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Microradiographic Investigation of Bone Grafts in Man

By

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Introduction

In the literature little has been written on microradiography of bone grafts.

Because of our previous interest in this problem, because of the encouragement given by Prof. Scaglietti and because of the fine equipment available in the Clinic, we decided to study this subject further.

Experimental bone grafts, *i.e.* bones transplanted experimentally in animals, often in their general aspects and in many details resemble those in man. Yet investigators hesitate in general to draw too far-reaching conclusions from findings in animals with regard to the situation in man, and this is particularly true of bone grafts because of the importance here of the size of the graft and the bed, and of the length of time of evolution. Clearly, we cannot entirely compare vascular invasion in a 2×0.5 cm fragment in a rabbit with that in a large cortical graft measuring 20×3 cm in man.

We therefore thought it wiser to use only human material for our study. As far as we have been able to find out very rare reports of microradiographic studies of bone grafts in man have as yet been published. We had at our disposal the vast material of the Orthopaedic Clinic of Florence and we were able to study a number of human autografts which had been removed after an interval of time for various reasons, such as tumour recurrence, death of the patient, etc.

The following pages report the results of several years work; we trust that we may claim that the material is original and its preparation accurate. As regards the interpretation of our findings we realize that the various attempts we have made bear, of necessity, the stamp of our own training and orientation which is largely that of the practising clinician. Still, we dare hope that some of these attempts at interpretation have not been entirely without merit and that some of the postulates arrived at may prove tenable.

THE AUTHORS

CHAPTER I

Generalities

Microradiography has gone through a process of laborious but continuous progress until at present it ranks technically as well as scientifically alongside histological technique, and now that the situation for normal bone has been worked out new possibilities emerge for the study of biological processes both in normal and pathological bone tissue.

Amongst the authors who have studied this subject some of the most important are Amprino and Engström who, between 1950 and 1954, evolved a satisfactory technique for the preparation of material and a system for the interpretation of results. Others who have made important contributions are Lacroix, Vincent, Engfeldt, Zetterström, Nilsonne, Marshall, Rowland, Jowsey, White and Cohen.

The subject of bone grafts was studied by Holmstrand, in 1957: he has published a monograph based on the results of experimental work in animals (rabbits and dogs) in which he states that: a) all bone grafts, whether autoplastic, homoplastic or heteroplastic, undergo approximately the same process of absorption and reconstruction once they have become fused to their beds; b) the length of time required for a complete "take" is shortest in the case of autografts; c) the arrangement of structures of the second order in the graft is not determined by mechanical factors but by the structure of the bed; d) mineralization of the bone, until a normal density is reached, is a very slow process; e) in accordance with what had been asserted earlier by Amprino and Engström, the deposition of calcium proceeds initially from the haversian canal out to the periphery centrifugally.

One year later Vigliani (1958) confirmed in the main the above-mentioned findings with a series of similar experiments also in dogs and rabbits.

CHAPTER II

Techniques

PREPARATION OF THE MATERIAL FOR MICRORADIOGRAPHY, AUTORADIOGRAPHY AND EXAMINATION IN POLARIZED LIGHT

The bone fragments used in our study were taken from the tibia, the femur, the radius, etc.; after their removal all adherent soft tissue was carefully removed. The samples were then examined by radiography after insertion of metal pins perpendicular to the horizontal axis of the specimen and situated at regular intervals. This was to enable us to determine transverse and longitudinal planes in the radiograph and to plan the destination of the various segments (one or other form of examination).

We preferred to make the sections with a fine handsaw rather than by a circular saw so as to reduce to a minimum the generation of heat during sawing and the damage done to the finer structures by the rotating saw.

Of each segment (as indicated above) sections were taken along the main axis of the bone and transversely.

Subsequently, the material obtained was divided into two equal portions which were then treated in different ways; the material for histological examination was treated in the usual manner, the material to be examined by microradiography was fixed in 80° alcohol (in a few instances only with acetone).

Although the material contained very little water it was nevertheless subjected to careful, protracted dehydration since any trace of water might disturb the polymerization phase by the formation of air bubbles.

For the imbedding we have as usual selected methyl methacrylate, although nowadays there are a great many other products on the market. Use was made of the well-known method of Puchett: a) first phase of the polymerization of the methyl methacrylate without inhibitors (hydroquinone) and with the addition of the catalyser (in this case, benzoyl peroxide) is in a water bath at 70° C for 30 to 45 minutes at the longest until a liquid of the consistency of heavy syrup is obtained; b) interruption of polymerization and the introduction of the biological material (previously dehydrated and kept in monomerous methyl methacrylate for at least 24 to 48 hours) in Petri dishes filled with methacrylate in the process

of polymerization; c) second phase of the polymerization in the Petri dishes prepared in this way in the thermostat at 50 to 55° C for approximately 24 hours.

In two respects the method has been slightly modified:;

1) during the first polymerization phase we have, as suggested by Vincent, several times lowered the temperature of the methyl methacrylate in the water bath in order to obtain a more homogeneous polymerization;
2) subsequently, the dishes were placed under a glass case at a reduced pressure for 15 minutes to allow the complete penetration of the imbedding medium, just as is done in the preparation for electron microscopy.

The embedding material was subsequently cut into sections of 0.5 to 1 mm thick, again with a fine jeweller's handsaw.

These sections were ground by hand to the desired thickness. First (to a thickness of 250 μ) with silicon carbide abrasive papers of a progressively finer grit; for this purpose the section was attached to an object glass of a microscope by double-coated scotch tape, by the method of Hammarlund-Essler. Subsequently, it was treated with extremely fine ground glass (Amprino) and during this grinding the section was not attached; it was moistened with 80 per cent alcohol and pressed against the grinding surface under slight constant pressure by a piece of cork (Amprino).

The greatest care was taken to keep the two surfaces of the sections as accurately parallel as possible. The thickness achieved was frequently measured by an optical system as well as by a mechanical system (Mauser precision gauge). The average thickness was kept at 50 μ, with minimal thicknesses of 30 μ for sections with smaller surfaces and of 70 to 100 μ for sections with a larger surface.

The same sections were used, for the micro-radiographic examination, for the autoradiographic examination, for the examination in polarized light and for the histological examination, without further processing.

The embedding medium was not removed (as many authors have done) in order to avoid crumbling, nor was decalcification carried out prior to the examination in polarized light or the histological examination. This aspect will be further discussed below.

MICRO-RADIOGRAPHIC STUDY

In order that the result might be as true to life as possible a number of difficulties had to be overcome:

a) it was necessary to use film with a very finely granular emulsion (in order that the granules would be appreciably smaller than the structures to be shown);
b) the sections of bone had to be extremely thin, as thin as histological sections or even thinner in order to avoid overlapping of the images;
c) use had to be made of sections of bone tissue with parallel surfaces, not calcified, in order to allow the differentiation of the calcified structures from the soft tissues;
d) special X-ray apparatus had to be used to provide X-radiation of a calcium-absorption wave length and an exposure time long enough to mark the film; the film having a very fine grain it was not very sensitive and required a long exposure time.

Of the various systems of micro-radiography possible we selected the contact method in view of the type of equipment at our disposal and because with this method the sharpest micro-radiographic film images are produced. Also the dimensions of the micro-radiograph were the same as those of the histological and autoradiographical images with which it had to be compared. The sections were brought into close contact with the emulsion of the film in special holders.

As X-ray sources, two different apparatus were used: the General Electric XRD-3F and the Philips C.M.R.5 apparatus. The former was used with three different tubes, viz. with a copper, chromium or tungsten anode. Radiation of a monochromatic type was obtained by filtering through a beryllium screen, using nickel, vanadium or aluminium, depending on the type of tube used. The wave length was kept between 1.54 and 2.3 Å with use of 12 kV and 20 mA, 10 kV and 25 mA, and 10 kV and 15 mA respectively.

The Philips C.M.R.5 apparatus was used for wave lengths of more than 2.3 Å and especially in those cases in which we required detail of less calcified structures, *e.g.* newly formed bone at the periphery of grafts. The following films were used:

a) Maximum Resolution Kodak
b) 649 GH Kodak
c) Scientia Lippman 5E 56 Gevaert.

The photometric densitometry was carried out by the Amprino-Engström system with a few modifications: the Zeiss Ultraphot N.2 microscope was used with a tungsten-wire lamp fed by a highly stabilized unit.

A mirror with total reflection projected the micro-radiographic image onto a white screen. An opening in the centre of the screen made it possible for a photo-multiplicator cell to catch the light signal and, after its conversion and amplification by a special amplifier, to put it through an electronic volt meter with a scale with 100 subdivisions. We preferred to use an opening, the diameter of which could be adjusted according to the enlargement used for the projection and an electronic volt meter of the ARCA W V 84B type because of the multiple measurements it allows and because of its excellent sensitivity. The micro-radiograms were printed with considerable magnification; each micro-radiogram showed its own scale of comparison, consisting of ten sheets of thin celluloid; the calculation was then done by Amprino's system.

AUTO-RADIOGRAPHIC STUDY IN VITRO

For the auto-radiography we used Amprino's technique using the same sections previously used for the micro-radiographic examination. These sections were subjected to the following processing stages:

a) the sections were thoroughly washed in distilled water and then immersed in a weakly acid aqueous solution of radioactive $CaCl_2$ (pH between 6.3 and 6.7). On Amprino's advice, the solution was kept very much diluted and the duration of the immersion fairly long in order to get a good uptake of the radio-isotope in the receptive areas, without affecting its distribution. We used the $CaCl_2$ in a watery solution of 0.5 g per 1000 ml, as supplied by the Amersham Radio-Chemical Centre (G.B.) (specific activity per gramme of Ca = 5–10 mC); the sections were immersed for 5 hours;

b) thorough washing in distilled water (changed several times), then drying at room temperature;

c) auto-radiography—in special holders the sections were brought into contact with films sensitive to beta-rays. The best results were obtained with Ilford C2 film. Exposure time: 5 to 7 days. Development with Kodak D.19b with a large proportion of K; (the results were excellent).

Washing and fixing with Ferrania Fl, again washing and drying. Just as during the micro-radiography the emulsion was protected by a cover glass attached with a drop of Canada balsam.

EXAMINATION IN POLARIZED LIGHT

In order to determine accurately the direction of the collagen fibrils in the bone sections of the autografts examined we also studied these sections with the polarized-light microscope.

We used the same sections for histo- and auto-radiography; no decalcifying treatment was applied nor did we remove the methyl methacrylate used for the imbedding, because of the danger of the sections crumbling. We have compared the results of polarized-light examinations with imbedded and with non-imbedded bone and have seen no differences.

The sections were placed between an object glass and a cover glass, attached with a drop of Canada balsam.

The microscope used was the Zeiss-Winkel Standard polarizer model GF 679, with apochromatic objectives.

By turning the stage of the polarizing microscope the direction of the optical axis for the collagen fibres in different structures was determined between crossed Nicol prisms, in relation of course to the longitudinal axis of the bone.

CHAPTER III

Material Studied

A detailed description is given below of the cases studied: we discuss human autografts exclusively, recovered after they had been in the body for a longer or shorter period of time, and removed because of complications or other reasons.

Case 1 (Figures 2–5)

A man aged 50 years. Pseudarthrosis of the tibia and fibula. Operation: Removal of a 20 cm bone graft from the crest and anteromedial surface of the contralateral tibia. This graft was introduced through an opening made across the site of the pseudarthrosis into the medullary canal. Other onlay grafts were also applied.

The operation wound healed by first intention. The patient was discharged. At home, 4 months after the operation, he started to use the operated upon leg for weight-bearing still in its plaster cast. Five months after the grafting the patient suddenly died from a cerebral haemorrhage.

At autopsy the tibia was removed. After it was cleaned of soft parts it felt solid. Radiographic examination showed that the transplanted bone had remained almost unchanged in the medullary canal of the tibia (Figure 2). The paracortical graft had fused with the tibia and had brought about clinical union. Around the site of the pseudarthrosis some callus formation was noted.

On macroscopical examination the graft still looked much the same as on the day it had been taken. It was entirely covered by a fibrous sheath (Figure 2) which here and there adhered to it fairly closely but could always be easily detached; at other places, and this applied to most of its surface, there was hardly any contact and no adhesion at all so that the graft could be slipped out of the sheath as is a finger from a glove.

For the present study a segment of the tibia was used which was 10 cm long and included the site of the pseudarthrosis. One section was cut in a coronal plane and two transverse sections were cut one from either end of the segment (Figure 2).

Case 2 (Figures 6–10)

A woman aged 48 years. The entire femoral diaphysis, which was affected by a parostal sarcoma, was resected and replaced by a cortical autograft from the tibia of a stick-like shape 25 cm long. Proximally it was introduced into the cancellous bone of the trochanteric region which had been left in place and distally into the medullary cavity of the metadiaphysis.

During the next few months the patient wore an extensive plaster cast from the hip downwards and weight-bearing was started 6 months after the operation.

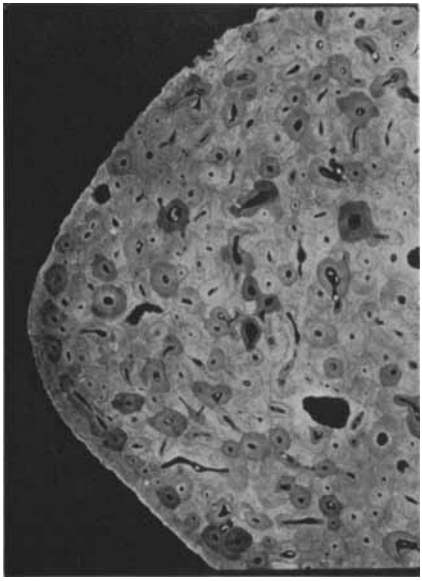


Figure 1. Section of an anterior tibial crest of a normal man aged 25 year. The predominance of osteons with a high mineral content is clear. There are also a certain number of osteons with a low level of mineralization.

Eleven months after the operation clinical and radiographical signs of local recurrence were seen in the upper third of the thigh, corresponding to the trochanteric region. Disarticulation of the hip was decided upon. A solution of Berlin blue was injected into the common femoral artery immediately after the disarticulation for visualization of the blood vessels.

The segment was examined radiographically (Figure 6): the graft had fused with the femur at its distal end but at its proximal end was entirely free and eroded by the tumour. Over its entire length it still looked more or less the same as it had originally: —a stick the size of a little finger with here and there a somewhat festooned outline, in some places fairly broad and a little porous in others narrower and of greater density.

Macroscopically, the musculature was found to be adherent directly to the graft over short lengths each of a few cm (Figure 7). In other, larger areas a fibrous sheath, loosely adhering to the graft, separated it from the soft parts. In most places there existed an actual cavity between the graft and the fibrous sheath and in these places the graft showed a glassy, white, ivory smooth surface. It is mostly to these areas of the graft, without connection with the bed, that we refere when below we speak of the "excluded" graft; the radiographic-macroscopic condition of the graft will be referred to by the name of "concentric sclerotic atrophy".

Figure 2. Case No. 1. Intramedullary cortical graft in pseudarthrosis of the tibia. A man aged 50 year, operated on 5 months prior to his death from cerebral haemorrhage. a) X-ray with the segment examined indicated. b) high transverse histological section; the graft is only loosely connected with the bed. c) sagittal histological section: in the centre of the medullary canal is the graft covered by a fibrous sheath. d) low transverse histological section.

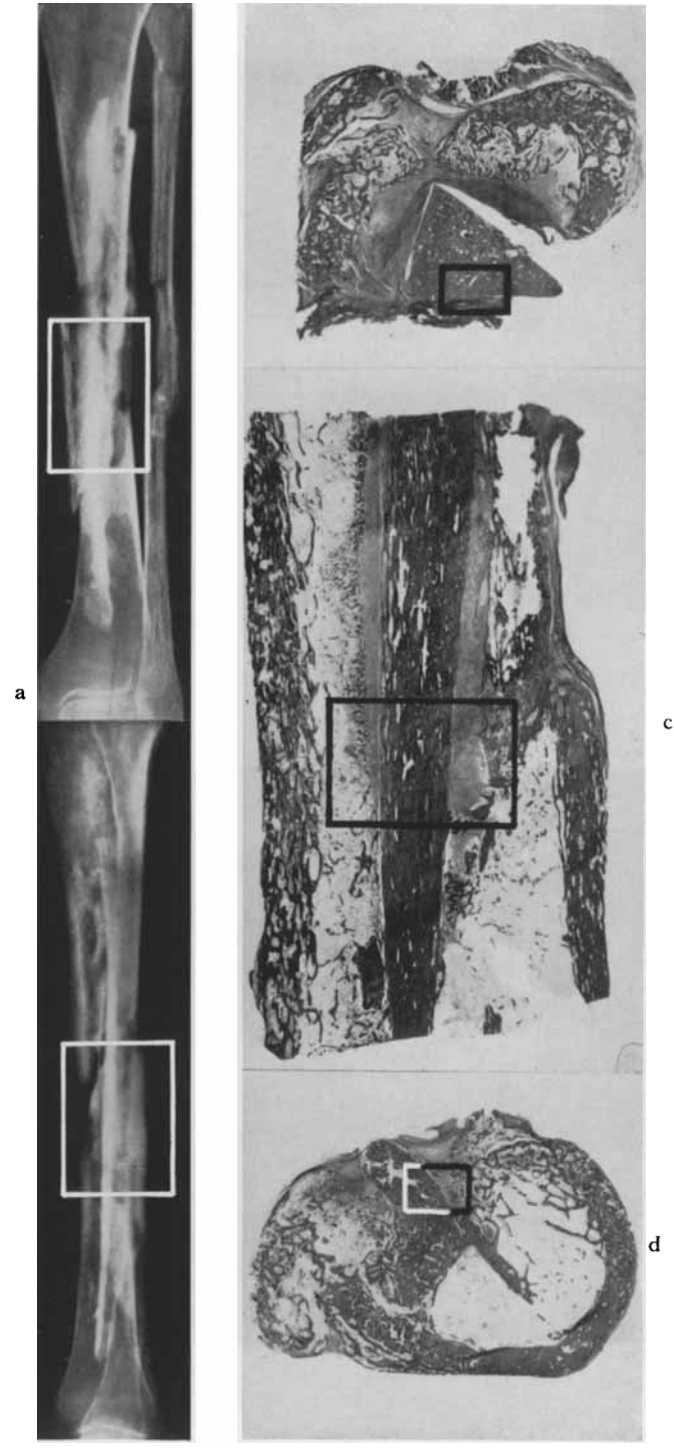
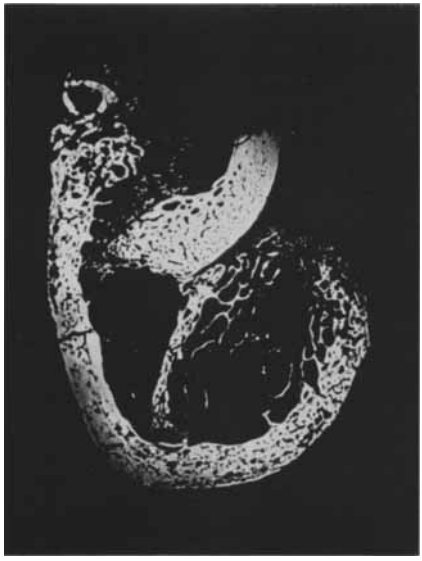
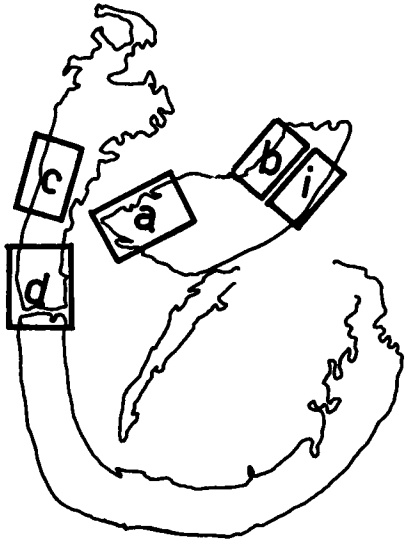
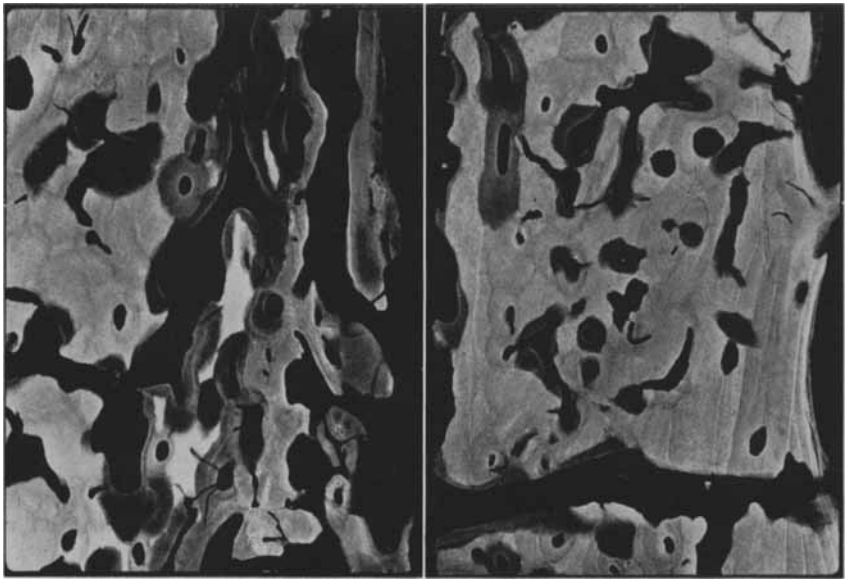


Figure 2







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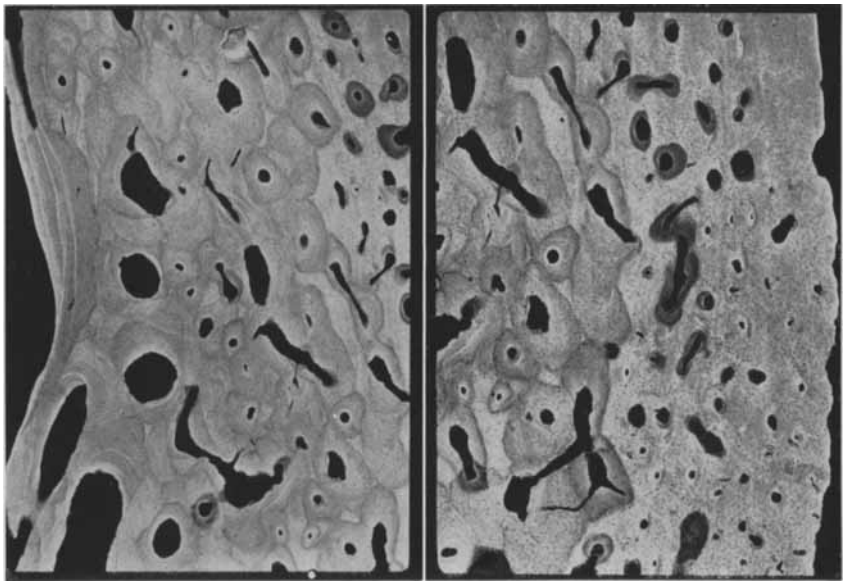

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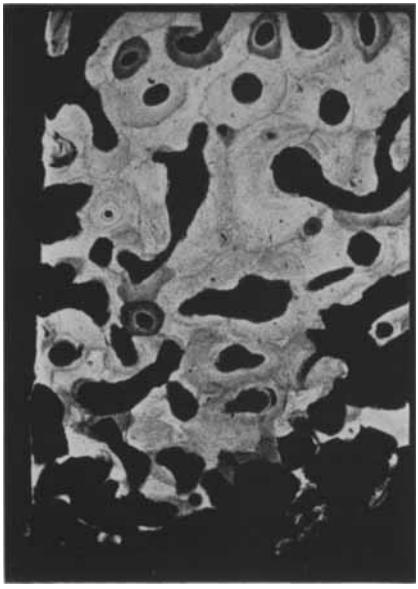
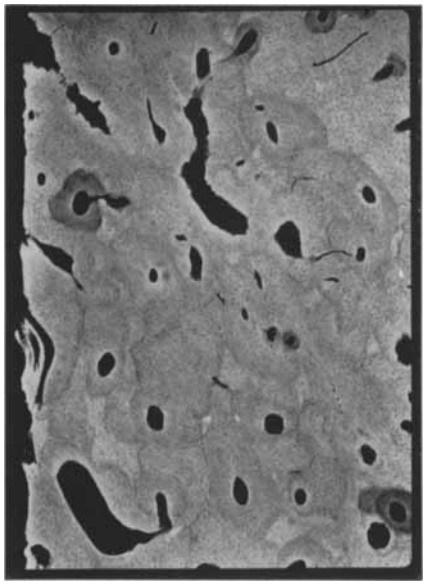


a

Figure 3. Case No. 1. The graft is situated at the centre, it has a semilunar shape in the opening of the tibial cortical layer that surrounds it. The scheme indicates the areas in the microradiographs below. c, d) the bone of the bed. Destruction precedes the reconstruction which, however, can already be discerned in many areas. The bed is in the process of altering and on macroscopic examination appears to be porotic. b, i) When these figures are compared with figure a, they show the difference in the behaviour of the graft at different sites. Both b and i refer to the area corresponding to the cortical layer through which the weight-bearing lines pass, and the graft already shows the formation of new osteons. Area a on the other hand mostly shows destruction of bone, vide the large diffused cavities, and this is because it is situated in the medullary canal which, physiologically, does not tolerate foreign bodies.

Figure 4. Case No. 1. The same case as shown in Figure 2; a section that lies farther distally than the one shown above. The graft has a triangular shape and is outlined by the tibial cortical bone of the bed. The scheme next to the fig. shows the areas on the microphotographs below. e) an example of an area in which the density of 100 osteons was measured by the photo-densitometric method. They are grouped in the diagram next to the fig. in batches of increasing density so that the diagram plots the numbers of the osteons against their density (0.91–1.00) and shows the osteons to be really old. In this area the graft is not in contact with the tissue of the bed, there has been no creeping invasion and it remains excluded and unchanged from the moment of operation. a) in this area we find a number of osteons of low or very low density (0.60–0.80), which shows that there has been creeping invasion and osteogenic regeneration.

Figure 5. A direct comparison of the density of the primary bone of the bed with that of the primary bone of the graft showing that the graft, especially if it is excluded, has on the average a 10 to 13 per cent higher density than the living bone of the bed. The scheme indicates the areas from which the samples have been taken (L = the bed; T = the graft).—Both are included in the same fragment (b), above the graft, below the bed. a): the photometric curve of the scale of comparison; c) the densitometric figures. As can be seen the primary and interstitial bone shows the higher values in the section of the graft. Since the two sections have precisely the same thickness (100 μ) we can conclude that the density of the graft is greater than that of the bone of the bed. *(See page 22–23).*



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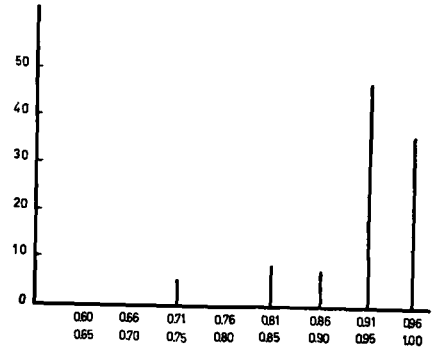
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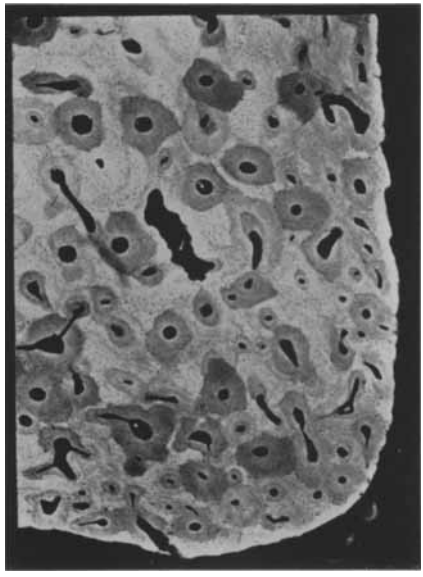

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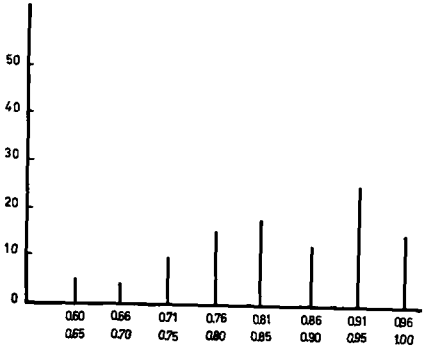
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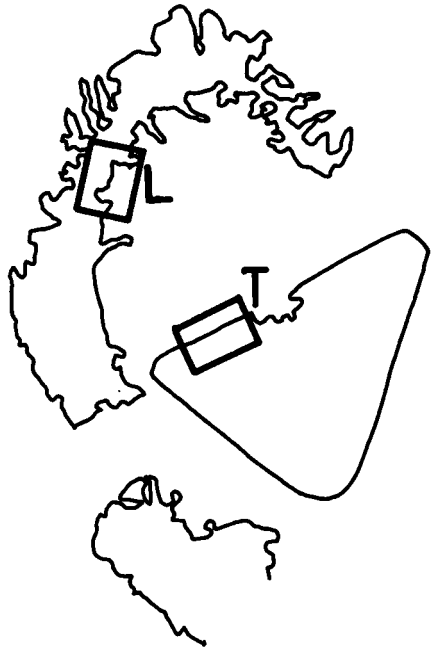

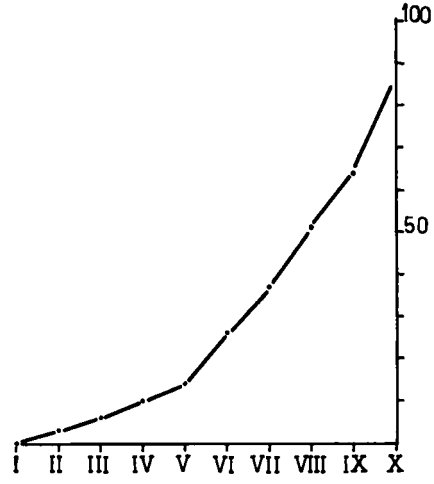

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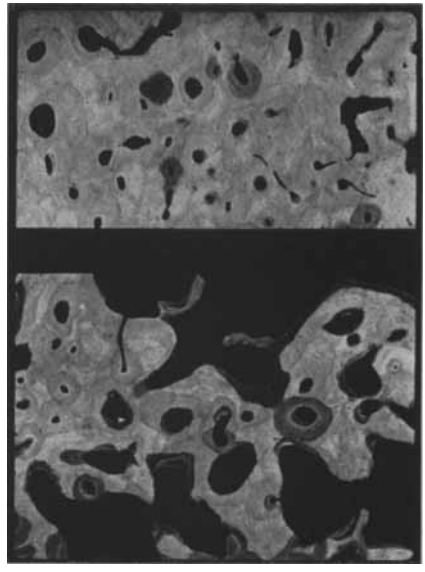

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Figure 5 (For text see page 20).

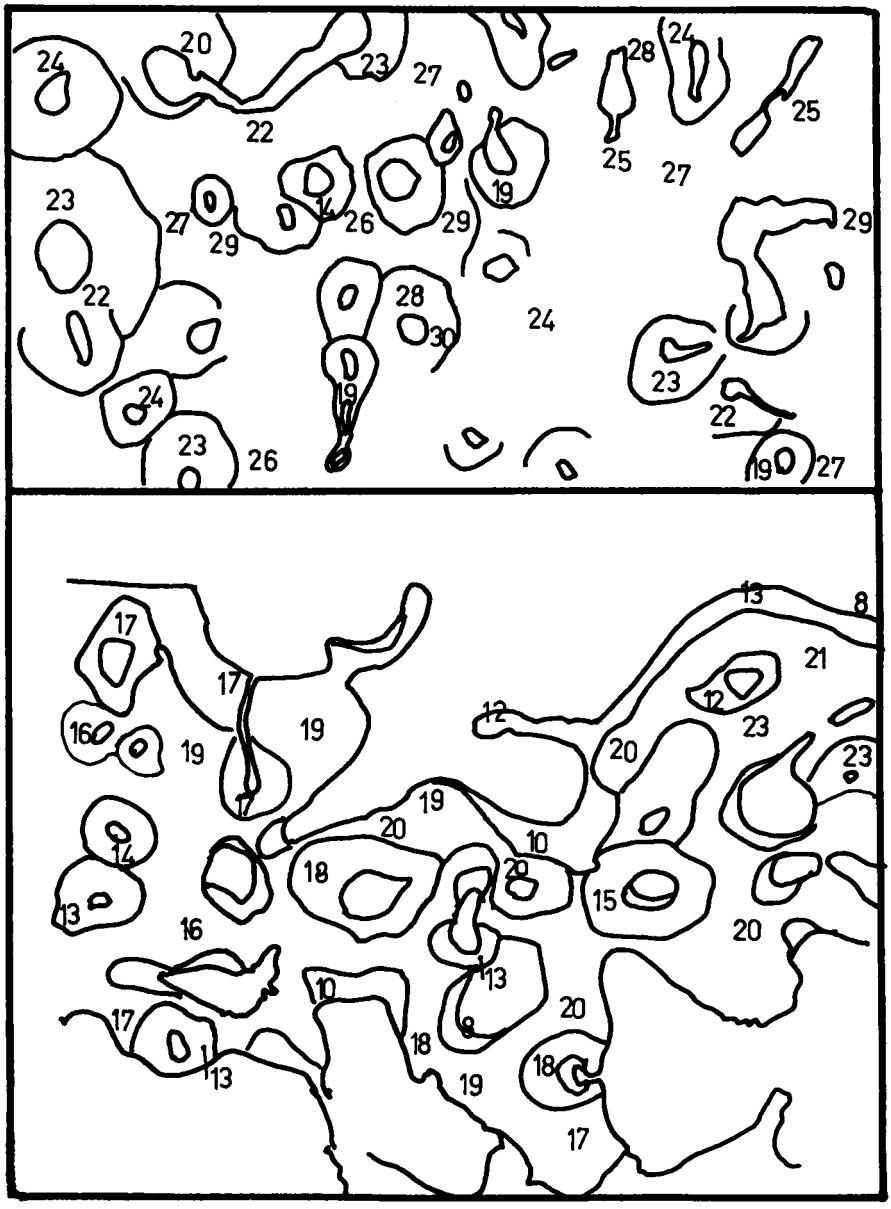

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Figure 5 (For text see page 20).

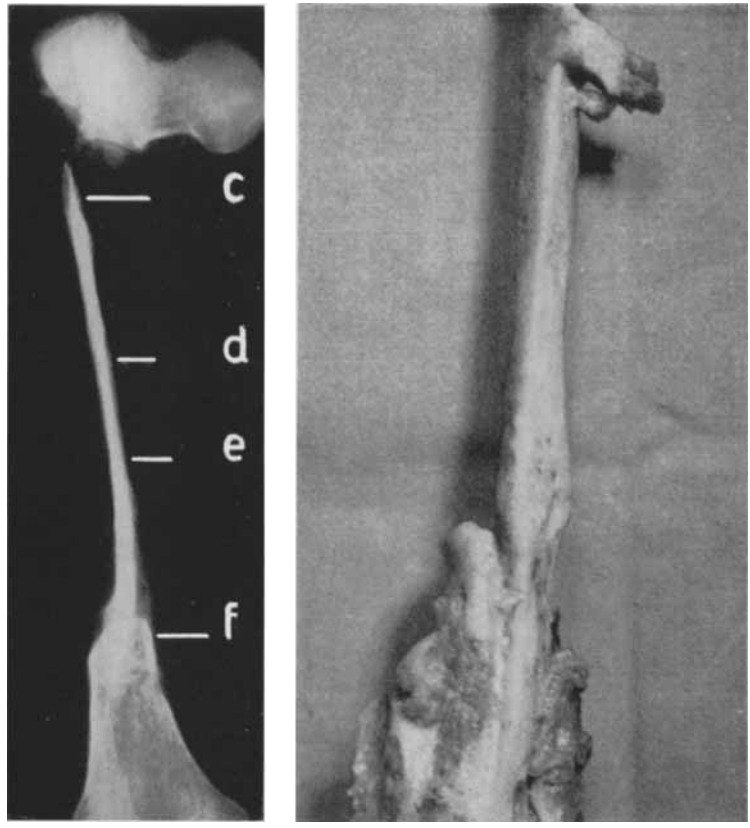


Figure 6. Case No. 2. A woman aged 48 years subjected 11 months previously to a femoral resection for parostal sarcoma. Disarticulation for recurrence. The levels of the various sections are indicated. b) Appearance of the fragment lying between levels e and f, largely free from connections with the surrounding soft parts, in other words, excluded.

For the present study we prepared 6 transverse sections taken from different levels, plus fragments for micro-radiographic and histological examination and for examination with the Ca^{45} isotope in vitro and in polarized light.

Case 3 (Figures 11–17)

A woman aged 36 years. The lower third of the tibia was resected because of a giant-cell sarcoma (October 1957). A 25 cm bone graft was taken from the contralateral tibia; it included the anterior crest. The graft was inserted into the diaphyseal medullary canal of the tibia and into the body of the astragalus and calcaneus. A plaster cast was fitted. Three months later, weight-bearing was allowed, still in the plaster cast. After 9 months, a fracture occurred at the upper pole of the graft. After one year the patient was allowed to walk with bivalved plaster cast.

Sixteen months after the operation, a local recurrence was diagnosed with extensive

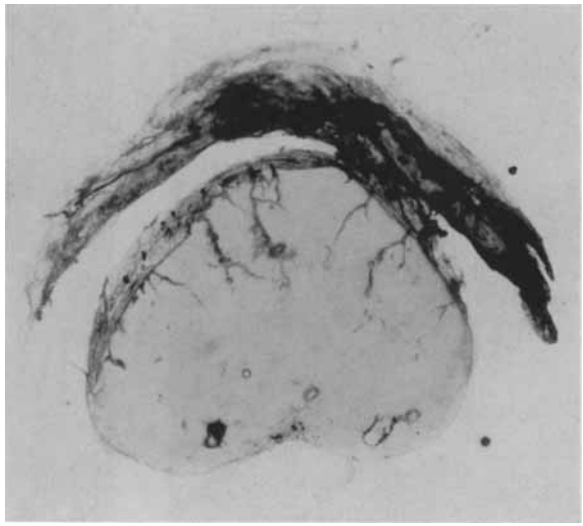


Figure 7. A section of the graft at the level corresponding to section d. Immediately after the disarticulation the femoral artery was injected with soluble Berlin blue. The cross section shows the vascular penetration (the injected vessels stand out against the transparent fragment) from the soft parts—connective tissue and loose muscular tissue which adhere to the graft; in the course of preparation of the fragment the soft parts have been partially detached from the graft.

involvement of the soft parts of the lower third of the leg. On X-ray examination the graft did not appear to be affected and indeed it proved to be free during the subsequent macroscopical examination (Figure 11). Amputation through the thigh was performed, so the graft was recovered.

On macroscopical examination the graft was found to have become firmly fused at both the proximal and the distal end, in the tibial medullary canal and in the astragalus respectively. Its central part was enveloped by thick fibrous tissue which, in only a few spots, connected with the graft itself so that some manoeuvering was required before it could be detached with a knife. In most places, however, the sheath was loose from the graft although it was lying against it without free space between.

Five transverse sections were taken at different levels, for the microradiographic and histological examination.

Case 4 (Figures 18–23)

A boy aged 11 years. Myxoma of the lower metaphysis of the left femur. In May 1958 the area of the tumour was resected and in the opening two grafts of compact bone from the tibia were introduced. Healing was by first intention. The patient walked without a plaster cast 5 months after the operation (Figure 18). Eighteen months after the operation there were signs of local recurrence of the tumour, clinical as well as radiographic signs, the latter consisting in two fairly small lacunae in the spongy bone of the metaphysis that had been left in situ. Total resection of the metaphyseal segment was carried out. The segment was freed from the soft parts which adhered firmly to it. The graft was found to have become adapted to its new situation to a considerable extent:; its outline had changed and now resembled the cortical bone which it had replaced (Figures 19 and 20). In coronal section the graft still retained a more eburnated appearance, more compact and denser than the rest of the cortical bone. Two transverse sections and one coronal section were made for microradiographic and histological examination.

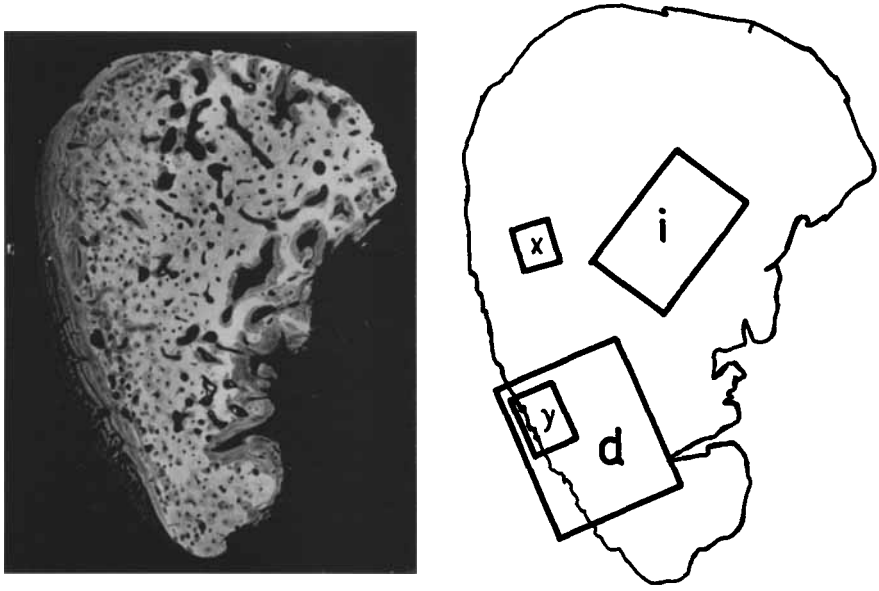


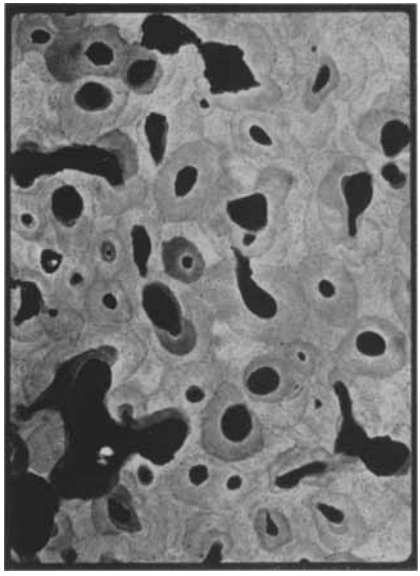
Figure 8. Case No. 2. Cross section in the process of creeping invasion and regeneration. The scheme shows the areas reproduced with greater magnification. i) Predominance of osteons with high density. However, there are also specimens of very young osteons (0.60–0.75) and areas of creeping invasion; most of the area is excluded with the exception of a few parts showing activity. d) Interstitial, osteonic and appositional regeneration on the surface of the graft. The fascia shows active participation, as indicated by the diffuse areas of creeping invasion and destruction and by the large proportion of young osteons of low density.

Case 5 (Figures 24–26)

A woman aged 32 years. In December 1956, 7 cm of the diaphysis of the radius was resected as it was affected by an as yet well-delimited reticulo-sarcoma. The bone was replaced by a cortical graft taken from the anterior surface of the tibia which was introduced above and below the defect into the medullary canal of the radius. A plaster cast was applied.

Three months later, the graft was fused at its proximal end but at the distal end there was a tendency to pseudarthrosis. Ten months after the operation the distal pole of the graft was inspected: it was as thin as a glass rod and there were no connections with the soft parts. 9 months later again distinct signs of recurrence of the tumour were seen at the lower pole. Twenty-two months after the grafting the forearm had to be amputated, so that the graft was recovered.

The radiographic examination (Figure 24) showed that the graft was in an advanced stage of adaptation as far as its upper two-thirds were concerned: the formation of a medullary canal could be seen as well as the external deposition of a cortical layer in continuity with the cortical layer of the radius; the excentric image of greater density corresponding to the original shape of the graft could still be made out. Its distal



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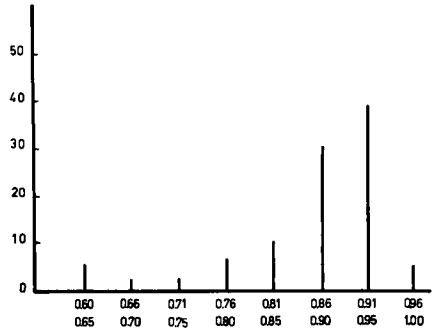
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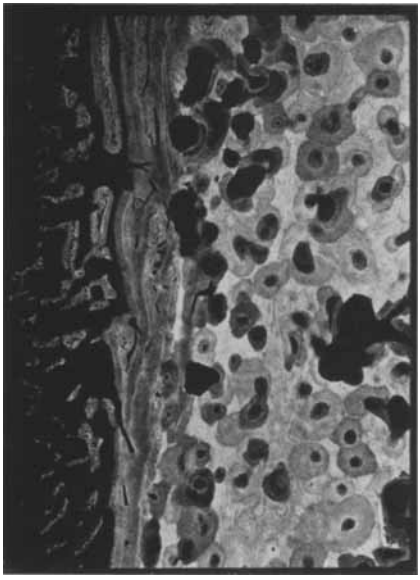

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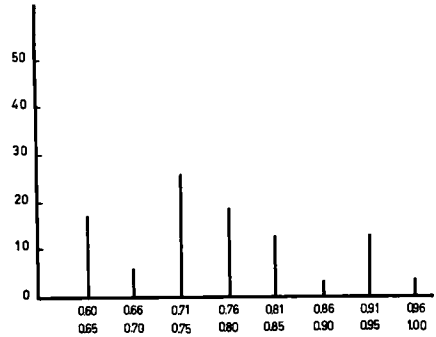
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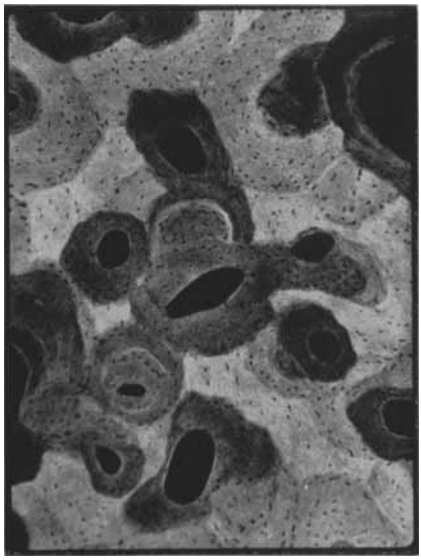


Figure 9. Young osteons with outer layer of low density. The reconstruction has run a fairly irregular course and has given rise to bizarre figures with considerable variability in the degree of mineralization in the same osteonic layer.

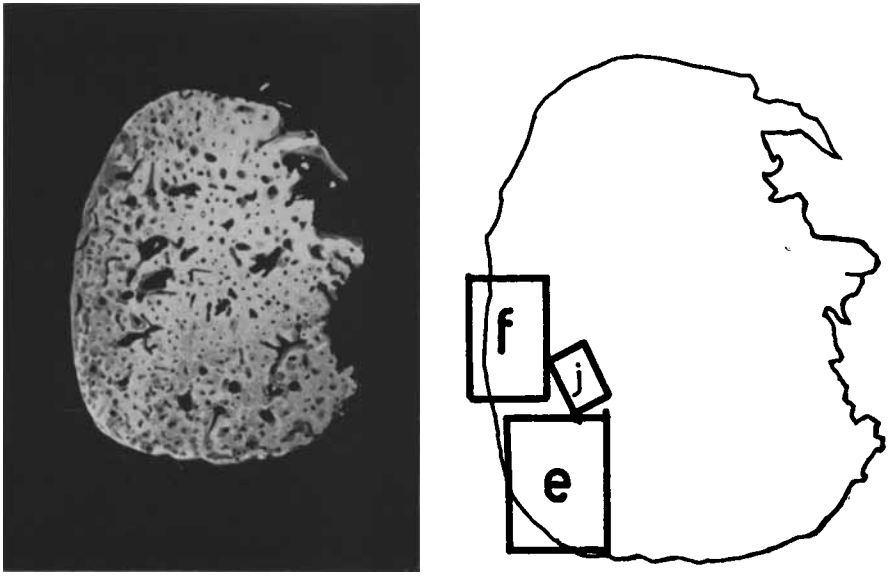
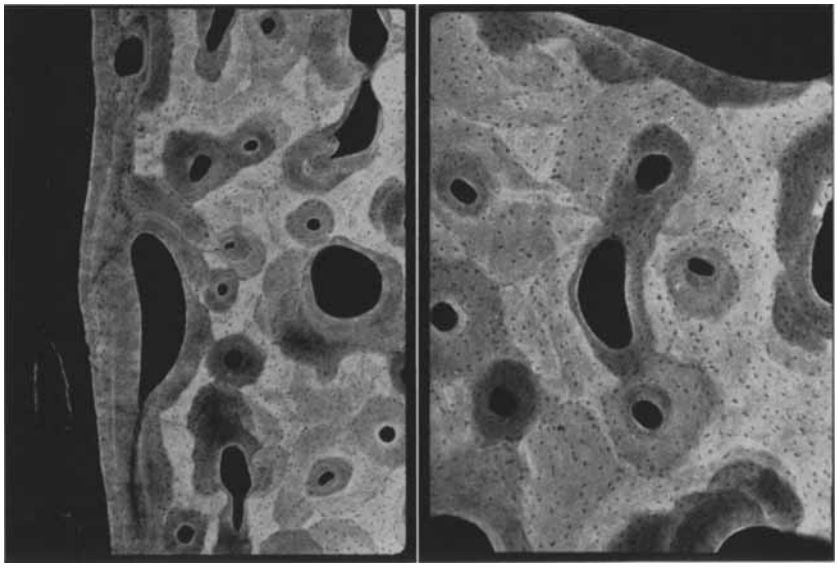


Figure 10.



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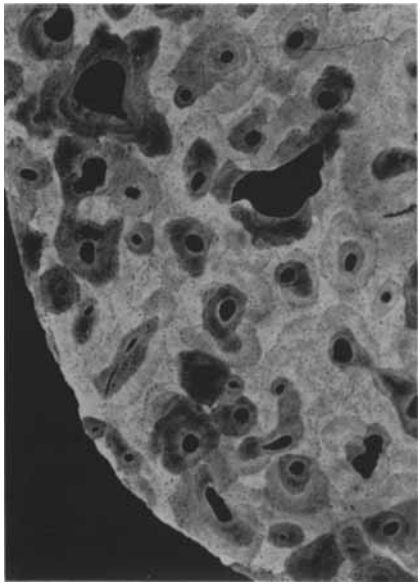

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Figure 10. Case No. 2. Peripheral surface shows osteogenic regeneration, while in the centre the graft has not been influenced by the bed and has remained unchanged. The scheme shows the areas represented with greater magnification: f) the periphery of the graft with relatively young osteons and large areas lined with newly-formed bone. j) Osteons with an irregular course; e) shows the initial stage of the creeping invasion; all the osteons of this section have already been destroyed and replaced by new systems with a low calcium content. Initially, it is just the blood vessels guided by the haversian canals that determine the reconstruction. Later on weight-bearing and function exert their influence.

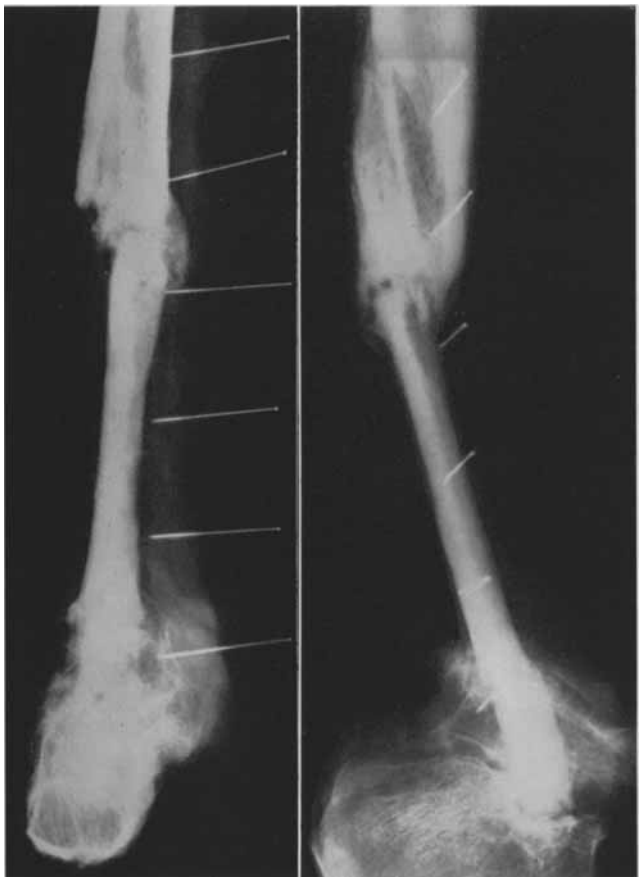


Figure 11. Case No. 3. A woman aged 36 year subjected 16 months previously to rescction of the lower end of the tibia for a giant-cell sarcoma and to tibial autografting. The levels of the sections are indicated.

portion was eroded by the recurrence of the tumour. Macroscopical examination of the fragment revealed total adhesion with firm, non-detachable connections to the musculo-aponeurotic planes around the proximal part of the graft. Toward its distal part the graft was lying free in a sheath that had formed around it from the tumour capsule, while its distal extremity was invaded and eroded by the neoplasm and was without a capsule.

Six transverse sections were made at various levels for histological, micro-radiographic and auto-radiographic examination.

COMMENT

For this study we have used five grafts of cortical bone taken from the antero-medial surface or from the crest of the tibia.

The patients were adults between the ages of 32 and 50 years, with the exception of one 11-year-old child.

In one case grafting was carried out for pseudarthrosis of the tibia; in the other cases the primary lesion was of a neoplastic nature and the resected segment was therefore replaced by a long graft. The grafts had remained in the body for 5, 11, 16, 18 and 22 months, respectively.

As regards the causes of failure and hence the necessity for reoperation and the recovery of the grafts, these were in one case the death of the patient and in the others, recurrence of the tumour. The recurrences affected only limited portions of the grafts or the soft parts so that large parts were available for the study of the evolution of the transplanted bone.

CHAPTER IV

Histological and Micro-Radiographical Features of the Various Phases in the Evolution of the Graft

After these elaborate preparations we had at our disposal 24 graft sections ready for micro-radiographical study and an equal number of sections for histological examination.

WORK PROGRAMME AND SUB-DIVISION OF THE MATERIAL

Every micro-X-ray picture section was studied with the microscope and photographed with a magnification of 20 ×, to obtain an orientation map. This micro-X-ray map was compared with the histological section and considered in the light of the macroscopic findings, taking into account its relation with adjacent soft parts and bones (adhesions, firm or loose connections, bone fusion, the situation in regard to the medullary canal or cortical layer, etc.) and it was further compared with the X-ray of the graft (density, rarefaction, festooning, thinning, hypertrophy, etc.).

The histological fragments were taken from areas immediately adjacent to the micro-radiography sections so that they showed very nearly the same general pattern and often the same osteons, some were taken from the same sections that had been used previously for the micro-X-ray pictures and were treated by the special methods described above.

The first factor taken into account was the presence or absence of osteocytes within the lacunae; if they were absent over a large area it was a fairly reliable sign of necrosis in that area. Further, we took into account the histological signs of resorption or new deposition of bone, of fullness of vessels and of connections with adjacent parts.

Each entire section was then sub-divided into parts (at least 20 areas for each section) and each of these was photographed separately with a greater magnification (30–70 diameters). More than 400 micro-photographs were taken covering the entire series of sections and each showing fields approximately 300–1000 μ wide.

In the course of this study we gradually began to distinguish micro-radiographic fields with particular features which corresponded to the histological, macroscopical and radiographical aspects. In this way it was found that a group of micro-radiographic fields, selected for particular factors, always presented histological and radiographic parallelism.

The key to this sub-division lay in the *stage of evolution* of the graft. We may here briefly repeat certain ideas and terms used in connection with the evolution of a graft. In a monograph published in 1958 one of us (Stringa) wrote: "In the evolution of a graft three phases can be distinguished:

1. creeping invasion;
2. osteogenic regeneration;
3. functional adaptation.

This division has a histological justification based on study of grafts in personally conducted experiments, on study of the literature and of grafts in human patients at different intervals after operation. It has also radiographic justification, as proved by the examination of thousands of radiographs. Every radiographic aspect of a graft can be defined and classified in one of these three phases and shows histological correspondence defineable in the same terms.

The term *creeping invasion* means the vascularization and mesenchymal penetration of the dead graft from the bed of the graft, which corresponds to radiographic rarefaction.

The term *osteogenic regeneration* means the new formation of bone on the inside and periphery of the graft, which corresponds to the radiographic signs of interstitial and peripheral bone apposition.

The term *functional adaptation* is used to indicate the structural reorganization of the graft (according to the local requirements) by the normal processes of histological modification which correspond, in the radiograph, to modifications in and different arrangements of the original graft structure.

The three phases follow one another in the order mentioned, but they overlap; there are no sharp dividing lines either in time or in space".

To return to our present study, we can confirm that each field of the sections examined, whether small or large, could be classified under one of these three headings: creeping invasion, osteogenic regeneration or functional adaptation, with their several histological and radiographic analogies and parallelisms. The stages of evolution of the graft have therefore been used as the basis of the classification, the various aspects have been listed

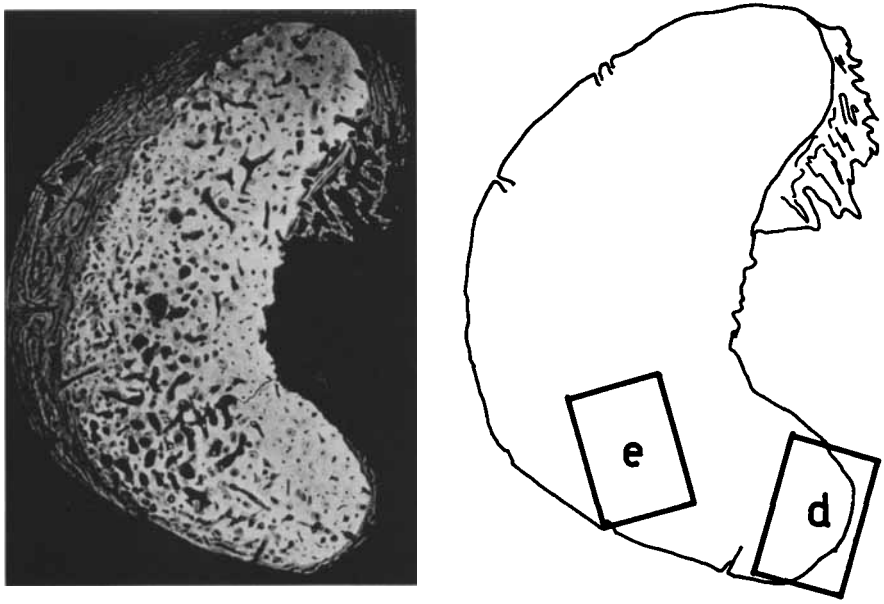


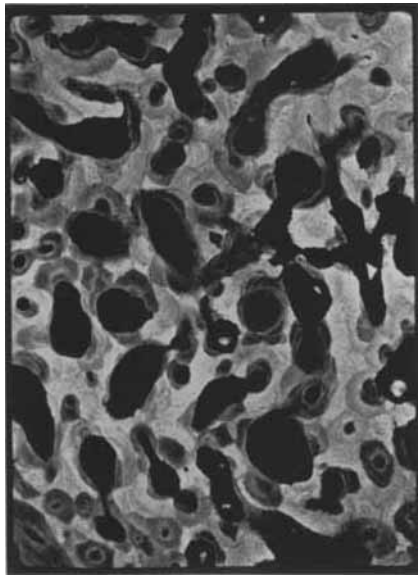
Figure 12. Case No. 3. Extensive appositional regeneration; diffuse areas of creeping invasion in a central fascia. The fascia over the right part of the graft has remained completely excluded, probably due to the effect of the electric saw. e) Area of creeping invasion with destruction cavities, with regeneration and newly-formed osteons. The scheme shows that the osteons of high density have nearly all disappeared and that the greater part of the osteons have a medium density (0.76–0.80): this shows that the process has already been active for some time (a few months); d) a largely excluded part with old unchanged osteons of high density. Still there are some zones showing reconstruction and some apposition.

under the corresponding chapter headings and a differential description has been attempted.

Of course, the various fields were examined not only for their structure but also for their density: the mineral density of the separate parts of the bones was measured by the methods and apparatus described elsewhere.

We also encountered and described certain pathological aspects of the evolution of the bone grafts in our cases. One of these may be present side by side with normal evolution: *resorption* of the graft, *i.e.* its disappearance in places where it is useless or even harmful to the economy of the skeletal segment in question. A second pathological aspect is *exclusion, i.e.* the complete isolation of the graft in the organism, and finally, we also describe the features of *neoplastic invasion* of the graft.

A last chapter deals with the study of the bone of the graft bed.



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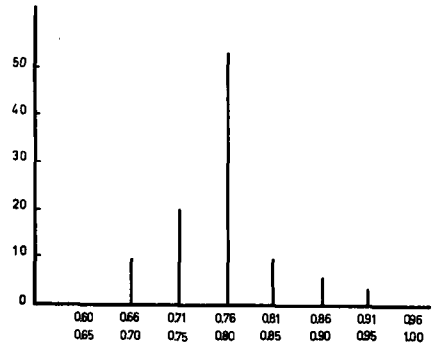
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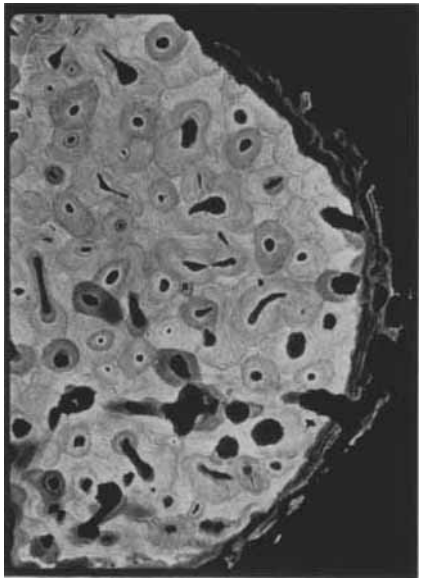

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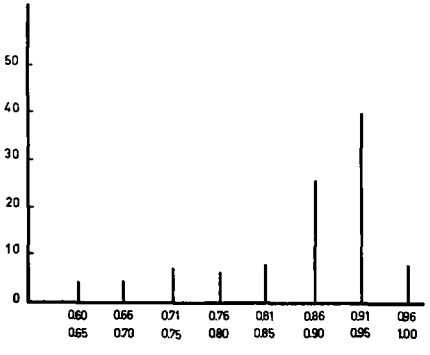
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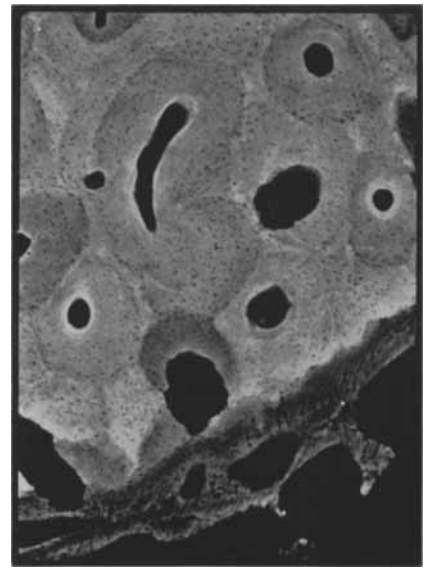
Figure 13. A comparison of the micro-radiograph (a), the auto-radiograph in vitro (b) and the examination in polarized light (c). The lower the degree of mineralization in the micro-radiograph, the higher the degree of uptake of radioactive Ca.



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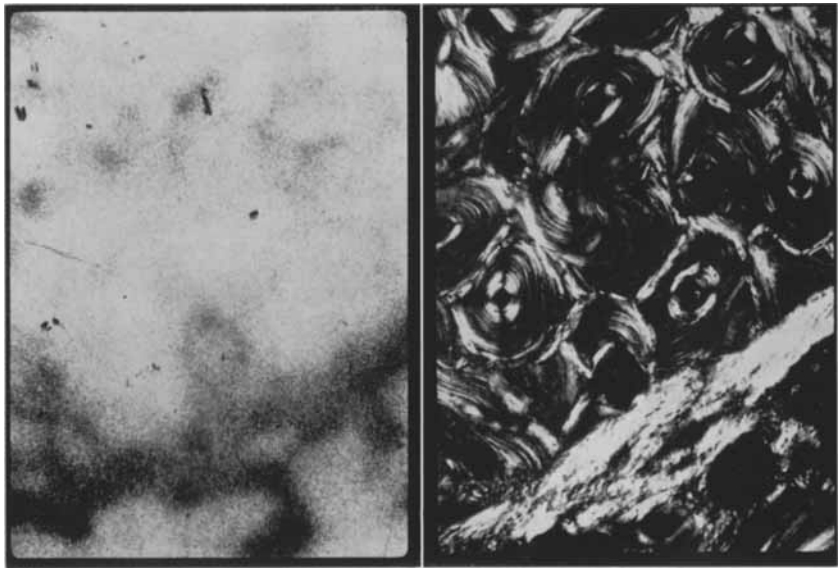
I. THE GRAFT IN THE STAGE OF CREEPING INVASION

We have defined above what we mean by creeping invasion of a graft: the vascularization and penetration by surrounding connective tissue into the graft; the graft itself is essentially dead, *i.e.* it is itself incapable of reaction or change. Once the mesenchyme has come into contact with and has penetrated the graft, the mesenchyme cannot remain indifferent to it: depending on the local and general situation as its first reaction it attempts to destroy the graft. On the micro-X-ray picture, the process of creeping invasion therefore appears as areas of destruction.

Normal microscopical anatomy and micro-radiographic studies of normal tibial (Figure 1) have taught us that the crest of the adult tibia shows a structure of fairly uniformly compact bone with a few rather small areas of destruction and with little difference in degree of mineralization between osteons. We learn about the process of creeping invasion by comparing these normal appearances with our grafts.

We have studied the shapes, sizes, sites, depths and patterns of these areas of destruction.

One might object that a study that essentially concerns "empty" areas, *i.e.* areas of bone destruction, benefits little from micro-radiography and



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there is a great deal of truth in this objection: this chapter might, also up to a point, have been written only on the basis of classical histological methods.

However, there are two aspects that can be elucidated only by microradiographic examination:

1) the degree of calcification (which means the age) of the osteon or of the bone in the process of being destroyed, so that we can be sure that we are not looking at bone that has only just been deposited and will be resorbed again directly;
2) the determination of the state of calcification of the bone that constitutes the absorption front around the destruction cavities so we learn new facts in regard about possible alistheresis.

Factors of the creeping invasion. A frequent observation is the following: the same graft, originally of a uniform calibre and structure, shows different degrees of spread and different types of creeping invasion in places quite close to one another. For instance, in one place the graft may have remained unchanged from the moment of the operation, in an adjacent section only a narrow peripheral tract may have undergone creeping invasion, while in still another section there may be diffuse creeping invasion. What are the causes of these differences?

Clearly certain factors regulate this phenomenon. The factor that exerts its influence first, that is the most important one and the one best proved by observation, is the firm and permanent adhesion to the graft of richly *vascularized tissue:* the anatomical study of the graft and the examination by the classical histological methods show that the areas of creeping invasion belong to a section of the graft that has close organic connections with adjacent muscular structures or with the tissue of the graft bed. Conversily, graft will remain unchanged, without creeping invasion, when a part is free from its environment without stable adhesion to the bed.

Mention may here be made of an interesting observation an our preparations. In all cases we saw a stretch of creeping invasion on the convex side of the graft (Figures 4, 7, 10, 12 and 14) whereas the concave or flat side remained quite unaltered due, in part, to lack of contact with the bed. Appositional regeneration might be seen but no creeping invasion.

It cannot be that in all cases there was a special situation in the limb as the result of which vascularization on one side was much more favoured than on the other. A much more credible factor, present in all cases, was the following: the surface showing creeping invasion was the original surface of the bone graft, *i.e.* the anterior tibial crest with its adjacent surfaces, deprived only of its periosteal lining; the surface showing no creeping invasion was that made with the power saw during the taking of the graft. A possible explanation—and we are not the first to think of it—is that the heat generated by the sawing brings about changes in the bone (*e.g.* coagulation of the canals) as the result of which contact with the bed is impaired.

The second factor is *function*; however, function in these cases at least at the beginning is not weight-bearing with transmission along the graft, because the graft consists of dead bone and accordingly, by definition, does not respond or react to external stimuli in the normal manner. The function

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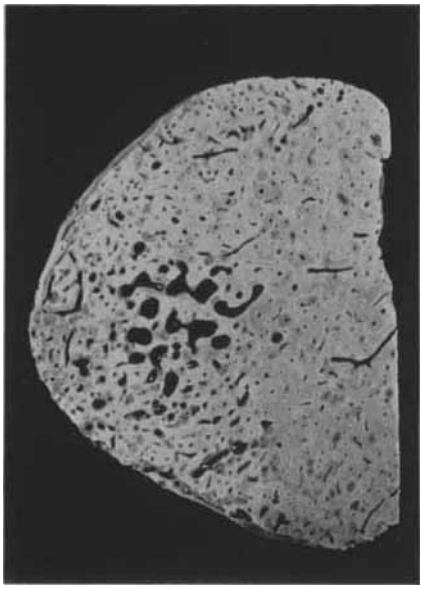
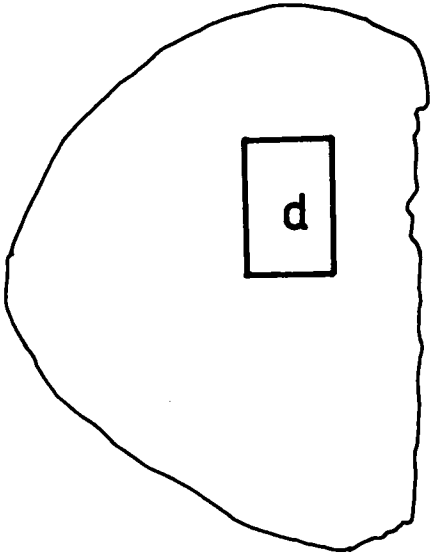
Figure 14. Case No. 3. Very slight central creeping invasion and regeneration in a cross section. We can see clearly the differences of the reaction of the convex side, which is the natural surface of the tibia deprived only of its periosteum, and the concave side which is the surface cut by the power saw: here no changes have taken place 16 months after the operation! d) Excluded area with old osteons of high density. The central part shows creeping invasion; we can see fairly small round areas caused by widening of the haversian canals and irregular, diffuse areas due to extensive destruction and confluence. In view of the time interval since the operation (16 months) and the beginning of function we may speak of adaptation of the graft leading to the formation of a central medullary canal.

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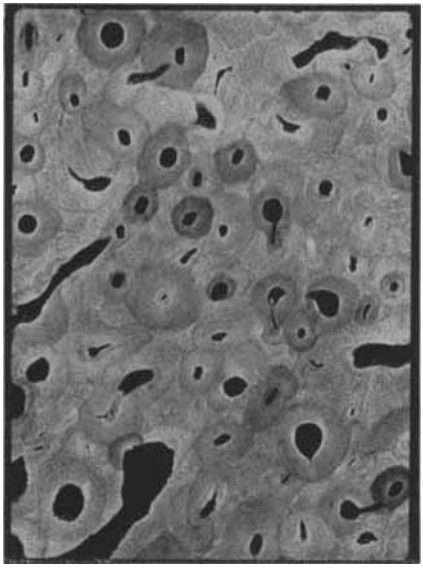

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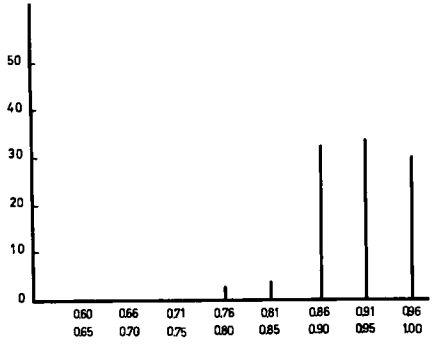
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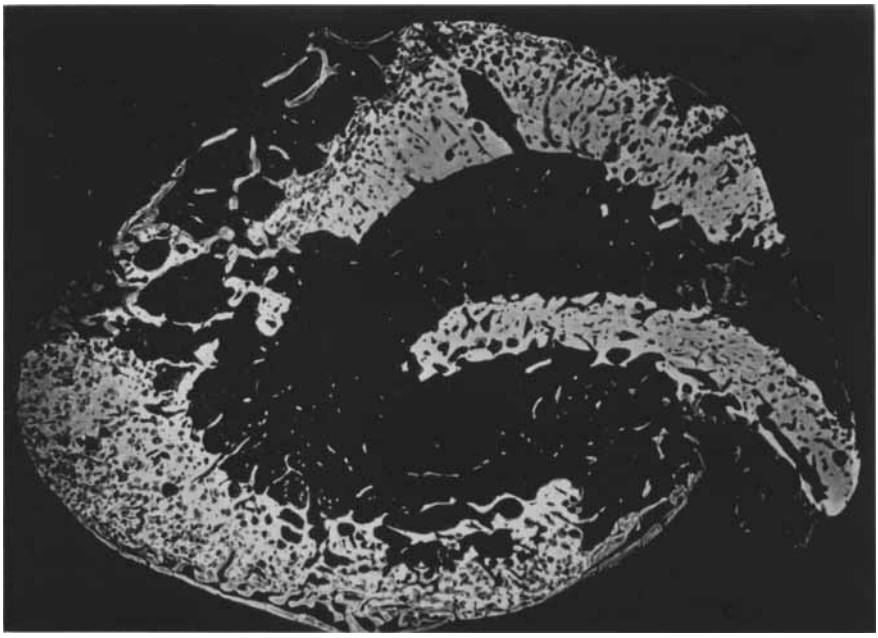
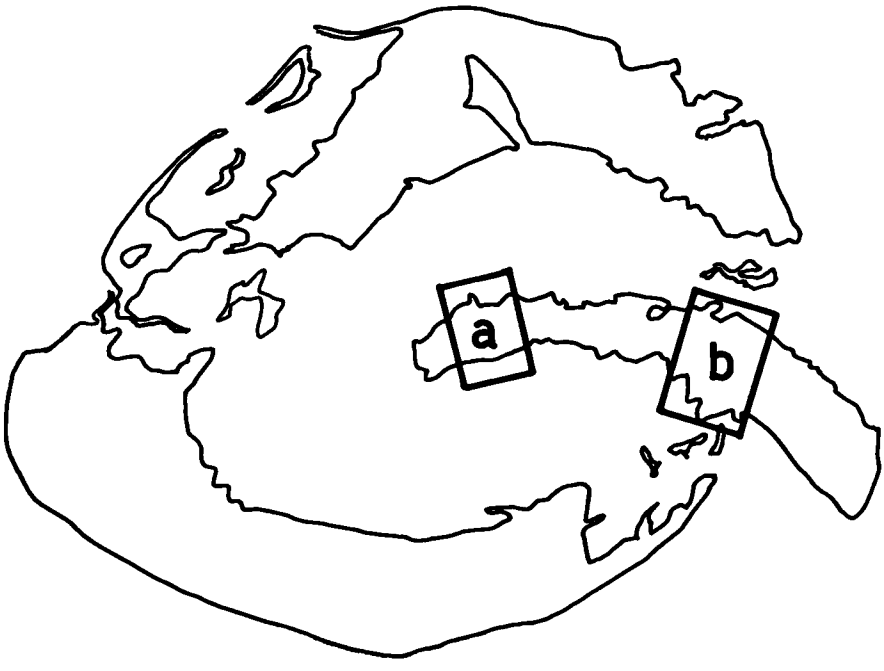


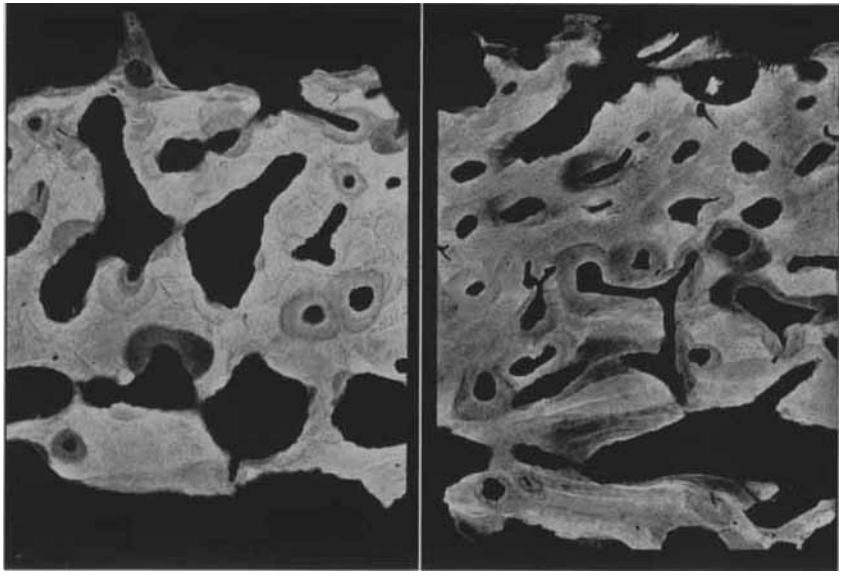
Figure 15. Case No. 3. Semilunar graft straddling an opening made in the cortical layer of the tibia. Note the different behaviour of the graft in different sites. a) In the intramedullary position there is only resorption without new formation. b) In the area of the cortical layer there is advanced regeneration.

is to anchor the surrounding muscular and osseous parts; the more and better-toned the musculature, or the ampler the vascular network of the adjacent living spongy or compact bone, the more intense will be the vascularization that invades the graft, accompanied as it is by young tissue with a high capacity of regeneration.

Patterns of the creeping invasion. The destruction of the graft does not proceed in a straight line from its external surface; on the contrary, the surface in contact with the bed remains intact for a long time while the first osteons extend, and the first newly-formed cavities appear, at some distance from the surface: this confirms the classical conception of "creeping substitution", or "schleichender Ersatz".

Micro-radiographical examination of a section of a graft shows three different features of the creeping invasion (they clearly correspond to the creeping invasion because the cavities are not visible in normal tibiae, and further because histological examination shows them to be lined or filled with young living mesenchyme): a) isolated osteons with a widened cen-





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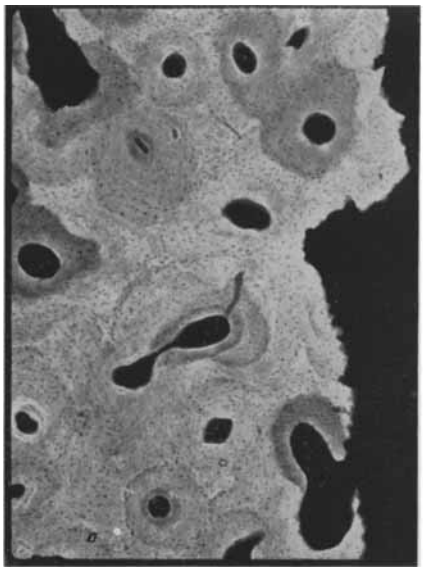


Figure 16. Excluded surface of the graft, without connection with the bed except in small areas where small resorption niches can be seen.

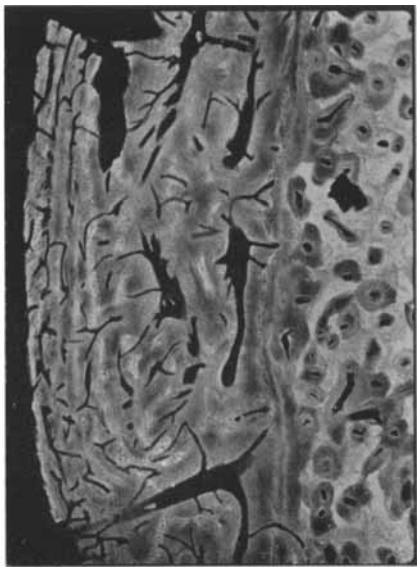


Figure 17. Osteogenic regeneration on the surface of the graft. The black lines indicate the tortuous course of the vessels around which the new bone has been deposited, in some places with interwoven fibres in others with parallel fibres.

tral canal; b) round areas of small size; c) diffuse round or elongated areas of considerable size which may involve half or two-thirds of the entire section. These features of increasing intensity clearly correspond to the duration and quality of the process, mostly to the latter, and the better the adhesion to the bed and the penetrating vascularization, the larger and more wide-spread the areas of destruction.

In a graft with good adhesion and functional stimulation, osteons without creeping invasion are a rare finding. Conversily, few enlarged osteons will be found in grafts that have inadequate contact with their bed.

Inside the osteon the destruction as a rule takes place centrally, viz. the haversian canal grows progressively wider, so that at the periphery only one or a few lamellae remain in contact with the boundary of the system.

The largest areas of destruction are clearly connected with the osteon; one of their ends consists in a haversian canal: evidently the destruction has started at this canal and has progressed excentrically until it passes beyond the confines of the haversian system (Figure 8d); the absorption

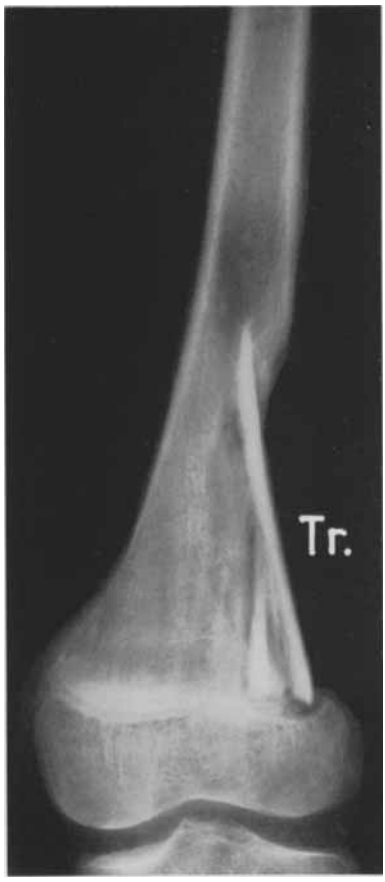


Figure 18. Case No. 4. A boy aged 11 years subjected two months previously to excision of a myxoma of the lower metaphysis of the femur, with transplantation of two cortical tibial autografts.

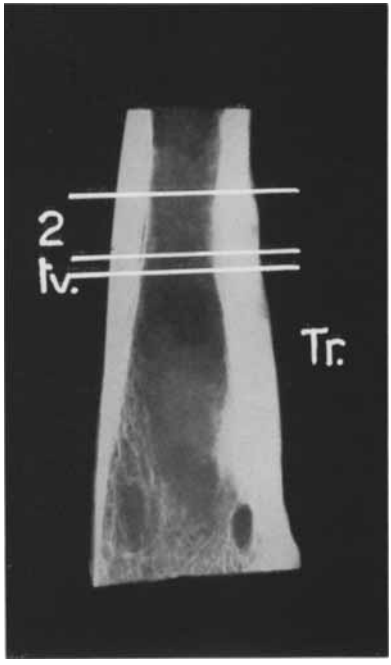


Figure 19. Because of local recurrence the entire segment had to be removed 18 months after the first operation. Tr indicates the site of the graft. The numeral 2 indicates the area shown in Figure 20. Tv indicates the cross section shown in Figure 22.

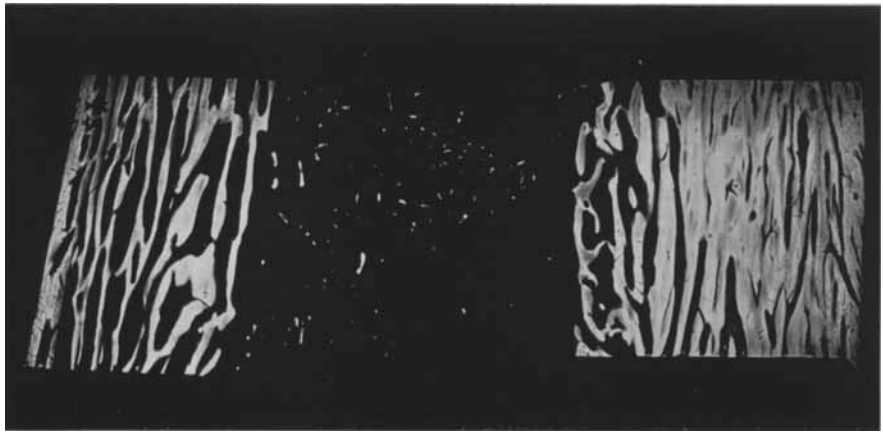


Figure 20. Coronal section of the diaphysis. On the right we can see the graft which is more compact whereas the cortical layer of the bed, on the left, is porotic.

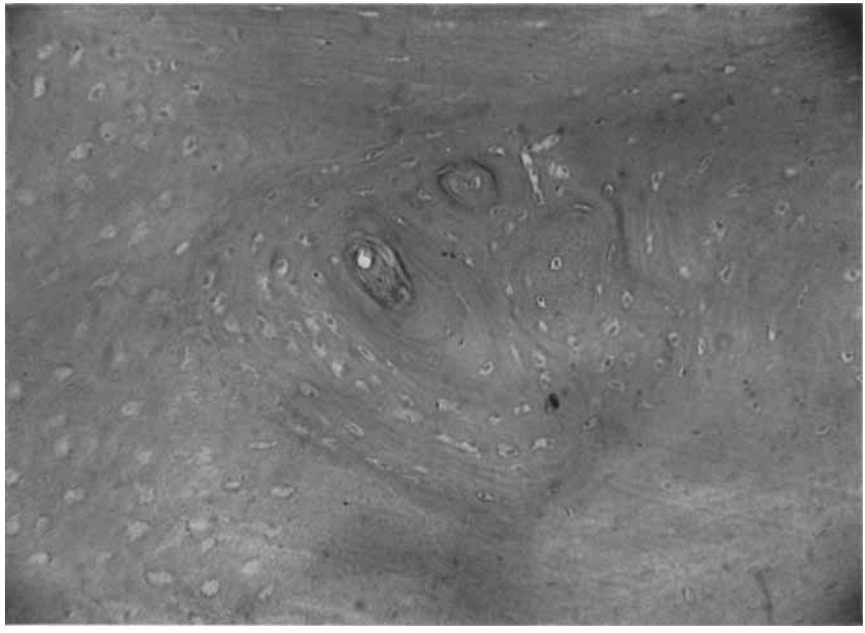


Figure 21. Microphotograph of the same case: in the centre, an island of newly-formed bone in which we can discern the osteocytary lacunae containing the osteocytes, whereas the lucunae of the surrounding bone are empty and show the death of the graft.

areas have highly irregular contours, as the destruction affects primary-breach bone and other systems. The cementing lines of adjacent osteons are not spared and destruction can spread taking no account of pre-existent structures.

The pattern of these cavities is variable and irregular (Figure 10); is sometimes elongated in the direction of a diameter of the graft and sometimes in other directions; sometimes parallel to the surface and sometimes perpendicular to it or oblique. No explanation has been found as to why the destruction proceeds in one direction rather than in another. Outside the haversian canals, from which the process originates, the pre-existent structures do not determine the progress of the destruction; we have no reason to believe that other factors, such as the general mechanics of the segment or conditions in adjacent soft parts, are of decisive influence.

There are a few clear exceptions only where we see how the cementing line of a system halts the destruction surrounding it and makes it to proceed in another direction. The same sometimes happens when the bone is periosteal: then areas of destruction extend (Figure 26 f) along two confining lines of the lamellar stratification and do not cross these lines. However, this is only seen in advanced cases in which functional adaptation is undoubtedly already exerting its influence. In cavities that have opened up and that are in the process of destruction the edge is not of a greater density, as is the case in the central canal of an osteon in the process of deposition.

Degree of spread and velocity of the creeping invasion. The creeping invasion begins in a peripheral tract of the graft; this tract varies depending on biological and mechanical conditions.

A long cortical tibial graft with little adhesion to the soft parts and little mechanical stimulation (for instance a graft replacing a segment of the femur) shows creeping invasion after 11 months (and then only irregularly in isolated osteons) in a peripheral tract 150–300 μ wide; the central part of the graft may still be regarded as excluded.

A similar graft 1½ years after transplantation shows widening of the creeping-invasion tract to half a millimetre with the exclusion of the centre still present. When an identical cortical graft is transplanted into a better bed (the forearm) it may present complete creeping invasion after only 6 months.

The creeping invasion (we shall continue to use this term for the destruction that is the only alteration visible in the micro-X-ray picture) continues for months and years after the operation such as to fully utilize the graft. Once living tissue has penetrated the graft, this tissue can no longer

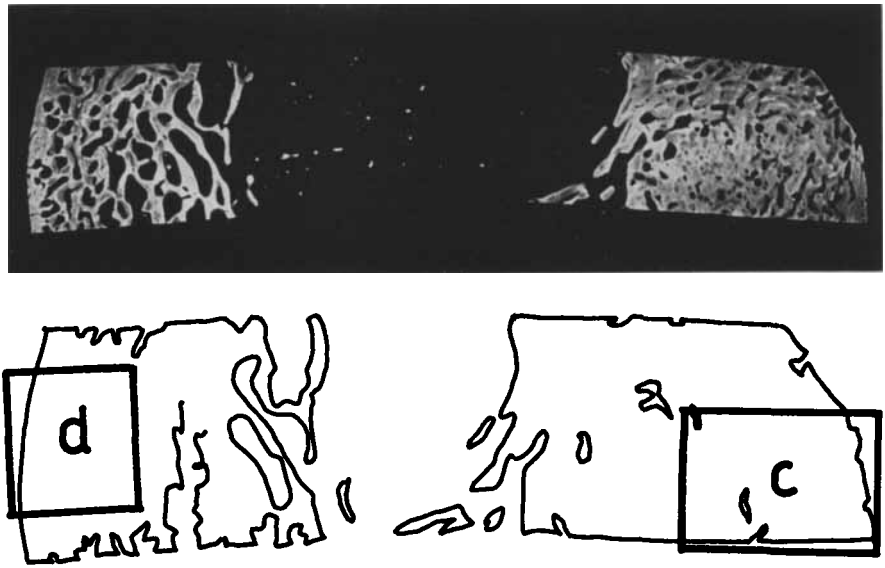
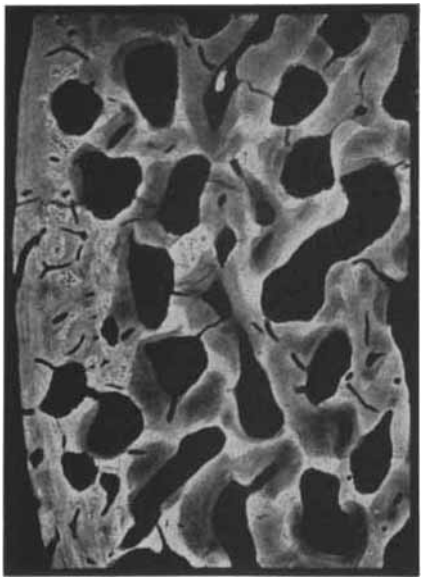


Figure 22. Case No. 4. Cross section: on the right, the more compact area that represents the graft surrounded by newly-formed bone; on the left (d) we can see the cortical bone of the bed. d) Osteoporosis and new formation of bone (osteons of lamellar bone with a low degree of mineralization) in the cortical layer of the bed. c) Newly-formed bone at the periphery of the graft. The graph shows that the phase of reconstruction predominates because of the large number of osteons with a low degree of mineralization.

remain indifferent; if the graft has a function, new bone will be deposited on it (this is the phase of osteogenic regeneration); if it has no function, the organism destroys it completely (this is the resorption phase).

Some 3 to 5 months after the operation we begin to see signs of the deposition of new bone on the cavity walls; when some 12 months have passed, three-quarters or four-fifths of the cavities show new bone at their margins, a sign that the construction process has for some time been predominating over destruction.

How long and how far does the destruction go on? The extension and duration of the creeping invasion vary greatly, depending on the patient's age and the site of the graft. A cortical graft transplanted into a femoral diaphysis of an adult may still show attempts at creeping invasion after 5 years, while at least 50 per cent of the primary bone of the graft will still be present. In a child there may be only 10 per cent of the primary cortical bone left 12 months after the operation.



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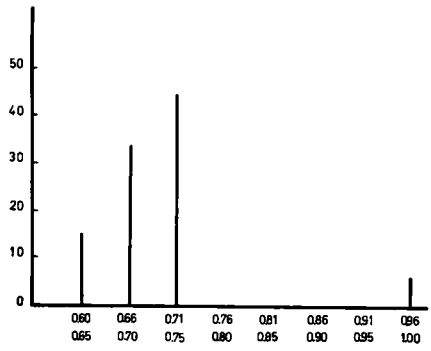
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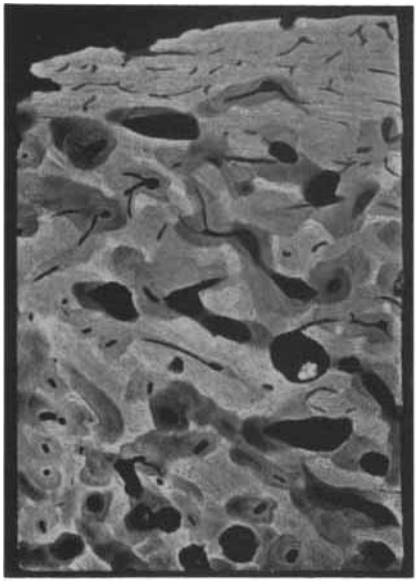

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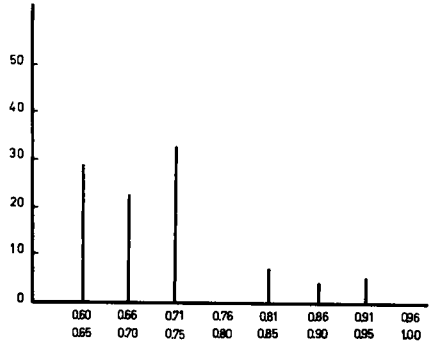
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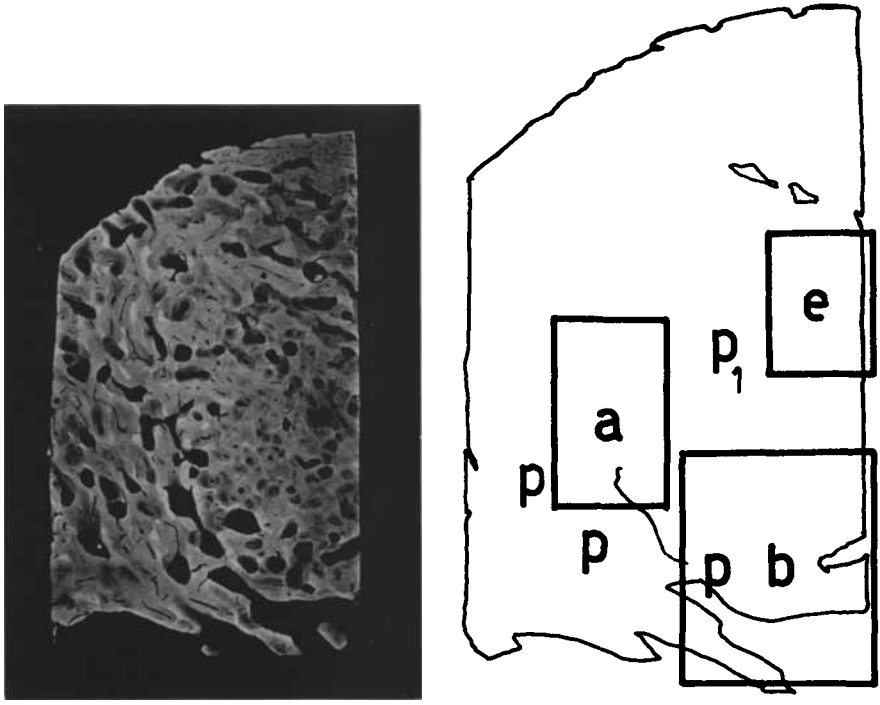
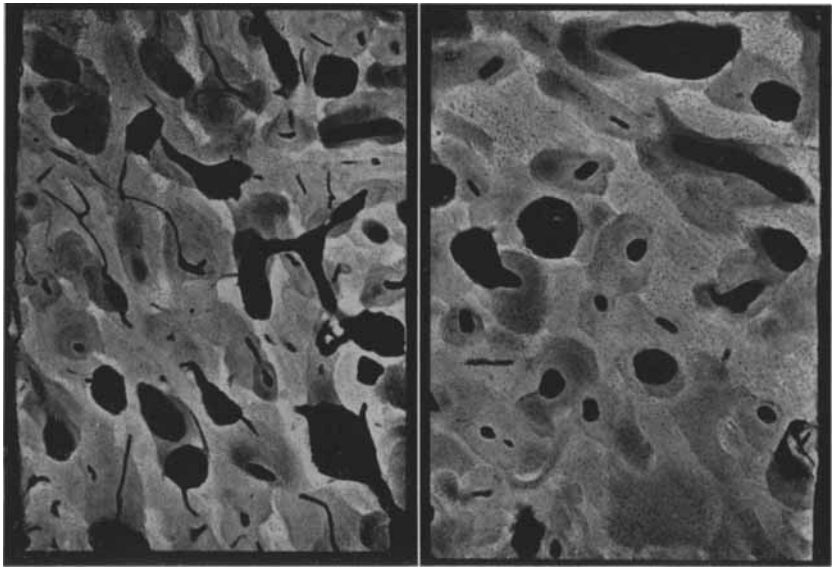


The extension of the creeping invasion naturally also varies according to site and mechanical requirements. If a graft is completely subjected to stress lines it will become entirely occupied by creeping invasion and subsequently "processed" until it has completely disappeared, having been gradually replaced by new living structures that form in and around it.

If the graft, although situated in the stress lines, is surrounded by weight-bearing lines, it may remain annexted indefinitely without tissues being able to penetrate into it as the creeping invasion. This phenomenon can clearly be seen in one of our cases (Figure 23).

Densitometry. Important information can be obtained from study of the density of the bone around the resorption cavities: the zone of bone in contact with the destruction line shows no demineralization and has the same mineral content as the adjoining areas. This indicates that the absorption takes place frontally, cell by cell, similarly to that which has been described by Amprino and Engström (1952) for the normal tibia, without any preceding demineralization of a tract adjacent to the resorption front (Figure 15 a).



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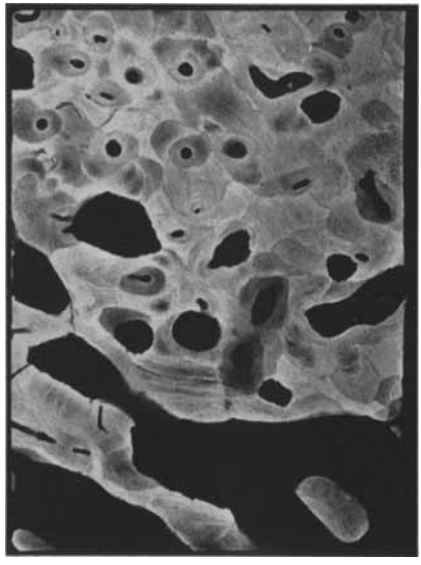

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Figure 23. Case No. 4. The central part, compact, with old osteons, represents the original graft which has changed only little and may be regarded as a scar in the new cortical bone that surrounds it. a) Newly-formed area. e) The periphery of the old graft. b) Another peripheral area of the old graft (above).

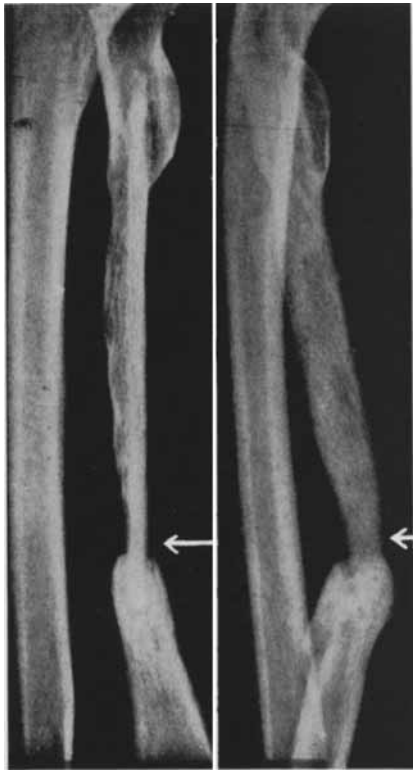
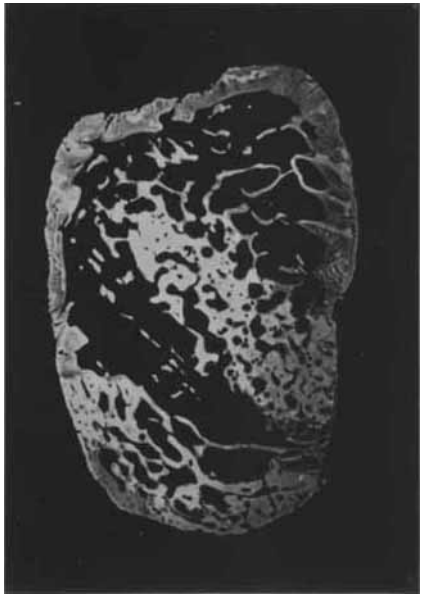


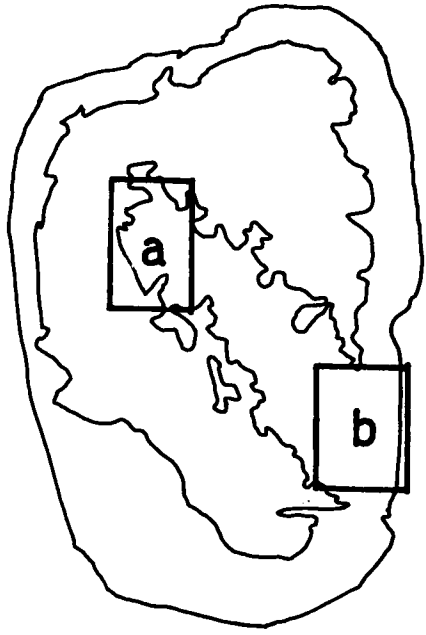
Figure 24. Case No. V. A woman aged 32 yr. subjected to resection of the radial diaphysis for a reticulo-sarcoma, followed by the insertion of a cortical tibial autograft. Amputation of the forearm for recurrence 22 months after the first grafting.

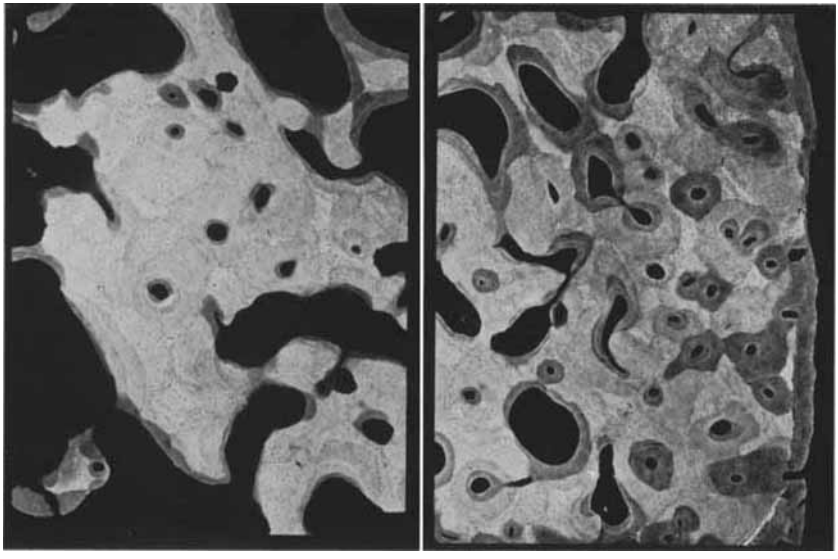
II. THE GRAFT IN THE STAGE OF OSTEOGENIC REGENERATION

When we begin to make out newly-formed bone on the surface of or behind the transplanted bone, we may speak of the phase of "osteogenic regeneration of the graft". This phenomenon can be seen on examination of histological preparations stained by classical methods, in that there is a difference between the appearance of the transplanted bone which shows empty osteocytary lacunae and is therefore dead, and the newly-deposited bone which presents living osteocytes and the surfaces of old bone covered with newly-formed bone or noncalcified margines. In the micro-X-ray

Figure 25. The graft in the cortical bone of the bed. The scheme shows the two areas under greater magnification. a) Intramedullary area with diffuse resorption; b) here the weight-bearing function has caused adaptation and diffuse reconstruction of bone can be seen (young osteons with a very low degree of mineralization).







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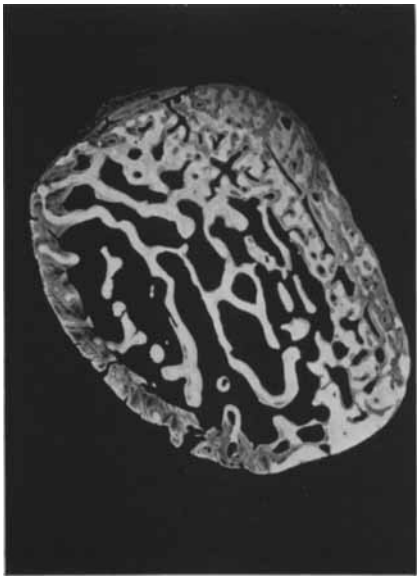

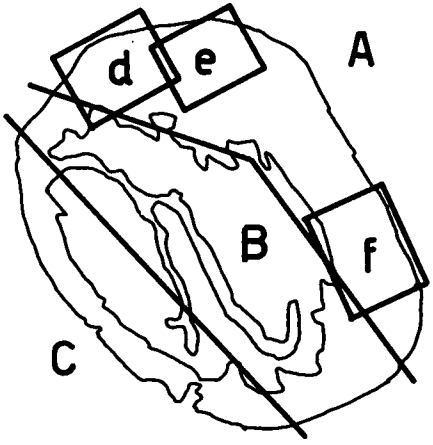

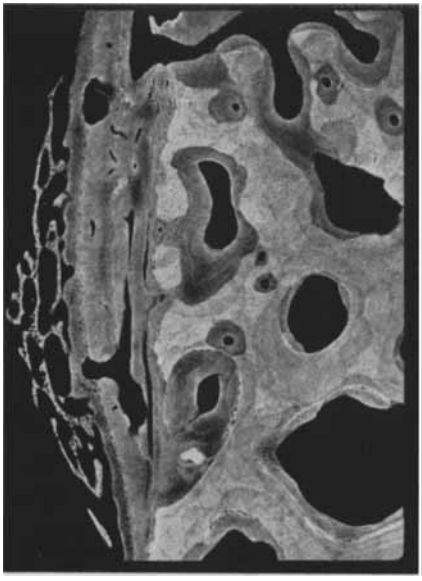

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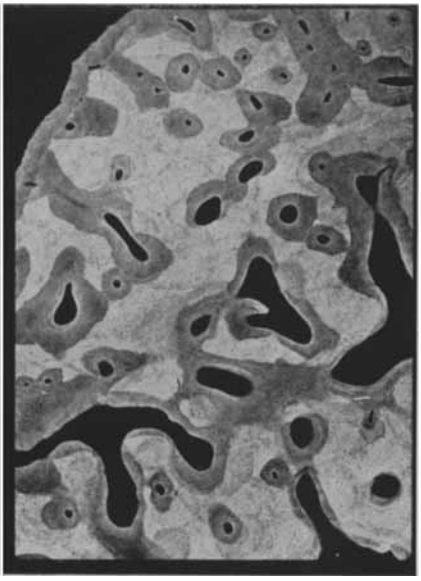

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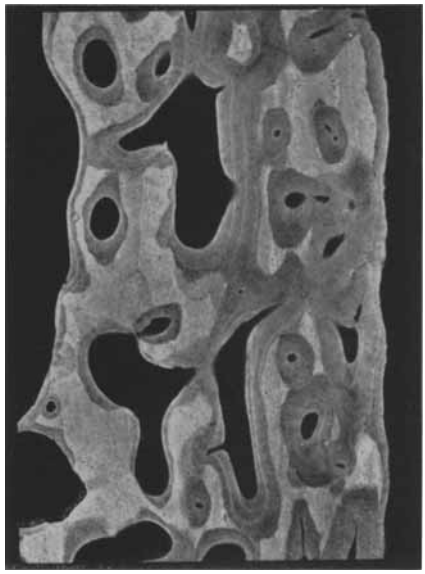

f

Figure 26. Case No. 5. Late stage of functional adaptation of the graft. Zone A represents the old part of the graft which, because it lies in the weight-bearing line, has been subject to creeping invasion and to interstitial and appositional regeneration (d, e, f); zone B shows that part of the graft that lies in the centre of the medullary canal and is therefore doomed to be destroyed. Zone C is an entirely new part which has formed a new cortical layer.

picture the osteogenic response differs from normal bone of corresponding site and age in that it shows an extraordinary abundance of young osteons and of periosteal bone which has a low calcium content, and is thus in the process of being formed.

In this chapter, reference is made to the newly-formed bone of a graft in relation to various factors: vascularization, the site of the graft, the function of the graft, the time since the operation, and the density and mineral content of the individual newly-formed parts.

Even during a first cursory examination we can easily make out osteogenic regeneration in the thickness of the graft (interstitial regeneration) and outside the margins of the graft (appositional regeneration).

1. THE INTERSTITIAL REGENERATION

a) *Structural aspects.* The study of the preparations shows that it is the vascular connection with the bed and nothing else that predominantly determines the new formation of bone during the initial period (the first few or months, depending on the rapidity of the process). In short, osteogenesis begins at the periphery and is well-developed there while still absent at the centre. Five months after the operation, a peripheral tract of 0.3 mm width

in the cortical graft shows diffuse signs of new bone deposition in the areas where there is good vascular contact whereas the centre of the graft, which may present some areas of resorption, shows no osteogenesis at all (Figures 8, 8 d, 10 e).

What has been said in the preceding chapter about the adverse influence of the power saw on the creeping invasion of the surface of the graft applies to regeneration as well since there can be no worth-while regeneration where there has been no creeping invasion. The parts of the graft adjacent to the sawn surfaces therefore remain cold, without colonization or osteogenesis (Figure 14). There may, however, occur appositional regeneration of which we shall speak below.

During the earliest stages after operation regeneration bone can be seen to form in areas where it can no longer be found later on, for instance in the medullary cavity of a hollow bone (Figure 4 a) or in the spongy bone of a metaphysis. This proves that the earliest stages of the development of a graft are determined exclusively by circulatory conditions and local metabolism and not by the mechanical laws of skeletal function. Later on however it is these mechanical laws that exert their decisive influence, during the phase that we call the adaptation phase which is marked by the destruction of all osseous formations inside a medullary cavity and of all areas of compact bone inside spongy bone (in accordance with the absolute norms of the economy of the skeleton (Figure 15 a)). Osteogenesis is guided by the blood vessels:; one or more such vessels will be found in a destruction cavity as soon as that cavity has been formed in the dead bone of the graft. The longest axis of the cavity runs parallel to the longest axis of the skeletal segment, *i.e.* parallel to the direction of the haversian canals because the vessels can easily penetrate along these pre-formed channels. The deposition takes place in the form of a lining to the walls of the cavities, sometimes with large, elongated lamellae and sometimes in genuine concentric systems. Typically is seen: an osteon of the graft, which has just been destroyed, being replaced by a new haversian system; this process takes place simultaneously for numerous osteons in the many destruction cavities which have opened up (Figures 8 d, 9, 10 j).

This first stage of regeneration, accordingly, consists only in the replacement of dead osteons by new osteons, without changes in the remaining structures of the interstitial lamellae and of the remnants of the haversian canal.

The blood vessels and the original structure of the graft determine the new deposition of bone during the first stage. The line of stress does not as yet exert any influence. This explains how Holmstrand, in a graft in a

rabbit, could find newly-formed osteons perpendicular to the long axis of the bone and therefore to the weight-bearing direction. All our grafts were transplanted with their length, and therefore their osteons, directed along the axis of the bone; a study of the effect of weight-bearing on the new osteons would therefore be of no value in our cases.

If the process is sufficiently active creeping invasion may destroy isolated osteons and in that case regeneration will be associated with the development of new osteons in the same cylinders which have remained empty; alternatively larger cavities may develop involving several osteons, together with interstitial bone and that of remnants of the haversian canal, in which case the process of regeneration lines the cavity walls with lamellar bone, and deformed osteons are formed with wide coats, either incomplete or delimited by common cementing lines (Figure 10 f). In these larger cavities the vessels run an uneven course and the deposition of new bone is irregular, precisely the same as it is during the first period of the growth of human bone, as described by Amprino and Bairati (1936); using the nomenclature of these authors, the structure in question is frequently defined as "lumpy" (Heuler).

When the process is more intense and the destruction more extensive, blood vessels are very numerous; the number of reconstructed osteons per surface unit is then larger than in the as yet unchanged graft. The ratio may be 2:1 or 3:2. Therefore, in the new bone the surface of the fundamental lamellae is fairly small when compared with that of the osteonic surface (Figure 8 d).

After this initial period, structural changes take place in the graft but it is clear that these are no longer determined, as before by simple first contact with the bed but are related to the function of the segment, *i.e.* the weight-bearing line. For this reason, these alterations are not discussed in this chapter but in connection with functional adaptation, which will be considered below.

b) *Densitometric aspects.* In this paragraph, we have adopted the same classification and ideas that Amprino used in his excellent publication of 1952.

Structures of the 3rd order

The relatively thick sections and the size of the emulsion granules of the films used makes the study of structures of the 3rd order, *i.e.* of the fibre bundles and of the osteo-mucoid between them, next to impossible.

It can be confirmed that the afibrillary osteomucoid contains larger

quantities of minerals than do the bundles of collagen fibres; this can be deduced from the observation that where there are areas of bone with interwoven fibres (in other words bone rich in osteomucoids), especially at the periphery of the graft, the absorption of X-rays is greater than takes place in the areas of lamellar bone.

Structures of the 2nd order

A) *Primary, periosteal bone.* Two fundamental questions arise in the study of primary transplanted bone: the density of the primary bone of the graft as compared with that of the primary appositional bone deposited after the operation; the density of the primary bone of the graft as compared with that of the primary bone of the bed.

With regard to the first question, when the degree of calcification of the transplanted primary bone is the same or slightly greater than the degree of calcification of the primary appositional bone deposited secondarily, we can quote from Amprino's article (1952): "even in the bones of animals examined during the period of the fastest appositional growth of the diaphysis, the trabeculae and the bone spicules in the process of formation below the periosteum have a calcium content per surface unit only slightly less than that of the deeper zones".

However in pathological conditions, as we have seen, this is not the case. The original primary bone of the graft has a much greater density than has the newly deposited appositional bone.

This difference may be even as high as 35 per cent but very often it is about 15. The densitometric difference between the two tissues persists for a long time so that we see newly deposited areas that are already quite a distance below the surface and therefore no longer young and yet still have a low mineral content only (Figures 17, 22 c). We cannot say whether this difference is permanent, in other words whether a stratification persists as Amprino has seen in certain species of animals. Apparently, appositional bone that develops in pathological conditions such as prevail here (below a graft), even though it is of periosteal origin as usual, behaves in a manner that cannot be compared to the process that is normally seen in a growing animal.

The same periosteal appositional bone that is found below the surface of the graft shows different densities; analogous to what can be seen in mammals and also in man during the first two years after birth it often presents a trabecular bone structure with interwoven fibres on which are deposited layers of bone tissue with parallel fibres, or lamellar bone, which form the

primary osteons. This trabecular structure of fibrous bone tissue is shown by densitometric examination to have a higher calcium content than the adjacent primary osteons.

The second question concerns the density of the transplanted primary bone as compared with the primary bone of the bed. In other words, does the detachment of a fragment from its site (in this case, the tibial crest) and its transfer to another site (soft parts of the lower leg or of the thigh, etc.) with immediate, practically total and final interruption of its blood supply, bring about a change of the mineral content of the primary bone as compared with the normal bone of the bed?

We have seen that there is only one way to obtain an answer to this question: by direct comparison of the graft with the normal bone of the bed. Above, a description has been given of the technique which involves the densitometric measurement of the two fragments in sites only a few tenths of a micron distance from each other in sections of the same thickness. In the case studied, the comparison was made between a cortical tibial graft inserted 5 months previously into the medullary canal of a pseudo-arthrotic tibia and excluded for the greater part, and the bed of this graft, *i.e.* the diaphyseal cortical layer of the same pseudo-arthrotic tibia (Figure 5).

Our measurements revealed that the primary bone of the graft had a 10 to 13 per cent higher density than the living bone of the bed. If the density of the primary transplanted bone is considered to equal 100, the calcifications of the primary bone of the bed reached values of 0.90 to 0.87. Also, the osteonic secondary bone of the haversian canal proved to have a higher calcium content that the corresponding haversian canal bone of the bed, the difference always being approx. 7 to 10 per cent (Figure 5).

In this pages we shall not attempt to interpret these differences of mineralization or to reopen the old, but still topical and still unsolved questions of the greater macro-and microscopical density of necrotic bone, whether it is a case of a passive mineral deposit or of changes in the crystallinization of or alterations in the protein matrix and the osteomucoid showing themselves as mineral modifications; it may suffice that we have established, we believe definitely, that there is a difference in the mineral density between transplanted bone, especially if excluded, and living bone.

B) *Secondary, osteonic bone.* As we have seen, the creeping invasion leads to more or less extensive destruction of the osteons of the graft and the regeneration to reconstruction of the new ones in the cavities vacated by the originals. This statement is founded on the densitometric study of the new osteons.

Naturally, the newly deposited osteon of the graft also obeys the general law in that it is mineralized to a lesser degree than the primary bone and than the older osteon. Examination of a transverse section of a graft in the regeneration phase shows therefore, against a background of whole or broken-up old osteons which have formed prior to the operation and which show almost 100 per cent calcification, a series of osteons with a very low mineral content newly formed in the new bed.

Various transplantation sites were selected which show a particular behaviour (in the phase of initial regeneration, or of regeneration by fits and starts, or of advanced and extensive regeneration) and with the aid of the densitometric method the calcium content of every osteon was determined, as compared with the calcium content of the original primary bone which always considered to be 1. The details of the method used for this study have already been described. The 100 osteons per field that were studied in this manner were classified according to their density: the result was a diagram which shows the variations of the osteonic calcification and which clearly indicates a particular evolutive stage so that the different stages can be easily distinguished from one another. It will be clear that the value of such diagrams presupposes the existence of a diagram for normal bone (tibia) of adult man, showing a relatively constant arrangement of the osteon groups of different densities. This has been confirmed by Amprino (1952, see Figure 19 on page 164 of his study) and we were able to confirm it on the basis of studies of normal tibiae made before we began our present investigation.

Below, we shall describe a number of fields which may serve as bases for numerous others found in the present study. Figure 8 d shows a peripheral area in the phase of regeneration with a large percentage of osteons which have a low mineral content and are therefore newly formed (0.60–0.75): these account for approx. 60 per cent. Very scarce as compared with the normal situation are osteons with a high degree of calcification (0.86–0.96) because these have been destroyed and replaced by new ones. We see regeneration that is not yet complete and runs an interrupted course, with phases of arrest and phases of continuation. Actually, in the scheme, there is no regular distribution of the osteonic groups but empty spaces are present; this indicates that while a certain group of osteons has already reached a certain age and has reached a certain degree of calcification (group 0.71–0.75) a second later group, with a lesser degree of calcification (0.66–0.70) is numerically smaller and is almost in a state of exhaustion; then appears a group that is more numerous and has an even lower degree of calcification (0.60–0.65) and which is therefore of an even

younger age, evidently having formed in a diffuse manner, whereas the previous groups had already been deposited some time ago and are in a state of advanced calcification. All this goes to show that in the present case the osteogenic regeneration occurs in fits and starts, with phases of arrest and of sudden resumption which are largely under the influence of the local circulation.

A similar pattern can easily be seen in all grafts including those which run a more favourable course. Figure 22 c shows a section of a cortical tibial graft in a boy aged 11 years, 18 months after his operation. We can see an analogous grouping, with a few old osteons of the graft and many newly formed osteons with their low concentration. The fact that this graft has developed more favourably than the one discussed above appears from the larger percentage of these newly formed osteons, which reaches 84 per cent, indicating that more than four-fifths of the old osteons of the graft have been replaced by new ones.

For purposes of comparison it is worth while observing the reconstruction of a normal segment of femur in the vicinity of the graft (Figure 22 d) (same case as the previous illustration, boy aged 11 years, after 18 months). This part of the femur has been altered by the reaction nearby. We can see a fairly regular distribution of osteons in the groups with a lower density (0.60–0.75) all of which are newly formed. Most of them have a higher density, as a sign that the alteration process has been active for some considerable time (18 months) and is becoming exhausted as shown by the uniform percentile decrease of the two groups with lesser density. All the bone has been reconstructed, as appears from the almost complete absence of old osteons. The difference in the distribution from the transplanted bone can be clearly seen.

When regeneration is fairly widespread and the process goes on with sufficient intensity for a sufficiently long time without much interruption or impairment, the diagram shows the highest percentage of osteons of approx. 0.75–0.80 *i.e.* those with a relatively low mineral content and a progressive and regular decrease in younger osteons; further, isolated osteons of the old bone can be observed with their high concentration (Figure 12 e).

In other cases, the regeneration is highly isolated and limited to a few osteons. The large majority of the original systems of the graft are grouped around the high densities (0.86–0.99), while a few groups of newly formed units represent the lower densities (0.60–0.75), as an indication of their youth (Figure 8 i).

As regards the minimal density encountered, osteons with a calcification of less than 0.60 can be found, for instance several with values of 0.57–0.58.

However, the systems with densities between 0.60 and 0.70 are more frequent.

In our opinion, we have data to support a hypothesis for the rate of mineralization of an osteon. In a graft of 11 months the greater proportion of the new osteons show a calcification of 0.71–0.75. At 16 months, the greater proportion of osteons have a calcification of 0.75–0.80. At 22 months, the osteons that can be recognized as belonging to the first regeneration, and not to the adaptation, have densities of approx. 0.92. If we take into account the fact that the deposition of osteons begins some two months after the operation, it may be argued that the osteon reaches 75 per cent calcification after approx. one year, with the calcification increasing only slowly thereafter and reaching 90 per cent in approx. two years. These data are in agreement with others that have been reported in the literature; all authors have pointed out the extreme slowness of the complete mineralization of the Haversian system. According to Holmstrand (1956), in the rabbit there is a rapid increase up to the 5th week, but thereafter 90 weeks, *i.e.* almost two years, are necessary before complete mineralization is reached. According to Vincent, in the dog at least 4 months are required to obtain some degree of calcification but this is then still far from complete.

In the Haversian system differences of calcification can often be noted. We can see the cockade type of osteons with a clear line separating the two concentric layers with their different degrees of calcification; particularly frequent, however, are the haloes with more intensive calcification touching the internal margin of the system (Figure 8 i, 9). This layer is usually thin: 1/5–1/10 of the whole radius of the system; its contour is often vague and its thickness is not always regular. It is not a photographic artefact.

Its interpretation is still a subject of investigation and we shall not attempt it here.

Often, within the individual concentric system, we can distinguish areas with different mineral concentrations:; these may be seen as spots occupying some part or sector of the osteon which thus looks marbled (Figure 16). Densitometric differences of up to 6 to 8 per cent may be present. The relatively high frequency of this finding, which is in sharp contrast with the low incidence encountered in normal adult animals, confirms the hypothesis advanced by Amprino (l.c.) that this is a phenomenon of transiently retarded deposition corresponding to periods of intense bone reconstruction.

2. EXTERNAL APPOSITIONAL REGENERATION

This term indicates osteogenesis in layers above the surface of the graft beyond its original limits. In its earliest stages it is dominated, just as is the interstitial regeneration, by the vascular connections and not by the mechanical factors. Thus we see peripheral zones which have vascular continuity with the surrounding bone lining with newly-formed bone layers of from 50 to 500 μ in thickness.

They are often absent in the excluded zones without creeping invasion. However, fairly frequently newly-formed appositional bone with interwoven fibres or lamellar structure can also be found adhering to the surface of an excluded graft (Figure 12 d); this is especially the case with areas cut with the electric saw, as has been discussed above. The mesenchyme, which in some way adheres to the graft, is induced to ossify but develops no vascular connections with the underlying tissue.

The apposition is seen (Figure 10 f) as a layer of lamellae lying directly on the graft without the presence of a cementing line, or there may be several layers in the thickness of 70–100 μ divided by interruption lines and furrowed, parallel to the circumference of the graft, by vascular lacunae. In other cases the lacunae may be highly irregular in their course and intersect the bone in all directions often with the formation of fairly large cavities (Figure 12). Above these structures we may see trabecular bone in direct continuity with it (Figure 8 d). During examination in polarized light we see the deposition of the fibres: the trabecular structure consists of interwoven fibres and this type of bone is encountered in the first place when the regeneration is rapid and intense. The more regular layers and those that the the line the primitive Haversian spaces possess parallel fibres. Owing to the progressive deposition of the latter type of bone the cavities grow smaller and smaller until large surfaces of primary bone are formed practically without concentric systems and with many longitudinal and transverse lacunae brought about by blood vessels (Figure 17).

The subsequent stage of the evolution is already a part of the process of adaptation and it is characterized by replacement of the primary bone by secondary osteonic bone, which already fulfils the mechanical requirements of function (orientation, etc.).

It will readily be recognized that the process of development of the appositional bone of the graft, as described above, is the same as that of bone growth in mammals and man.

As regards the densitometric findings, this appositional bone for a long time naturally retains the lesser degree of calcification of newly-formed

bone. When it has only just been deposited it has very low indices (0.57, 0.64) but, as stated above, there are differences between the central trabecular bone with its interwoven fibres, which from the beginning shows a very high density due to higher calcium level (0.70), and the lamellar bone that forms the primary osteons.

3. AUTO-RADIOGRAPHY OF THE OSTEOGENIC REGENERATION

The technique used for in-vitro auto-radiography has been described elsewhere. Our findings have shown constant characteristics: when micro-radiography revealed a lesser density, auto-radiography revealed a greater fixation of radioactive calcium (Figure 13). The osteons with a low mineral content showed very clearly on auto-radiography because of calcium fixation in a precisely corresponding pattern. The layers of newly deposited bone at the periphery of the graft with a low mineral content were shown by the auto-radiogram to contain much radioactive calcium.

Briefly, there is inverse proportionality between the mineral content and the fixation of radioactive calcium so that the structural pattern is the same in the two preparations. The principal disadvantage of auto-radiography in vitro is that its findings are static, so to speak: it does not reveal whether a positive osteon is in the process of deposition or has not taken up any calcium for a long time:; all that the fixation in vitro shows is that at such and such a moment a given structure has an incomplete calcium deposition.

III. THE GRAFT IN FUNCTIONAL ADAPTATION

Our observations as laid down in this chapter are based on a graft of 16 months and one of 22 months; the latter was in such an advanced stage of development that the original graft, intended to replace a radius, had grown into a hollow bone with the beginning of a central canal.

At this stage, which may be reached in a few months or in several years depending on local factors, on contact, on functional requirements and on the subject's age, it is clear that osteogenic regeneration and reabsorption, *i.e.* the deposition or destruction depend on mechanical factors.

Those parts of the graft in the area between the external and the internal circumference of the cortical layer, *i.e.* where weight-bearing is felt, show newly-formed osteons and bone deposition (Figure 15 b, 25 b); a graft in such a place is intended to be used.

Outside this sector, in the zone corresponding to the medullary canal,

there is no osteogenesis but only absorption (Figure 15 a, 25 a). A graft in the medullary canal is therefore doomed to disappear because the economy of the skeleton makes it necessary for there to be an empty canal behind the diaphysis of a long bone. A cortical graft situated in spongy bone is destroyed, because the meta-epiphysis must contain not compact but spongy bone only.

A section from case no. 5, seen 22 months after the operation, gives a clear impression of the adaptation stage (Figure 26). This section may be divided into three parts, A, B and C. Parts A and B represent the old grafted bone but both have undergone changes, each in a different way, depending on site and function.

Part A, at the periphery of the cortical layer, has a weight-bearing function; accordingly it has only partially been destroyed and the destroyed portions have immediately been replaced by newly-formed bone, at first also parallel to the surface (Figure 26 f) but later with the osteons arranged according to the axis of weight-bearing (Figure 26 e).

Part B, in the central zone, has been largely resorbed; new depositions, in the form of thick trabeculae, are in the process of disappearing so that the medullary canal gradually emerges.

Part C is completely newly-formed; it surrounds the graft in a semicircle and constitutes an entirely new cortical layer.

At left end also (Figure 26 d), we can see new bone deposition which is intended to regularize the shape of the diaphysis.

The "purpose" of the organism during the adaptation phase is, therefore, to align the new bone with the cortical lines that were previously present in the resected segment, *i.e.* the weight-bearing lines. If the graft lies inside these lines apposition will refill the open space, first with bone of a coarse texture of interwoven fibres and then supplemented with lamellar bone. Subsequently, while this bone undergoes calcification, there appear the first primary osteons and then the secondary osteons with their development determined by mechanical requirements.

When the graft lies outside the lines of the cortical layer it is gradually destroyed until the surfaces are once more level. If the graft lies between the lines in question its fundamental bone remains intact for a long time and only the osteons, none of them excluded, are replaced by new concentric systems. Examination of our own and of other preparations gives the impression that the original fundamental bone of the graft may for years fulfil its new functions in the state in which it has been transplanted with modification of the osteons only. Thus, in certain areas persistence of as much as 60 per cent of the original graft was observed for as long as two

years after the grafting, and this with an excellent evolution which had led to perfect anatomical and functional reconstruction. This explains certain histological pictures of dead bone and certain areas of radiographic density persisting in preparations and in radiograms of grafts many years after transplantation. In one of our own cases this phenomenon can be clearly seen (Figure 23).

Undoubtedly, the arrangement of the osteons parallel to the axis of weight-bearing is of essential importance in connection with function (Tagliapietra & Vigliani 1958).

This view has nevertheless been contradicted, among others by Holmstrand, on the basis of his own experimental preparations in which he observed how osteons re-formed in a direction at right angles to the weight-bearing lines. However, an objection can be raised to these experiments in connection with the technique used: the bone fragments transplanted by Holmstrand (discs that were rotated through 90° and realigned without interrupting the tibial diaphysis) were not subjected to obligatory weight-bearing or at least not for some months; the weight-bearing could bypass the discs which lay within the outlines of the normal un-interrupted tibia; therefore the evolution of the graft was rather slow and the phase observed by Holmstrand was only the regeneration phase (not influenced by weight-bearing). The situation is different for a cortical tibial graft which replaces an entire skeletal segment and which is immediately subjected to weight-bearing, as in our cases. For the adaption phase we may therefore conclude definitely that it is essential for the osteons to be arranged in the direction of the weight-bearing axis.

We have insufficient preparations to prove that the arrangement of the osteons, their grouping according to a particular design, is determined by mechanical factors; we therefore have no comment to make on the assertion made by Amprino and Bairati (1936), by Amprino and Godina (1947) and by Vigliani (1950) that factors of a non-mechanical nature play an essential part in the determination of the structural renewal of the bone. The long-term study of bone grafts will undoubtedly yield more information in this respect relating to the density of the concentric systems per unit of surface, their union and their reciprocal relationships.

IV. THE GRAFT IN THE PROCESS OF RESORPTION

We speak of resorption if the destruction goes beyond a certain limit and proceeds progressively and intensively while there is no simultaneous osteogenesis.

A graft in the process of resorption shows an entirely familiar radiographic picture: diffuse porosis which grows gradually more pronounced until the segment has disappeared.

All our preparations, micro-roentgenograms made 5, 11, 16 and 22 months after grafting, show zones of resorption. Let us examine, for example, a graft situated in the medullary canal 5 months after operation (Figure 3 a): in this case it is the site in the medullary canal that causes the resorption because the graft represents a foreign body. It is significant that a few mm laterally the same graft, where it corresponds to the limits of the cortical layer, shows osteogenic regeneration (Figure 3 i).

Resorption has been seen in a graft buried in spongy bone; the graft has been eroded at the surface, progressively, without the slightest trace of new deposition of bone. Resorption was further seen, after one and a half years of slow evolution, in the centre of a long bone replacing a femur: functional adaptation makes it necessary for the long bone to be tubular and therefore the central portion of the graft disappears while at the same time bone is deposited on the outside. Considerable resorption could be seen after 2 years in a radial graft which had developed favourably: the new formation of bone in a circular pattern, together with the destruction of the internal layers of the graft, give the segment the shape of a tubular bone (Figure 26).

In other words, bone is resorbed when it is harmful or useless from the point of view of the economy of the skeleton: inside a medullary canal and in spongy bone it is harmful and is not functioning because it lies outside the weight-bearing lines and is not continuous with the skeleton.

The micro-radiographic image of the resorption is typical (Figure 15 a). The cut surface of the bone is composed of smaller and larger cavities and the outline of the graft shows irregular notches; there is no, or only very little, new bone formation so that the bone tissue that is not destroyed lies between the multiple resorption zones and is of a markedly uniform appearance of rather high density with its osteons a few per cent denser than those of the fundamental bone. The layers of newly-formed bone with a low degree of mineralization that line the cavities in creeping invasion are absent; also absent are the newly-formed osteons that are typical of a graft during the regeneration or adaptation process. The destruction of the bone, with the formation of larger and larger cavities, is not influenced by the osteons or by the boundary lines; it spreads independently of any-pre-existent structure, just as does the creeping invasion. The destruction surface in the indented and festooned preparations as a rule looks like a piece of paper nibbled by mice. The bone is completely inert without any modi-

fication: there are no layers of decalcification and every structure ends abruptly at the resorption line.

V. THE EXCLUDED GRAFT

To repeat, exclusion of a graft means its isolation from the organism so that it lies without developing direct vascular connections with the bed. A segment may be completely excluded and then it is enveloped by a fibrous sheath that encapsulates it without closely adhering to it; macroscopically it then looks like a stick of polished ivory and radiographically it shows the densification and thinning that we may call "sclerotic atrophy". Another possibility is the partial exclusion of certain areas so that in a section of the graft only some zones do not come into contact with the bed, while other zones nearby undergo creeping invasion and respond with osteogenesis.

In exclusion concave portions of the tibial graft appear which, in our cases, were cut with the electric saw; we can find no other reason for this reaction than the action of the saw, by the mechanisms described above.

Normally the surface of the excluded portion is smooth and rectilinear as it was at the moment when it was grafted after sawing, or its outline may be slightly festooned due to the resorption notches (Figure 12). Histological examination shows that there is no connective-tissue or vascular continuity between the bed of the graft and these portions of the graft; between the two there is a gap.

On the surface of the graft can sometimes be seen a deposition layer of newly formed bone: bone and interwoven fibres which are sometimes rather thick. In this case is there a distinct dividing line between the newly formed and the transplanted tissue. In the graft everything is uniform and dense; there are neither destruction cavities nor newly formed osteons. The newly formed bone simply adheres to the graft without establishing any vascular connection with it.

Furthermore, it cannot be excluded, and it may even be asserted that, there exist chemophysical relationships between the two tissues, with passage of minerals either actively or passively such that it would be justified to use the term osteogenic induction. However these are definitely not as pronounced as in a graft with a considerable degree of creeping invasion. It is not simply a question of the apposition surface (in the onlay graft only on the outer surface, in the graft with creeping invasion between the individual destruction cavities and the single osteons). The excluded graft has less active relationship with the bone which has newly formed on it than has the graft that has undergone creeping invasion and is in the pro-

cess of regeneration. This all for no other reasons than the difference in vascularization which leaves large zones of the excluded graft isolated.

Densitometric aspects. The areas of the excluded graft show as areas of normal non-transplanted bone. The concentric systems are grouped according to the higher densities (0.86–0.99) with younger, less calcified osteons hardly perceptible. As can be understood, after transplantation the appearance of the bone is arrested in the state it was at the moment of its dissection and this does not change with the passage of months (see Figure 14).

The results may be reported here of our studies of the density of the primary bone of the onlay graft as compared with that of the primary bone of the transplantation bed which were measured in adjacent sections of the same thickness; the primary bone of the graft had a 10–13 per cent higher density than the living bone of the transplantation bed.

VI. PARTICULAR PATHOLOGICAL ASPECTS OF THE GRAFT

The material on which this chapter is based consists of three sections in which graft was invaded by tumour recurrence: case no. 2 was a recurrence of an ossifying parostal sarcoma at the upper pole of the cortical graft, in contact with the neck of the femur; case no. 5 was a recurrence of a reticulosarcoma with involvement of the distal pole of the graft at the site of its fixation in the medullary canal of the radius.

The morphological picture was that of erosion destroying the newly-formed and the old surfaces of the graft regardless of differences in age and structure. The outlines were notched, the pattern of the destruction cavity irregular and indented. The difference between the cavities of creeping invasion and those of reabsorption lies in the shape of the empty spaces which in the normal process are round or oval in shape with small outlines, whereas in the presence of a tumour the destruction leaves behind angular, festooned empty spaces.

No other particular aspects of a strictly micro-radiographic nature can be made out; the destruction line cuts into the pre-existing structure without any modification in the degree of mineralization of adjacent zones. Farther distant, in the depth where the graft is surrounded by the tumour and the erosive process, no special features relating to density could be observed. In this respect it must, naturally, be remembered that these were delicate

observations and that sometimes no conclusions could be drawn from data collected from three sections if we do not know exactly the factors in question.

VII. THE BONE OF THE BED OF THE GRAFT

For our study of the reactions in the bone of the bed of the graft we had at our disposal sections of two tibial, two femoral and one radial diaphysis removed at periods of from 5 to 22 months after grafting.

We studied a section of a normal tibia of a male aged 25 years (Figure 1) in which can be seen the regular osteonic structure which, over a considerable length, has been replaced by fundamental periosteal bone along the antero-medial surface; resorption cavities are rare and osteons with a low degree of mineralization are not very frequent.

Destruction was the dominant feature in the bone of the graft bed 5 months after the operation. The operation itself, the presence of the graft, the inactivity resulting from lack of weight-bearing and the plaster cast were contributory factors to the destruction. Large resorption cavities made the cortical layer porous (Figure 3 c, d). The newly-formed bone was not very extensive in comparison with the destruction and had a rather low mineralization index.

In a segment 11 months after transplantation, that so far had been subjected to only a little weight-bearing, the destruction had reduced the cortical layer to a thin shell but there was also diffuse new formation of bone which showed a higher index of mineralization than the section described above. Nearly all the osteons appeared to be new. On the whole, at this stage osteogenic regeneration seemed to be dominant.

In still another segment, with slow evolution 16 months after grafting, considerable osteonic and periosteal deposition had to a certain extent reconstructed the previous outline of the bone (Figure 15). There were still large areas of destruction but reconstruction was predominant everywhere.

Eighteen months after transplantation the bone of the graft bed still did not have a normal appearance (Figure 20, 22d). Although this was a young patient who had completely resumed normal activity the presence of the, as yet, only incompletely adapted graft was probably still causing modifications in the circulation; these induce large resorption cavities and osteons that still ran in various directions, some oblique, as if the definitive balance of normal weight-bearing had not yet been reached.

Twenty-two months after the operation, in our case (Figure 25) the cortical layer of the graft bed still had not returned to normal because of

the continuing presence of the graft in the medullary canal even though this was being slowly resorbed; the cortical layer showed a very special structure with large medullary areas, newly formed osteons and periosteal bone deposition.

A description has already been given of the measurements of the density of the primary bone of the graft bed as compared with the density of adjacent zones of transplanted primary bone.

VIII. SUMMARY AND CONCLUSIONS

If we take into account the fact, which is confirmed by the micro-radiographic study of normal bone, that the cortical layer of the tibia in the adult shows a special structure with a predominance of osteons of high density and with few or no areas of resorption we can consider that every change in this aspect in a graft is due to the operation.

As regards the *creeping invasion,* this shows in the micro-radiographic sections in the form of resorption areas: the first chapter is concerned with this. Features of the creeping invasion are: 1) the close fitting and adhesion of the graft to a richly vascularized bed; if this area is small or absent the process is very limited or absent, 2) the function of the skeletal segment, not so much for weight-bearing which is very slight in the beginning but rather for vascularization, intensified by the laws of use.

As regards the pattern of the creeping invasion, it does not proceed from the periphery of the graft inwards, but shows first as a widening of the Haversian canals in peripheral bundles and then by the appearance of progressively wider and deeper cavities nearer and nearer to the centre of the graft. These cavities widen at the expense of the Haversian systems, of the primary and of the periosteal bone, regardless of cementing lines and without any obvious reason for the shape, direction and dimensions of individual cavities.

The size of the area of creeping invasion is always correlated with the conditions of supply and function of the graft: it ranges from only 150 μ at the periphery, after one year in unfavourable cases, to areas occupying the entire cross section of a graft of a diameter of 3 cm, after 6 months in favourable cases. The micro-radiographs show clearly that the absorption advances and affects the organic and mineral matrix without preliminary demineralization of peripheral bundles of the alistheretic type.

The *osteogenic regeneration* shows itself by the presence of new bone which, at first, shows only a low degree of mineralization. Interstitial re-

generation is that which takes place inside the bone and replaces it; appositional regeneration is that which takes place on the surface of the transplanted bone.

The interstitial regeneration is determined directly by vascular connections and occurs independent of functional requirements and mechanical laws. It is directed by the blood vessels and therefore forms its structures in the widened Haversian canals. Thus the process is one of replacement of old osteons by new osteons. If the process is more active the replacement is irregular such that, for instance, the place for one old osteon may be filled by several newly-formed ones.

Densitometric examination shows that calcification of the newly deposited primary bone takes place gradually and slowly, in contrast to the physiological deposition of bone during the growth.

It was further found that the density of the primary bone of the graft was 10 to 13 per cent greater than that of the living bone of the graft bed; in other words, the bone of the graft is denser that the living bone of the bed.

If we examine the density of 100 osteons in a selected field and we classify them in groups of progressively increasing density we obtain a diagram which indicates the age and the osteonic evolution in that field. If the predominant osteons have a very high density (0.86–0.96), this means that they are the old osteons of the old graft. If most of the osteons show a low degree of mineralization (0.71–0.75), the bone is in a stage of osteogenic activity.

The different arrangement of the group of osteons also makes it possible to reconstruct the history of the evolution of the field in question with its arrests and resumptions of osteogenesis. We find that an osteon achieves 75 per cent calcification in one year, while thereafter the increase is slow and 90 per cent is reached in approx. 2 years.

Differences of calcification of the cockade type and of other types described above, may be seen within an individual osteonic system.

Appositional regeneration may take place on the surface of a bone that has undergone creeping invasion and, more rarely, also on the surface of excluded bone. There appears a layer of lamellae and interwoven fibres when the regeneration is rapid (as can be seen in polarized light); this is rapidly replaced by parallel fibres and a trabecular system develops parallel and perpendicular to the surface of the graft and contains primitive Haversian spaces.

Autoradiography in vitro reveals that there is an inverse correlation between the mineral content and the fixation of radioactive calcium, in that

the layers of newly deposited bone with a low mineral content appear to have fixed the radioactive calcium.

During the phase of *functional adaptation* we can see destruction of areas of graft around the axis of the diaphysis where the medullary canal should be; we can further see the appositional and interstitial reconstruction of the areas along the weight-bearing lines where the cortical layer must come. This second generation of osteons begins to be directed along the axis of function, a factor which until then exerts no influence.

As late as two years after grafting, and perhaps indefinitely, there may still be many areas of bone belonging to the old graft which remain included and unchanged as a scar inside the new bone which is in the process of active transformation.

Resorption shows as diffuse destruction without corresponding reconstruction. It occurs when site or the function demand it (the centre of the medullary canal or positions outside the lines of functional requirements) except in the case of resorption due to pathological processes (tumours, etc.). The destruction is frontal, not alistheretic.

The *excluded* graft, *i.e.* one isolated from the organism, is seen mostly on surfaces cut with the electric saw as the result of the chemical and physical alterations brought about by the use of this instrument. The structure of such excluded bone remains the same as it was at the moment of the transplantation. However, and we believe this to be important, the density of excluded primary bone is approx. 10 per cent more than that of the living bone.

The bone of the graft *bed* passes through a phase of diffuse and intensive destruction with little new formation after the operation lasting for approx. one year. At approx. 16 months deposition begins to predominate and it is only after two years that something like a normal appearance can be seen again.

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