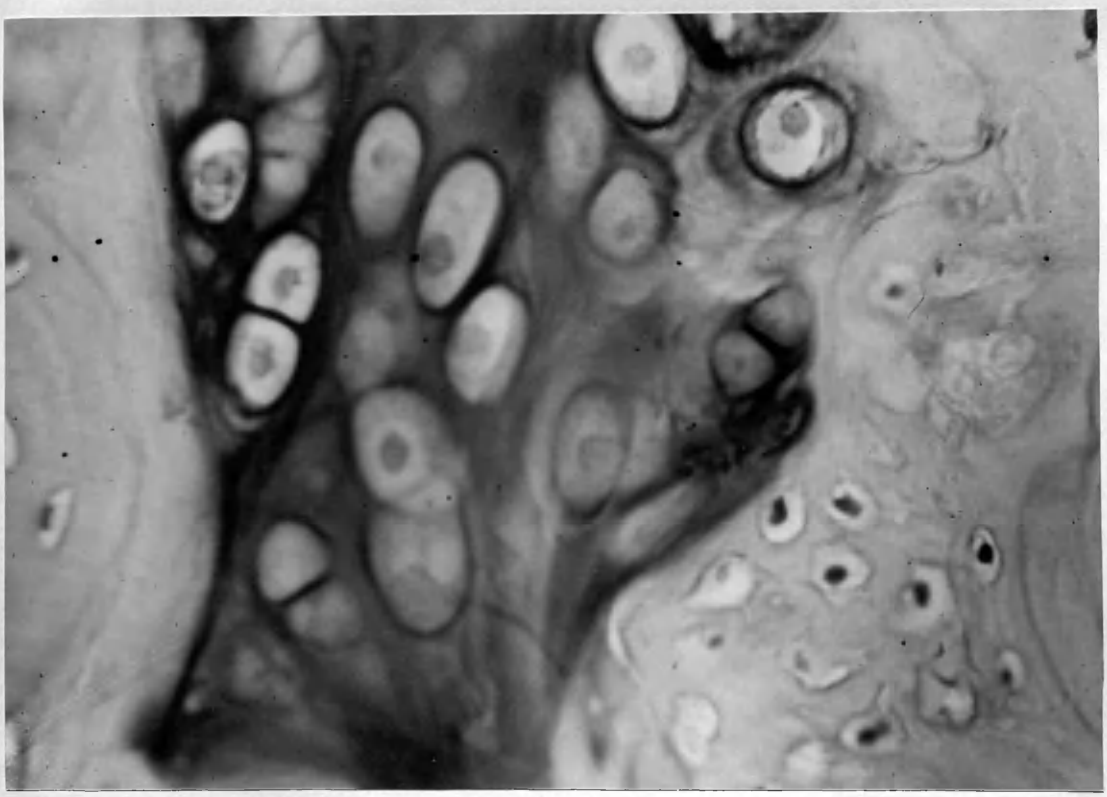

Fig:21 x 750. Young bone cells in close proximity to large cartilage cells. Elsewhere the matrix is comparatively a-cellular.

Homogenous Bone transplant into Muscle.

42 Days.

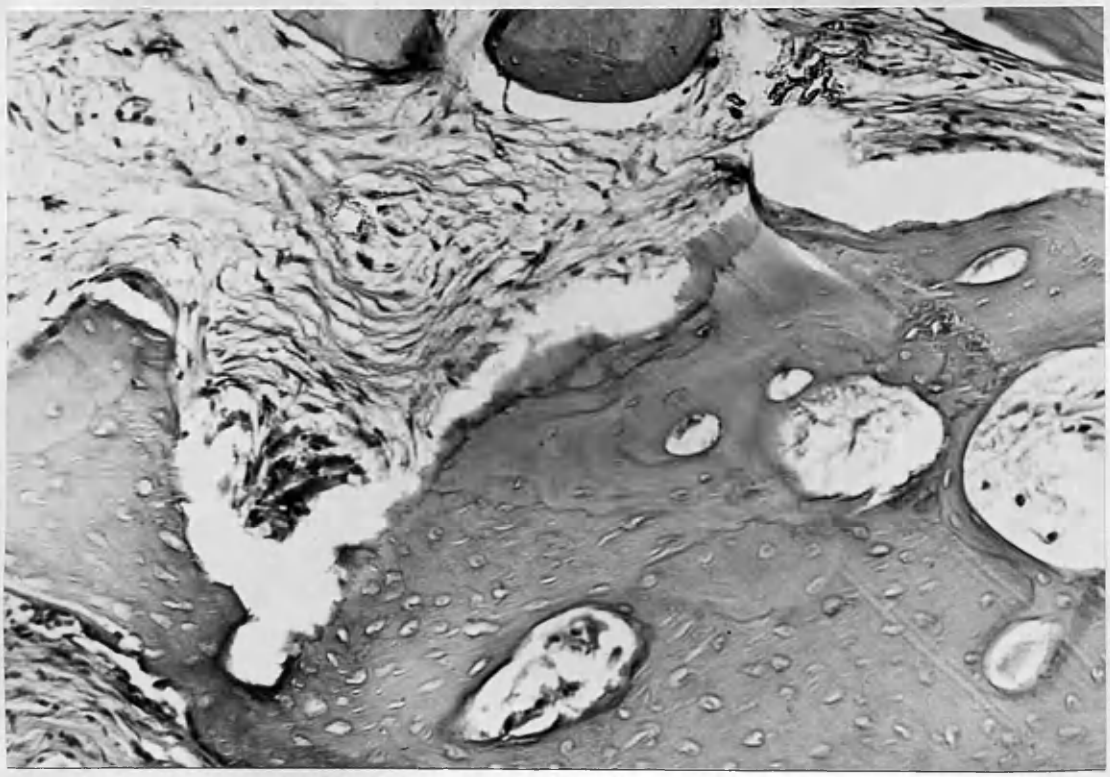

Fig:22 x 250. The transplanted bone tissue is dead. The irregular somewhat denser periphery appears to have no histological contact with the surrounding connective tissue. There is little or no osteoblastic activity in the endosteal spaces.

Homogenous Bone transplant into Muscle.

42 Days.

Fig:23 x 200. Dead sequestrum like bone tissue. The edge is sharply demarcated by a denser line due to commencing lysis.

Homogenous Bone transplant into Muscle.

42 Days.

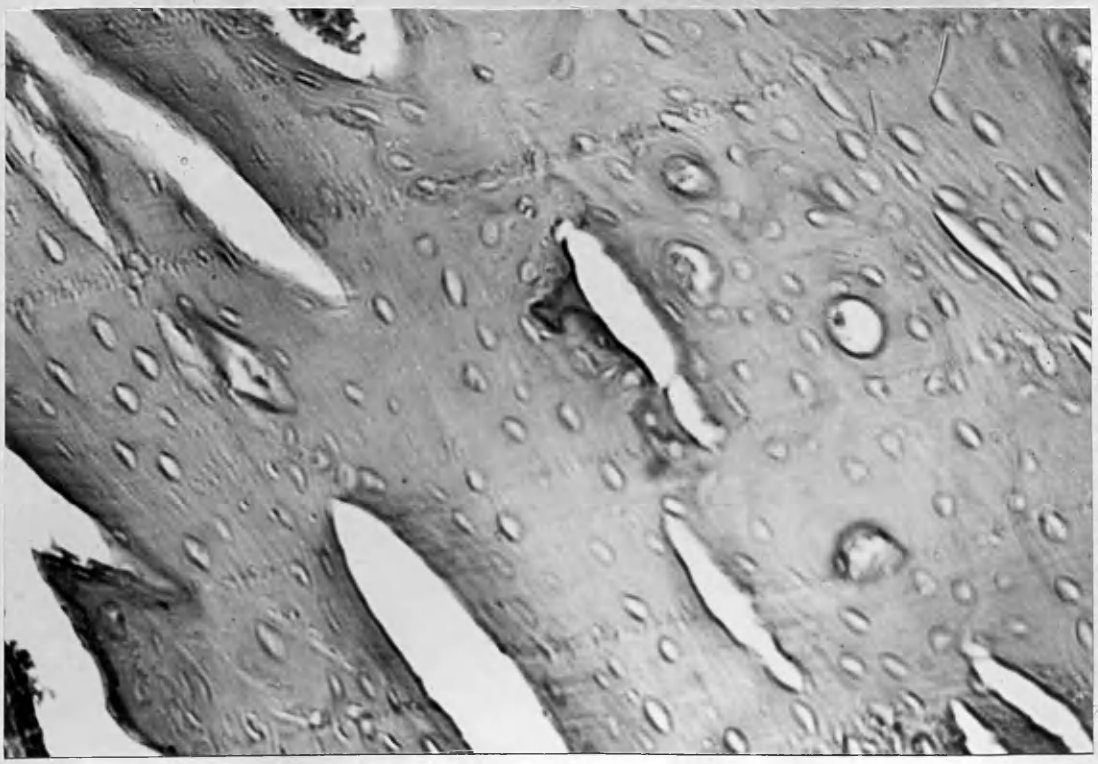

Fig:24 x 350. The empty lacunae of the dead homogenous transplant. There is no endosteal cell survival.

Homogenous Bone transplant into Muscle.

42 Days.

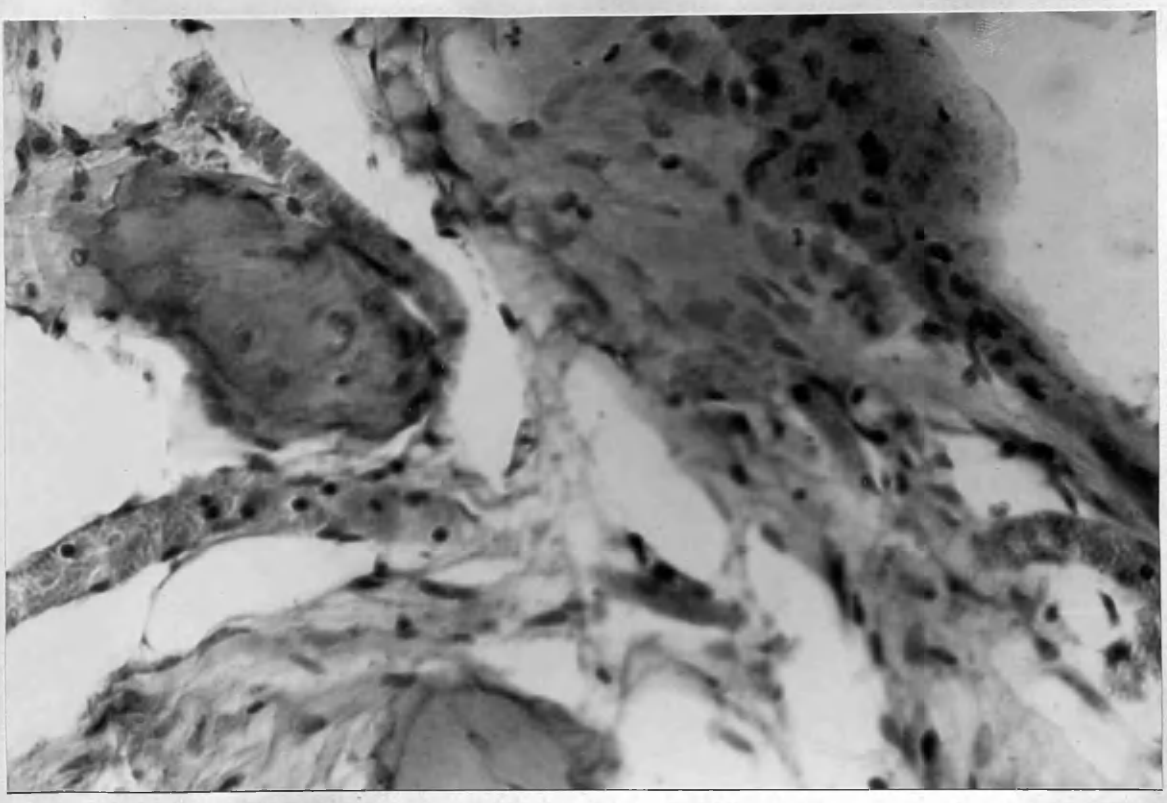

Fig:25 x 250. The extra skeletal connective tissue consisting of proliferating fibroblasts and capillaries. The early stage of osteogenesis with differentiation of cells into osteoblasts and deposition of the primary matrix is seen.

Homogenous Bone transplant into Muscle.

42 Days.

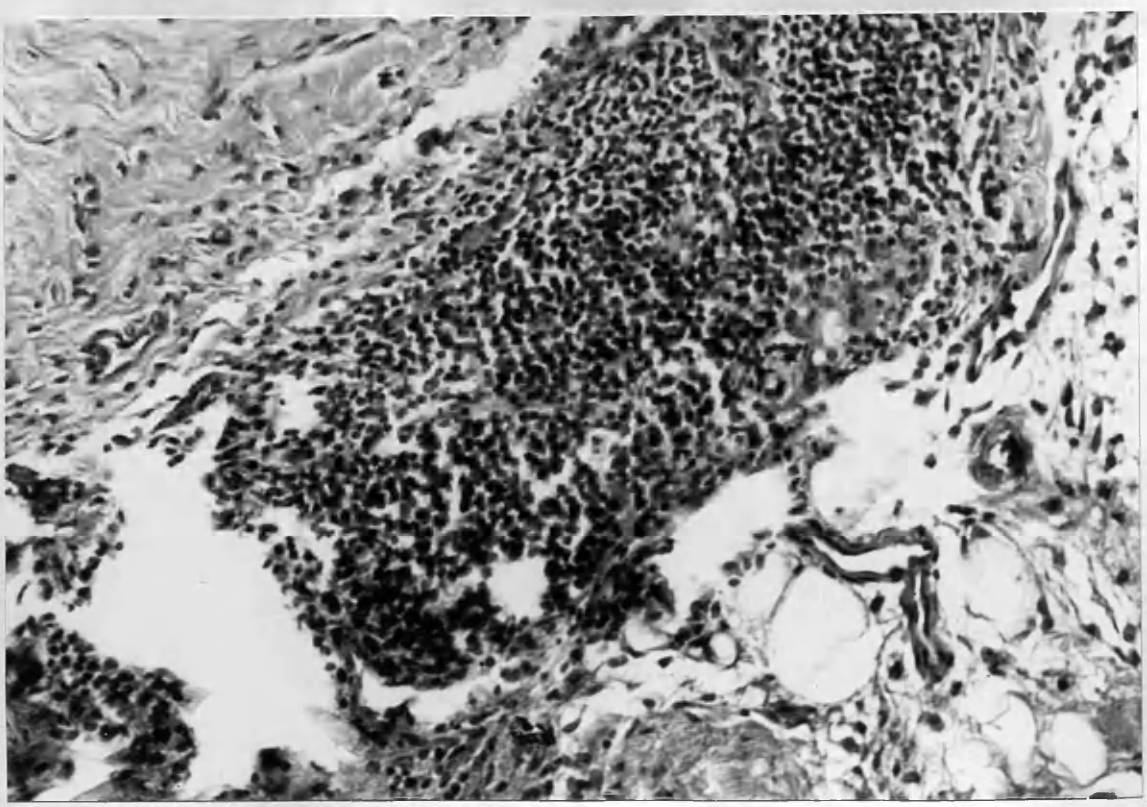

Fig:26 x 250. The dense lymphocytic infiltration which surrounds the homogenous transplant, together with capillaries and young connective tissue cells.

Homogenous Bone transplant into Muscle.

42 Days.

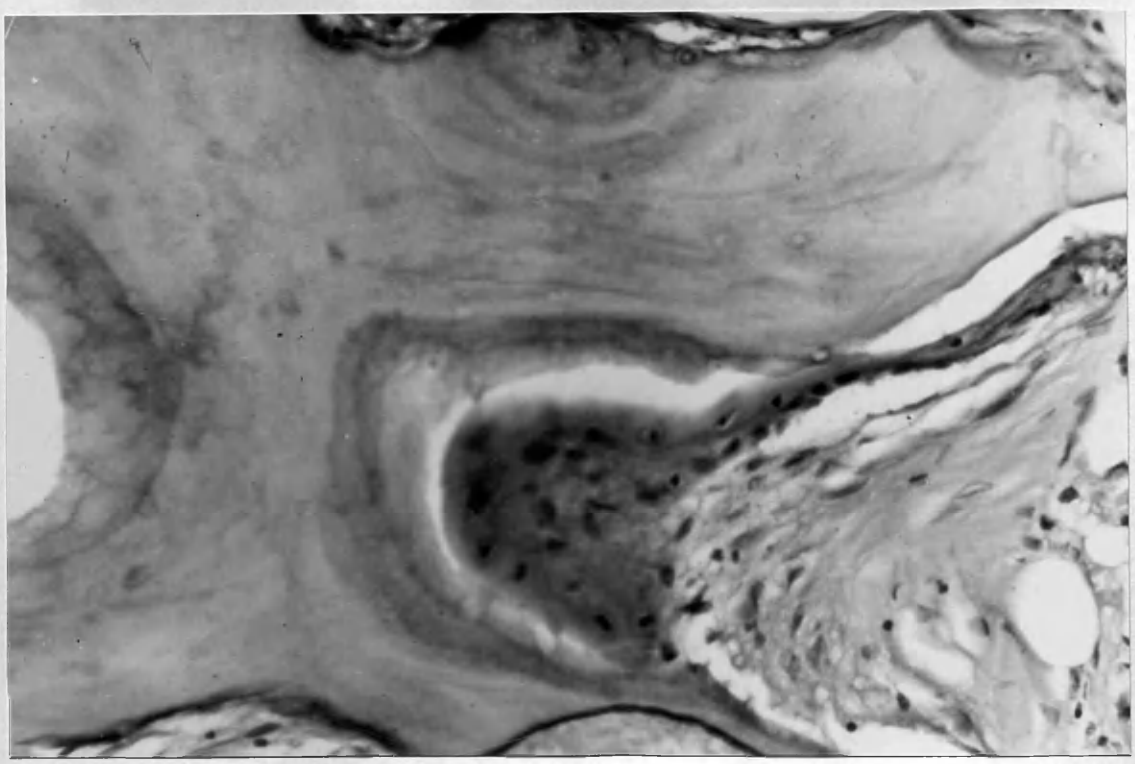

Fig:27 x 200. The host connective tissue cells differentiate into osteoblasts, lay down pre-osseous tissue and advance to replace the dead matrix which has undergone lysis — creeping substitution.

Homogenous Bone transplant into Muscle.

42 Days.

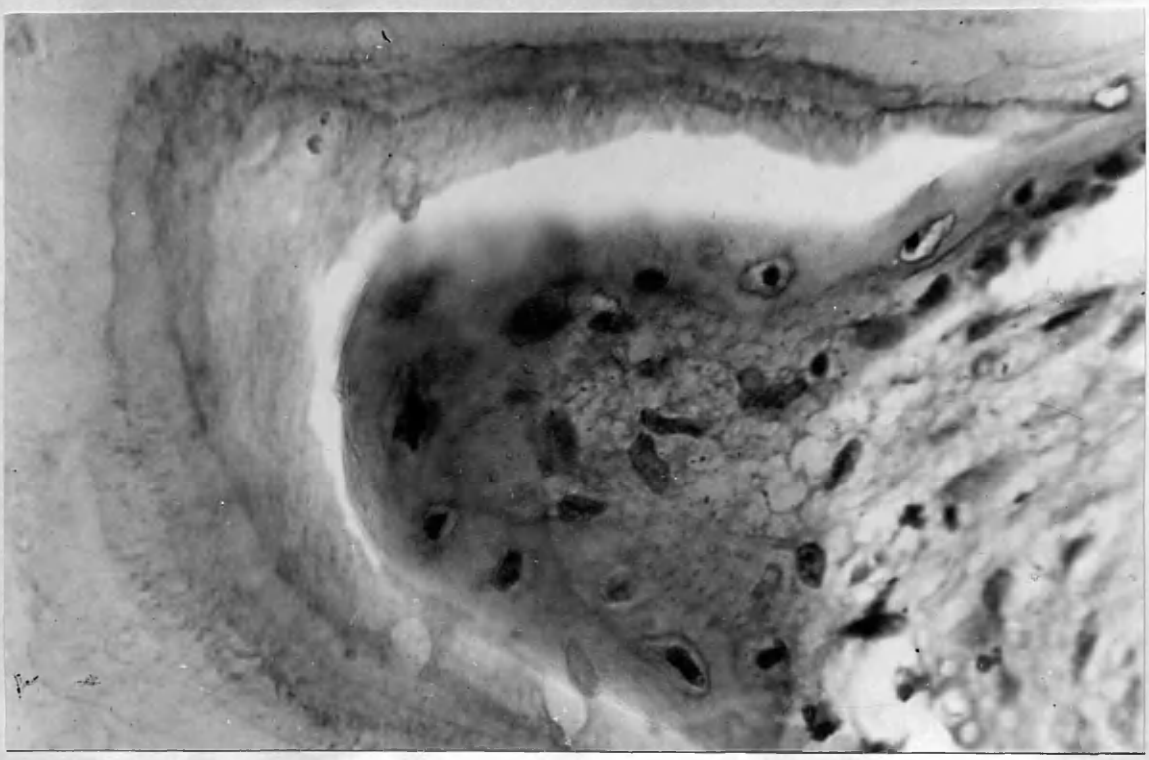

Fig:28 x 750. The transition from connective cells to osteoblasts and the wave like appearance of the dissolving edge of the matrix. This process of lysis accounts for the dense outline so characteristic of the dead homogenous transplant.

Autogenous Bone transplant into Muscle.

90 Days.

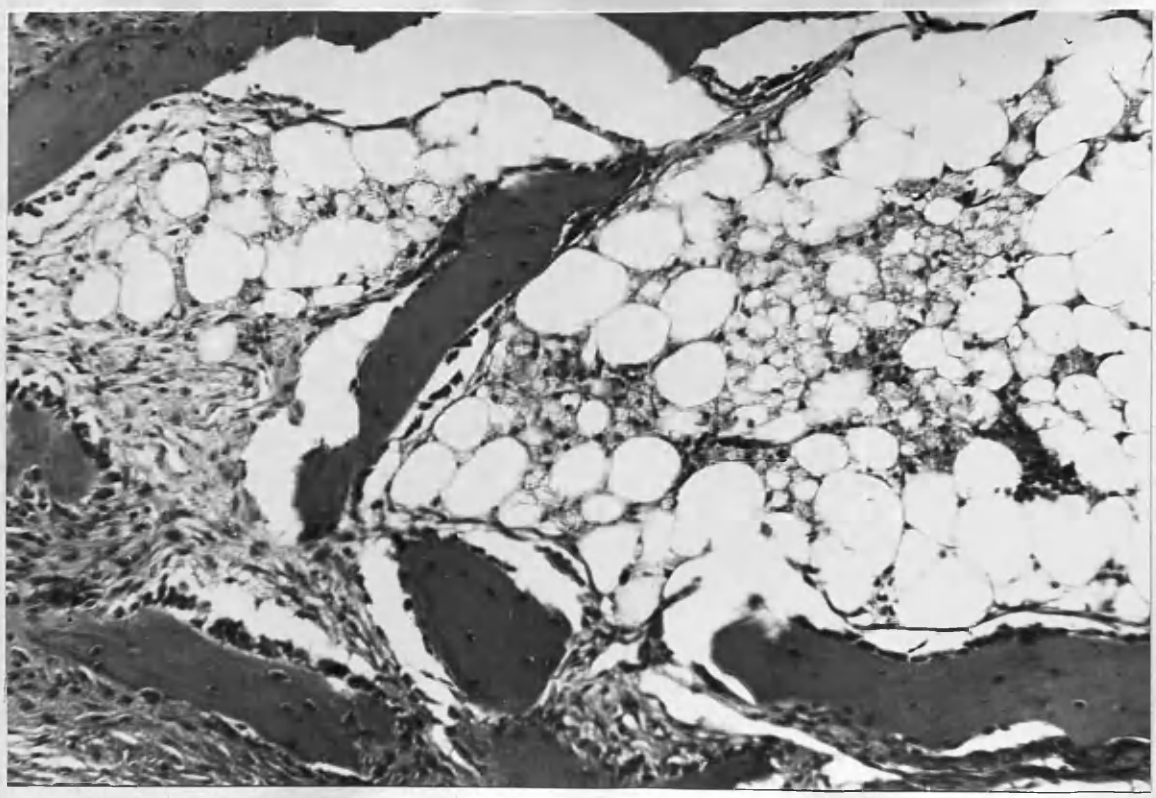

Fig:29 x 200. The transplant resembles normal rabbit bone with a well stained matrix, osteocytes of mature appearance and a single endosteal layer around the smaller trabeculae which are entirely repopulated.

Autogenous Bone transplant into Muscle.

80 Days.

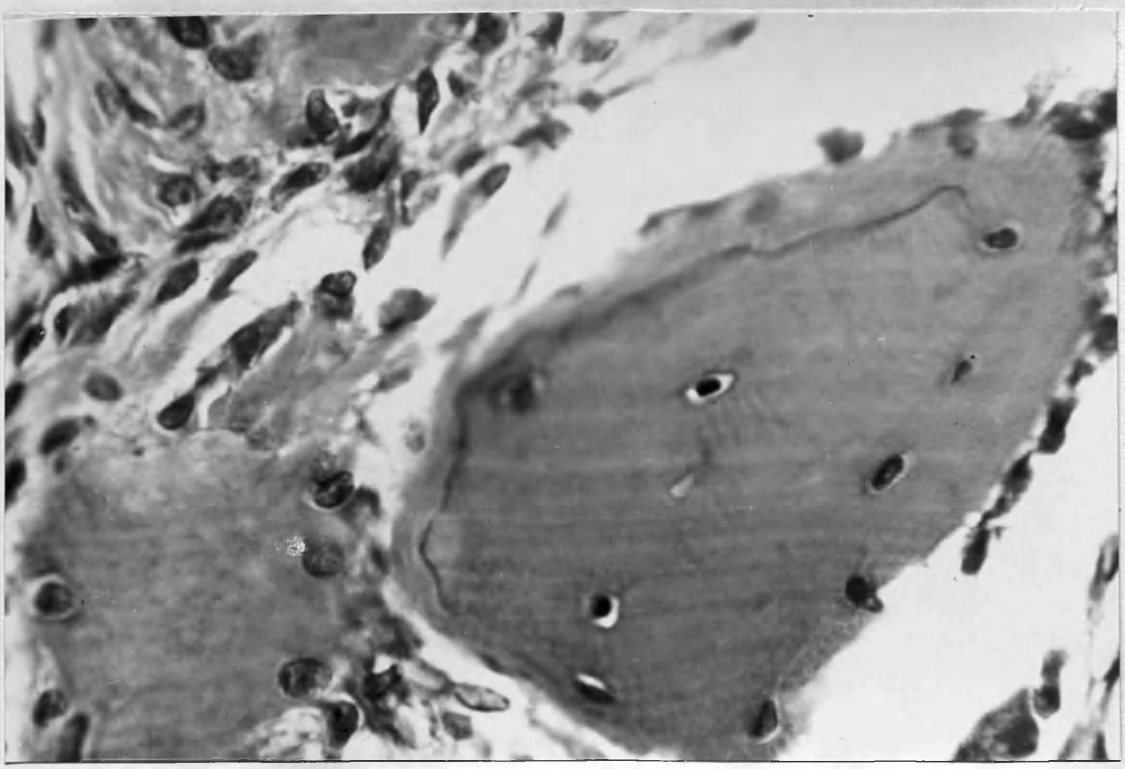

Fig:30 x 750. Active proliferation of endosteal osteoblasts which are repopulating the matrix of the transplant.

Autogenous Bone transplant into Muscle.

120 Days.

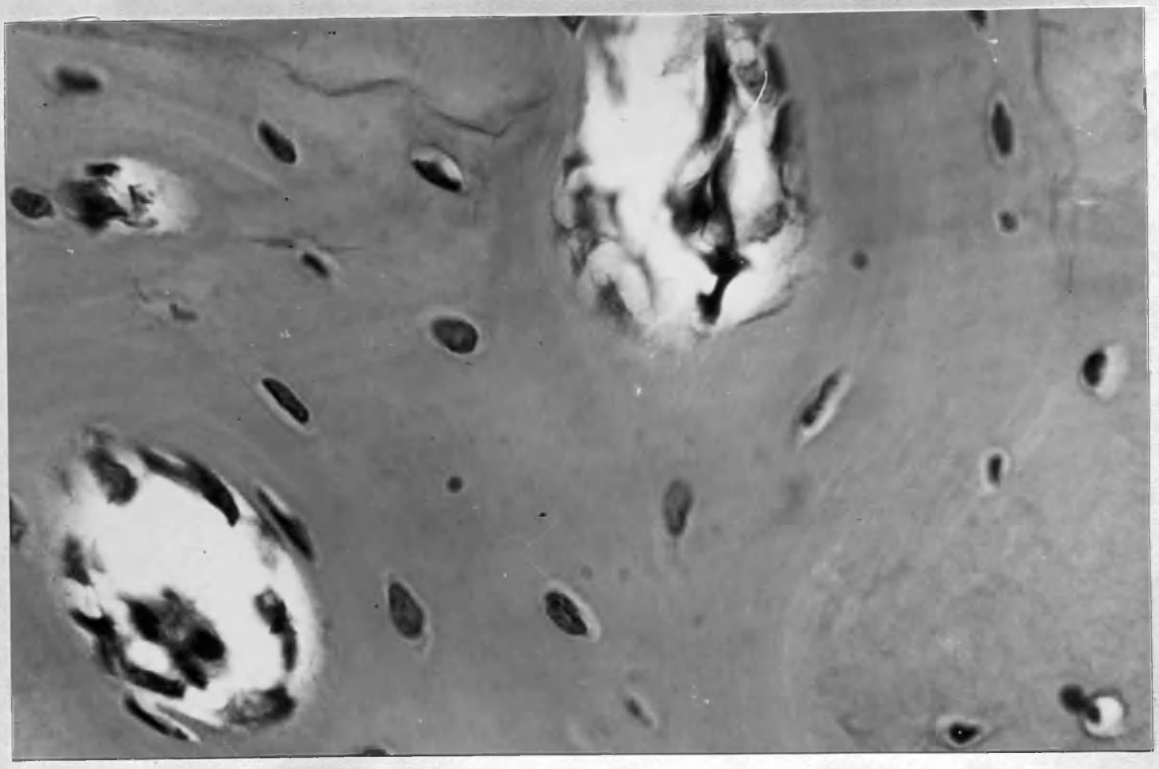

Fig:31 x 750. The maturing osteocytes of the recovering autogenous transplant.

Autogenous Bone transplant into Muscle.

80 Days.

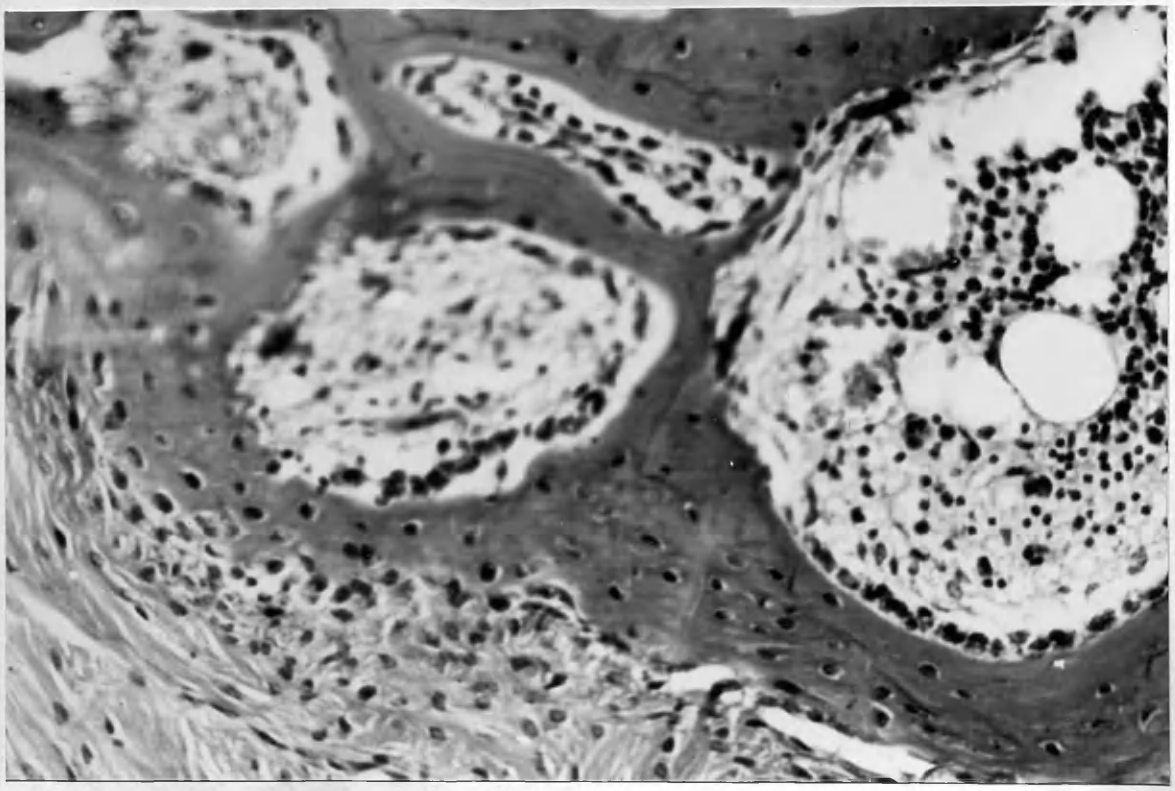

Fig:32 x 250. Osteogenesis in the host connective tissue. The three stages are seen: connective tissue cells, their differentiation into osteoblasts, and the deposition of bone trabeculae.

Autogenous Bone transplant into Muscle.

90 Days.

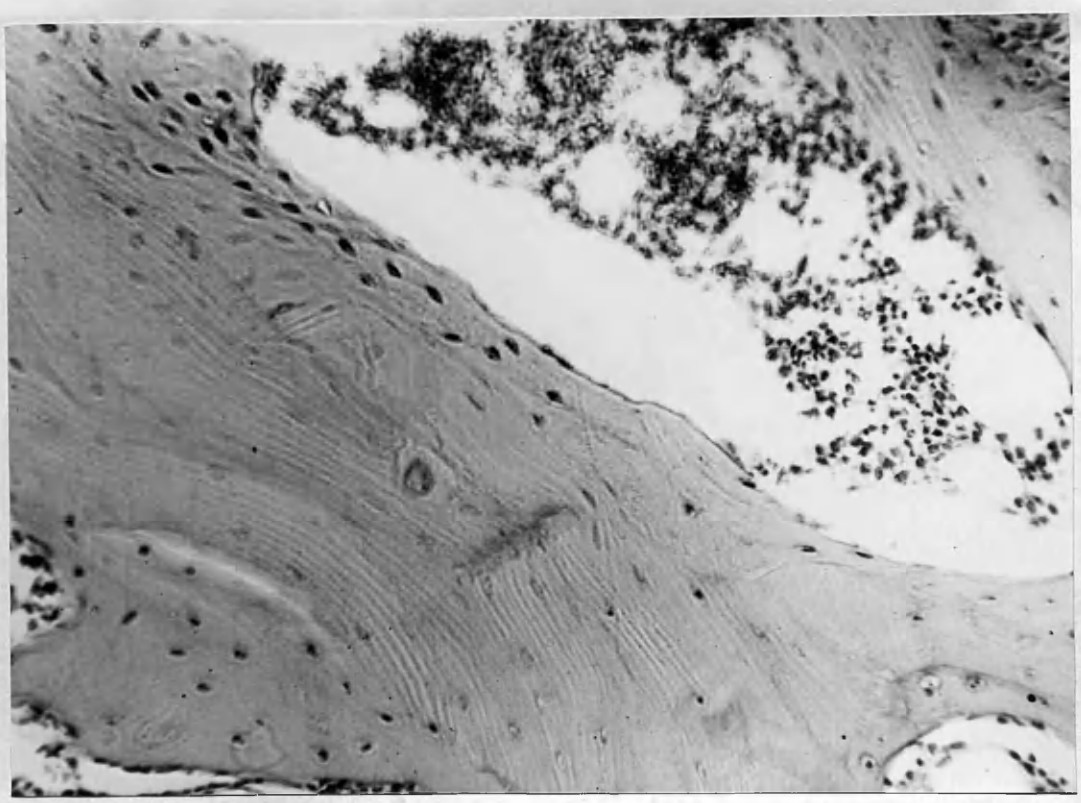

Fig:33 x 250. Autogenous cortical bone transplant. The periphery shows some cellular activity, and the regions surrounding Haversian canals and endosteal spaces have been repopulated, but the central areas are a-cellular.

Autogenous Bone transplant into Muscle.

100 Days.

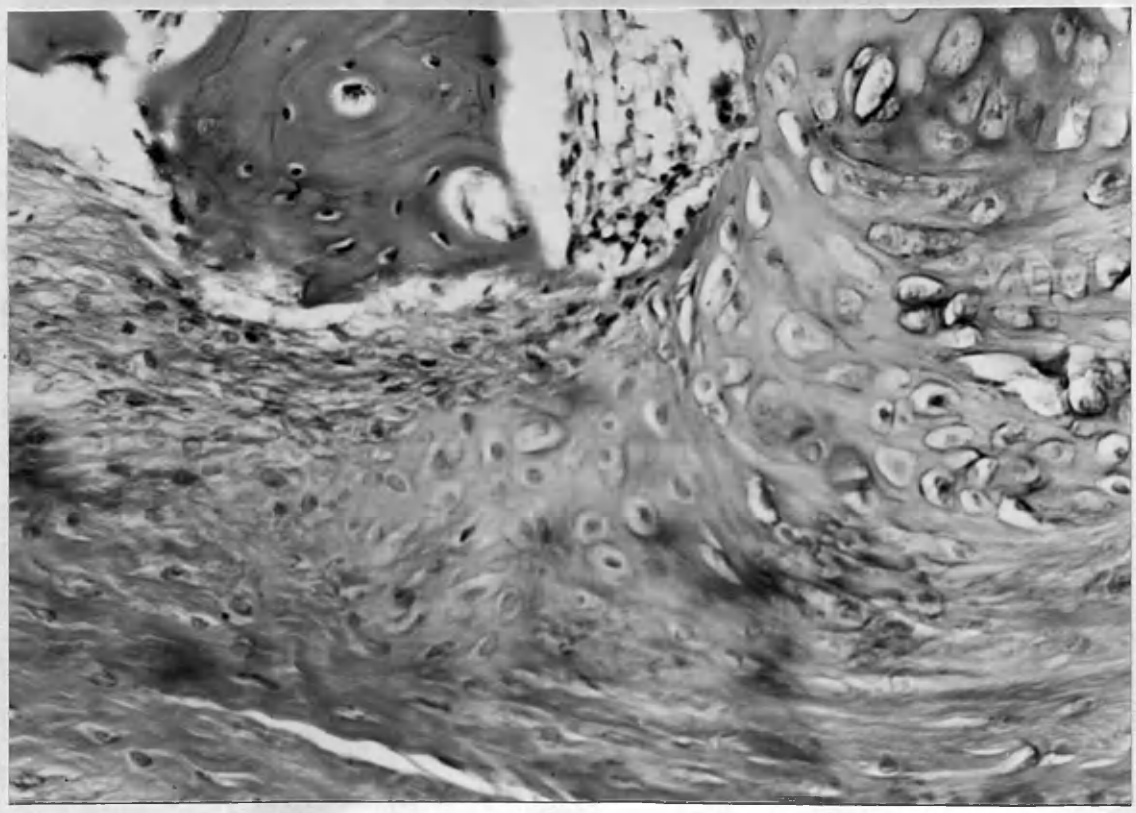

Fig:34 x 250. Cartilage tissue survives transplantation and in its immediate vicinity chondro-osseous tissue is formed from the host connective tissue.

Homogenous Bone transplant into Muscle.

80 Days.

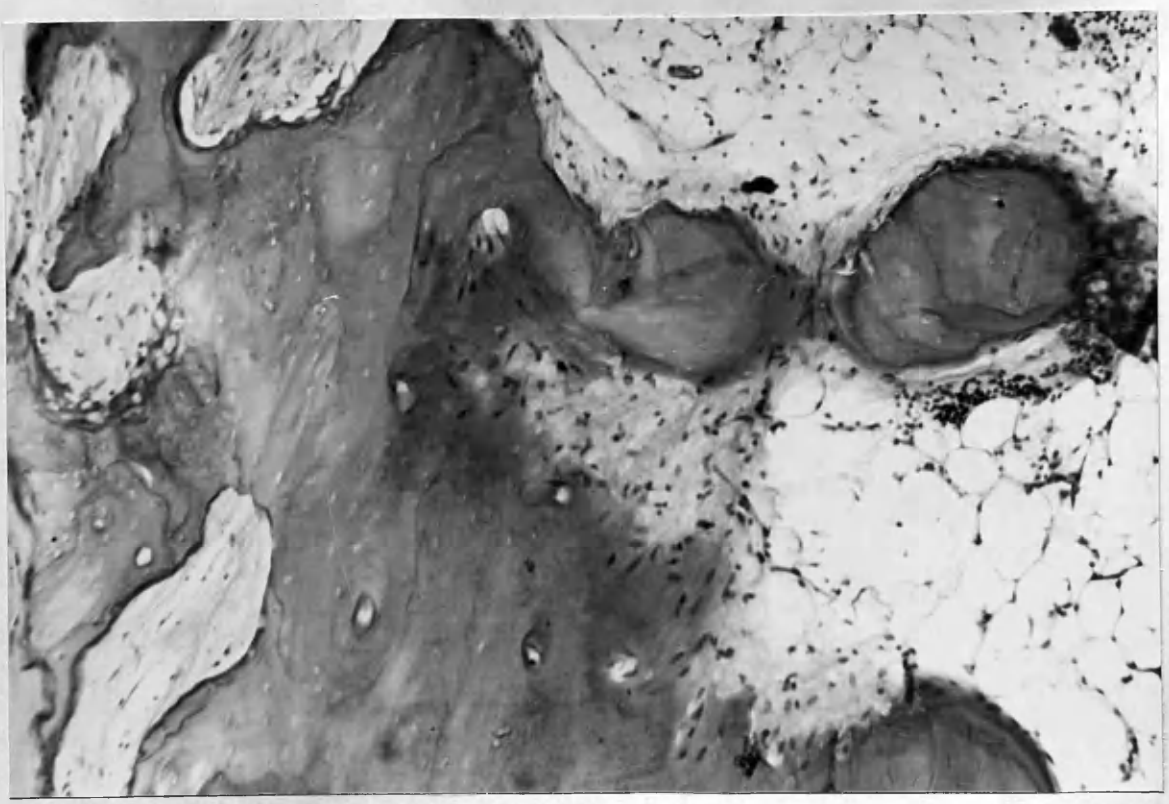

Fig:35 x 150. The transplant is dead. The outline of the matrix is sharply defined and the lacunae are empty. There is no endosteal cellular activity. Lysis and invasion by connective tissue cells is noted in one area.

Homogenous Bone transplant into Muscle.

80 Days.

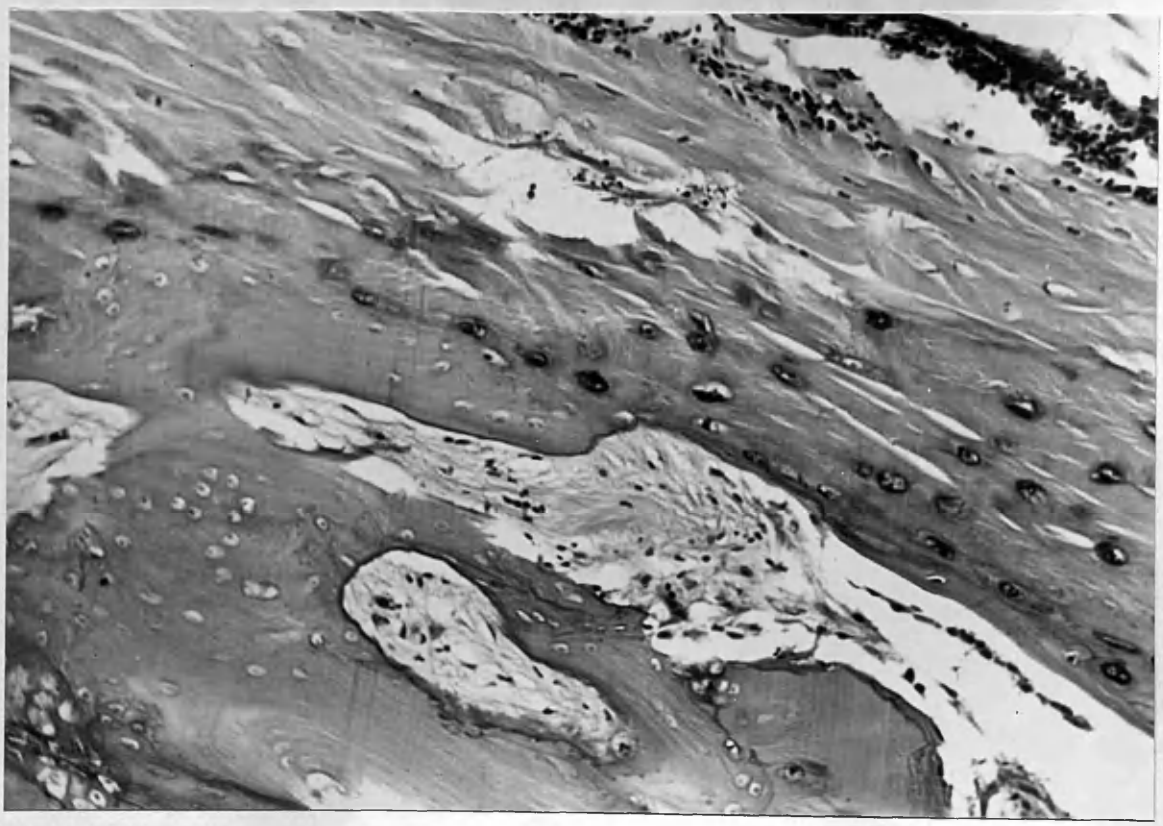

Fig:36 x 200. Cartilage tissue has survived, but the transplanted bone tissue in its immediate vicinity is quite dead. There is no evidence of osteoblastic activity in the proximity of cartilage.

Homogenous Bone transplant into Muscle.

100 Days.

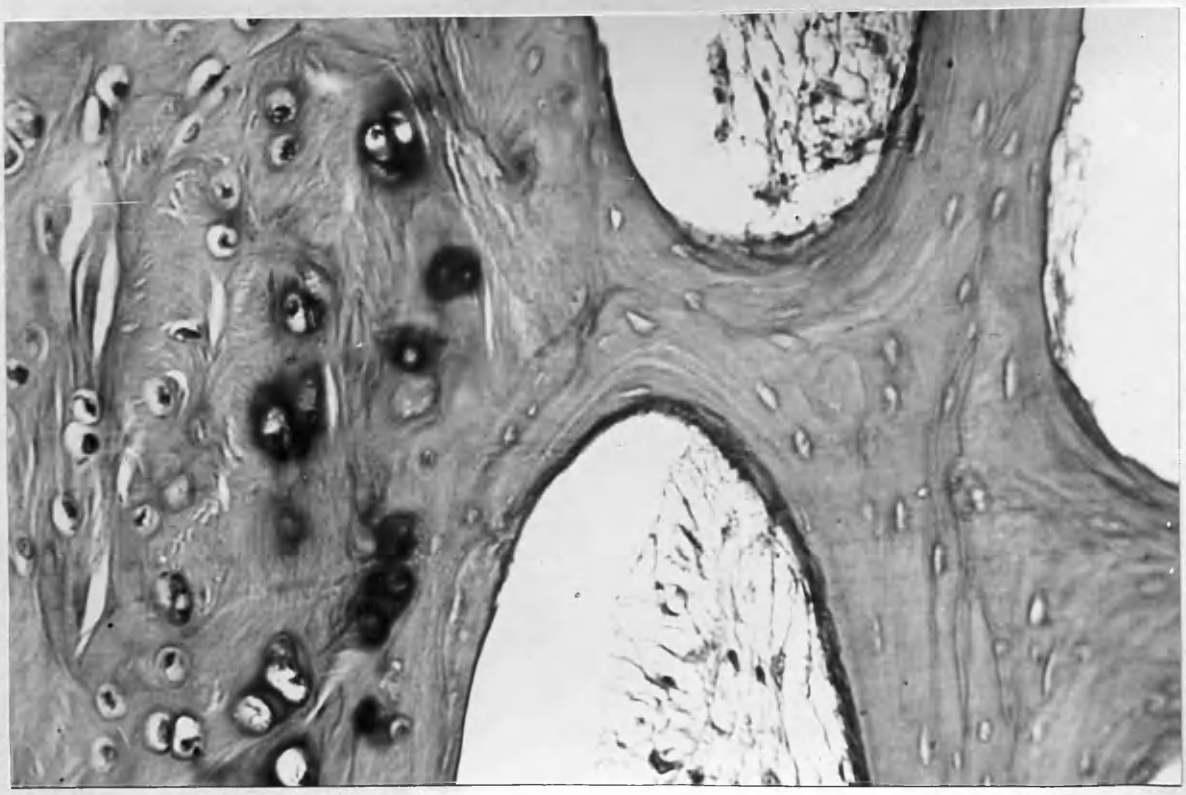

Fig:37 x 450. Dead bone tissue in close association with surviving cartilage.

Homogenous Bone transplant into Muscle.

100 Days.

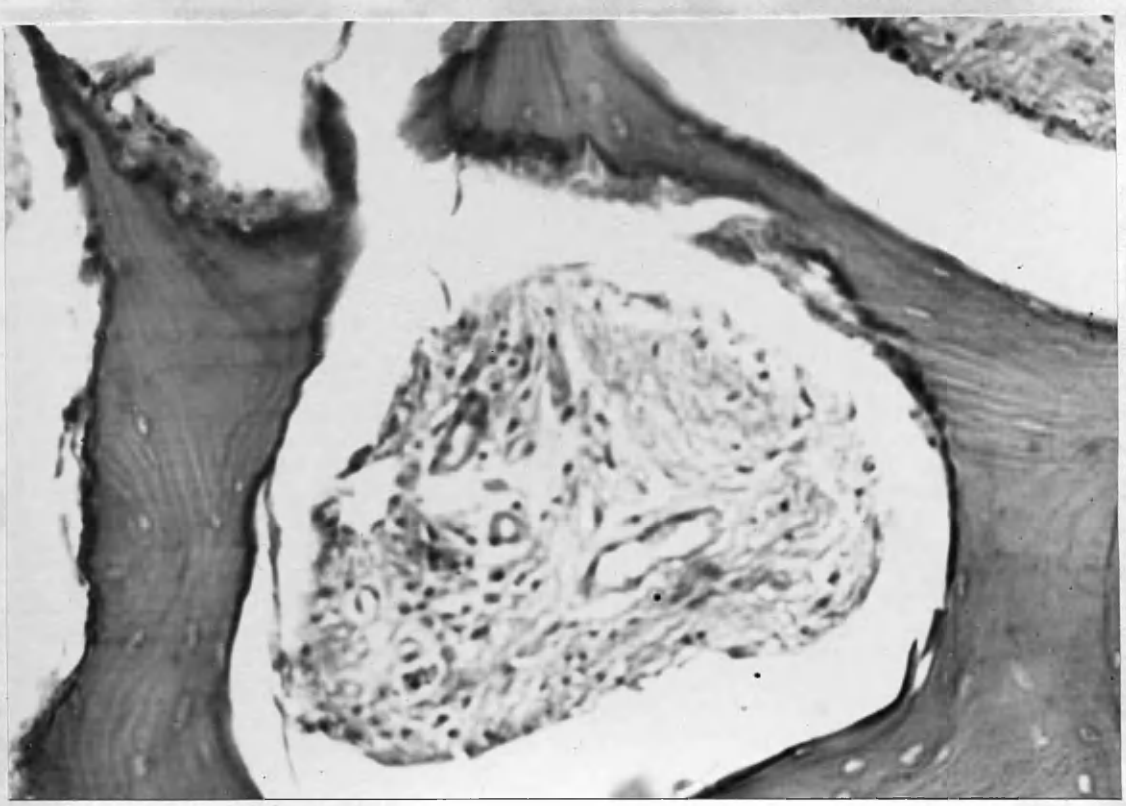

Fig:38 x 250. Dead sequestrum like bone tissue. The endosteal spaces are occupied by connective tissue which has no contact with the dead transplant.

Homogenous Bone transplant into Muscle.

80 Days.

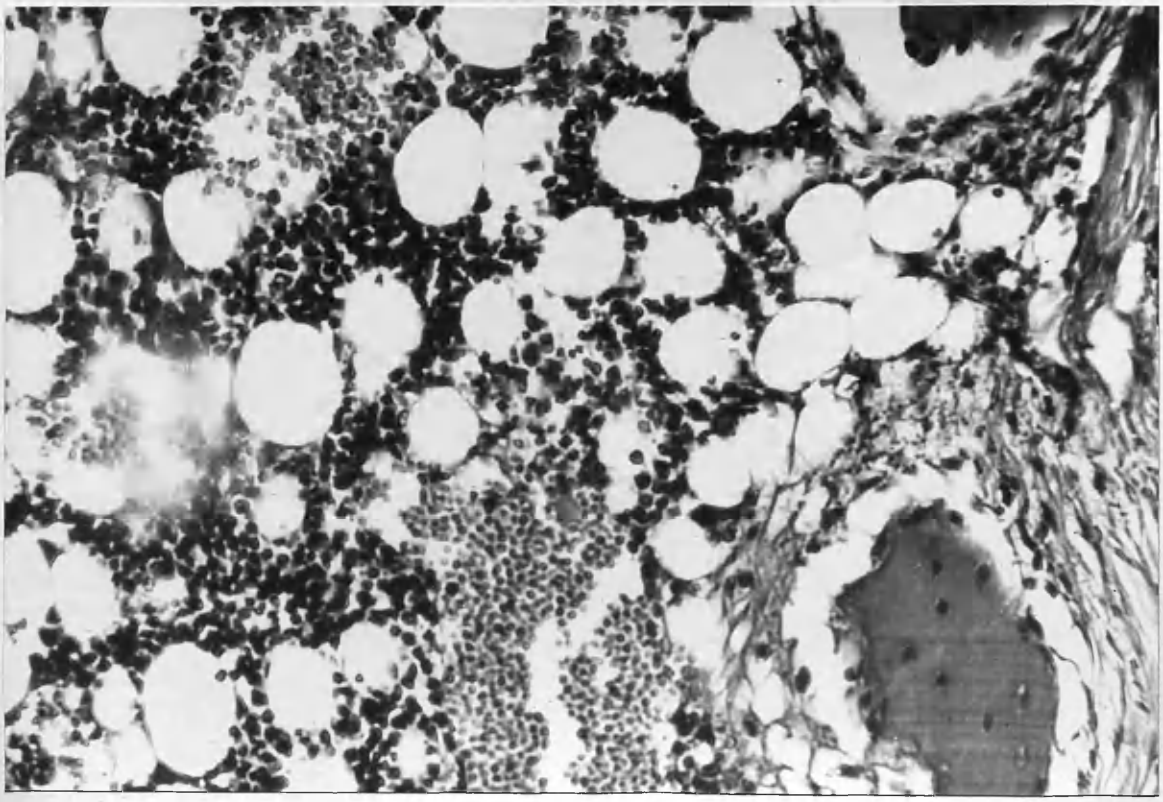

Fig:39 x 250. A syngenesious transplant showing the survival of marrow and bone tissue.

Homogenous Bone transplant into Muscle.

100 Days.

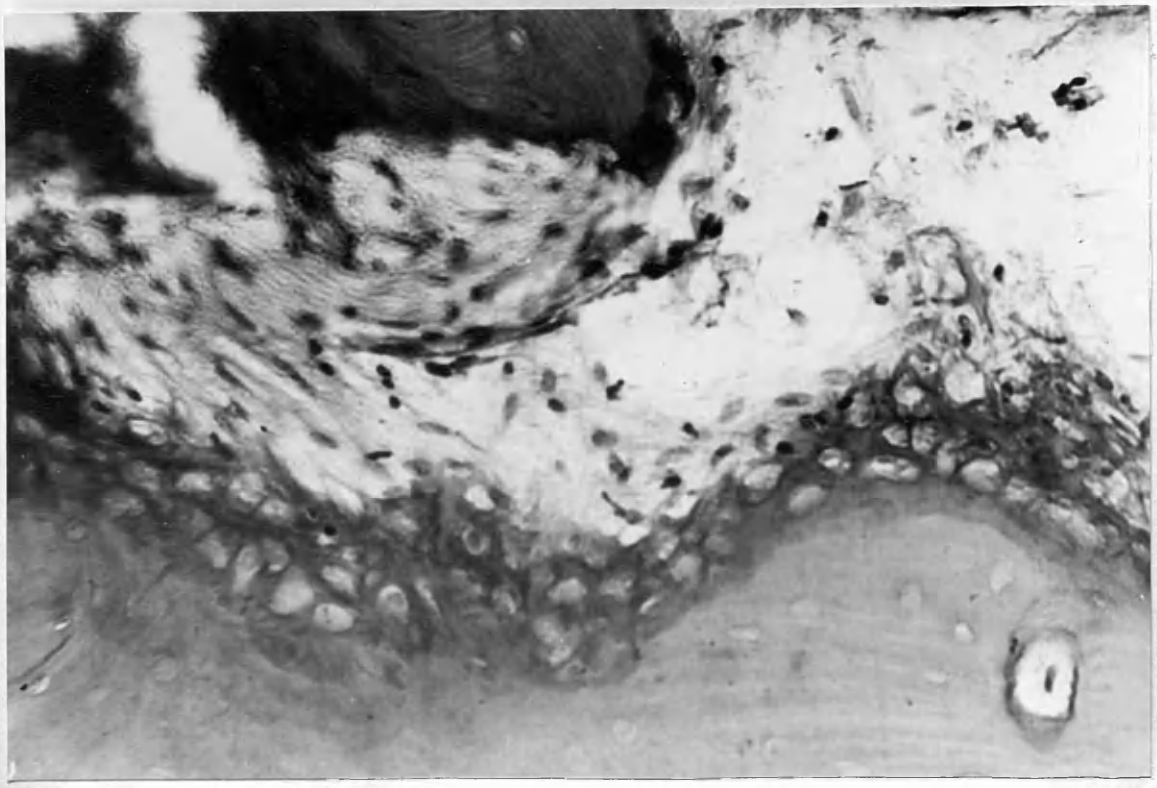

Fig:40 x 500. The peripheral layer of new bone laid down by osteoblasts of the host tissue is quite devitalised.

Autogenous Bone transplant into Muscle.

128 Days.

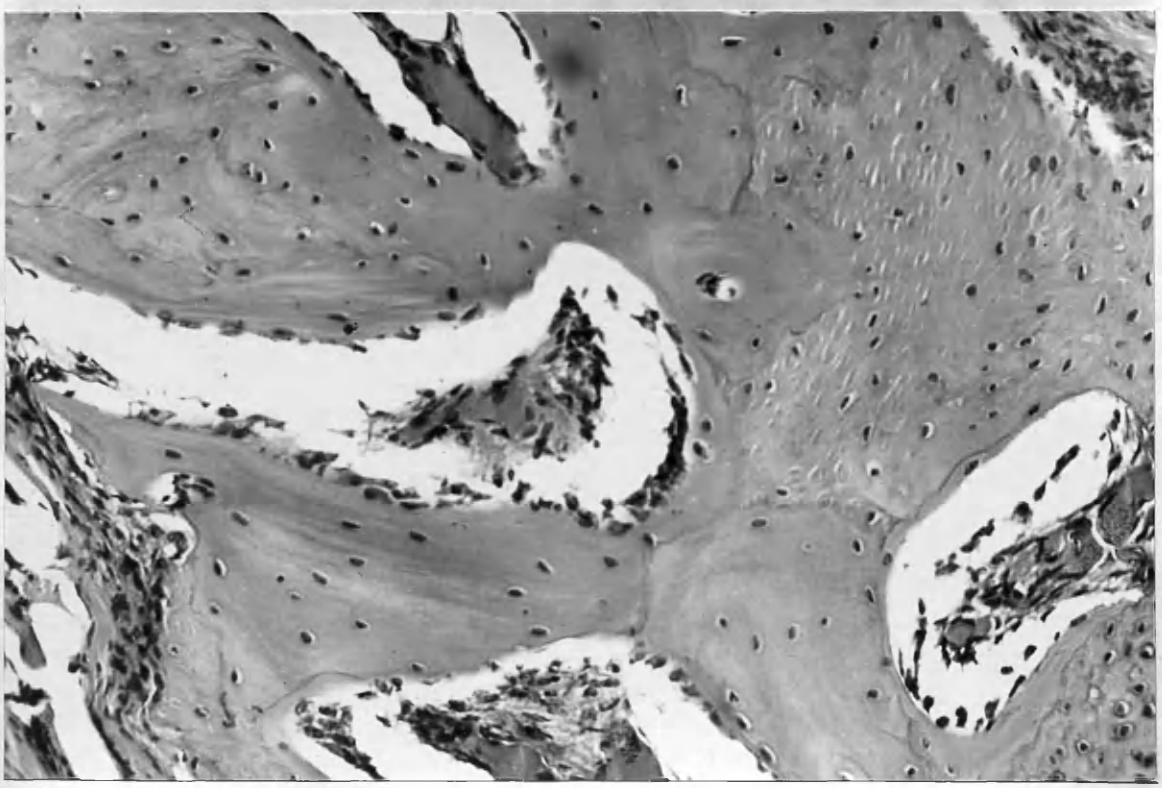

Fig:41 x 250. The transplant approximates to the appearance of normal rabbit bone. The matrix is well stained, populated by osteocytes and surrounded by a regular layer of endosteal cells.

Autogenous Bone transplant into Muscle.

120 Days.

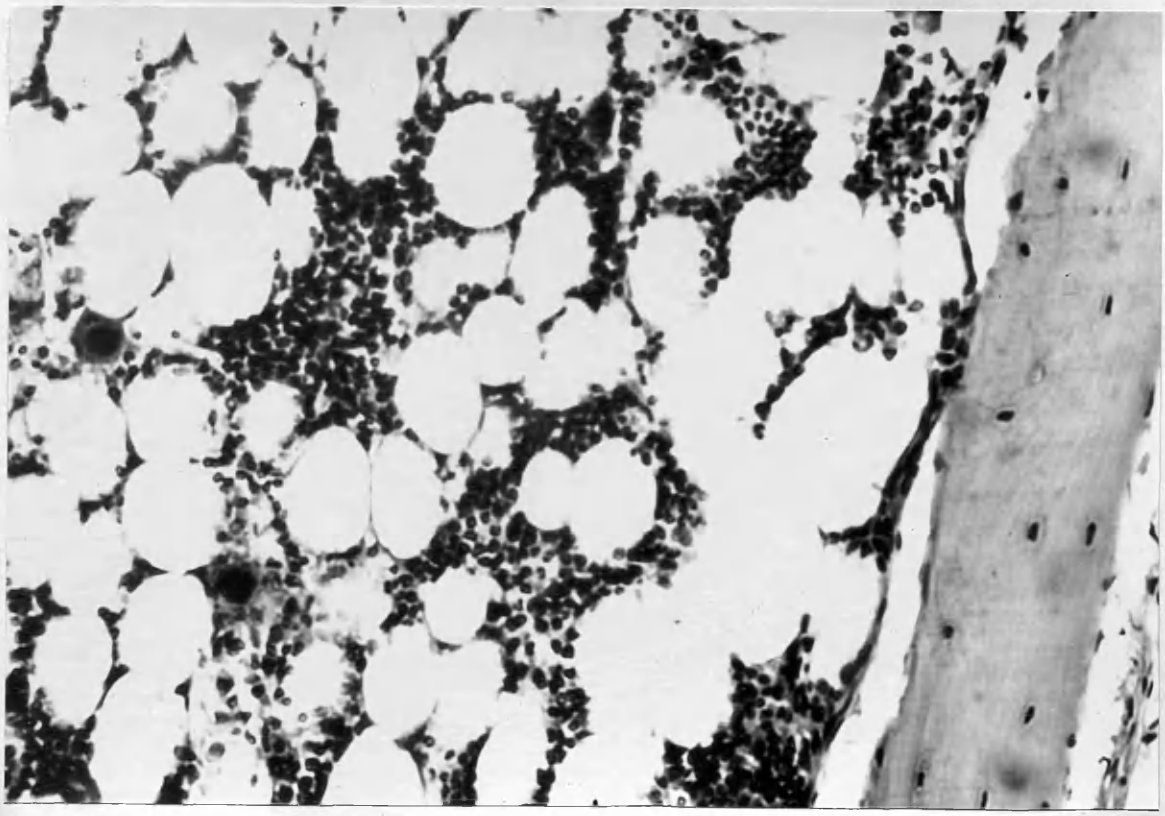

Fig:42 x 250. Marrow tissue of normal appearance occupies the endosteal spaces.

Autogenous Bone transplant into Muscle.

120 Days.

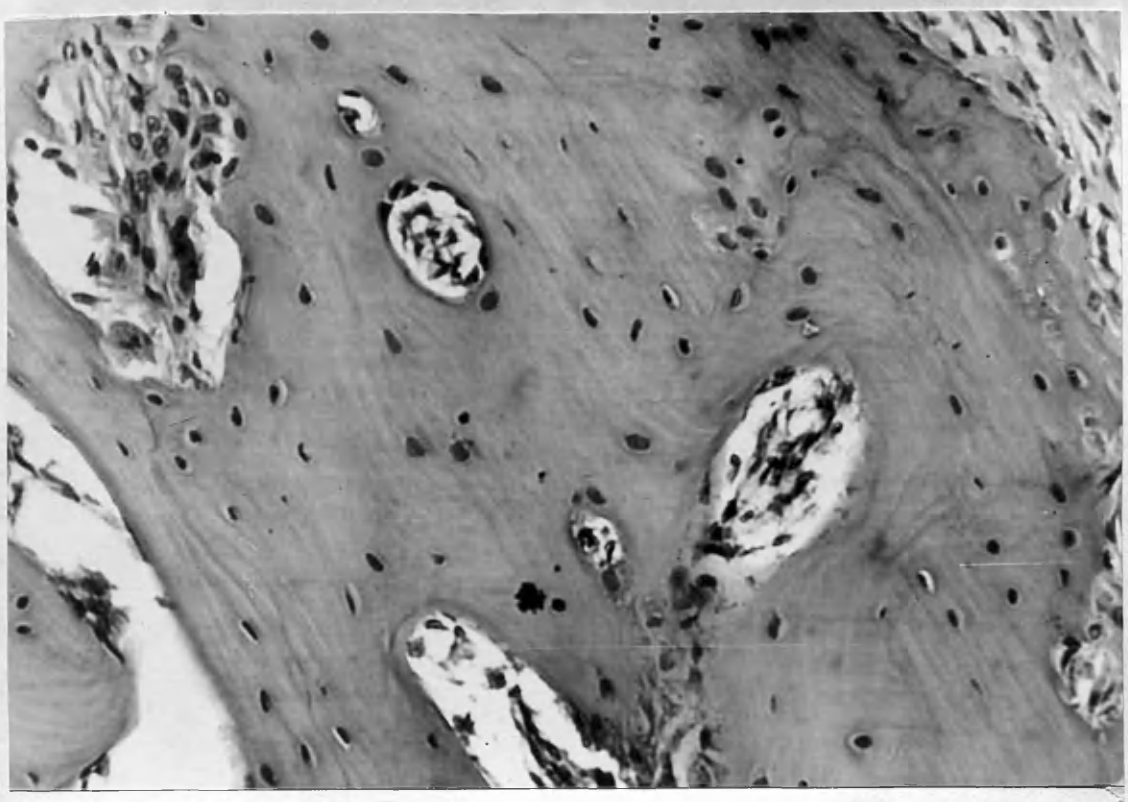

Fig:43 x 700. Autogenous bone tissue populated by mature osteocytes. The endosteal spaces are filled with young connective tissue cells and osteoblasts, some of which are invading the matrix.

Autogenous Bone transplant into Muscle.

140 Days.

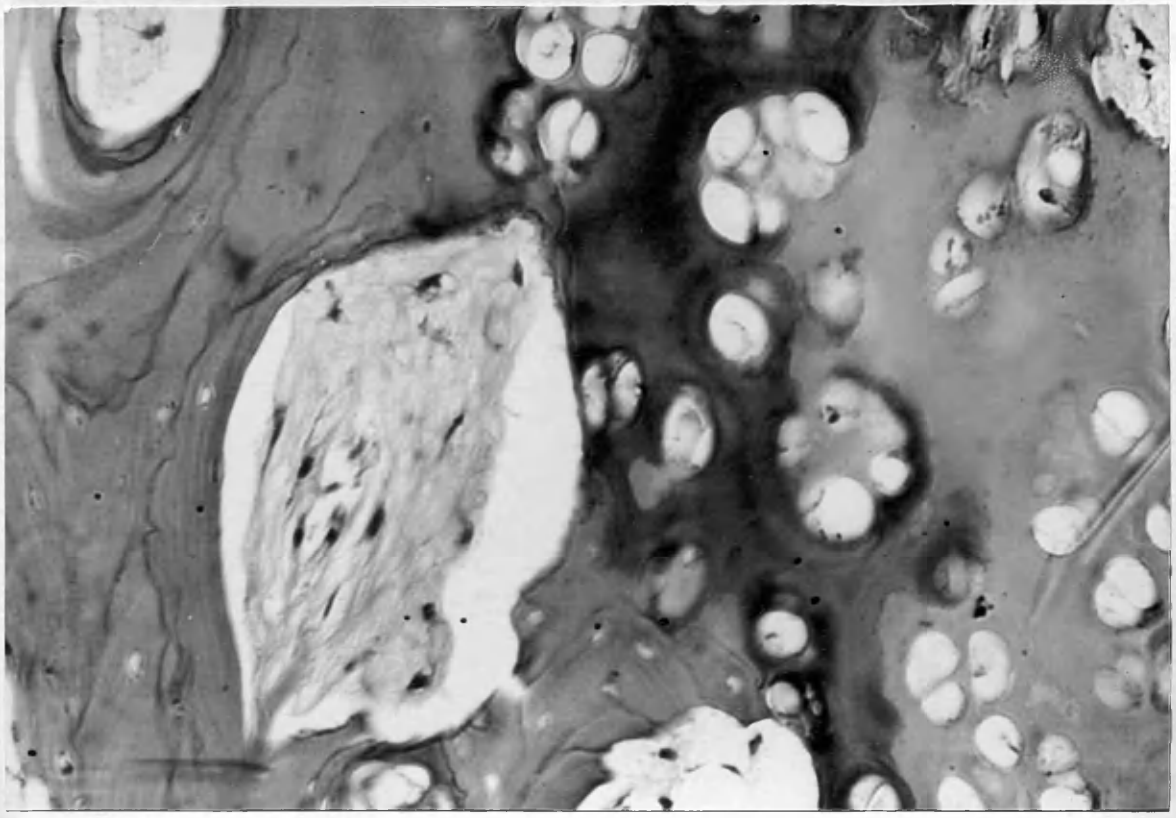

Fig:44 x 700. Dead bone and cartilage. The only completely devitalised autogenous transplant in the series.

Homogenous Bone transplant into Muscle.

140 Days.

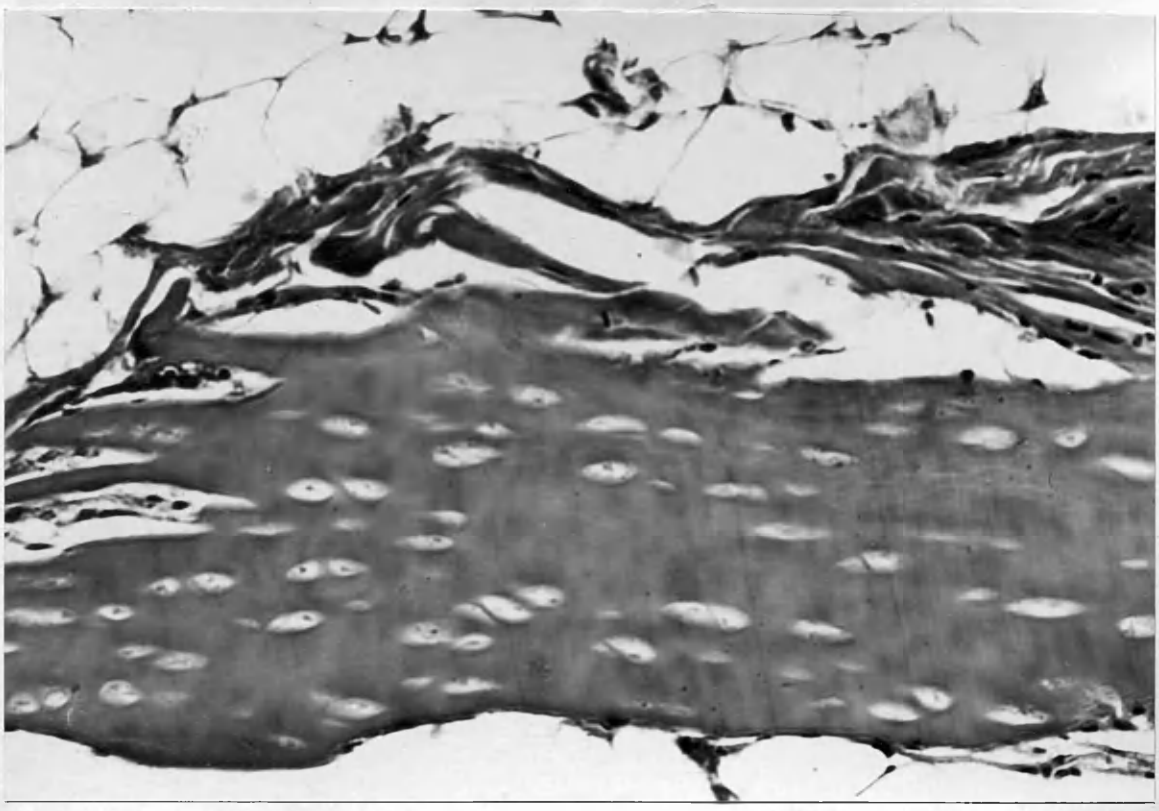

Fig:45 x 750. Sequestrum like remains of homogenous transplant. The matrix with empty lacunae has a necrotic appearance and is isolated from the surrounding tissue.

Homogenous Bone transplant into Muscle.

180 Days.

Fig:46 x 700. The remains of an homogenous transplant consisting of a space containing crystalline spicules surrounded by lymphocytes and fibrous tissue.

Homogenous Bone transplant into Muscle.

140 Days.

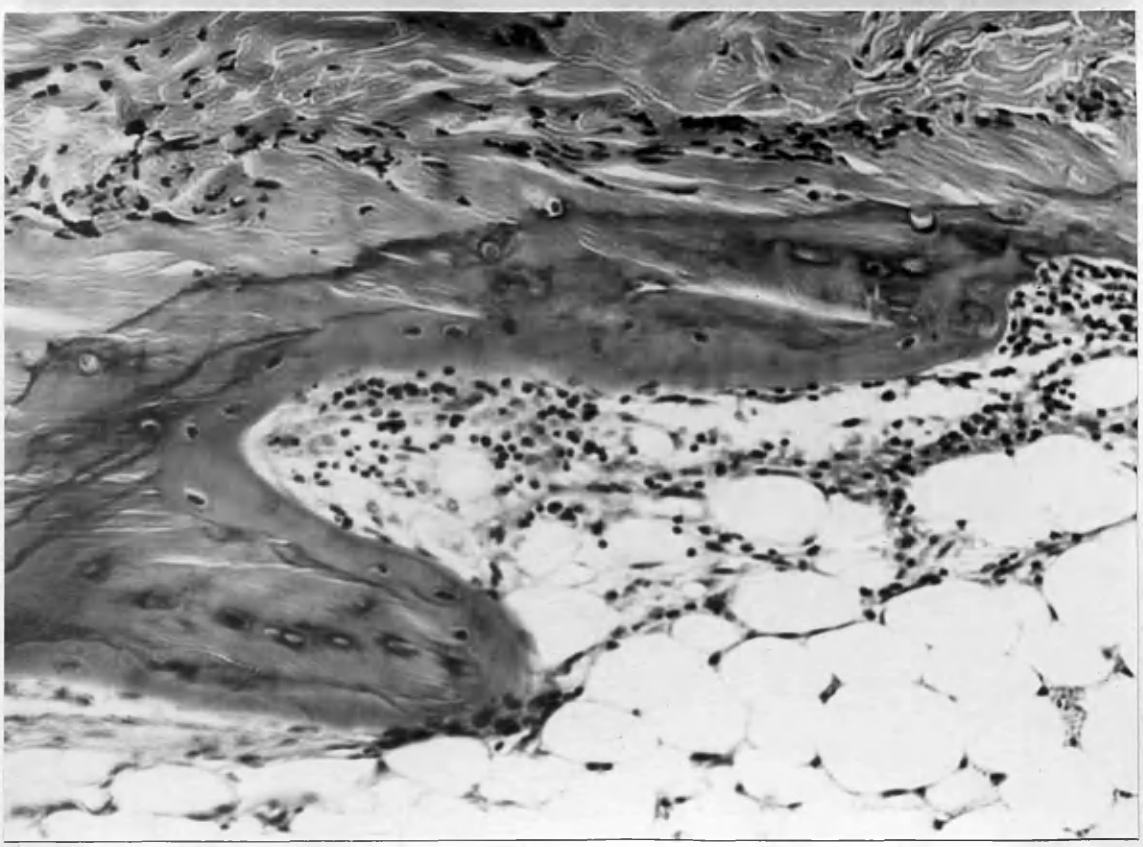

Fig:47 x 250. A syngenesious transplant in which living bone tissue and cartilage is seen.

Autogenous Bone transplant into Bone.

10 Days.

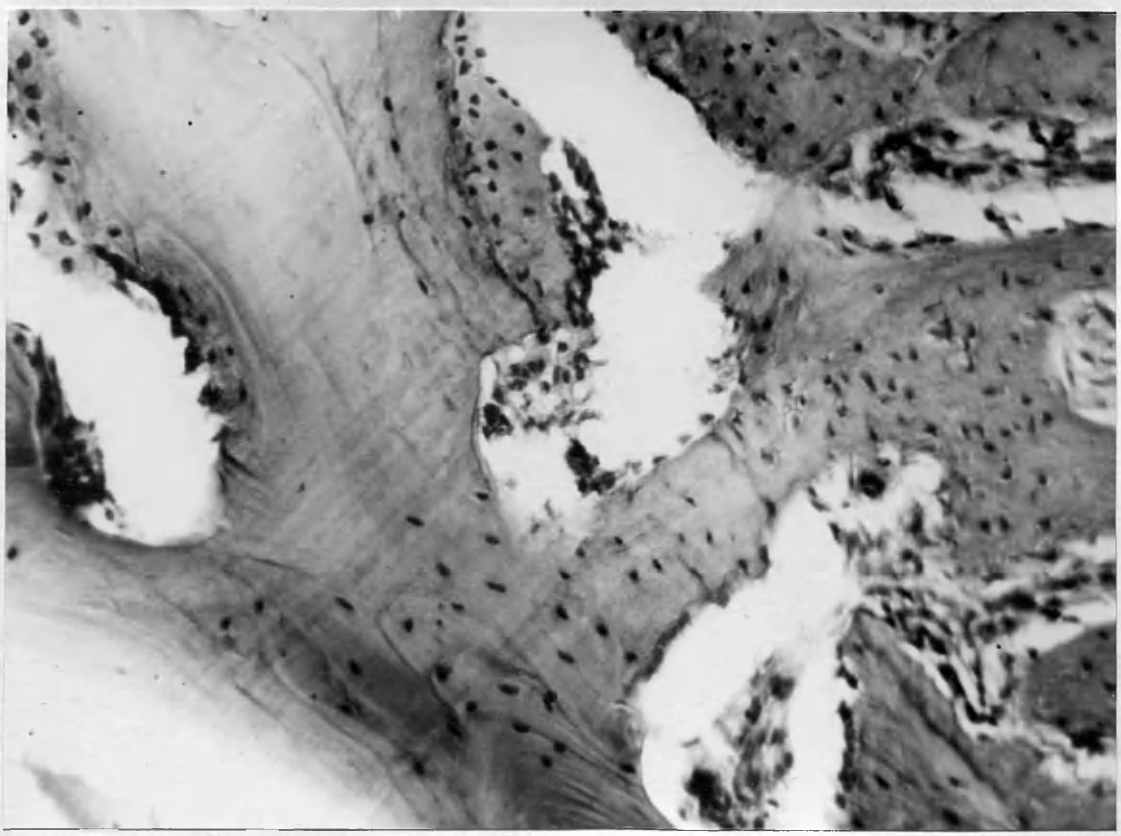

Fig:48 x 250. There is some cell survival, but many, especially those more centrally placed have died. Endosteal proliferation is noted.

Autogenous Bone transplant into Bone.

10 Days.

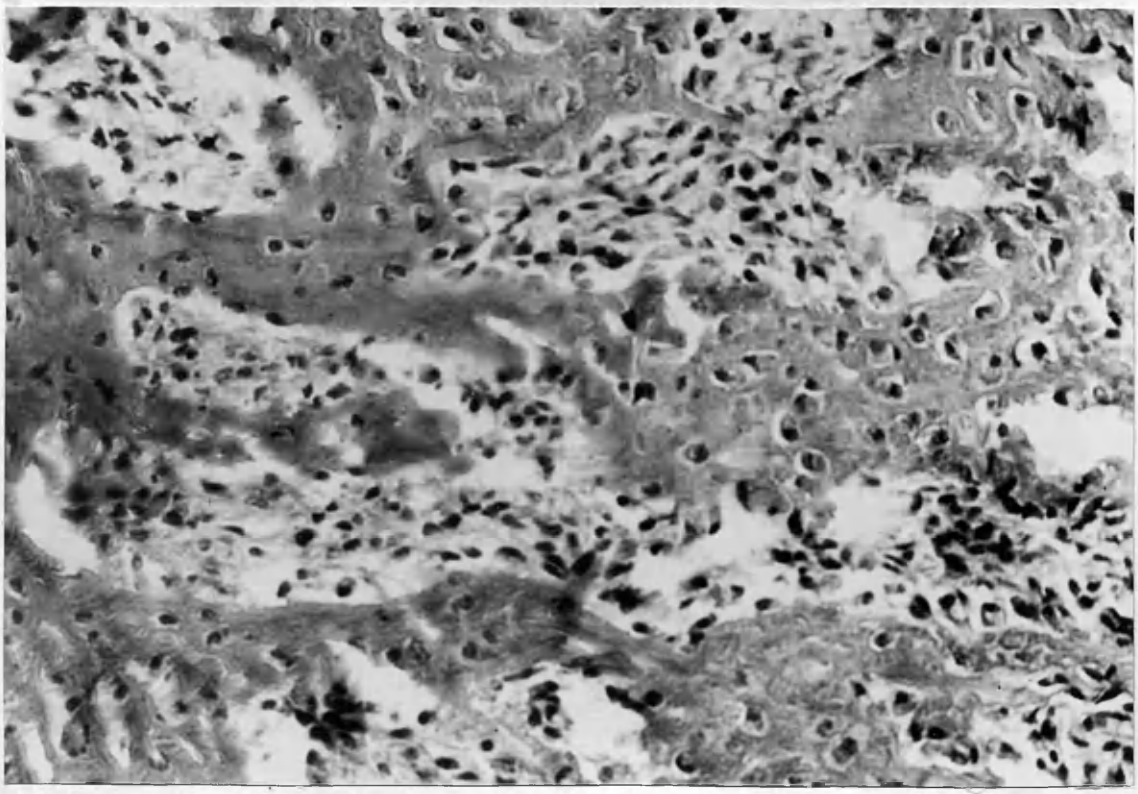

Fig:49 x 250. Host connective tissue showing fibroblasts differentiating into osteoblasts and laying down trabeculae of young bone tissue.

Homogenous Bone transplant into Bone.

10 Days.

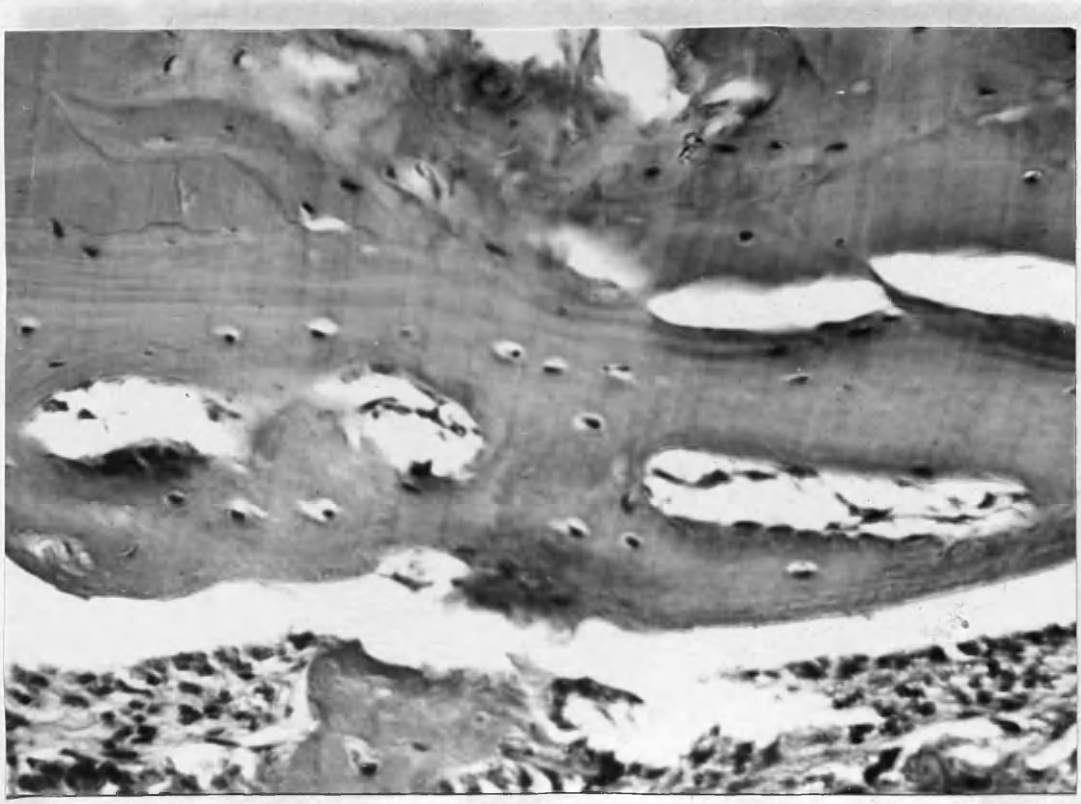

Fig:50 x 250. Homogenous transplant of cortical bone. The nucleiare small and devitalised, many having disappeared entirely.

Autogenous Bone transplant into Bone.

21 Days.

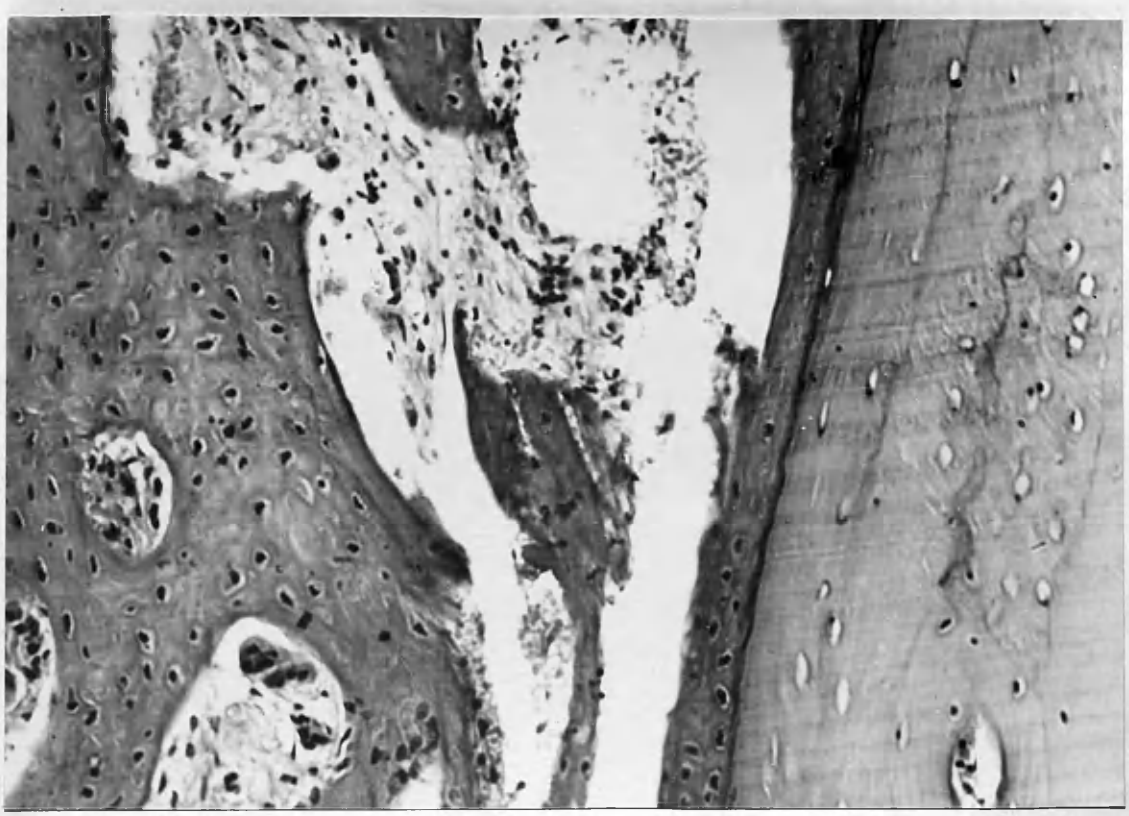

Fig:51 x 250. The transplant is less vital. The endosteum of the transplant has laid down an apposing layer of new bone and it is surrounded by trabeculae of new bone developed from the host tissue.

Autogenous Bone transplant into Bone.

21 Days.

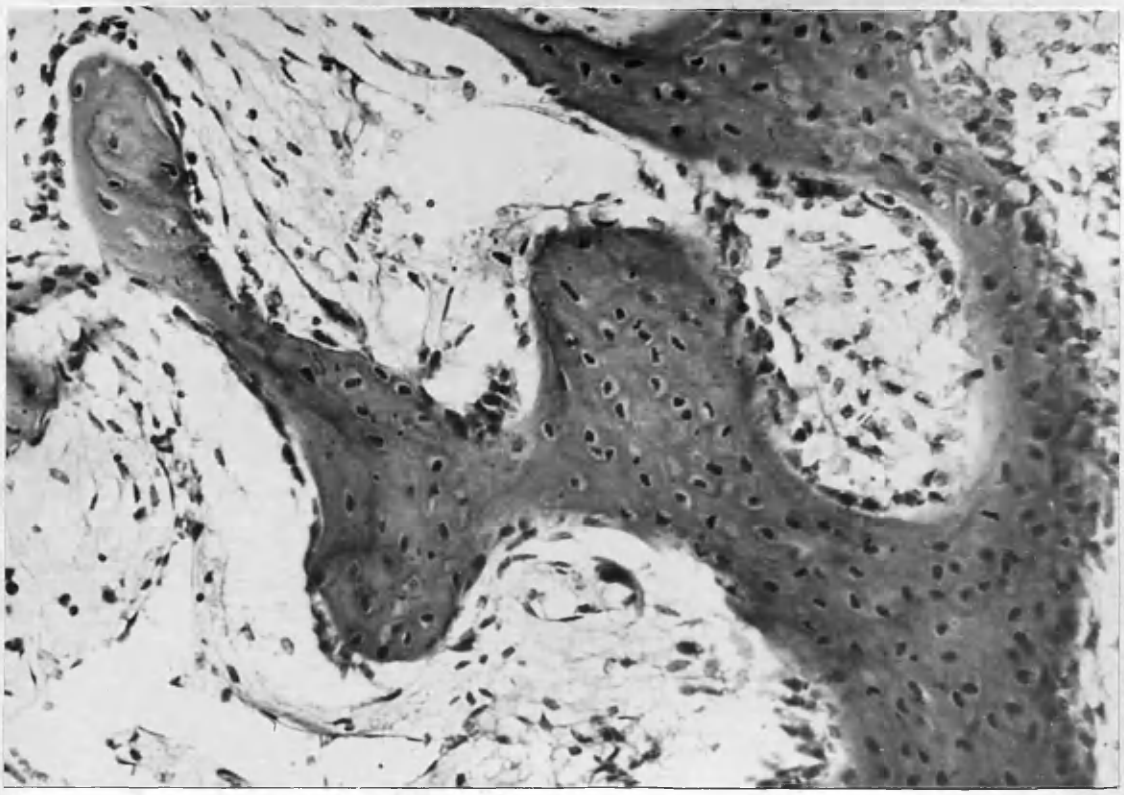

Fig:52 x 250. New bone formation in the host tissue showing the transition from undifferentiated connective tissue to well formed trabeculae.

Autogenous Bone transplant into Bone.

21 Days.

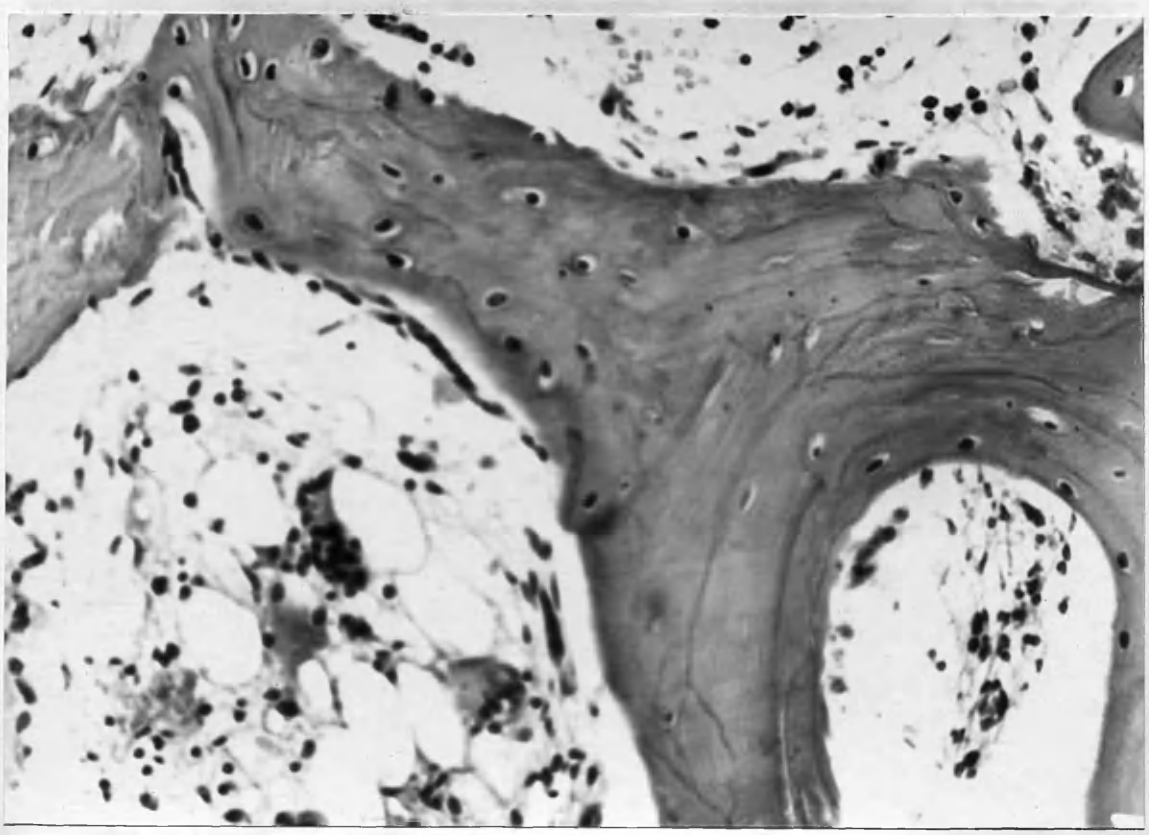

Fig:53 x 250. Autogenous transplant populated by large osteocytes. These cells appear to arise from endosteal osteoblasts which invade the matrix.

Homogenous Bone transplant into Bone.

21 Days.

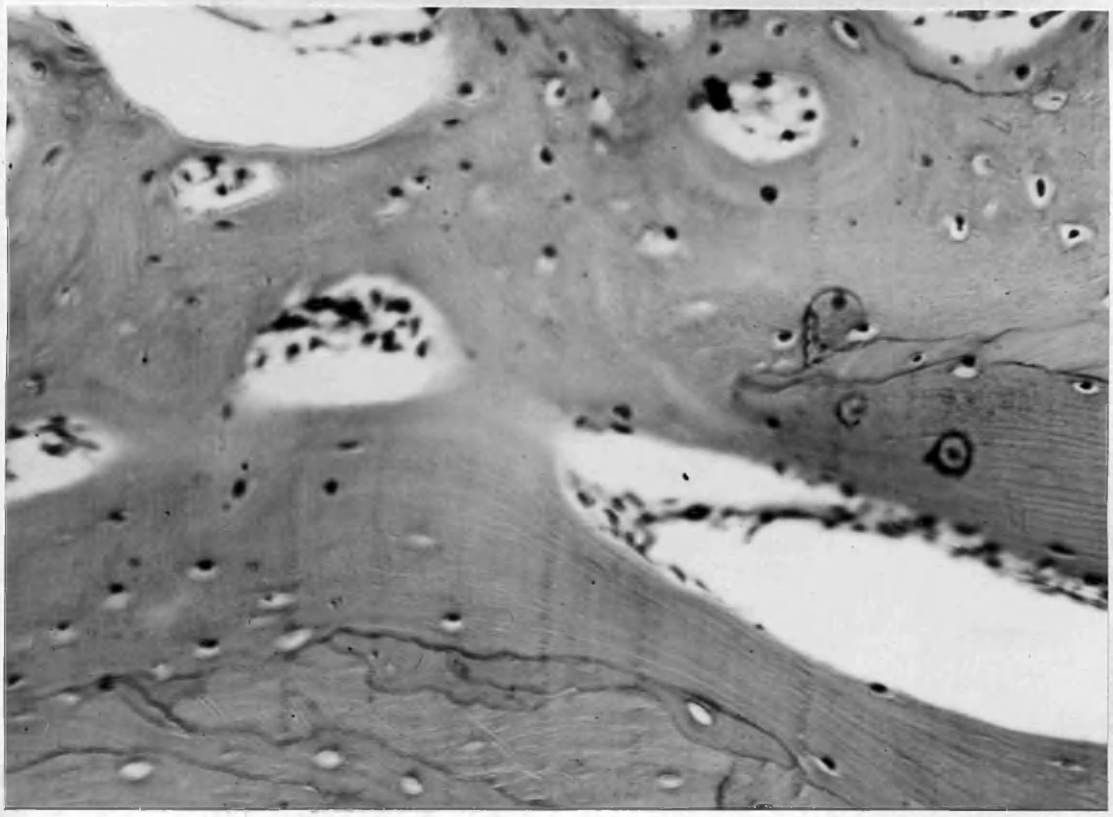

Fig:54 x 250. The bone tissue is devitalised, the remaining nuclei are smaller and many of the lacunae are empty, particularly in the larger trabeculae. There is no evidence of endosteal osteoblastic activity.

Homogenous Bone transplant into Bone.

21 Days.

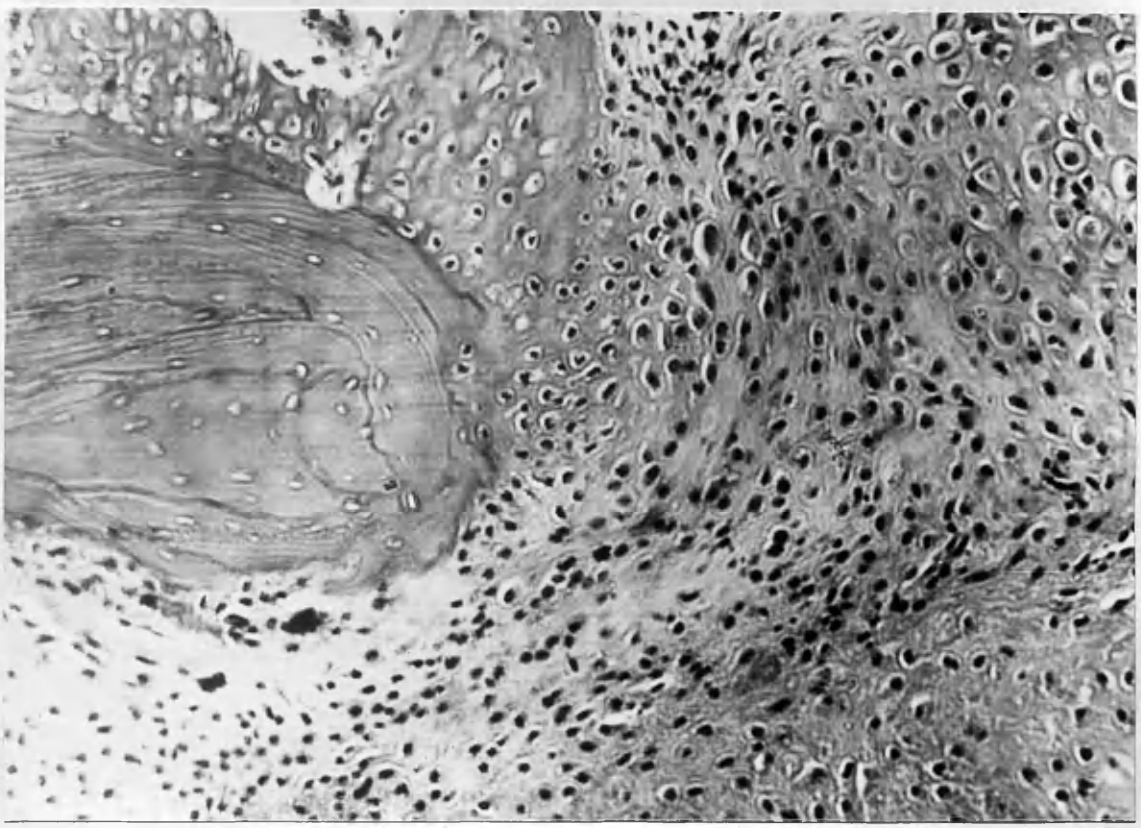

Fig:55 x 250. Homogenous transplantation into a young animal showing active chondro-osseous tissue formation surrounding the dead transplant, the edge of which has been invaded by chondroblasts.

Homogenous Bone transplant into Bone.

21 Days.

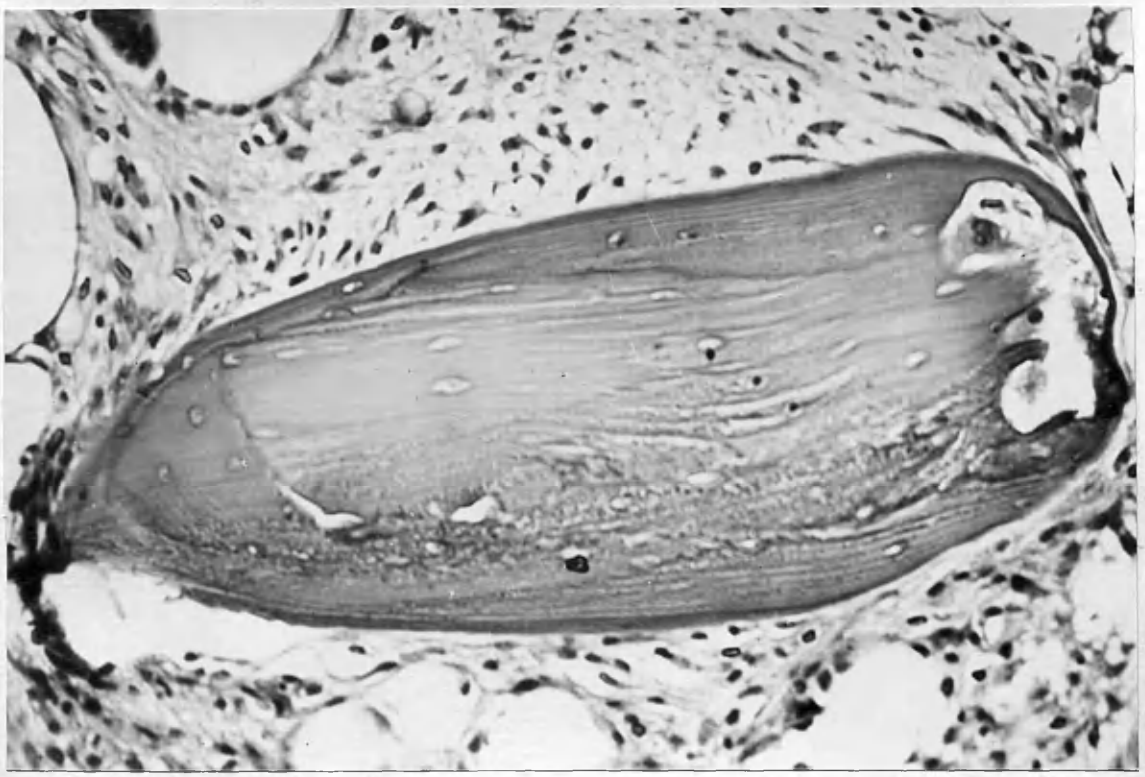

Fig:56 x 250. Sequestrum like fragment of transplanted bone. There is complete absence of periosteal osteogenesis, the surrounding connective tissue cells having no contact with the dense periphery of the transplant.

Autogenous Bone transplant into Bone.

42 Days.

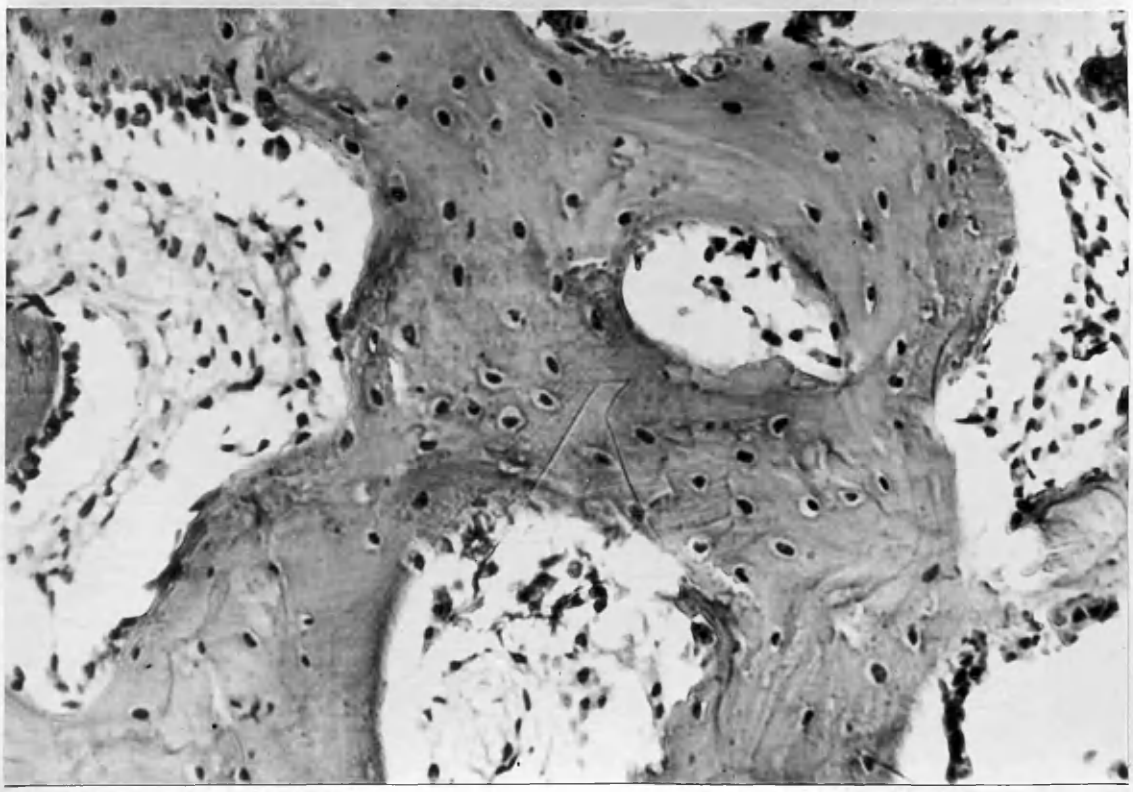

Fig:57 x 250. Transplant of cancellous bone tissue well populated by young osteocytes. The connective tissue of the endosteal spaces differentiates into larger osteoblasts which are seen to migrate into the matrix.

Autogenous Bone transplant into Bone.

42 Days.

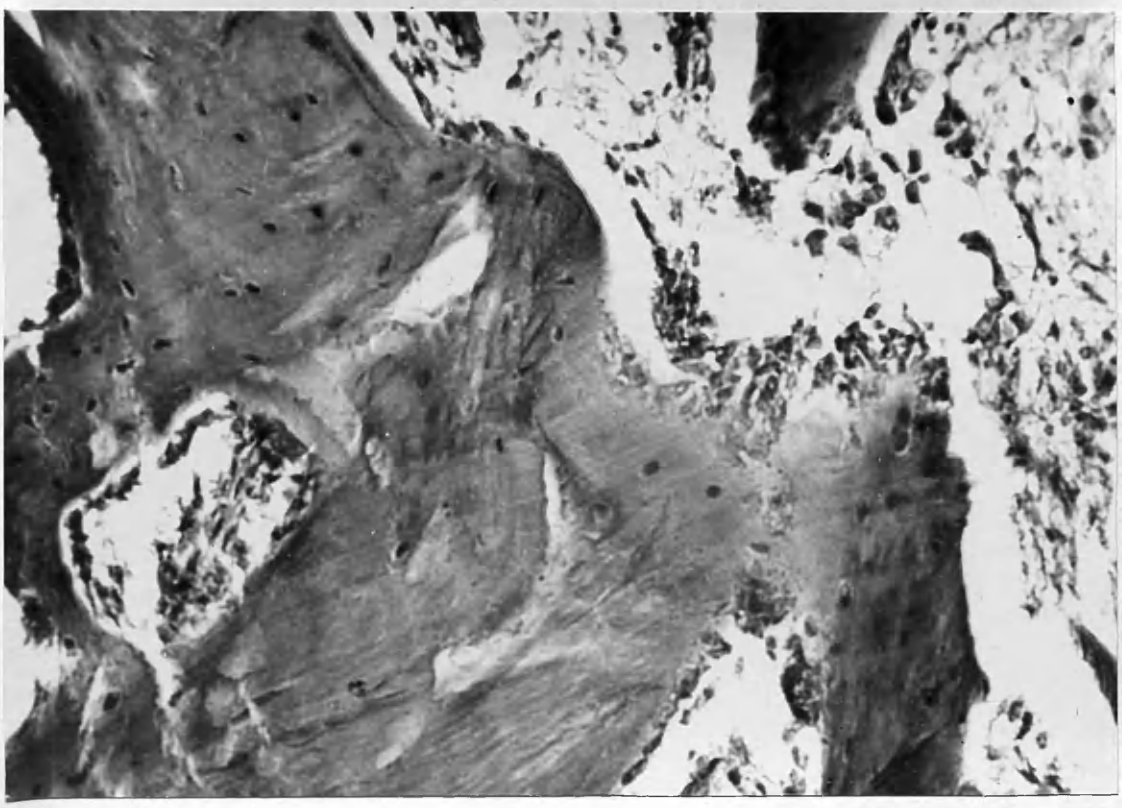

Fig:58 x 250. An autogenous transplant of cortical bone showing much less cellularity, but living osteocytes are present and peripheral osteoblasts are in close contact with the transplant.

Homogenous Bone transplant into Bone.

42 Days.

Fig:59 x 250. A homogenous transplant of cortical bone. The cells are devitalised; the edge of the bone tissue has a dense outline and has no osteoblasts in contact with it.

Homogenous Bone transplant into Bone.

42 Days.

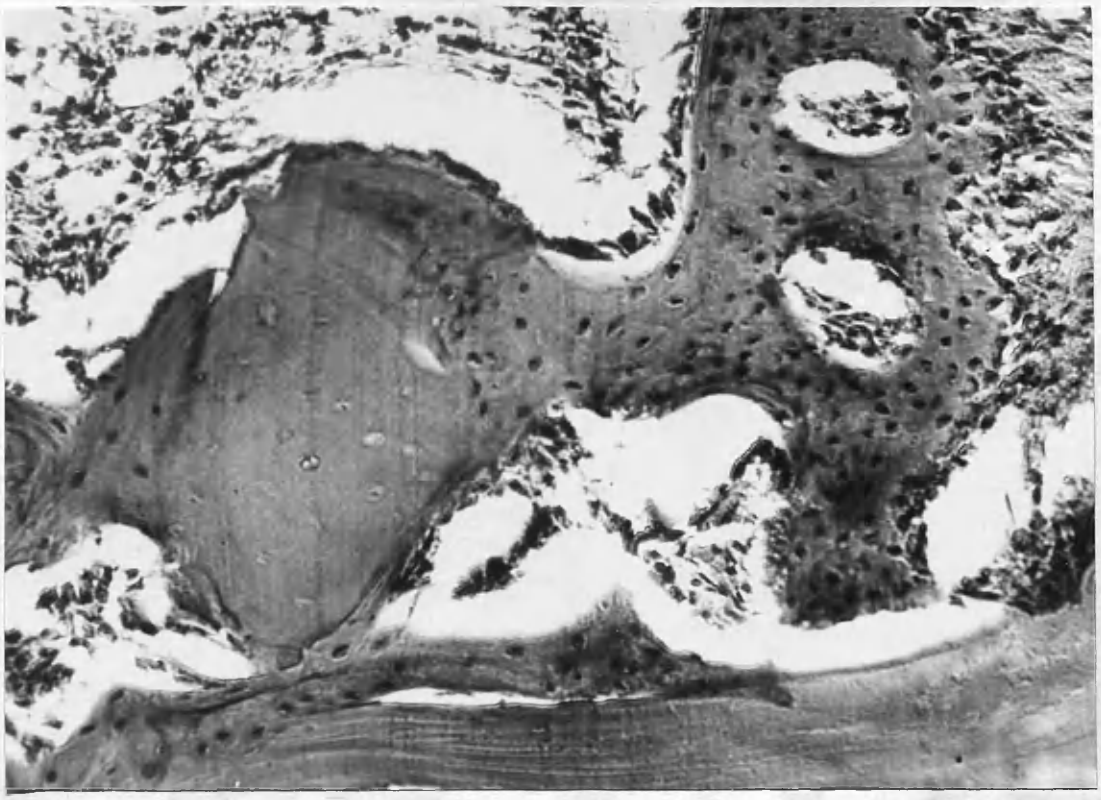

Fig:60 x 250. The dead bone tissue of the homogenous transplant is seen surrounded by new bone trabeculae formed from the host tissues.

Autogenous Bone transplant into

Anterior Chamber of the Eye.

10 Days.

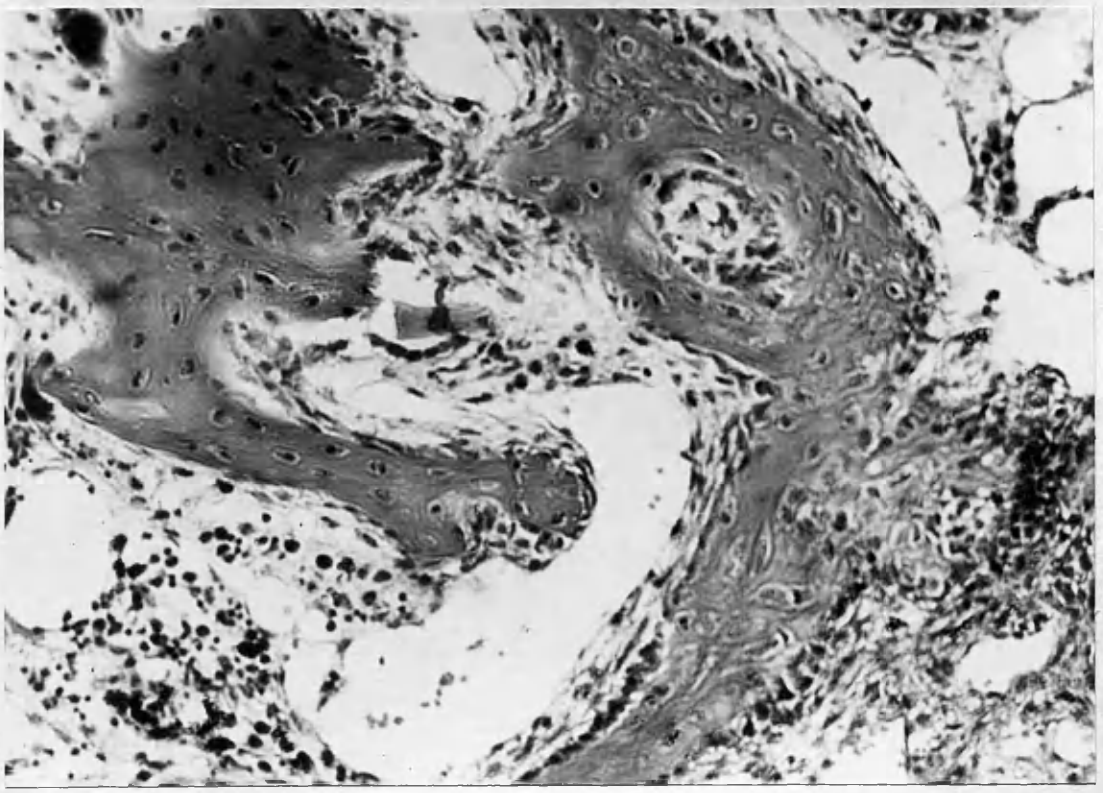

Fig:61 x 300. Transplanted cells have survived at the periphery of the trabeculae, but those more centrally placed have died. The endosteal osteoblastic proliferation is very marked.

Homogenous Bone transplant into

Anterior Chamber of the Eye.

10 Days.

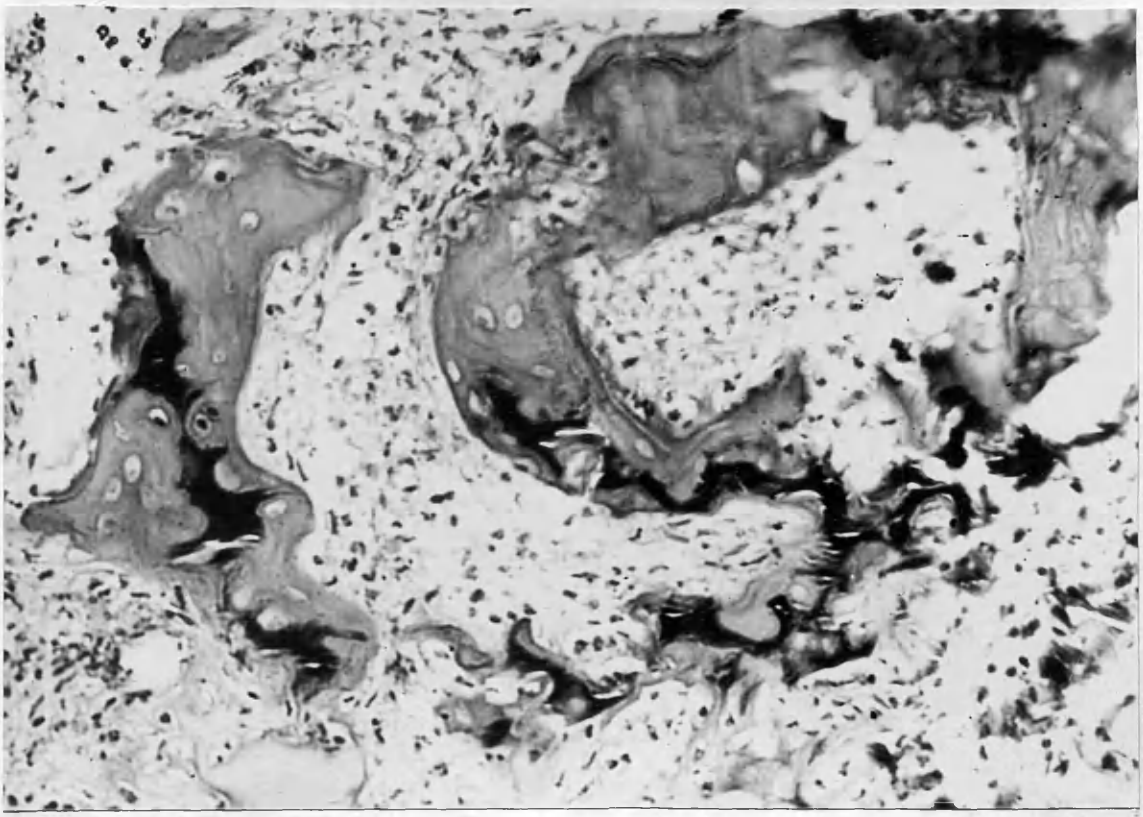

Fig:62 x 200. The transplanted bone tissue is quite devitalised. There is no periosteal reaction corresponding to that seen around the autogenous transplant.

Autogenous Bone transplant into

Anterior Chamber of the Eye.

21 Days.

Fig:63 x 300. The osteocytes in the centre of the trabeculae have died leaving empty lacunae, but more peripherally young osteocytes are present. The endosteal connective tissue differentiates into osteoblasts which appear to invade the matrix.

Homogenous Bone transplant into

Anterior Chamber of the Eye.

21 Days.

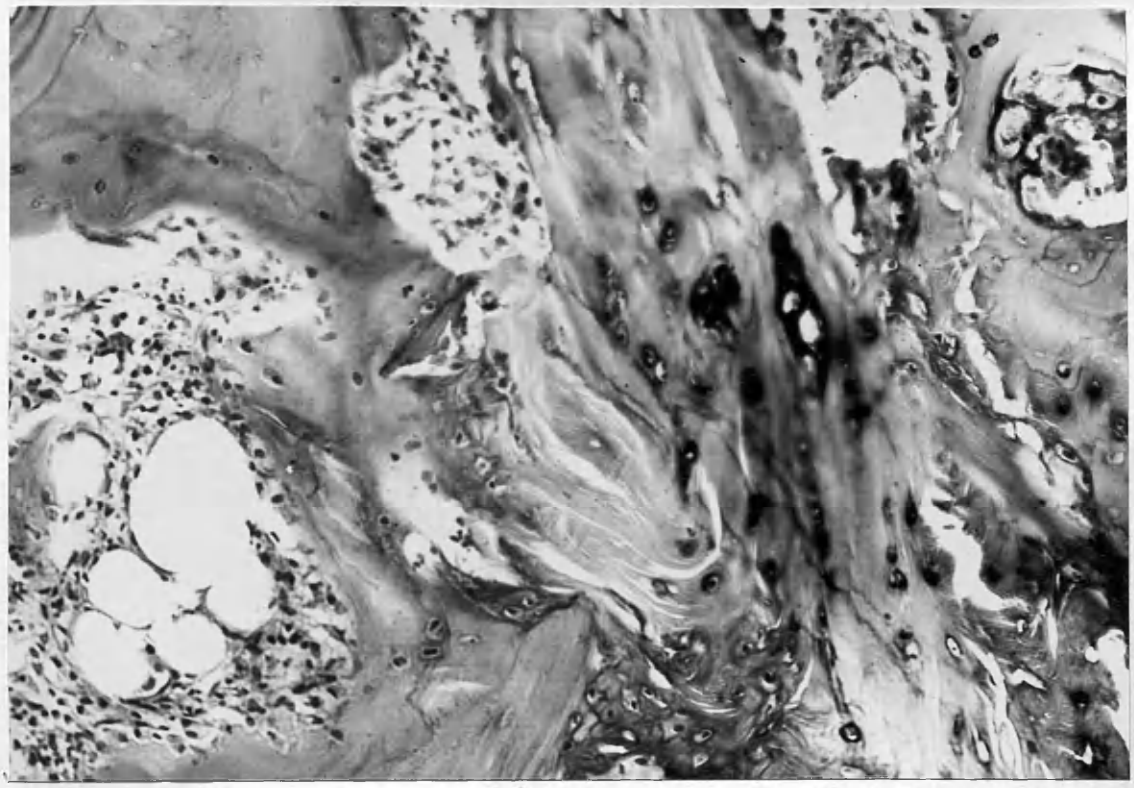

Fig:64 x 250. The majority of the osteocytes are dead, but cartilage and marrow tissues have survived. There is no endosteal proliferation as seen in the autogenous transplants.

Homogenous Bone transplant into

Anterior Chamber of the Eye.

21 Days.

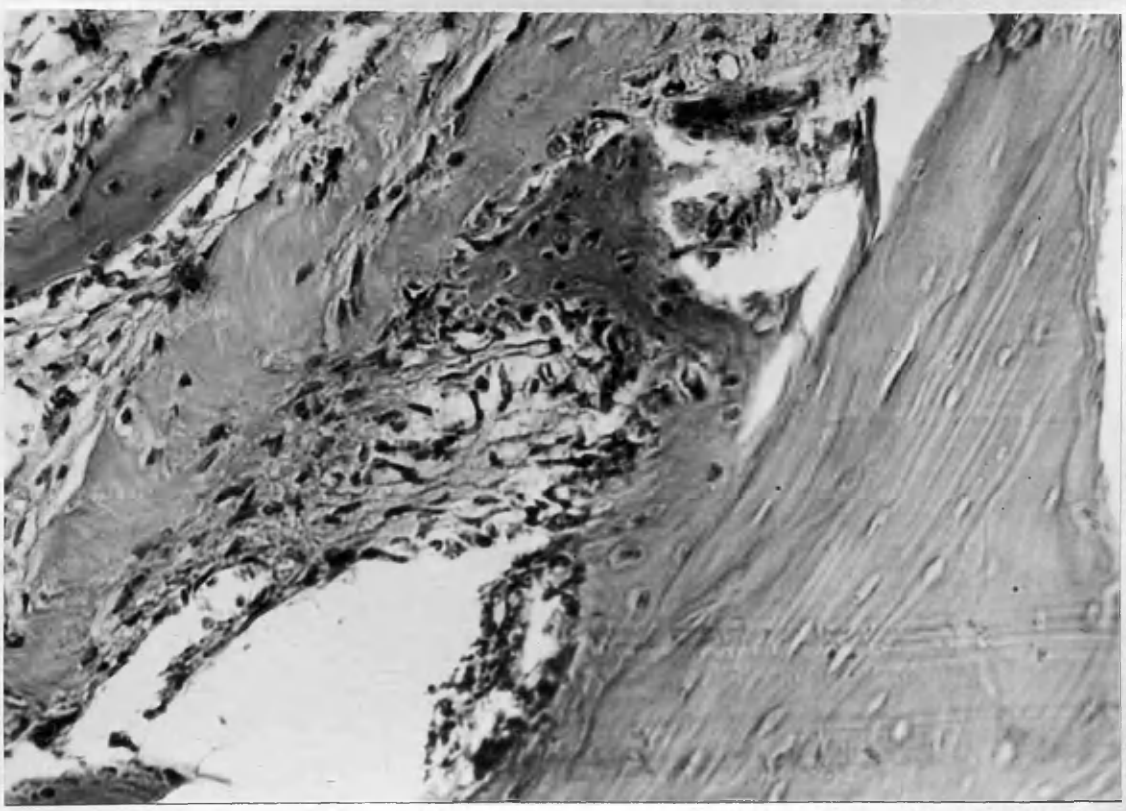

Fig:65 x 250. New bone being formed in the host connective tissue adjacent to the dead transplant.

Autogenous Bone transplant into

Anterior Chamber of the Eye.

42 Days.

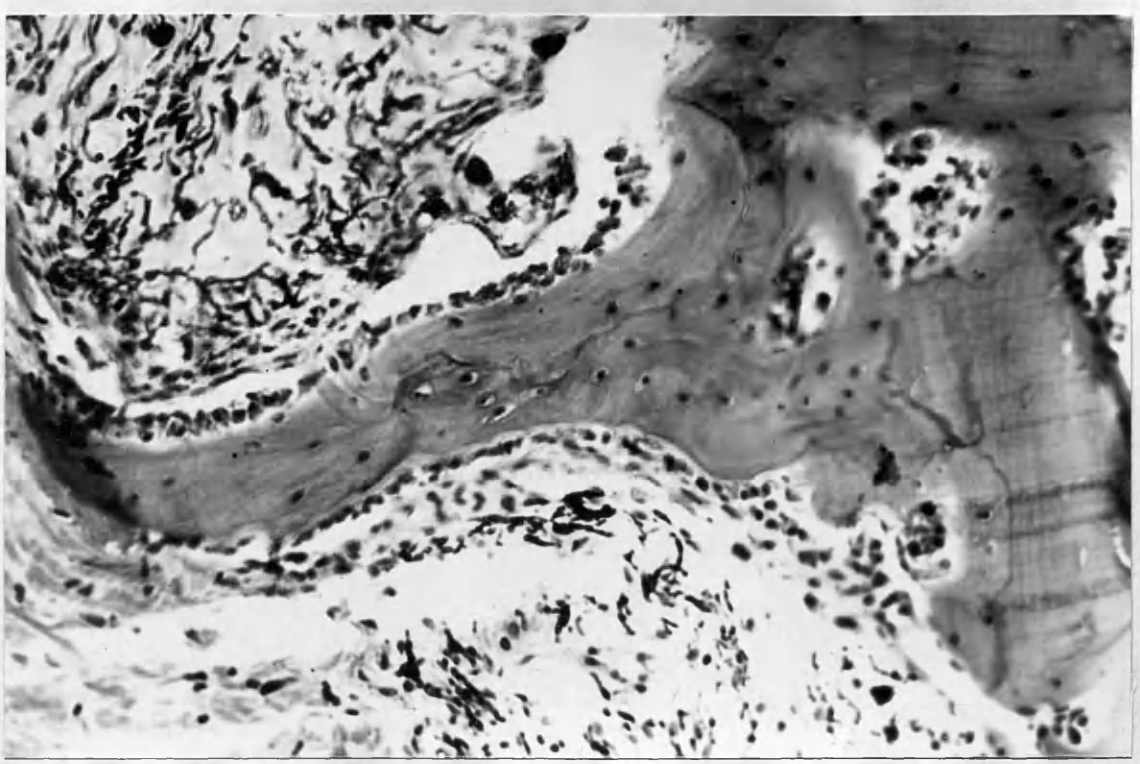

Fig:66 x 250. The transplant is now well populated and there is a regular endosteal layer surrounding it.

Autogenous Bone transplant into

Anterior Chamber of the Eye.

42 Days.

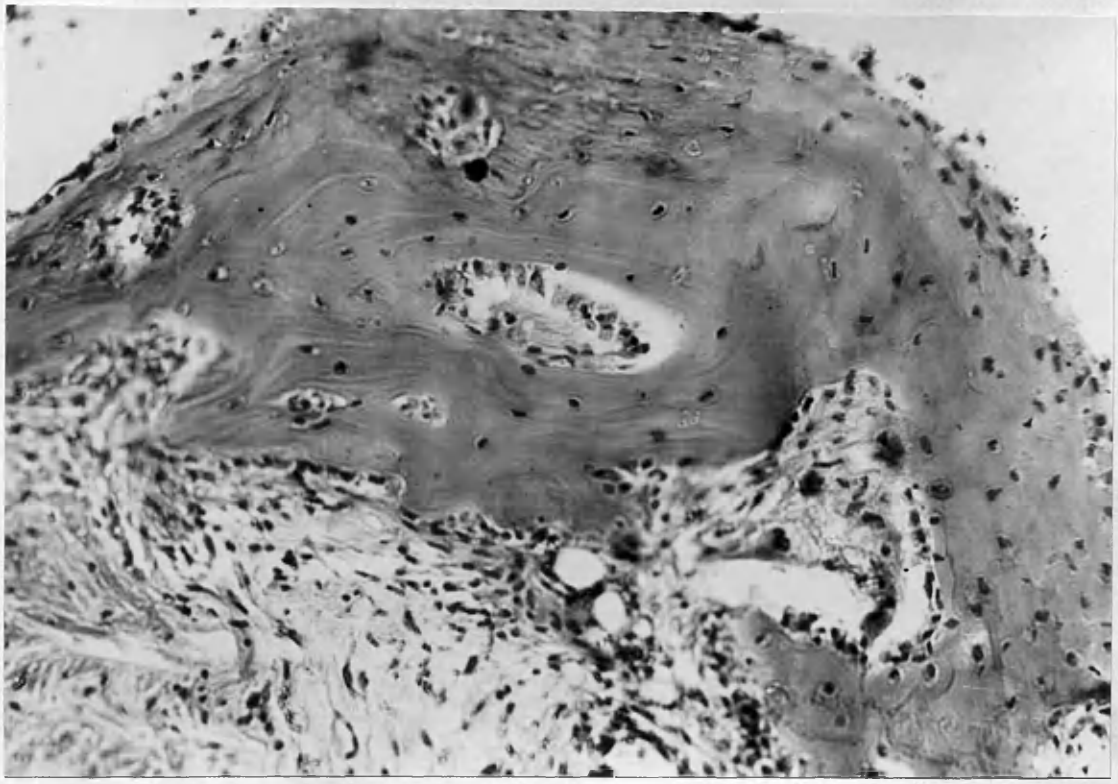

Fig:67 x 250. The migration of osteoblasts into the matrix of the transplant is seen on all sides. The surface of the transplant lying free in the aqueous humor of the anterior chamber also has a layer of osteoblasts surrounding it, proliferating and migrating.

Homogenous Bone transplant into

Anterior Chamber of the Eye.

42 Days.

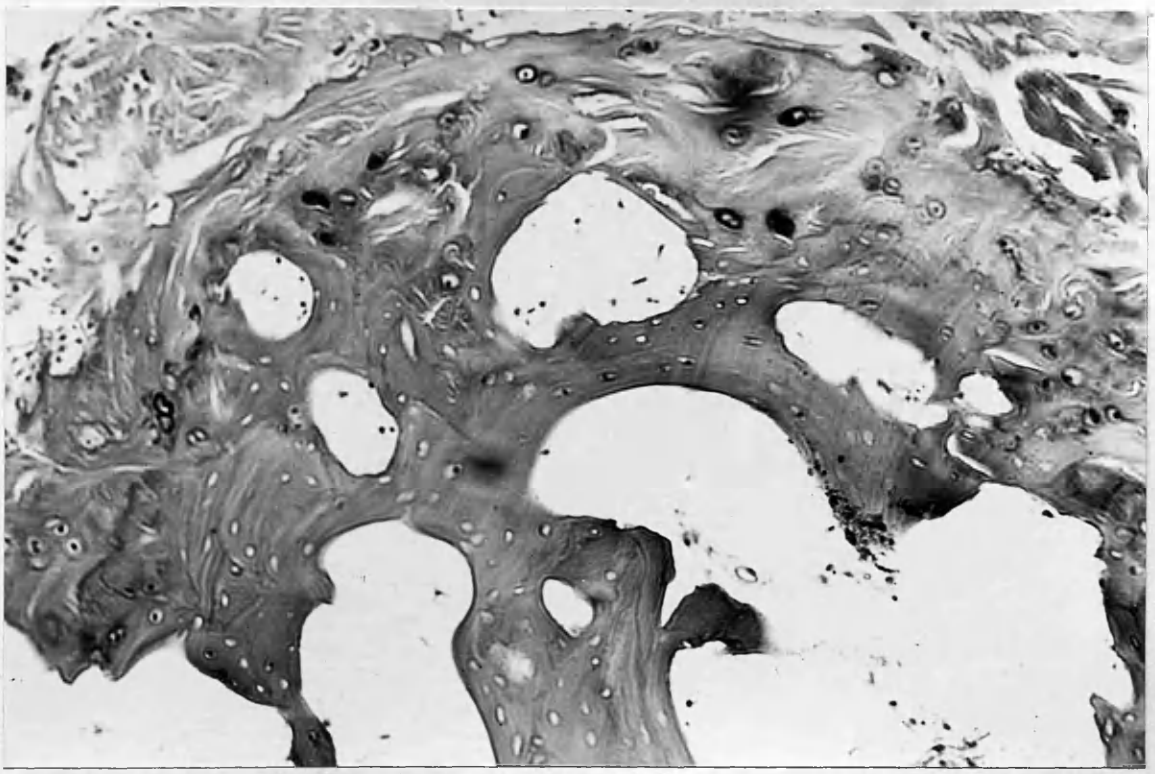

Fig:68 x 200. The bone tissue is quite dead with empty lacunae and sharply demarcated edges, including the surface in contact with the host connective tissue. A few cartilage cells survive.

Homogenous Bone transplant into

Anterior Chamber of the Eye.

42 Days.

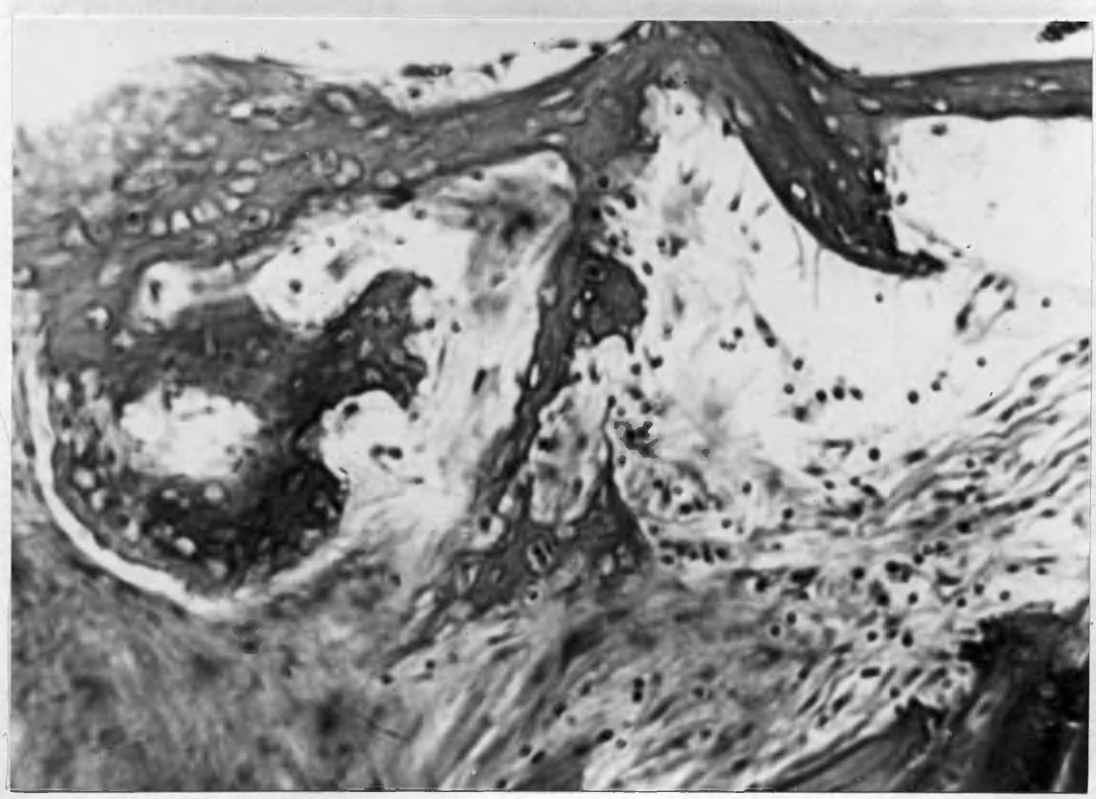

Fig:69 x 250. New bone formed in the host connective tissue has become completely devitalised and has a sequestrum like appearance similar to the transplant itself. Round cells are noted in the surrounding connective tissue.

Autogenous Bone transplant into

Anterior Chamber of the Eye.

80 Days.

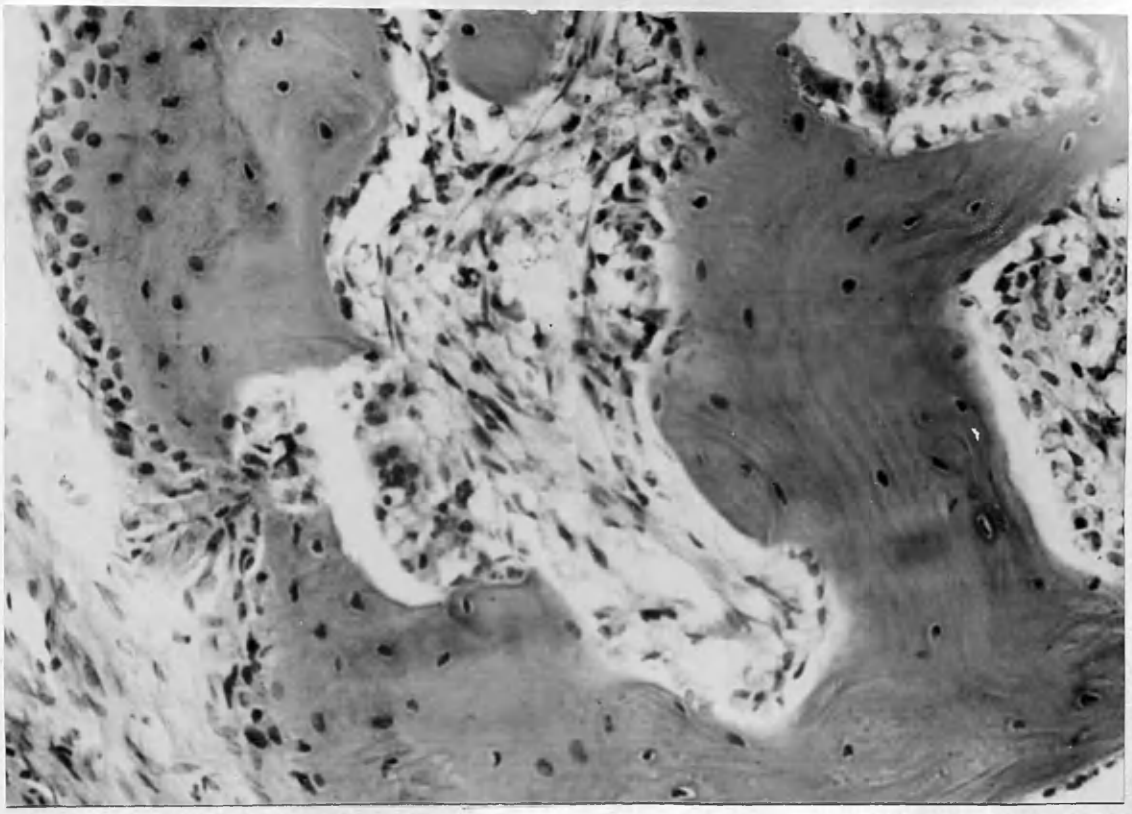

Fig:70 x 250. Endosteal and periosteal osteoblastic activity and migration still present. The transplant is becoming well populated.

Autogenous Bone transplant into

Anterior Chamber of the Eye.

100 Days.

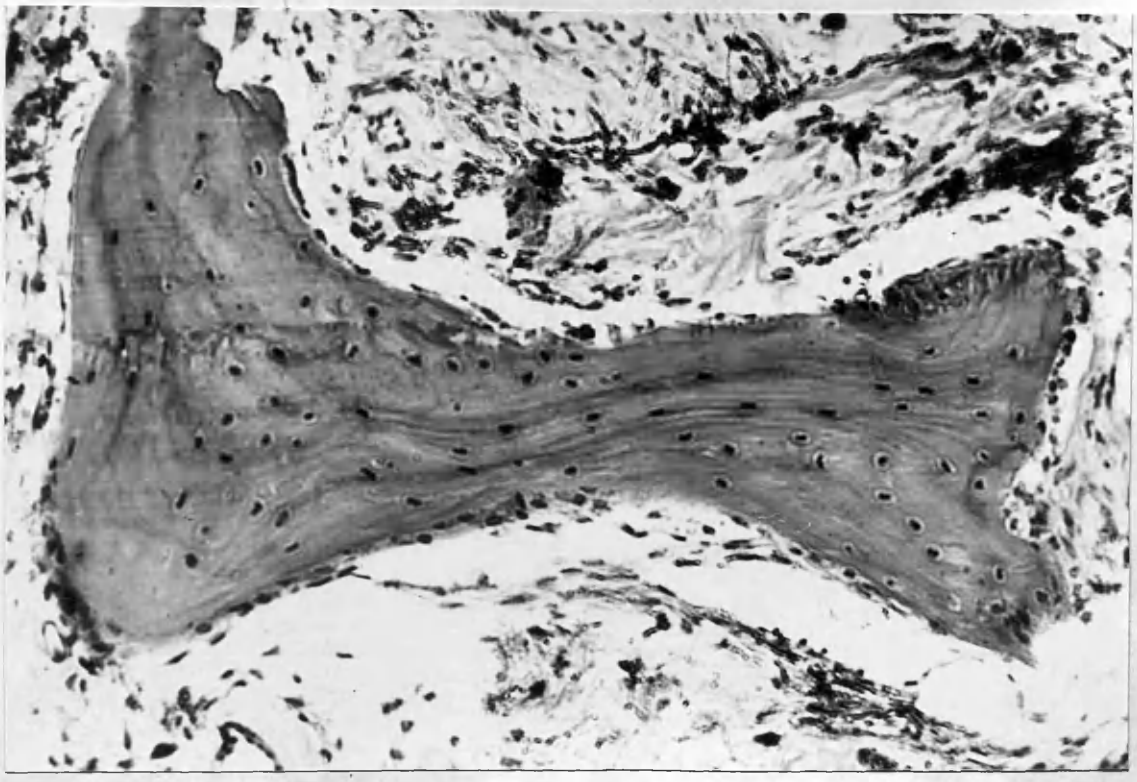

Fig:71 x 200. The transplant is well populated. The osteocytes have a more mature appearance and the periosteal layer of osteoblasts is thinned out and more regular.

Homogenous Bone transplant into

Anterior Chamber of the Eye.

100 Days.

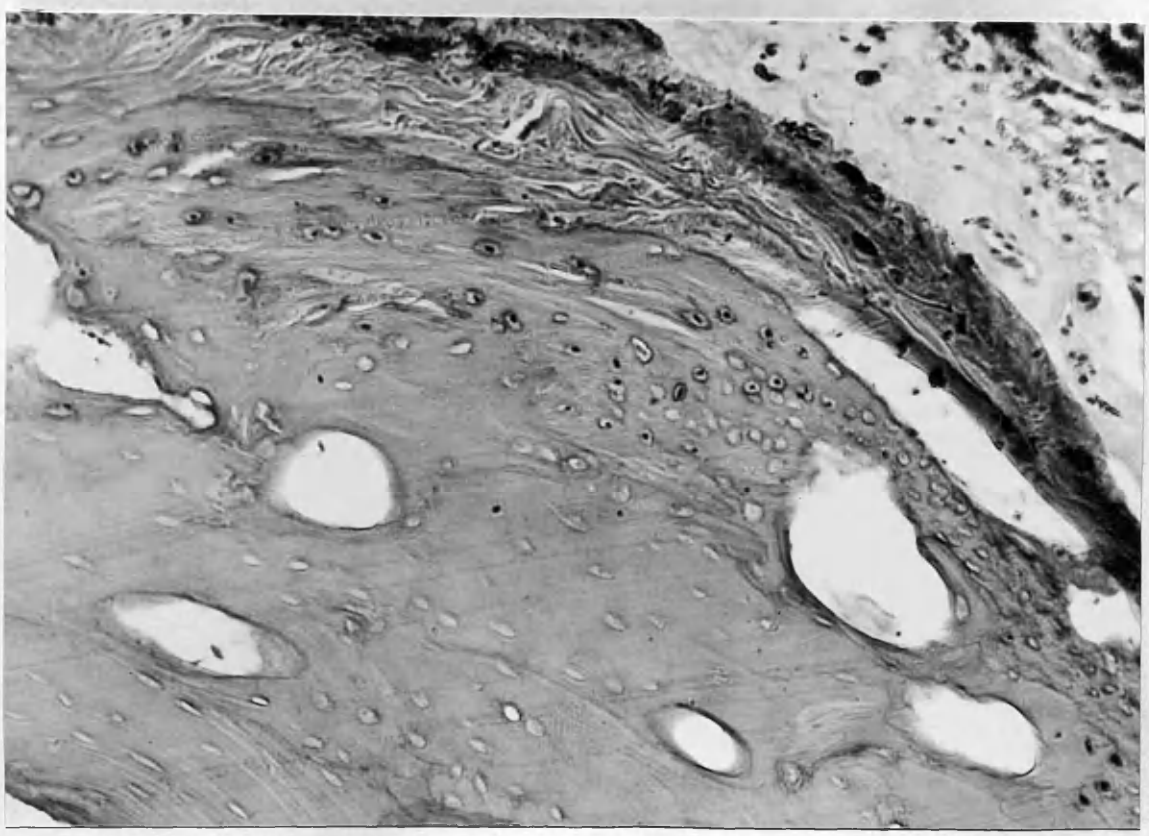

Fig:72 x 250. The transplant consists of sequestrum like dead bone, the edge of which is clearly demarcated from the surrounding connective tissue. Cartilage cells have also become devitalised.

Homogenous Bone transplant into

Anterior Chamber of the Eye.

100 Days.

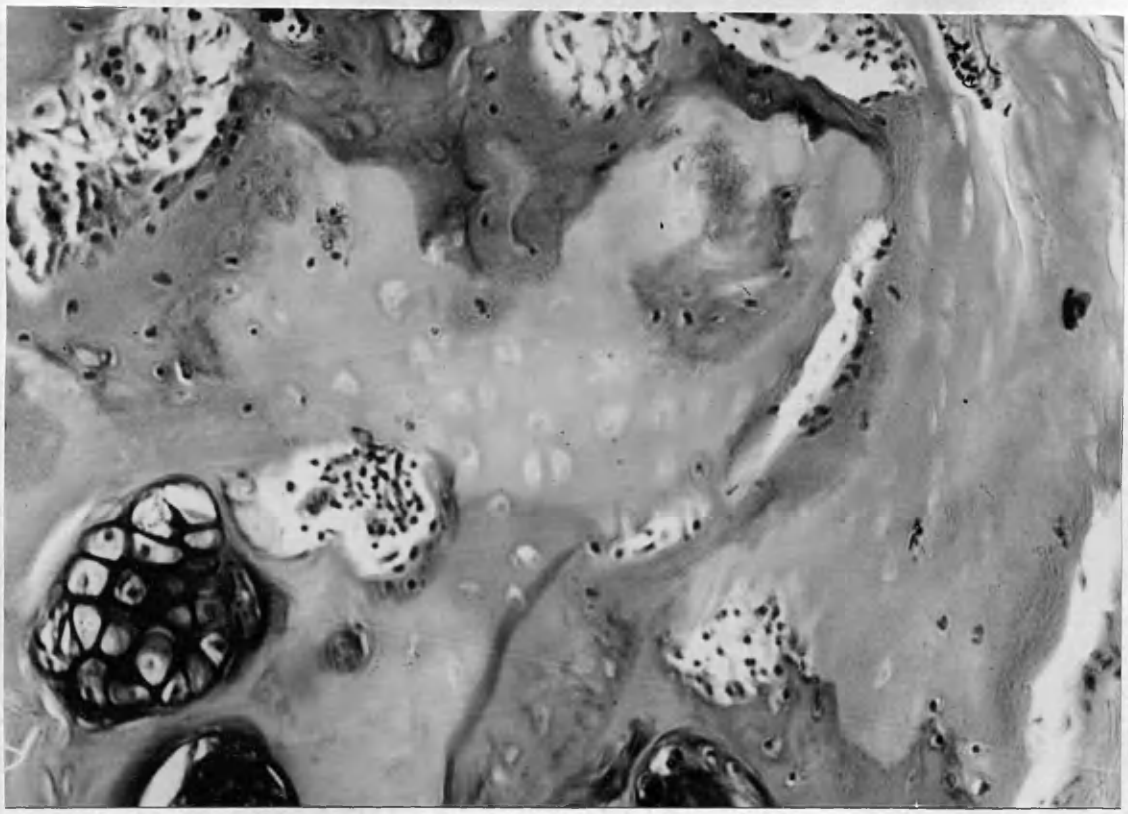

Fig:73 x 250. Devitalised homogenous bone and cartilage. Some new bone indicated by its more deeply staining matrix has substituted the transplant but it is now also devitalised.

Autogenous Bone transplant into

Anterior Chamber of the Eye.

160 Days.

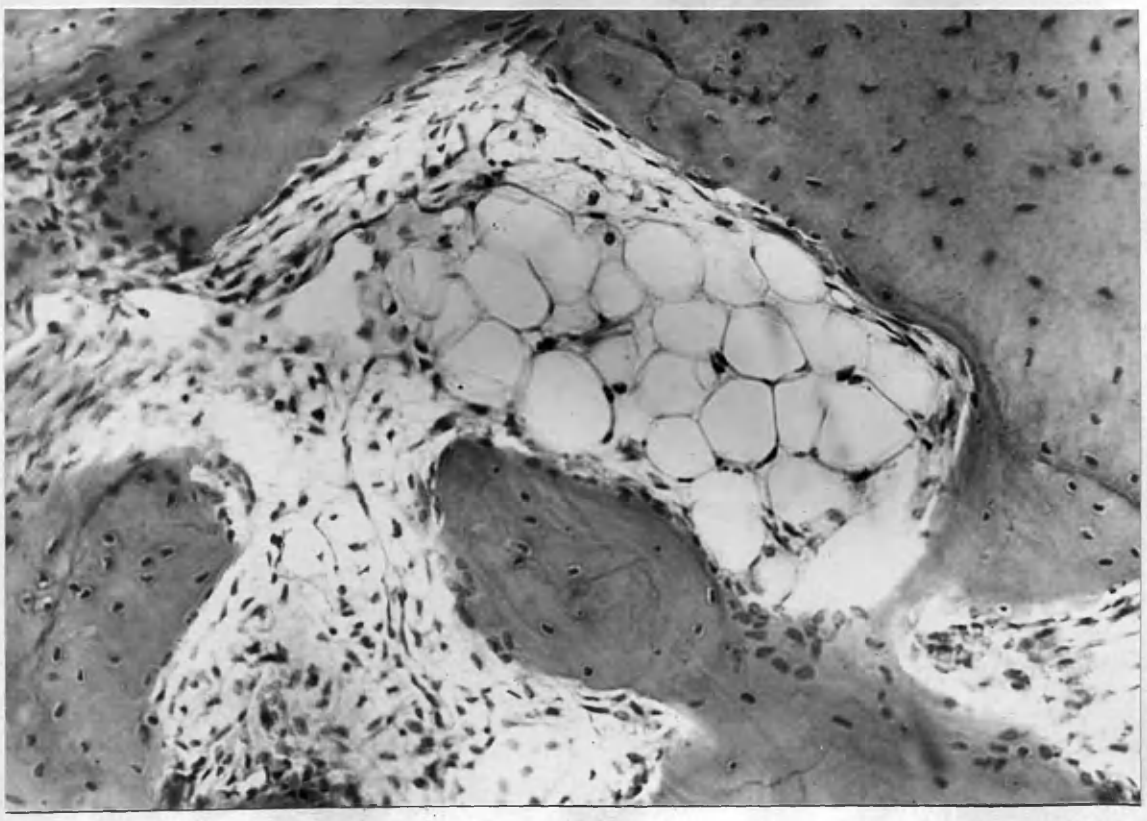

Fig:74 x 200. Bone tissue of mature appearance populated by osteocytes, the endosteal layer is less active.

Homogenous Bone transplant into

Anterior Chamber of the Eye.

160 Days.

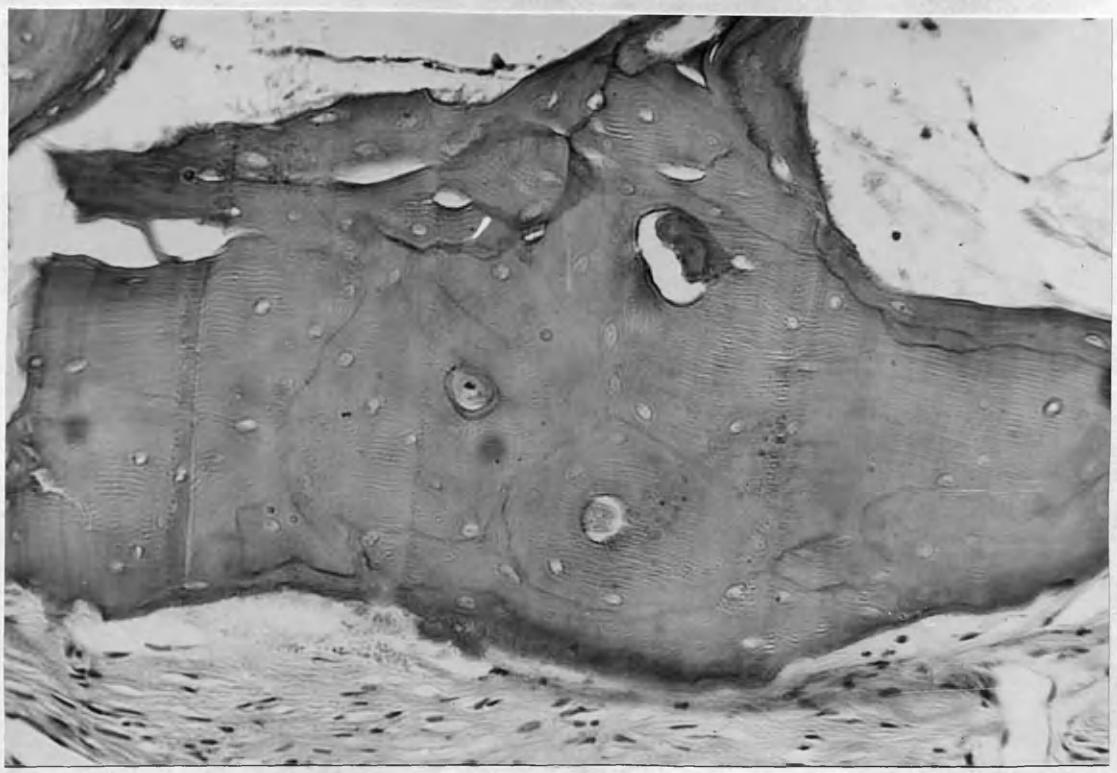

Fig:75 x 200. Sequestrum like dead bone tissue with empty lacunae, and no surrounding connective tissue reaction.

Homogenous Bone transplant into

Anterior Chamber of the Eye.

180 Days.

Fig:76 x 200. The transplant is quite devitalised in spite of the presence of cartilage tissue. The new formed bone tissue with its more deeply staining matrix has also undergone necrosis.

Heterogenous Bone transplant into Muscle.

21 Days.

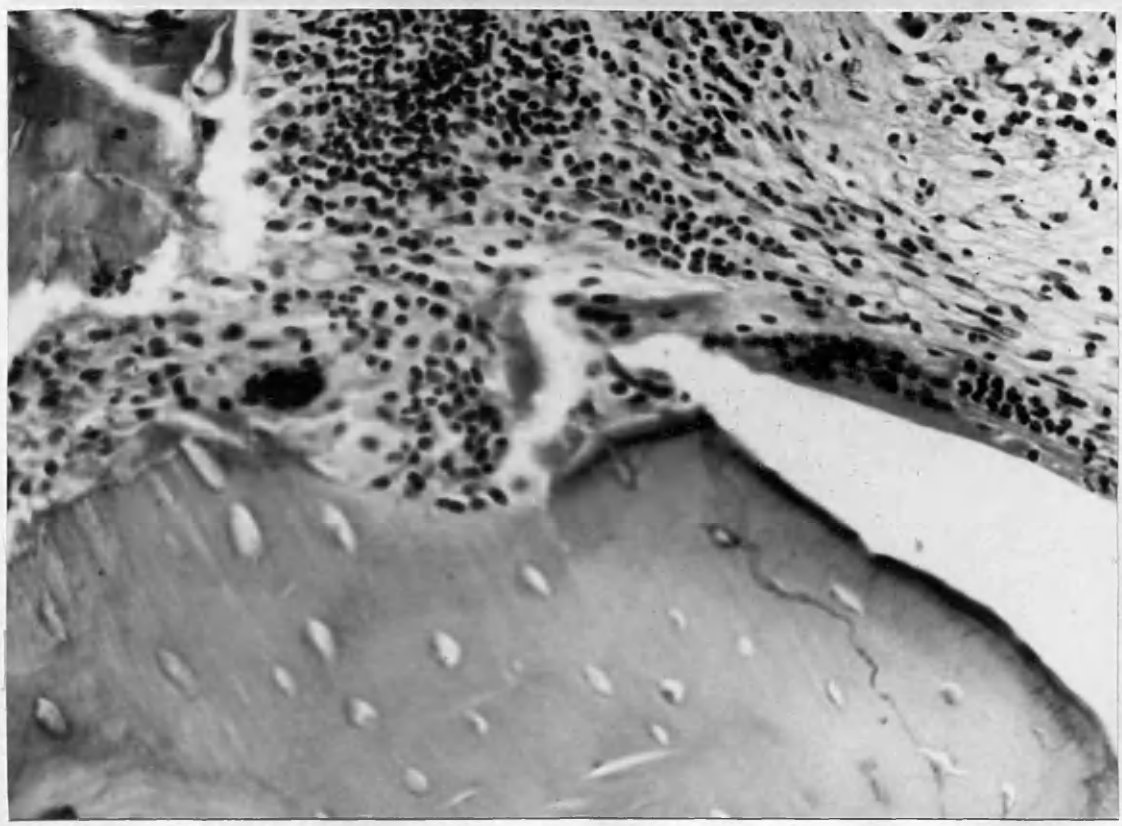

Fig:77 x 300. The transplant is quite dead. The matrix showing large vacant lacunae is surrounded by an intense round cell infiltration consisting of lymphocytes and polymorphonuclear leucocytes.

Frozen Autogenous Bone transplant into Muscle.

42 Days.

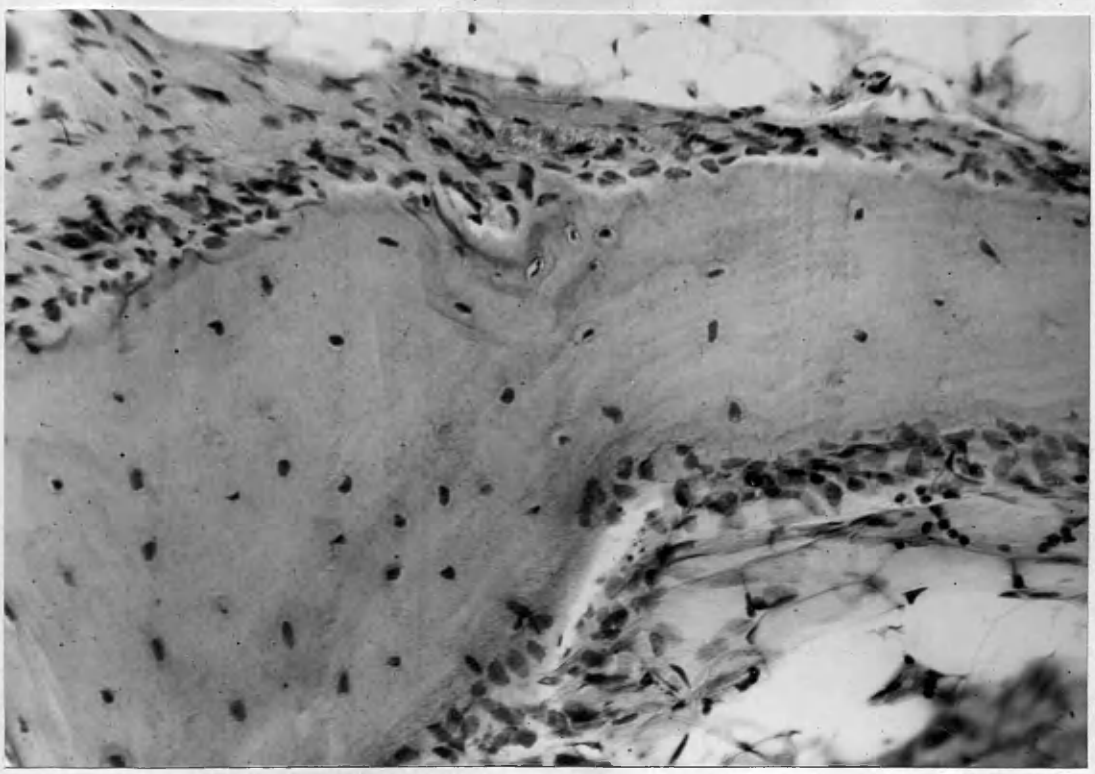

Fig:78 x 250. Healthy bone with a regular endosteal layer enclosing marrow tissue.

Frozen Homogenous Bone transplant into Muscle.

42 Days.

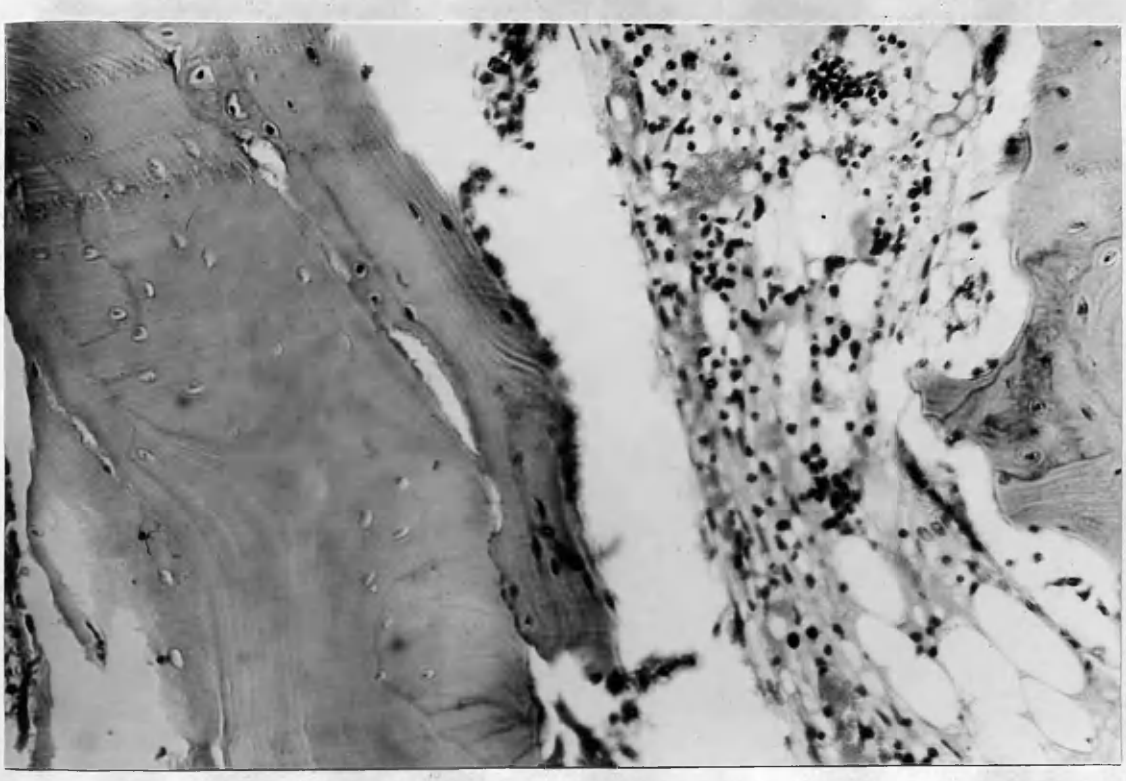

Fig:79 x 250. The transplant which is almost completely devitalised has a surrounding round cell reaction.

Frozen Homogenous Bone transplant into Muscle.

80 Days.

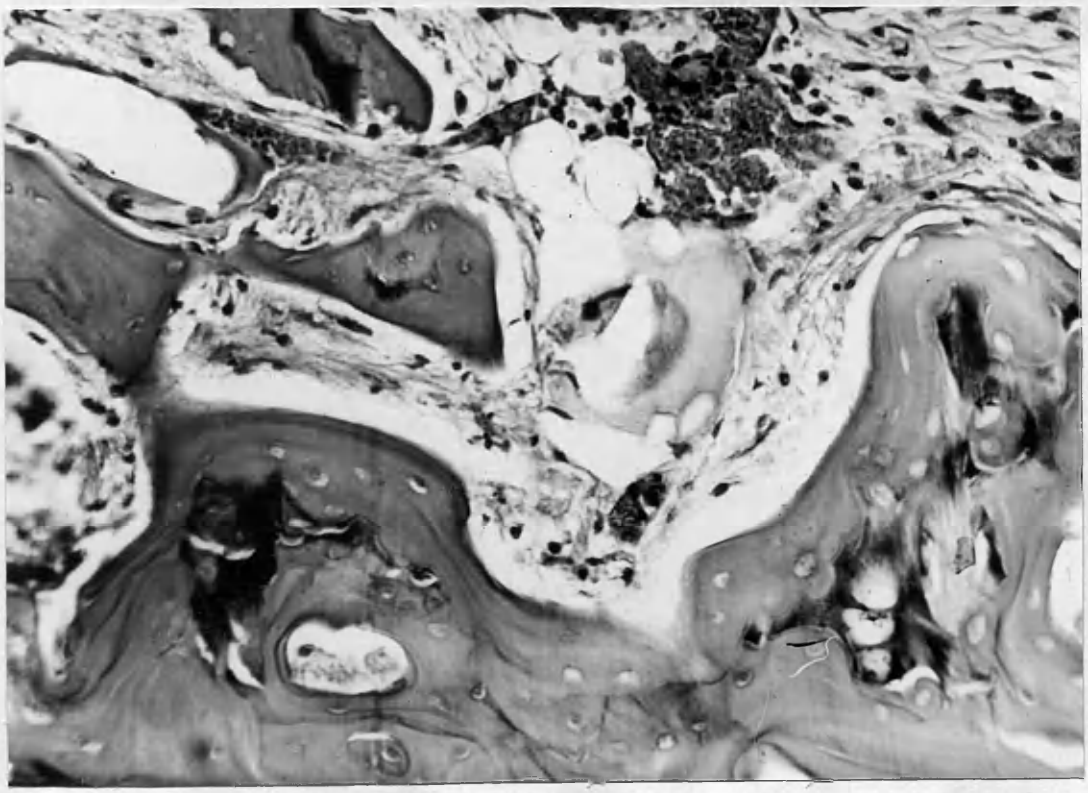

Fig:80 x 200. The bone tissue is completely devitalised. Its outline is dense indicating superficial lysis, and there is no evidence of osteogenesis either from the transplanted tissue or from the host tissue.