From the Ortopaedic Hospital of the Invalid Foundation,
Helsinki, Finland (Head: Professor A. Langenskiöld, M.D.)

REORGANIZATION OF FRESH AND PRESERVED BONE TRANSPLANTS

AN EXPERIMENTAL STUDY IN RABBITS USING TETRACYCLINE LABELLING

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ACADEMIC DISSERTATION

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INTRODUCTION

Bone transplantation is a common procedure in orthopaedic surgery. In order to speed up the operation and to minimize the operative risk it would be advantageous if it would not be necessary to take the bone required for grafting from the patient himself, especially when a great amount of bone is needed as e.g. in spinal fusion involving several vertebrae. Therefore, homogenous bank bone is often used in bone transplantations, either as such or together with autogenous fresh bone. Bone may also be taken from the patient some weeks before operation and preserved in the bone bank to await the grafting. This method was used some years ago at the Orthopaedic Hospital of the Invalid Foundation, when much bone was needed for spinal fusion in severely paralyzed patients. It was thought that autogenous bank bone is more valuable than homogenous bank bone.

The prerequisite of the use of bank bone and abandoning autogenous fresh bone is, however, that the operative results do not deteriorate. During recent years many clinical materials have been published, in which the value of bank bone in demanding transplantations has been questioned. Also, at the Orthopaedic Hospital of the Invalid Foundation, *Riska* (1964) found, during follow-up examinations of patients operated upon for scoliosis, that non-union had occurred more often following spinal fusion with autogenous frozen bone than when fresh autogenous bone had been used. On the basis of this observation it was found necessary to elucidate experimentally whether the difference between autogenous fresh and autogenous bank bone is so great that it would be better to abandon the practice of taking bone from the patient and preserving it in bone bank before implantation. The tetracycline method, based on labelling new bone with tetracycline, was chosen for the study of this question. In the course of the investigation the primary subject widened to include the comparability of bone preserved in another way with fresh autogenous bone.*)

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*) A preliminary report was presented at the Annual Meeting of The Finnish Orthopaedic Association, November 13, 1964.

REVIEW OF LITERATURE

Theories of osteogenesis in bone transplants

Since *Ollier* laid the scientific foundation for the study of transplanted bone in 1858, literature concerning this subject has been abundant. In a way it is understandable that bone grafts, which play such a major role in orthopaedic surgery, have provided the impetus for thorough research, but actually the great number of publications is explained by the fact that the problems involved in bone transplantation are anything but unambigous. The results of various investigations differ from each other and opinions concerning the process of bone formation in the grafts are perpetually controversial.

One of the key questions in the study of transplanted bone is whether the resulting osteogenesis in the graft takes place through the agency of its own cells or of the host's cells. Not until this question is settled by a generally accepted scientific explanation will differences of opinion on different kinds of grafts die out.

In his wide and systematic investigations *Ollier* came to the conclusions that the periosteum plays the decisive role in the osteogenesis of bone transplants. He believed that, under favourable conditions, periosteum-covered bone may survive and that the graft receives its nutrition through the periosteum.

He was also the first to make a distinction, in principle, between autogenous, homogenous and heterogenous bone. In his opinion, in homogenous grafts the periosteum might survive but not in heterografts. Therefore he firmly rejected the use of heterogenous bone for transplantation.

The doctrines of *Ollier* were prevalent until 1893 when *Barth* demonstrated that transplanted compact bone dies and is eventually replaced by bone-forming surrounding tissues. According to him, the periosteum plays no part in reorganization but perishes in the same way. Neither did he find any significant differences between different kinds of bone grafts. While he had some supporters, he was soon subjected to widespread criticism, also from practicing surgeons.

G. Axhausen, with his extensive and scientifically planned studies (1907, 1909), made a kind of synthesis of the views of *Ollier* and *Barth*. He supple-

mented his experimental work with rabbits and rats by histological studies of material from operated patients. He showed that the bone substance in periosteum-covered transplants dies but that most of the periosteum survives and possesses strong osteogenetic properties. In his view autogenous and homogenous transplants do not differ in principle but only in the speed of regeneration. He emphasized his preference for fresh, periosteum-covered autogenous bone for clinical transplantation. On the other hand he completely condemned the use of heterogenous bone in surgery.

The osteoblast theory is based on the investigations of *Ollier* and *G. Axhausen*. According to this theory, new-bone formation in a transplant mainly takes place through the agency of its surviving cells. This view was generally accepted for years until *Levander* presented the results of his investigations in 1934. He had effected new-bone formation by injecting alcoholic extracts of bone into the muscle of rabbits. On the basis of his observations, *Levander* believed that the reorganization of transplants occurs from the surrounding pluripotential connective tissue cells which are induced into osteogenic cells by the substances released from the dead tissue. This opinion, known as the induction theory (theory of metaplasia), aroused wide enthusiasm, which is reflected in the literature. Several investigators (*Annersten* 1940, *Bertelsen* 1944, *Oberdalhoff* 1947, *Lacroix* 1947, *Hartley et al.* 1949, *Willestaedt et al.* 1950, *Roth* 1950) performed various types of extraction experiments, trying to isolate the substance called osteogenin by *Lacroix*. The existence of such a substance was questioned by *Heinen et al* (1949) after they had demonstrated new-bone formation in the muscle of rabbits following the injection of pure alcohol. In experiments with other animals, *Lindahl* and *Orell* (1951) as well as *W. Axhausen* (1956) showed that alcoholic bone extracts and bone fragments kept in alcohol had no effect on osteogenesis.

These extraction experiments have, however, not proved convincing, and no osteogenetic substance has been isolated. On the other hand, the metaplasia theory is better supported by the observations which have been made in connection with transplantations of pieces of bone. As early as in 1912 *Baschkirzew* and *Petrow*, on the basis of their experiments, concluded that reorganization of bone transplants occurs from the surrounding tissues. *Engström* and *Orell* (1943) implanted bone grafts frozen to -190°C into the subcutaneous tissues of humans and observed reorganization after 55 days. They were certain that no living cells could have been preserved in the bone grafts, and since they had also obtained similar results with fresh autogenous bone transplants, they felt that new-bone formation in bone grafts occurs exclusively from the surrounding young proliferative cells which through the action of dead bone tissue become transformed into osteoblastic cells.

Unequivocal evidence of induction by bone tissue has been lacking. *Duthie* (1958), *Hurley et al.* (1959) and *Danis* (1960) did not see any induction in the surroundings when the transplant was encased in a millipore filter; new-bone formation occurred only inside the casing. Research on osteogenesis is, however, not unambigous since *Goldhaber* (1961) under similar experimental circumstances has been able to cause bone-formation in the surrounding host tissue as well.

Urist in 1965 presented new evidence in favour of the theory of induction. In his experimental work, he suggests that bone formation in the interior of devitalized, decalcified bone implant occurs by autoinduction in which both the inductor cells (wandering histiocytes) and the induced cells (perivascular connective-tissue cells) are derived from ingrowing cells of the host bed stimulated by degradation products of dead matrix. The osteogenic cells arise through interaction of these cells.

The osteoblast theory and induction theory have so much in common that, according to them, the actual hard bone substance dies after transplantation and has an activating effect on osteogenesis. Opinions differ, however, in explaining where this bone formation originates. The subject remains a controversial one, although an increasing number of works have been published suggesting that at least some osteogenic cells are surviving in bone transplants (*Fell* 1928, *Vainio* 1948, *Phemister* 1951, *Ray et al.* 1952, *Campbell et al.* 1953, *Ham* and *Harris* 1956, *Bonfiglio* 1958, *Chalmers* 1959, *Heslop et al.* 1960, *Anderson* 1961, *Burwell* and *Gowland* 1961, *Burwell* 1964, *Deleu* and *Trueta* 1965).

In his extensive histological experiments with dogs, *W. Axhausen* (1956) has found that reorganization of bone grafts preserved at −15°C and transplanted into the muscle begins after three weeks, but very slowly. On the other hand, he demonstrated that new-bone formation in fresh autogenous bone already starts within a week. In his view, this occurs through the surviving cells of the transplant. At the same time he stressed the significance of the periosteum in osteogenesis. On the basis of these investigations he came to the conclusion that reorganization of the transplant takes place in two osteogenetic phases. In the first and most important phase, rapid new-bone formation begins in the fresh autogenous graft within seven days, originating in pre-existing specific cells of the periosteum, marrow and bone canals. In the second phase some osteogenesis appears, after 3 to 4 weeks at the earliest. This originates in the non-specific pluripotential connective tissue cells of the host which differentiate into osteogenic cells. In bony surroundings this second phase develops more rapidly, leading to a more rapid reorganization of the transplant.

W. Axhausen thus combined the osteoblast theory and the induction theory into an entity, and gave to experimental and clinical results an explanation

which eliminates the old dualism. *Vainio* in 1948 and *Urist* and *McLean* in 1952, in principle, arrived at similar results on the basis of their histological studies. The view that both the graft and the host are capable of taking part in osteogenesis is supported by study of *Ray* and *Sabet* in 1965 who used tritiated thymidine and by that of *Arora* and *Laskin* in 1964 who used sex chromatin as a cellular label.

Different bone transplants

The first autogenous bone transplantations were performed in 1820 by *von Walther*, and the first transplantation of homografts in 1878 by *Macewen* (quoted by *Chase* and *Herndon* 1955). Not until after the experimental work of *Ollier* and *G. Axhausen*, however, did the use of bone transplantation really start in surgery. In 1923 *Albee* published his experiences with 5000 and in 1924 *Lexer* his with 1000 bone transplantations. *Albee* accepted only the autogenous graft. According to *Lexer*, a fresh, autogenous, periosteum-covered transplant is also superior because it most certainly contains surviving cells. Homogenous bone may be used if the surrounding tissues are capable of replacement. Heterogenous bone, on the other hand, was completely condemned by *Lexer*. In the beginning of the twentieth century, both autogenous and homogenous bone was used as transplants. The results obtained, however, were better with the former. Besides, fresh homogenous bone was not always readily available, and it was feared that diseases could be transferred as a result of possibly inadequate sterilization. Therefore, homogenous bone grafting was given up for several decades in surgery.

New methods of preserving homogenous bone presented a solution to organizatory problems. The report of *Inclán* in 1942 gave a real impetus to the development of bone preservation. He had preserved both autogenous and homogenous bone in from + 2 to + 5°C in citrated blood and obtained favourable clinical results which were comparable with the new-bone formation in fresh autogenous grafts. American surgeons, especially, started to develop methods of preservation. In 1947 and 1951 *Wilson* published his experiences with homogenous bone transplantations in which he had used refrigeration at −20 to −29°C. In his opinion, bone-bank bone fully corresponds to the autogenous bone transplants. Several investigators (*Bush* and *Carber* 1948, *Weaver* 1949, *Hult* 1950, *Reynolds* and *Oliver* 1950, *Roth* 1950, 1953, *Schmid-Schmidsfelden* 1950, 1954, *Böhler* 1952, 1955, *Ehalt* 1954) stressed later that clinical results were equally good with fresh autogenous bone and bone-bank bone.

Later, other methods of storage have been used such as merthiolate solution (*Reynolds et al.* 1951), a polymerical plastic called Palavit (*Idelberger* 1956),

lyophilization, etc. Nevertheless, preservation of bone in a deep-frozen state is most widely employed in many parts of the world.

The doctrines of *Levander* (1934, 1938) provided a theoretical basis for the operation of bone-banks. The role of the bone graft was considered to consist of inducing the surrounding connective-tissue cells to metaplastic bone formation. This makes it understandable that, in the beginning, homogenous and heterogenous bone were so enthusiastically thought to fulfil the same osteogenetic function as fresh autogenous bone. *Judet* (1949), *Meiss* (1952) and *Guilleminet et al.* (1953) went even so far as to maintain that heterogenous bone-bank bone was almost equivalent to autogenous bone. It was found comparatively soon, however, that because of biologically unsuitable properties heterogenous bone was worthless for grafting purposes in human surgery. On the other hand, suitable material has been prepared from calf bone by removing all organic material from it. Immunologically, the bone thus obtained is completely neutral. Such bone, prepared according to *Maatz* and *Bauermeister* and called Kieler Span, has been energetically offered for sale in recent years, but the excellent results of the authors (1961) and others have not found convincing support.

Prior to the introduction of Kieler Span, *Orell* (1934), however, by macerating bovine bone, had already prepared material which he called "Os purum" and "Os novum". *Orell* had mainly used this in operations by the *Albee* method. At present these bone materials only survive as a curiosity in the literature.

During recent decades, comparative research has largely centered in autogenous bone and homogenous bank-bone. On the basis of clinical material, only a few authors (*Magnolfi* 1958, *Küppermann* 1959, *Arens* 1964, *Rehn* 1964, *Schwemmer* and *Contzen* 1964) have been completely content with the bone-bank. In 1959, when reporting on his large operative series, *Bürke de la Camp* maintained that although a fresh autograft is replaced by new bone more rapidly than frozen bone, the difference is nevertheless not striking and plays no essential role. Many authors emphasize that bone-bank bone cannot replace autogenous fresh bone and that the indications for its use must be carefully weighed (*Sieber* 1954, *De Tomasi* 1955, *Schoch* 1956, *Buser* 1959). In 1959, on the basis of their clinical experience *Carnesale* and *Spankus* stressed, that preserved homogenous bone may be used when the available supply of autogenous bone is not adequate for the particular situation or when the hazard of surgery is materially increased by obtaining the autogenous bone. The percentage of failures has generally been over 20 in published series where bank bone was used (*Report of the Committee to Study the Preservation of Bone* 1953, *Bosworth et al.* 1953, *Hackethal* 1956, *Moe* 1958, *Riska* 1964).

Since the enthusiasm prevailing at the time of establishing the bone-banks waned, in the opinion of most investigators, histological studies have demon-

strated that regeneration of fresh autogenous bone occurs more rapidly than that of homogenous bone. *Stringa* in 1957 and *Kingma* and *Hampe* in 1964 studied the rates of vascularization in autogenous and frozen bone by histological and injection techniques. They found that vascularization in homogenous bone was two to three times slower and less complete than in an autograft.

The actual controversy emerges in attempts to find a reason for the superiority of autogenous bone. The role of immunological activity has been widely investigated but without conclusive results. Some investigators (*Zeiss et al.* 1960, *Sabet et al.* 1961) believe that antigen-antibody reaction reduces revascularization of homogenous bone.

The possibility that the superior osteogenetic capacity of autogenous bone is due to relatively intricate immunological factors, has resulted in the use of various deproteinized and decalcified bones in order that antigen-antibody reaction may be avoided. In experiments with dogs, *Heiple et al.* (1963) compared different bone grafts. They found that regeneration in decalcified, irradiated frozen and deproteinized homogenous transplants occurred more slowly than in freeze-dried homogenous bone or frozen bone; of these two, new-bone formation was more rapid and more satisfactory in lyophilized bone than in frozen bone. In their work they established the antigenic status of different transplants. They found that freezing and lyophilization destroy the antigenicity of homogenous bone. In this way they arrived at the conclusion that perhaps the above-mentioned methods of preservation do not exert the same effect upon the inductor mechanism. In these investigations they employed as control fresh autogenous bone which proved definitely superior to other bone grafts. The results of the study of *Heiple et al.* suggest that antigenicity does not play any decisive part in the regeneration of bone.

De Bruyn and *Kabish* (1955) stress the importance in osteogenesis of osteogenetic material released from the dead bone transplant. They studied new-bone formation in transplants implanted in the muscle of rabbits; their observation period ranged from 6 to 129 days. New-bone formation occurred in 86 per cent of the fresh autogenous grafts, whereas in homogenous and autogenous frozen bone it could only be found in 0 to 8 per cent. They explained this remarkable difference by assuming that freezing destroys the osteogenetic inductor. On the other hand, no histological evidence could be found to suggest that surviving cells in autogenous bone contribute to the reorganization of the transplant.

Contrary to this latter investigation, an increasing number of investigators have now confirmed that osteogenic cells do indeed survive in fresh autogenous bone. In 1951, *Reynolds* and *Oliver* were still of the opinion that a slight time-factor was the only difference in reorganization between autogenous fresh bone and bank bone, but a few years later they agreed that the difference is evident.

and believed that this is partly due to the cells surviving in an autogenous graft. Thus. *Reynolds* (1953) said that he had almost entirely abandoned the use of bank bone in spinal fusion.

The difference between fresh autogenous and frozen homogenous transplants has been studied by *Vainio* (1957) in experiments with rabbits, imitating the technique of spinal fusion by the *Albee* method. After the interval required by the autogenous graft to become fully regenerated, the homograft had still preserved its size and original shape. Regeneration occurred most easily when the autogenous bone was covered with periosteum. On the basis of his experiments, *Vainio* believed that at least a part of the cells survives in autogenous transplants. Entirely similar results are reported by *W. Axhausen* (1962) in his extensive and thorough histological investigations. He concluded that the bone-bank in the course of this development will become unnecessary.

It would seem that after decades of investigations we are again returning to the basic views already presented at the end of the last century by *Ollier* and *G. Axhausen*. Bone-bank bone is nevertheless still used all over the world even in very exacting bone transplantations. Large parts of bones (e.g. the femur) may be replaced by homogenous bone-bank bone. Whether homogenous bone is preferable to a metallic endoprosthesis in replacing a major portion of a bone is a question which arises when the use of autogenous bone is impossible for quantitative reasons.

PROBLEMS

It is obvious that even in the future the use of bone transplants will retain a place in orthopaedic surgery, notwithstanding the fact that certain methods of metal fixation, e.g. in pseudarthroses, have reduced the need for bone grafting, just as drug treatment has often rendered arthrodesis or spondylodesis unnecessary. On the other hand, bone transplantation has gained new indications. Although the study of bone transplantation has aroused wide interest, the problems involved are anything but solved. Even though matters have been somewhat clarified in recent literature, so much controversy still prevails that further investigations are called for. Since bone preserved in a frozen state is continually used in many parts of the world, there is every reason to try to find out whether, and to what extent, such bone is equal to fresh bone in transplantation. Also, it is important to know how the handling of an autogenous bone transplant affects its osteogenetic properties. These questions are significant in surgery, because osteogenetically low-grade bone leads to poor operative results.

In surgery, the biological value of a bone transplant is determined by its osteogenetic capacity and by the quality and speed of its reorganization. Fresh autogenous bone has been considered to be the best for grafting purposes, but whether its osteogenetic capacity is essentially different from that of preserved bone, is a problem which can only be solved by attempting to provide answers to the following questions:

1) Are there any differences in reorganization between a fresh autogenous transplant and
   a) autogenous bone preserved in a frozen condition
   b) homogenous bone preserved in a frozen condition
   c) autogenous bone preserved in the open air or in normal saline solution at room temperature
   d) autogenous bone preserved in blood at 37°C?
2) What role in reorganization of the graft is played by the time factor?

The difference between a fresh and a preserved transplant is of theoretical interest. However, the main purpose of this study has been to try to elucidate the

question whether an immediately transplanted autogenous bone graft is much more quickly and completely incorporated into the skeleton than a preserved autogenous or homogenous graft; if so it makes the use of fresh autogenous grafts preferable in orthopaedic surgery whenever possible.

In the process of bone transplantation, new-bone formation occurs both in the graft and the host bone. In the former, bone undergoing necrosis is replaced by new bone. This process involving both resorption and apposition is called *reorganization of the transplant*. New-bone formation in the host bone, on the other hand, serves to fix the graft and finally enclose it in the host bone. In this investigation, the *osteogenetic capacity* of the transplant implies the ability of the graft to be reorganized and to stimulate new-bone formation in the host bed.

MATERIAL AND METHODS

Material

With the exception of six adult rabbits, the study was performed on rabbits aged 5 to 15 weeks. In young rabbits, the osteogenetic processes in bone are more rapid than in adults, so that regeneration of transplants occurs in them in a far shorter time.

A total of 136 rabbits were operated on, and 15 of them died postoperatively. This left 121 rabbits, of which three rabbits with a plastic tube as the transplant were added to the control series.

Thus there were 118 rabbits available for tetracycline and histological examinations. In nine rabbits two grafts were implanted in both femurs, in five one graft in the left femurs and the other in the right femurs, and in the remaining 104 rabbits two or three grafts were implanted in the left femurs only. Thus the material consisted of 127 experiments. 26 femurs with grafts were reserved for both tetracycline and histological examinations.

Distribution of the material according to the methods of study:

Experiments with	
tetracycline	94 (93 rabbits)
histological method	7 (7 „)
tetracycline and histological method	26 (18 „)
Total	127 (118 „)

Surgical technique

The operations were performed aseptically under local anaesthesia (0.25 per cent Xylocain-Exadrin®, Astra). The lowest ribs, either adjacent ones or from opposite sides, were used as transplants containing only diaphysial bone. The periosteum was occasionally preserved in order that observations could be made as to its role in reorganization (Table 3 and 4).

The transplants were grafted into the left femur, in some cases in both femurs. One tip of the transplant was lying on the surface of the femur

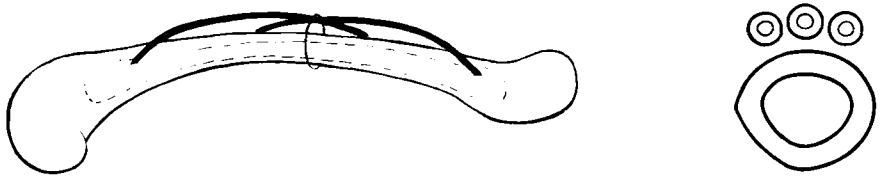

FIG. 1. — *Schematic drawing. a) Side view of the femur with two transplants. b) Cross section of the femur with three transplants.*

and the other was laid into an oblique hole (Fig. 1). This hole, reaching to the marrow cavity, was drilled either proximally or distally into the anterolateral diaphysial surface. Two transplants were usually grafted into the same femur and one of these was the fresh autograft. The ribs were placed to lie partially side by side, at which point they were fixed to the underlying bone with a nylon or catgut suture, in order to secure them firmly. Because of the curved form of the ribs the transplants were only in contact with the bone at both ends. If it was deemed desirable to graft a third rib, it was placed on the lateral side of the distal rib.

Except for the burr holes, the femoral periosteum was not damaged in any way. The fresh autogenous bone was transplanted distally or proximally in order to eliminate the effect of an eventually better osteogenetic capacity at either end of femur. The fresh transplant was preserved in physiological saline solution during the time it took to close the chest with some sutures and make the necessary preparations for grafting it to the femur, or about 5 minutes. It was not allowed to stay dry even for a moment.

A bony surrounding was chosen for the transplants because it explicitly corresponds to surgical practice. Since the bone transplants were grafted mostly into the same femur, as described above, the conditions for osteogenesis were uniform and thus observation of the reorganization of the bones presented no difficulty.

Handling of preserved bone transplants

Frozen bone. Both autogenous and homogenous frozen bone, mostly without periosteum, was taken from the rabbits usually 1 to 10 days before transplantation and placed immediately in a temperature of −15 to −17°C. According to *Roth* (1952), this is the most favourable temperature for the preservation of bones. It is true that the literature contains descriptions of even much lower temperatures

and lyophilization techniques for the preservation of bone, but different methods are not known to account for decisive differences in osteogenetic capacity. Prior to implantation the bone-bank bone was placed in normal saline solution warmed to room temperature.

Bone preserved in whole blood. Immediately after the removal of an autogenous rib with or without periosteum it was placed in a glass tube containing heparinized, penicillin-containing whole blood of the same rabbit. The tube was then kept in an incubator for 3 or 5 days at 37°C.

Autogenous bone preserved in the open air or in physiological saline solution at room temperature. Two adjacent ribs without periosteum were removed, one of which was immediately grafted to the femur and the other was left in room temperature either to dry in the air or immersed in physiological saline. The bone was kept dry for 1 or 2 hours and in saline solution for 1, 2 or 3 hours before it was grafted to the same femur, where the fresh bone had been previously grafted.

Investigation with tetracycline

In this study, the reorganization of bone transplants was followed by means of tetracycline deposited in the bone.

Tetracycline in bone labelling

A surgeon, *John Belchier*, in 1736, reported that the bones of pigs which had been fed on madder were coloured red with this dye. *Duhamel* between 1739—1745 and *Hunter* between 1760—1770 used madder as an aid in the study of bone growth (quoted by *Sissons* 1956). In 1957, *Milch et al.* reported, that antibiotics of the tetracycline group have a special affinity for bone tissue, where they have the ability to fluoresce in ultraviolet light. Because of its striking and clearly discernible yellow fluorescence and because of its lesser toxicity, tetracycline is superior to alizarin in bone research.

Tetracycline given by mouth or parenterally is distributed diffusely into the organism, but after 24 hours it has already disappeared from the soft tissues and only remains in calcified tissues. Tetracycline-labelled bone appears as yellow-stained, clear-cut bands which are parallel to and in the substance of the lamellae. The bands do not cross lamellae or cement lines. The labelled band-widths are a direct function of the duration of tetracycline medication and the rate of mineralization of osteoid. When life continues after withdrawal of the

tetracycline medication, the labelled bone and calcified cartilage become covered by unlabelled bone and calcified cartilage, unless appositional growth ceases during drug administration. Once deposited in bone tissue, the tetracycline and its fluorescence remain until resorption of the bone results in its disappearence. This implies that in adults the fluorescence may persist for years, but in a growing individual it may disappear fairly rapidly.

From the point of view of research on bone, the essential fact is that, like alizarin and radioactive calcium, tetracycline is taken up by the new bone. On the other hand, tetracycline is not deposited in cartilaginous or osteoid tissues which therefore, in ultraviolet light, show a bluish fluorescence. Neither has it been found that tetracycline is deposited in necrotic bone (*Rokkanen et al.* 1963).

According to *Harris et al.* (1962), tetracycline labels all bony surfaces, but surfaces undergoing resorption and inactive surfaces showing fluorescence immediately after tetracycline injection rapidly lose their fluorescence. On the other hand, *Harris et al.* found some diffuse distribution of tetracycline and presumed that it followed the distribution of calcium in the form of secondary mineralization and long-term exchange. According to *Hulth* and *Olerud* (1962), the diffuse distribution of the fluorescence is greatest during the first 24 hours, because the tetracycline is then not sufficiently firmly fixed to the bone, but is probably in "the hydration shell" surrounding the apatite crystals. Fluorescence may also appear around many lacunae, this phenomenon being in the opinion of *Harris et al.* probably an artifact. Fluorescence may also occur in connection with so-called marginal sclerosis.

The tetracycline molecule is bound in unaltered form to superficially situated calcium atoms in crystal nucleation sites in freshly formed bone. Polysaccharides and collagen may play a part in this binding. The relatively high content of tetracycline in freshly mineralized bone is explained by the fact that the crystals are then small and form a wide surface area suspended in tissue with maximum content of water and high reactivity of calcium-deficient apatite. In less than 24 hours after mineralization, calcium-deficient apatite is transformed into a less reactive form, mature hydroxyapatite. In addition, the crystals grow larger and the amount of water decreases and the matrix becomes compact, following which large molecules such as tetracycline can no longer penetrate into the organic matrix (*Urist* and *Ibsen* 1963).

Many writers (*Milch et al.* 1958, *Frost et al.* 1960, *Harris et al* 1962, *Hulth* and *Olerud* 1962) are of the opinion that the tetracycline method is most suitable for investigation concerning the appositional growth of bone. Despite the fact that tetracycline may also become deposited elsewhere than in new bone, this method nevertheless constitutes an interesting and adequately reliable means

of studying the reorganization of transplants. Histological methods that have mostly been used until now, leave much scope for interpretation, which is seen in the fact that in similar investigations different authors have arrived at opposite results. One drawback of the tetracycline method is that it is difficult to determine quantitatively the distribution of tetracycline (*Harris et al.* 1962, *Vanderhoeft et al.* 1962), but one must be satisfied with the qualitative demonstration of its deposition in the bone by means of fluorescence.

Own experiments with tetracycline

Since tetracycline marks the places where new bone is being formed, yellow fluorescence in a transplant signifies the occurrence of reorganization in the transplant. Reorganization proceeds through resorption and apposition (*G. Axhausen* 1907). At first, the new bone is woven bone, but after final reorganization the structure of the graft resembles the lamellary structure of the host bone. Therefore, fluorescence reflected by the tetracycline accumulated in host bone presents a point for comparison in judging the degree of reorganization of the transplant.

Oxytetracycline (Terramycin®, Pfizer*) was used in single doses of 50 mg per kilogram of body weight subcutaneously, this being the generally employed dose.

The tetracycline experiments were divided on the basis of the method of administration into two series, as follows:

Series I: the tetracycline injection was administered after the bone grafting.

Series II: the tetracycline was injected on two successive days two or three days prior to the taking of the rib for preservation.

Series I gives information about the new-bone formation (apposition) in the graft and Series II about the resorption of the original tetracycline-labelled bone transplanted into the femur. In this way, the reorganization can be studied from two opposite points of view. As tetracycline virtually labels the bone formed during eight hours after the injection (*Tapp* 1966), repeated doses had to be administered in Series I, usually at intervals of one week. Tetracycline was often administered on two successive days, especially if the time of observation was short.

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*) The author is much indebted to Pfizer for kindly supplying the Terramycin used in this study.

The distribution of tetracycline experiments according to the nature of the transplants is presented in the table 1.

TABLE 1. — *Experiments with tetracycline.*

Combination of rib transplants	Number of experiments	
	Series I	Series II
Autogenous fresh bone Autogenous frozen bone	59 (55)*	10 (10)
Autogenous fresh bone Homogenous frozen bone	12 (8)	
Autogenous fresh bone Autogenous bone preserved in whole blood Autogenous or homogenous frozen bone	5 (5)	
Autogenous fresh bone Autogenous bone preserved in the open air or saline solution	26 (25)	8 (8)
	102 (95)	18 (18)
Total number of experiments	120 (111)	

* In parentheses the number of rabbits.

Four autogenous bones preserved in blood and six homogenous frozen bones were compared with autogenous frozen bone; in these cases altogether three bones were grafted into the same femur because every combination included a fresh autograft. This was thought to be the best way to find out possible differences.

The rabbits in Series I were killed so that the time interval between the grafting of the ribs and the last tetracycline injection ranged from 6 days to three months, with at least 48 hours after the last injection in order to avoid short-term exchange. The rabbits in Series II were killed from 2 weeks to 3 months after the grafting.

Examination of the grafts and photography. Immediately after killing the animals, the femur with transplants was prepared free of soft tissues and preserved by deep-freezing. The specimens were examined with a stereomicroscope, in ultraviolet light (HBO 200 W), using UG 1 as exciter filter. From specimens, preserved in a dark deep-frozen state, the fluorescence did not disappear to any noteworthy extent over a year. The specimens were examined macroscopically both as such and as transverse and longitudinal sections. The sections were prepared by sawing through the femurs with the grafts and grinding the resulted surfaces. Tetracycline incorporation in the transplants

could be demonstrated clearly by low magnification of the section surfaces. Eight specimens were embedded in methyl methacrylate and ground to a thickness of 100 μ for microscopic examination.

The macroscopic specimens were photographed in reflected light with a Leica Aristophot macroscopic photographic apparatus, using Agfa daylight film CT 18 As exciter filter before the ultraviolet light a UG 1 was used, while in the camera a Wratten 2 B was used as the barrier and a CC20R as the compensating filter. Since demonstration of the results is based on yellow fluorescence, only colour photography could be employed. A black and white picture does not give a marked view of the reorganization of the bone transplant because the new-bone formation occurs diffusely without distinctive growth lines.

Estimation of tetracycline fluorescence. Reorganization of the transplant was estimated visually on the basis of yellow tetracycline fluorescence. The whole graft was assessed except the implanted part that was inside the femur. The scale used in estimating the relative amount or loss of fluorescent bone in the transplant was: none or minimal, scanty, considerable, abundant.

The whole tetracycline material is tabulated using the following scales (Tables 3, 4, 5):

Series I (formation of new bone labelled with tetracycline)

None or minimal	= hardly any yellow fluorescence
Scanty	= scattered strong yellow fluorescence
Considerable	= strong yellow fluorescence in large areas
Abundant	= strong yellow fluorescence throughout the graft

Series II (resorption of the original tetracycline-labelled bone)

Loss of yellow tetracycline fluorescence in the graft was examined by comparison with a rib labelled with tetracycline. This rib was taken at the same time with a future graft prior to the transplantation and preserved in a frozen state to serve as a control.

None or minimal	= hardly any loss of yellow fluorescence
Scanty	= scattered loss of yellow fluorescence
Considerable	= no yellow fluorescence in large areas
Abundant	= hardly any yellow fluorescence discernible.

Histological examination

Although the investigation was performed with the tetracycline method, it was found desirable to include histological examination as well (Table 2). Seven rabbits were reserved for histological survey only. On the other hand, 26 femurs with grafts were examined by both tetracycline and histological methods.

In 16 cases the femur with transplants was cut in the middle, one end being reserved for histological and the other for tetracycline fluorescence examination. Histological and tetracycline sections prepared from the place of the cross-cut present a mutually supplementary and comparable picture of the reorganization occurring in the grafts.

TABLE 2. — *Histological material.*

Preserved bone implanted into same femur with the fresh autograft	Number of experiments and time of observation									
	days		weeks					months		
	6–8	14	3	4	5	6	7	2	3	
Autogenous frozen bone	6 (1)*	1 (1)	1	3 (1)	1 (1)		2	2 (2)	4 (1)	20
Homogenous frozen bone	1		1	2		1				5
Autogenous bone preserved in blood		2**								2
Autogenous bone preserved in the open air				4		2				6
Totals	7	3	2	9	1	3	2	2	4	33

*) In parentheses the number of experiments with histological method only. In the remaining cases tetracycline method was used as well. In these experiments the time of observation was counted from grafting to last tetracycline injection. The rabbits used for these experiments were killed two days later in order to avoid short-term exchange.

**) As third implanted autogenous frozen bone.

In assessing the histological picture, attention was paid both to the topography of the specimen and viability of the bone. Bone tissue was considered vital when its lacunae contained an osteocyte whose nucleus was not pycnotic. The preparations were decalcified with EDTA and stained with Weigert — van Gieson's hematoxylin.

Control series

In order to make it possible to asses the role of the transplant in bone forming of the host bone, a piece of plastic tube was used instead of grafts. The ends of this plastic tube were implanted in a distal and proximal burr-hole on the ventral surface of the femur. Nine experiments were included in this series: in 3 rabbits the plastic tube in the both femurs (6 experiments); in 3 rabbits the plastic tube in the right femur, bone grafts in the left femur. Tetracycline injec-

tion was performed according to the method already described (Series I). The rabbits were killed after one to seven weeks.

The shape of the femur with the transplants was compared with that of the opposite femur without transplants. In this way an idea of the effect of the transplants on the host bone was obtained.

In Series II (resorption of the original tetracycline-labelled bone) a rib labelled with tetracycline was taken at the same time with a future transplant prior to the transplantation and preserved in a frozen state for comparison of the degree of resorption in the graft.

RESULTS

In the present experiments, two or three pieces of ribs, one of which was the autogenous fresh transplant, were grafted mostly into the same femur. This was thought to provide a reliable means for the comparison of the reorganization between the different transplants. The results are presented according to the combination of the transplants used in the experiments. As the material (page 19) indicates, particular emphasis was laid on the investigation with tetracycline. The transplantation of the fresh and frozen autogenous ribs into the same femur forms numerically the most common combination (Table 1).

Autogenous fresh bone and autogenous frozen bone

Comparison between autogenous fresh and autogenous frozen transplants was performed on the basis of the formation of the tetracycline-labelled new bone (Series I) and on the basis of the resorption of the original tetracycline-labelled bone (Series II). The former comprises the main part of the experiments. The results are presented in the table 3. The investigation with tetracycline was completed and controlled by histological examination.

Autogenous fresh bone

Formation of new bone labelled with tetracycline (Series I). In fresh autografts, lively reorganization began soon after the implantation. At 6 to 7 days some tetracycline was already incorporated in the fresh transplant. After this the tetracycline incorporation, which indicates new-bone formation, increased quickly (Figs. 2, 3, 5, 6). After a month the fresh autograft was already far reorganized (Figs. 7, 8, 10). In 2 to 3 months the transplants resembled the lamellary structure of the host bone (femur) (Figs. 12, 14).

In a fresh autograft the reorganization began in the end of the rib implanted in the femur. However, more tetracycline incorporation could be seen in the middle part of the transplant than in its ends (Fig. 9). There could even be more

TABLE 5. — *Autogenous fresh graft (aF) and autogenous frozen graft (aB).*
Series I (tetracycline injection after grafting)

No. of rabbit	Age at transplantation (weeks)	Time of observation (days) (from grafting to last inj.)	Autograft transplanted after being kept frozen (days)	Tetracycline fluorescence in the graft								Remarks
				Autogenous fresh graft				Autogenous frozen graft				
				None or minimal	Scanty	Considerable	Abundant	None or minimal	Scanty	Considerable	Abundant	
141 s*	6	6	3		+			+				
141 d*	6	6	3		+			+				
142 s*	6	6	3		+			+				pe (aF)
33	8	7	2		+			+				
135 s*	7	8	4		+			+				Fig. 16
135 d*	7	8	4		+			+				pe (aF)
1	5	10	3		+			+				pe (aF)
22	13	10	6			+		+				pe (aF) Fig. 2
26	13	10	6		+			+				Fig. 4
33	6	14	6			+		+				pe (aF, aB)
6	34	14	7		+			+				
38	7	15	2		+			+				pe (aF)
39	7	15	2			+		+				
40	7	15	3			+		+				pe (aF)
41	7	15	3			+		+				
42	7	15	4		+			+				
44	7	15	1			+		+				pe (aF) Fig. 5
45	7	15	1			+		+				pe (aB)
46	7	15	5		+			+				
47	7	15	5		+			+				pe (aF + aB)
12	8	15	3		+			+				aF→s, aB→d
11	9	18	3			+			+			
2	5	20	3		+			+				aF→s, aB→d
3	30	21	5			+		+				
8	30	21	3			+		+				
29	6	21	10			+		+				
137 s*	6	21	3			+		+				pe (aF)
3	5	22	3			+			+			aF→s, aB→d
32 d	7	22	19			+			+			pe (aF)
13	7	28	3			+		+				
17	7	28	7			+		+				Fig. 9
30	7	28	5			+		+				pe (aF)
35	7	28	5			+		+				
124	7	28	3				+		+			

Continued

Table 3. – Continued

No. of rabbit	Age at transplantation (weeks)	Time of observation (days) (from grafting to last inj.)	Autograft transplanted after being kept frozen (days)	Tetracycline fluorescence in the graft								Remarks
				Autogenous fresh graft				Autogenous frozen graft				
				None or minimal	Scanty	Considerable	Abundant	None or minimal	Scanty	Considerable	Abundant	
125	7	28	7				+		+			
126	7	28	3				+		+			
9	36	28	1			+		+				aF→s, aB→d
136 s*	6	28	3			+			+			
139 s*	6	28	3			+		+				
23	10	30	6				+	+				Fig. 8
10	7	35	3				+		+			aF→s, aB→d Fig. 10
32 s	7	35	6			+		+				pe (aF + aB) Fig. 11
57	10	42	12				+		+			Fig. 7 the third graft in the same femur was an autog. bone preserved in Palavit. It had scanty fluorescence like the frozen bone
133*	40	49	3			+		+				pe (aF)
134*	40	49	3				+	+				Fig. 20
16	7	60	6				+		+			
48	6	60	1				+			+		pe (aF)
49	6	60	1				+		+			pe (aB)
50	8	60	2				+		+			Fig. 12
51	8	60	2				+		+			pe (aF + aB)
54	7	69	6				+			+		pe (aF + aB)
15	7	84	6				+		+			
21	13	90	6				+		+			Fig. 13
27	6	90	6				+		+			pe (aF + aB)
131 s*	7	90	3				+		+			pe (aF + aB) Fig. 21
131 d*	7	90	3				+		+			pe (aF + aB)
132 *	7	90	3				+		+			
88	6	90	7				+		+			Fig. 14
92	6	90	7				+		+			

Continued

Table 3. – Continued

Series II (tetracycline injection prior to grafting)

No. of rabbit	Age at transplantation (weeks)	Time of observation (days) (from grafting to killing)	Autograft transplanted after being kept frozen (days)	Loss of tetracycline fluorescence								Remarks
				Autogenous fresh graft				Autogenous frozen graft				
				None or minimal	Scanty	Considerable	Abundant	None or minimal	Scanty	Considerable	Abundant	
73	8	14	3			+		+				
83	6	21	3			+		+				Fig. 15
74	8	25	3			+			+			
84	6	28	3				+		+			
75	8	30	3				+			+		
76	8	30	3				+		+			
77	8	42	3				+		+			
85	6	42	3				+		+			
86	6	42	3				+		+			pe (aF)
87	6	90	3				+			+		pe (aF)

Explanations:
s (sinister) or d (dexter) after number: in which femur the grafts were implanted
*: sections for histological examinations as well
aF→s: only aF implanted in the left femur
aB→d: only aB implanted in the right femur
pe(aF): periosteum in aF

tetracycline within the transplant than on its surface (Figs. 4, 6). Strong yellow fluorescence also occurred in the bone marrow (Fig. 3). Anywhere on the surface of the transplant new bone might be seen as a deposit resembling a gnarl on the side of a tree (Fig. 16).

In the fresh autograft the new-bone formation labelled with tetracycline progressed rapidly and the transplant was in the process of remodelling. So much new bone could be formed in the fresh transplant about to reorganize, that the graft became 2–3 times thicker than it had originally been, making it difficult to say where the original transplant had been (Figs. 7, 20). After the fresh autograft had been lamellarly reorganized, it became smaller and lost its original shape and curvature (Figs. 12, 13). It lay atrophied and thin on the femur, as far as it was not incorporated in the host bone.

Histological findings. 8 to 10 days after the transplantation, most of the lacunae of autogenous fresh transplants were empty. However, the fresh autografts exhibited a picture suggesting that vital tissue was present, scattered here and there (Fig. 17). Most commonly, bone tissue containing osteocytes was found at both the external and internal surfaces of the graft. Nucleus-containing lacunae might be seen in the bone marrow and around the haversian canals. New immature bone was often encountered in well-discernible layers on the external surface of the graft while especially in the superficial parts of the graft itself were living areas of lamellary bone (Fig. 16). In all, within 8 to 10 days vital bone tissue was found in the fresh autograft in the form of islands of immature bone and as normal bone with nucleus-containing lacunae. The occurrence of the latter suggested that some parts of the transplant could remain alive.

In the fresh autograft the changes occurred rapidly. Increasing porousness and vital bone islands modified the shape of the graft. Numerous trabeculae filled the bone-marrow cavity and the transplant could grow considerably larger than it was originally (Fig. 20). In about 4 to 6 weeks only a small amount of necrotic bone tissue could be discerned in the fresh graft. Within three months, the fresh graft was reorganized and its lamellary structure completely corresponded with that of the host bone (Fig. 21). At this stage the transplant had definitely attained the shape of a tubular bone but most commonly it was thinner and more flattened in comparison with the original transplant.

Apparently after 1 to 2 months, when the dead bone had almost entirely been replaced by living bone, immature bone tissue started to change into mature bone, the structure of the transplant became more dense and the graft thickened during reorganization, became smaller through resorption and again acquired distinct bone-marrow cavity (Fig. 21).

Autogenous frozen bone

Formation of new bone labelled with tetracycline (Series I). In the frozen autograft, scanty amounts of fluorescent bone were actually seen only after 3 weeks. The yellow fluorescence increased very slowly. Thus after about 6 weeks (Fig. 7) the degree of osteogenesis as indicated by tetracycline fluorescence in the frozen bone was not yet on the same level as that of the fresh autograft at 10 days (Figs. 2, 4), and during 3 months (Figs. 13, 14) not more tetracycline was incorporated in the frozen bone than during 2 weeks in the fresh bone (Fig. 3). In adult rabbits this difference in reorganization was perhaps still clearer. Thus after 7 weeks the fresh autograft was almost

completely reorganized, whereas the frozen autograft was as if recently transplanted (Fig. 20).

In the frozen autograft, tetracycline incorporation was seen first in the end of the transplant implanted into the femur and then in that end, which was lying on the femur. Reorganization of the transplant with the exception of the end parts, characteristically began from the part situated most closely to the fresh autograft (Fig. 7). Usually the frozen bone became attached to the fresh autograft, as the reorganization of the latter progressed, and obviously as a result of new-bone formation originating from the fresh autograft.

When the transplants were studied in low magnification, it was seen that the cortical layer of the frozen bone looked quite compact for several weeks, whereas in the fresh bone it was porous (Figs. 2, 15). On the other hand, in reorganization of the frozen bone the resorptive component was in the foreground. While in the fresh autograft, "creeping apposition" occurred concurrently with resorption, in the frozen bone the apposition phase lagged markedly behind.

Histological findings. 8 to 10 days after transplantation the frozen bone showed no living cells and empty lacunae made it porous (Fig. 16). In this phase the fresh autograft was porous too, but in frozen autograft this porousness then persisted without change for weeks. While reorganization was thriving in a fresh transplant, in frozen bone it was poor. Vital bone did not appear until after 3—4 weeks in small amounts. Within 3 months (Fig. 21) there was approximately as much vital bone in frozen bone as in a fresh transplant 2 weeks after implantation.

When a few weeks had passed since the transplantation, the fresh and frozen autografts had often grown firmly together. Histologically, it might be seen that the living bone tissue of the fresh transplant mostly ended with a sharp line of demarcation against the necrotic tissue of the frozen bone. The corresponding phenomenon is illustrated with homogenous bone in the figure 19. However, there might be scattered osteocyte-containing lacunae in the preserved bone, indicating the beginning of reorganization. In the frozen autograft, vital bone was mostly first seen in the shaft of the graft in the part that was next to the fresh transplant. In the bone marrow, living bone islands were also encountered earlier than in other parts of the graft.

The effect of the grafts on the host bone

Formation of new bone labelled with tetracycline (Series I). After the transplantation, bone formation also occurred in the host bone itself. This began immediately after the implantation of the transplants from the holes for fixing

them. This was proved when plastic tube was used instead of grafts in control experiments. Plastic tube had no osteogenetic effect on the femur between the holes. On the other hand, the bone grafts stimulated bone formation in the adjacent part of the femur, and even in the area between the ends of the transplant which was not touched by the grafts and which during the transplantation was not mechanically irritated. The fresh and frozen autografts differed from each other even in this respect. Almost consistently, bone formation of the host bone was more lively at the site of the fresh transplant (Figs. 2, 3, 5, 7). New bone originating from the host bone seemed to grow like a cuff along the surface of the graft (Figs. 5, 11). The line of demarcation between autogenous fresh graft and the host bone was broken quickly (Fig. 2), whereas in the frozen bone it was preserved for several weeks (Fig. 14).

The bone formation originating from the host bone seemed to have a clear drift towards the fresh autograft (Fig. 19). New-bone formation could be very lively, attempting to fill the space between the host bone and the transplant (Fig. 5). The fresh graft might become partly or completely incorporated in the host bone, and the graft could hardly be distinguished from it (Figs. 8, 10, 12). This generally occurred in about 5—7 weeks. It seemed that resorption of new bone began after about 2 months, because in 3 months the shape and thickness of the femur with grafts were generally the same as those of the femur without grafts. Then the fully lamellary reorganized fresh autograft could usually be discerned from the host bone even at a short distance, and the transplant, atrophied and thin, lay on the host bone, having lost its original form and curvature (Fig. 13). The amount of new bone derived from the host bone, however, exhibited considerable variations. Sometimes new-bone formation was rather scarce, although the reorganization of the transplant was already far advanced (Fig. 9).

Histological findings. Histologically, new bone formation in the cortical layer opposite to the grafts was seen to occur in the host bone. This obviously began immediately after implantation because after one week definite trabeculation (Figs. 16, 17) could be seen in the surface of the host bone. Characteristic of this phenomenon was the additional fact that new-bone formation occurred in greater amounts near the fresh transplant towards which it showed a tendency to progress. This new-bone formation might be so abundant that a separate cavity covered by new bone developed (Fig. 19). After 3 to 4 weeks the vital tissue could be seen to continue uninterrupted from the host bone into the fresh transplant.

Resorption of the original tetracycline-labelled bone (series II)

The differences in reorganization between autogenous fresh and autogenous frozen bone were not only studied from the point of view of formation of new bone but also as regards resorption of transplanted bone (Table 3). The bones of rabbits were labelled with tetracycline on two successive days. Two ribs, one for grafting, the other for the control series, were taken after 2 to 3 days and preserved in a deep frozen state. After 3 days a rib from the opposite side was taken and both the fresh and frozen ribs were implanted according to the method described above.

Since the transplant is replaced by new bone, resorption forms an essential part of reorganization. It removes the tetracycline deposited earlier in the bone. Thus, the sooner a bone graft is reorganized, the more quickly the tetracycline disappears. The loss of yellow fluorescence in the graft was compared with the rib labelled with tetracycline, taken prior to the transplantation. As can be seen from the figure 15 and table 3 resorption occurred definitely more rapidly in a fresh bone, fully corresponding with the observations in the preceding series based on the deposition of tetracycline in the bone after transplantation.

The effect of the time of preservation

Autogenous frozen bone was preserved for 1 to 19 days in a frozen state. The time of preservation within these limits had no effect upon the rate of uptake of tetracycline in the transplant. This observation was further verified in a series of 14 rabbits. In 10 cases (2 litters, rabbits Nos. 38–47) the time from grafting to last tetracycline injection was 15 days and in 4 cases (the same litter, rabbits Nos. 48–51) 2 months. In this series the time of preservation varied in pairs from 1 to 5 days.

Autogenous bone preserved in Palavit. For the sake of curiosity, an autogenous rib was preserved for 12 days in a plastic substance called Palavit. Autogenous frozen bone was implanted in the same femur as a third graft in addition to a fresh transplant. This result did not differ from that observed in other methods of preservation (Fig. 7).

Comments

Although new-bone formation varied to some extent depending on individual factors and the different specimens are not absolutely directly comparable with each other, it was nevertheless proved that a fresh autograft without exception incorporated tetracycline decidedly faster than a frozen autograft.

The histological observations are in agreement with those obtained by means of the tetracycline method. Of those preparations of which both histological and tetracycline sections were taken from the same place, it could be seen that bone containing osteocytes was present in the place where yellow tetracycline incorporation was seen. However, 8—10 days after implantation, osteocyte-containing lacunae were seen in the histological section, especially in the superficial parts of the transplant where no tetracycline had occurred. This observation suggests that, in immediately transplanted autogenous grafts, osteocytes might survice after transplantation (Fig. 16). Some investigators (*Albee* 1944, *Vainio* 1950, *Vainio* and *Solonen* 1957) hold the view that a part of the fresh autograft is able to survive and even to become a part of the regenerated transplant.

Autogenous fresh bone and homogenous frozen bone

In the present study 14 homogenous frozen bones were grafted. The material (Table 4) provides the possibility of comparing homogenous frozen bone, not only with autogenous fresh bone, but also with autogenous frozen bone, bone preserved in whole blood, and bone preserved one hour in the open air (spec. 140 d in the table 5). In this way, the mutual differences between these types of bone were most easily recognized.

As regards the deposit of tetracycline, homogenous frozen bone was not essentially different from the other preserved bones (Fig. 6). The histological findings in homogenous frozen bone (Figs. 17, 19) were quite the same as those in autogenous frozen bone described on the page 33.

Autogenous fresh bone and autogenous bone preserved in whole blood at 37°C

As a counterbalance to frozen bone it was thought relevant to investigate whether preservation of bone in the experimental animal's own blood at 37°C prevents loss of its osteogenetic capacity. In order to make comparison with frozen bone also feasible, frozen bone was grafted into the same femur as a third transplant (Table 4). Two of the five rabbits were chosen for histological survey as well; the results are presented in the table 4. In the light of this small experimental series (5 rabbits), no differences from frozen bone could be observed either with tetracycline (Figs. 5, 6) or with histological methods (Fig. 18).

TABLE 4. — *Autogenous fresh bone (aF) combined with one or two of the following preserved bones:* { *autogenous frozen bone (aB)*, *homogenous frozen bone (hB)*, *autogenous bone preserved in whole blood (aBl)*

No. of rabbit	Age at transplantation (weeks)	Time of observation (days) (from grafting to last inj.)	Autograft transplanted after being kept frozen (days)	Autograft transplanted after being kept in blood (days)	Homograft transplanted after being kept frozen (days)	Tetracycline fluorescence in the graft								Remarks
						First graft				Second graft				Third graft			
						None or minimal	Scanty	Considerable	Abundant	None or minimal	Scanty	Considerable	Abundant	None or minimal	Scanty	Considerable	Abundant	
						aF				hB				aB				
142 d*	6	6			7	+				+								Fig. 17
143	7	7			120	+				+								
45	7	15	4		7		+			+				+				pe (aF)
60	8	15	3		3	+				+				+				pe (aF + aB)
72	8	13	5		14	+				+				+				pe (aF)
62	7	21			25		+			+								pe (aF)
137 d*	6	21			10		+			+								
136 d*	6	28			10		+				+							Fig. 19
139 d*	6	28			10		+				+							pe (aF) the distal part of the hB had considerable fluorescence
55*	9	42	5		13		+				+				+			
90	6	90	7		7				+		+				+			
91	6	90	7		7				+			+				+		
										aBl				aB or hB				
68*	7	14	3	3			+			+				+				Fig. 18
69*	7	14	3	3			+			+				+				pe (aF, aB, aBl)
70	7	14	3	3			+				+				+			pe (aF)
71	7	14	3	3			+			+				+				pe (aB + aBl) Fig. 5
67	7	21		5	8		+			+				+				pe (aF + aBl) Fig. 6

Explanations: d(dexter) after number: in which femur the grafts were implanted
*: sections for histological examinations as well
pe(aF): periosteum in aF

Autogenous fresh bone and autogenous bone preserved in the open air or in normal saline solution at room temperature

In orthopaedic operations where bone transplantations must be employed, it is often customary first to remove the bone to be transplanted and then keep it in room temperature either in the open air or in normal saline solution. After preparatory measures have been taken the transplant is grafted to its new location. This may well take 1 to 2 hours, especially in spinal fusion. In a survey of patients after spinal fusion for scoliosis at the Orthopaedic Hospital of the Invalid Foudation the suspicion arose that bone transplants, when preserved in the open air, may lose a part of their osteogenetic capacity.

This question was studied with the method described above in both tetracycline series. The material consists of 34 experiments. Six specimens were reserved for histological survey as well. All the grafts were implanted without periosteum. The material and the results are presented in the table 5.

It was found out that one hour's preservation in the open air was enough to change the properties of the bone transplant to such an extent that the bone began to resemble frozen bone (Figs. 22, 23, 25, 27, 28).

In normal saline solution the bone graft retained the properties found to be characteristic of fresh autogenous bone for a more prolonged period (Fig. 29), but a difference began to appear within 2 hours (Fig. 26), and after three hours the bone behaved like bone preserved one hour in the open air in comparison with freshly transplanted bone.

All tetracycline (Figs. 22–29) and histological (Fig. 30) preparations in these experiments gave fully uniform results.

Comments. It was unexpected that bone tissue is so sensitive that one hour's preservation in the open air was enough to make the autogenous bone similar to the frozen bone. Dryness, lack of oxygen etc. are obviously the reasons for which, after 4 weeks, the formation of the tetracycline-labelled bone and bone tissue containing osteocytes were seen to be scanty in such bone. The same reasons probably prevented the formation or influence of some factor stimulating osteogenesis, because there was less bone formation in the host bone at the site of such a transplant in comparison with a fresh autograft.

No attempt was made to make any distinction between time intervals of less than one hour of preservation in the open air, because many other factors, such as air humidity etc. may contribute to the time factor in changing the properties of a bone graft.

From the practical point of view it seems important that one hour was sufficient to reduce the osteogenetic properties of fresh autogenous bone, if preserved

in the open air, to the level of those of frozen bone. This suggests that in clinical surgery it may be wise to transfer the bone immediately to its final site or, if this is impossible, at least to preserve it in normal saline solution.

In the literature, not enough attention has been paid to this question, nor have experiments elucidating this matter, as far as known, been previously made.

TABLE 5. — *Autogenous fresh bone and autogenous bone preserved in the open air or in normal saline solution.*
Series I (tetracycline injection after grafting)

No. of rabbit	Age at transplantation (weeks)	Time of observation (days) (from grafting to last inj.)	Autograft preserved in the open air (hours)	Autograft preserved in saline solution (hours)	Tetracycline fluorescence in the graft												Remarks
					Autogenous fresh graft				Autograft preserved in the open air				Autograft preserved in saline solution				
					None or minimal	Scanty	Considerable	Abundant	None or minimal	Scanty	Considerable	Abundant	None or minimal	Scanty	Considerable	Abundant	
119	6	15	1			+			+								
65	6	15	2				+		+								Fig. 24
66	6	15	2				+		+								
80	6	21	1					+		+							Fig. 25
81	6	21	1					+		+							
120	6	22	1			+			+								
121	6	22	1					+		+							
122	6	22	1					+		+							
125	6	22	1				+			+							
105	5	28	1				+			+							
127 *	6	28	1				+		+								
128 *	6	28	1				+			+							
140 s*	6	28	1				+			+							
140 d*	6	28	1							+							a frozen homograft as the second graft. Scanty fluorescence
93	5	35	1					+		+							
94	5	35	1					+		+							Fig. 23
95	5	35	1					+	+								Fig. 22
96	5	35	1					+		+							
129 *	6	41	1					+		+							Fig. 30
130 *	6	41	1					+		+							
63	6	15		2			+							+			Fig. 26
64	6	15		2			+							+			
78	6	21		3			+						+				
79	6	21		3			+							+			
102	6	28		1				+							+		
103	6	28		1				+								+	

Continued

Table 5. — Continued

Series II (tetracycline injection prior to grafting)

No. of rabbit	Age at transplantation (weeks)	Time of observation (days) (from grafting to killing)	Autograft preserved in the open air (hours)	Autograft preserved in saline solution (hours)	Loss of tetracycline fluorescence												Remarks
					Autogenous fresh graft				Autograft preserved in the open air				Autograft preserved in saline solution				
					None or minimal	Scanty	Considerable	Abundant	None or minimal	Scanty	Considerable	Abundant	None or minimal	Scanty	Considerable	Abundant	
109	5	30	1				+		+								Fig. 28
110	5	30	1			+			+								
111	5	42	1					+		+							
112	5	42	1					+		+							Fig. 27
114	5	42	1					+		+							
106	5	30		1			+								+		
107	5	42		1				+								+	
108	5	42		1				+								+	Fig. 29

Explanations:
s(sinister) or d(dexter) after number: in which femur the grafts were implanted
*: sections for histological examinations as well

DISCUSSION

For almost a hundred years, experimental studies on bone transplantation have been carried out in various parts of the world. Mice, dogs and especially rabbits have been employed as experimental animals. Bone has been transplanted into soft tissues, into the anterior chamber of the eye and into bony surroundings, using widely different techniques. On the basis of such investigations, quite contradictory views have been expressed concerning the fate of the cells in transplanted bone tissue. This may partly be due to the diversity of the methods, but it is highly probable that, after all, the main reason for this controversy has been the lack of adequate methods of demonstrating the fate of transplanted bone. The interpretation of the histological appearance may give rise to differences of opinion. The more unambiguous a method of investigation is, the more reliable are the conclusions. In recent years, isotope studies have been successfully employed in studies on bone transplantation. Still simpler than the isotope technique is supravital labelling of bone formation with tetracycline, used since 1957 in bone research. As far as is known, this is one of the very first times that the tetracycline method has been used in studies of bone transplantation.

With the aid of the yellow fluorescence caused by the tetracycline, the sites of new-bone formation may be readily and unambiguously distinguished. Although tetracycline may also be deposited elsewhere, as was stated above, this occurs in such low concentrations that misunderstanding will not arise, provided that time interval between the last tetracycline injection and killing of the animal is at least 48 hours. In this case, no tetracycline will be seen in the place of resorption and the short-lived fluorescence of inactive surfaces has disappeared (*Milch et al.* 1958, *Harris et al* 1962, *Hulth* and *Olerud* 1962). Many investigators (among others, *Saxén* 1965) have observed that tetracycline has an inhibiting effect on osteogenesis. In the present study, tetracycline doses were usually administered at intervals of one week. Therefore the inhibiting effect of tetracycline is hardly of any consequence, because the inhibition is reversible and tetracycline will be eliminated in 24 hours (*Saxén*).

The surgical technique employed in the present experimental work is simple. Naturally there are individual differences in new-bone formation between

different rabbits, but this is of no significance, since the differences in osteogenesis were studied in bone transplants grafted into the same femur.

Reorganization of bone grafts occurs through resorption of the original graft and formation of new bone. Both of these events were studied with tetracycline in a way described on page 23, and complementary pictures of the reorganization of the bone transplant were thus obtained.

The present investigation has proved beyond dispute that a fresh autograft possesses the greatest tendency to be reorganized and incorporated as a part of the living skeleton, not attained by preserved bone even in a bony environment. There were no differences worth mentioning between autogenous frozen bone, homogenous frozen bone and autogenous bone preserved in whole blood (Figs. 3, 6).

In a fresh autograft, new-bone formation was seen within one week, whereas preserved bone began to show some slight osteogenesis only after 3 weeks. Reorganization of the fresh autograft progressed quickly (Figs. 2, 5, 7, 8, 12). Within 3 months (Figs. 14, 21), the fresh autograft completely resembled the lamellary structure of the host bone, whereas in the preserved bone not more yellow tetracycline fluorescence could be seen than after 2 weeks in fresh autogenous bone (Fig. 3). New-bone formation in the fresh autograft could be so abundant that the transplant grew 2—3 times larger than it was originally (Figs. 7, 20), and it was impossible to say where the original transplant lay. This suggests that the fresh autograft actively participates in the bone formation.

Reorganization of the preserved bone occurred very slowly. For a long time the reorganization was therefore restricted merely to parts close to the surfaces of the graft. Within 3 months, the preserved bone retained its shape and never grew larger than it was originally. The resorption was more strongly apparent in the preserved bone than in autogenous fresh bone, because in the former new-bone formation lagged clearly behind.

Besides reorganization of the bone graft, in these preparations, new-bone formation could be seen on the surface of the host bone (femur) in the immediate neighbourhood of the transplant. The new-bone formation originating from the host bone had a clear drift towards the fresh autograft (Figs. 19, 24). In about 5 to 7 weeks the fresh autograft was generally becoming embedded in bone formed by the host bone and obviously also by the graft itself, and sometimes the graft could hardly be distinguished from the host bone (Figs. 8, 10). New-bone formation at the site of preserved bone, on the other hand, occurred mostly slowly and the preserved graft could easily be detached from the host bone even after several weeks.

The present investigation also produced evidence to the effect that it made no difference whether bone was preserved in the bone-bank for one or several days, because it reorganized equally poorly. The time factor, however, played an important role in the handling of autogenous bone. Keeping it for one hour in the open air at room temperature already meant that the bone decidedly lost its osteogenetic capacity and most closely resembled frozen bone (Figs. 22–25). If preserved in normal saline solution, however, definite differences in osteogenesis became apparent after 2 hours (Fig. 26), and after 3 hours it was equal to frozen bone. Autogenous bone preserved for 3 to 5 days in the animal's own blood at 37°C behaved in the same manner as frozen bone (Fig. 3).

In this study, the pieces of ribs utilized always contained also bone marrow. Both tetracycline and histological findings showed early bone formation in the bone marrow of the autogenous fresh graft. The role of the fresh autogenous marrow in reorganization of the transplant is supported by *Burwell*'s experiments (1964) with the composite homograft-autograft. He found that most of the new bone formed in this kind of composite graft is derived from the autogenous marrow. In his opinion, the primary wandering cells (called the intermediate cells by *Trueta* 1963), derived from the littoral cells of the surviving marrow, are differentiated into osteoblasts by the osteogenetic substance liberated from the dying bone and marrow tissues of the transplant.

The role of the periosteum in osteogenesis, emphasized by *Ollier*, *G.* and *W. Axhausen* and *Vainio*, was not systematically studied, since this question has been competently dealt with in earlier investigations (*Vainio* 1948). In the present study the general impression has also been that fresh bone transplanted with its periosteum will regenerate more rapidly than if the periosteum is absent. On the other hand, it seemed that periosteum-covered preserved bone does not undergo osteogenesis any better than when transplanted without the periosteum.

What causes the difference between fresh autogenous bone and preserved bone? It becomes obvious that antibody reaction cannot play any significant role, as apart from homogenous frozen bone, only autogenous preserved bone was used. Besides, freezing has been found to diminish the antigenic status of the homogenous bone, and in the present investigation no differences could be found between autogenous and homogenous frozen bone.

When looking for the cause of the more rapid reorganization of a fresh autogenous transplant, two questions must be answered:

1) Are there, in a fresh autograft, surviving osteogenic cells?

2) Are factors stimulating bone formation and important to the process of the reorganization of the transplant destroyed during the preservation?

The present series would suggest that freezing destroys the cells in a graft. 3 weeks following transplantation the lacunae of frozen bone were still histologically empty, nor had any tetracycline incorporation worth mentioning occurred. Several previous investigations corroborate these observations, and physico-chemical incidents in the frozen bone make them understandable. Drying, lack of oxygen etc., on the other hand, are obviously the reasons why a bone preserved for one hour in the open air acts like frozen bone.

It seems obvious that osteogenic cells survive in a fresh autogenous transplant. This is supported by the following observations:

- New-bone formation could already be seen after one week.
- New-bone formation did not always appear first in that part of the graft that had been inserted into the burr-hole of the femur. It could also arise from a part of the transplant separated from the host bone and sometimes even from the interior and not from the outer surface of the graft.
- In addition, histological findings suggested that some part of the fresh autogenous bone survived after transplantation (see comments, page 35).

The idea according to which osteogenic cells survice in a fresh transplant, is supported by recent investigations. *Ray* and *Sabet* in 1963, using tritiated thymidine, and *Arora* and *Laskin* in 1964, using sex chromatin as a cellular label, found that in an isograft (naturally also in an autograft) both the cells of the graft and those of the host are capable of taking part in new-bone formation. Even recent immunological investigations have supported this view (*Chalmers* 1959, *Burwell* and *Gowland* 1961, *Mawdsley* and *Harrison* 1963). *Chalmers* found that after implantation into the muscle, new-bone formation occurs in two phases in fresh homogenous cancellous bone: in the early phase new bone is already present on the fourth day and dies out on the eighth day, while in the late phase, new bone will appear after four weeks, albeit in only some transplants. According to his interpretation, the destruction of the bone formed in the early phase proves that these osteogenic, proliferating cells survive in the homograft, resulting in acquired immunity in the host. In the late phase, on the other hand, new-bone formation starts from the surrounding connective tissue as a result of induction. *Chalmers* as well as *Burwell* and *Gowland* made the observation that if the host bone is sensitized to the donor beforehand, this will prevent early bone formation in homogenous graft. In their opinion this supports the view that the new-bone formation in the early phase is derived from the cells of the transplant. They found that in the fresh autograft this early bone formation is more abundant and progresses without interruption because there is not immunological reaction to destroy the cells.

To the first question whether osteogenic cells are surviving in a fresh autograft, one is thus justified to answer in the affirmative. As regards the second

question, it is obvious that bone degeneration and necrosis always occur in free bone transplantation when blood circulation to the transplant is interrupted. This results in the release of biochemical agents which, in the view of many authors, are responsible for the reorganization of the transplants since these agents stimulate surrounding pluripotential cells to form bone. According to *Trueta* (1963), such a substance would arise after the death of chondrocytes, osteocytes and endothelial cells of the graft, exerting a stimulating influence upon angioblasts in the vascular system of its environment. This leads to vascularization of the graft and, unless there is an antibody reaction as occurs when homogenous bone is used, the development of osteoblasts from endothelial cells begins.

Freezing always causes alterations in the biochemical structure of the bone (*Sieber* 1956). So having compared fresh and frozen autogenous and homogenous bone grafts with each other, *De Bruyn* and *Kabish* (1955) found that fresh autogenous bone is definitely more valuable. They concluded that freezing destroys the osteogenetic substance. They also explained that at the moment of transplantation there are still cells surviving in an autogenous fresh bone that are capable of producing such a substance, which induces the mesenchymal cells to form bone. Since homogenous fresh bone cannot do this, they believed that this substance is partly specific.

However, attempts upon isolating some osteogenetic substance have not been successful and *Urist* (1965) does not even believe in its existence. According to him, bone induction is more complex than a simple chemical stimulus and direct cellular response. On the basis of *Spemann*'s theory of induction he suggests that cellular differentiation occurs by autoinduction: proliferating, pluripotent, ingrowing connective-tissue cells interact and produce osteoblasts. However, the evidences for induction by bone tissue are not beyond dispute and many investigators (*Danis* 1957, *Duthie* 1958, *Bridges* 1959) question its value in osteogenesis

The present investigations demonstrate:

- There was no early bone formation in preserved bone, such as there was in fresh bone After 3 weeks, however, the reorganization of preserved bone started but it progressed slowly.
- The transplant was also found to exert a definite osteogenetic action on the surface of the host bone. There, even vivid trabeculation occurred in that area which was not in contact with the transplant and which had not suffered any mechanical damage during transplantation.
- It was also found in the experiments that a fresh transplant had a definitely stronger influence upon the trabeculation of the host bone than preserved bone.

— The fresh autograft has obviously some osteogenetic effect on preserved bone, because new-bone formation in the latter almost regularly started in the part that was next to the fresh transplant (Figs. 7, 25, 30).

These observations would suggest that there exists some system stimulating osteogenesis with an effect on the reorganization of the transplant, even in the case of preserved bone. In addition, it is plausible that the preservation of the bone destroys some sensitive factors stimulating osteogenesis. This deleterious effect on the autogenous bone is already produced by one hour's preservation in the open air. However, the nature of the system stimulating osteogenesis is obscure. Solution of this question is difficult, because obviously osteogenesis is a result from interaction of many biochemical factors.

On the basis of the results of the present investigation, it is apparent that the rapidity with which immediately transplanted autogenous bone reorganizes as compared with the preserved bone, is due to the survival of a part of the grafted bone transplant, and to the influence of some easily destructible factors stimulating osteogenesis.

Sir Reginald Watson-Jones said when speaking of bone grafting at the American College of Surgeon's meeting in San Francisco in 1951 that, if he had to perform a bone-grafting operation he would require grafts, the bone cells of which would fight for him (quoted by *Wilson* 1953). This is exactly what bone transplanted from the donor site without delay does. It would not merely serve as a guiding structure but actively participates in the bone formation.

SUMMARY

In this experimental work, the reorganization of autogenous fresh bone was compared with that of autogenous and homogenous frozen bone and with that of autogenous bone preserved in the open air, in normal saline solution or in whole blood, after free transplantation. Attention was also paid to the role of the time factor in reorganization.

The property of tetracycline to form a deposit in newly-formed bone and to produce yellow fluorescence in ultraviolet light was utilized in the examination of reorganization. The study was performed mainly on young rabbits. Ribs were used as transplants, which were implanted in the femur (Fig. 1, page 20). The material comprises 118 rabbits (see page 19). The rabbits were killed 8—90 days after operation.

By the tetracycline method it was possible to study both components of reorganization: the formation of new bone and the resorption of the original transplanted bone. New bone formed after transplantation (apposition) was made visible by injecting tetracycline in rabbits at intervals of about one week after the bone grafting. Resorption could be estimated by observing how the rib labelled with tetracycline before grafting lost its yellow fluorescence when it was serving as a transplant. In addition, histological examination was used as a parallel method. The results obtained with both methods are in conformity with each other.

In the experiments performed, the difference in osteogenetic capacity between autogenous fresh bone and any other kind of bone graft proved to be very great (Figs. 2, 5, 24). First, fresh autogenous bone became reorganized decidedly more rapidly, and second, new bone was also formed from the host bed in greater amounts at the site of the fresh transplant than when preserved bone was used.

There were no differences worth mentioning between homogenous and autogenous frozen bone and autogenous bone preserved in whole blood (Figs. 3, 6).

It could not be shown here that the time of preservation of the bone in a frozen state could have any essential effect on its capacity of reorganization.

Autogenous bone preserved in the open air at room temperature for about one hour lost its osteogenetic properties to the extent that it almost resembled

frozen bone (Figs. 22, 23, 25). On the other hand, if bone was preserved in normal saline solution, this degradation did not occur until after about 2 hours (Figs. 26, 29).

The difference between fresh and preserved autogenous bone may be due to the fact that living osteogenic cells, even some osteocytes, survive in fresh bone after transplantation. A fresh autograft would thus not merely serve as a guiding structure but actively participates in the bone formation while, at same time, it gives rise to stronger bone formation from the host bone than preserved bone. The possibility cannot be excluded that the tranplant contains or develops some inductive substance which is rapidly destroyed or whose development is readily inhibited.

The observation that one hour's preservation in the open air in the experiments resulted in diminished osteogenesis of the bone might be significant from the point of view of clinical practice.

The main focus of this study has been to try to elucidate the question whether an immediately transplanted autogenous bone graft is more quickly and completely incorporated into the skeleton than a preserved autogenous or homogenous graft. The experiments strongly support a preference for immediate transplantation of autogenous bone.

REFERENCES

Albee, F. H.: Fundamentals in bone transplantation. Experiences in three thousend bone graft operations. J.A.M.A., 17. *81*:1429, 1923.

Albee, F. H.: Evolution of bone graft surgery. Amer. J. Surg., *63*:421, 1944.

Anderson, K. J.: The behavior of autogenous and homogenous bone transplants in the anterior chamber of the rat's eye. J. Bone and Joint Surg., *43-A*:980, 1961.

Annersten, S.: Experimentelle Untersuchungen über die Osteogenese und die Biochemie des Frakturcallus. Acta Chir. Scand., *84*: Suppl. 60, 1940.

Arens, W.: Erfahrungen und Folgerung aus 500 Knochenverpflanzungen. Langenbecks Arch. Klin. Chir., *308*:1024, 1964.

Arora, B. K., Laskin, D. M.: Sex chromatin as a cellular label of osteogenesis by bone grafts. J. Bone and Joint Surg., *46-A*:1269, 1964.

Axhausen, G.: Histologische Untersuchungen über Knochentransplantation am Menschen. Deutsch. Z. Chir., *91*:388, 1907.

Axhausen, G.: Die histologischen und klinischen Gesetze der freien Osteoplastik auf Grund von Thierversuchen. Langenbecks Arch. Klin. Chir., *88*:23, 1909.

Axhausen, W.: The osteogenetic phases of regeneration of bone. A histological and experimental Study. J. Bone and Joint Surg. *38-A*:593, 1956.

Axhausen, W.: Die Bedeutung der Individual- und Artspezifität der Gewebe für die freie Knochenüberpflanzung. Mschr. Unfallheilk., Heft 72, 1962.

Barth, A.: Über histologische Befunde nach Knochenimplantationen. Langenbecks Arch. Klin. Chir., *46*:409, 1893.

Baschkirzew, N. J., Petrow, N. N.: Beiträge zur freien Knochenüberpflanzung. Deutsch. Z. Chir., *113*:490, 1912.

Bertelsen, A.: Experimental investigations into post-foetal osteogenesis. Acta Orthop. Scand., *15*:139, 1944.

Bonfiglio, M.: Repair of bone-transplant fractures. J. Bone and Joint Surg., *40-A*: 446, 1958.

Bosworth, D. M., Wright, H. A., Fielding, J. W., Goodrich, E. R.: A study in the use of bank bone for spine fusion in tuberculosis. J. Bone and Joint Surg., *35-A*: 329, 1953.

Bridges, J. B.: Experimental heterotopic ossification. Int. Rev. Cytol., *8*:253, 1959.

Burwell, R. G.: Studies in the transplantation of bone. VII. The fresh composite homograft-autograft of cancellous bone. J. Bone and Joint Surg., *46-B:* 110, 1964.

Burwell, R. G., Gowland, G.: Studies in the transplantation of bone. II. The changes occurring in the lymphoid tissue after homografts and autografts of fresh cancellous bone. J. Bone and Joint Surg., *43-B:*820, 1961.

Buser, P.: Erfahrungen mit Kältekonservierten Knochenspänen. Langenbecks Arch. Klin. Chir., *290:* 289, 1959.

Bush, L. F., Carber, C. Z.: The bone bank. J.A.M.A., *137:* 588, 1948.

Bürkle de la Camp, H.: Knochentransplantationen. Verhandlungsbericht d. XVIII. Kongr. d. Société Internationale de Chirurgie. S. 169–205. Impr. Med. et Scient., Bruessel, 1959.

Böhler, J.: Erfahrungen mit der Knochenbank. Chirurg, *23:*255, 1952.

Böhler, J.: Banksverpflanzung bei frischen Schaftbrüchen der langen Röhrenknochen. Chirurg, *26:* 76, 1955.

Campbell, C. J., Brower, Th., MacFadden, D. G., Payne, E. B., Doherty, J.: Experimental study of the fate of bone grafts. J. Bone and Joint Surg., *35-A:* 332, 1953.

Carnesale, P. L., Spankus, J. D.: A clinical comparative study of autogenous and homogenous-bone grafts. J. Bone and Joint Surg., *41-A:* 887, 1959.

Chalmers, J.: Transplantation immunity in bone homografting. J. Bone and Joint Surg., *41-B:* 160, 1959.

Chase, S. W., Herndon, C. H.: The fate of autogenous and homogenous bone grafts. A historical review. J. Bone and Joint Surg., *37-A:* 809, 1955

Danis, A.: Étude de l'ossification dans les greffes de moelle asseuse. Acta Chir. Belg., Suppl. 3, 1957.

Danis, A.: Après une greffe de tissu squelettique ostéogène c'est a partir des cellules transplante'es que se contitué l'os de nouvelle formation. Bull. Soc. Int. Chir., *19:* 647, 1960.

De Bruyn, P. P. H., Kabisch, W. T.: Bone formation by fresh and frozen, autogenous and homogenous transplants of bone, bone marrow and periosteum. Amer. J. Anat., *96:* 375, 1955.

Deleu, J., Trueta, J.: Vascularisation of bone graft in the anterior chamber of the eye. J. Bone and Joint Surg., *47-B:*319, 1965.

De Tomasi, L.: Gli innesti d'osso: ulteriore contributo clinico e sperimentale alla banca dell'osso. Arch. Ortop., *68:* 242, 1955.

Duthie, R. B.: A histochemical study of transplanted skeletal tissue during tissue culture *in vivo*. Brit. J. Plast. Surg., *11:* 1, 1958.

Ehalt, W.: Ergebnisse bei der Verwendung konservierter Knochen. Langenbecks Arch. Klin. Chir., *279:* 44, 1954.

Engström, H., Orell, S.: Über Regeneration rings um subcutane Knochenimplantate. Z. Mikr. Anat. Forsch., *53:* 285, 1943.

Fell, H. B.: Skeletal development in tissue culture. *In* Biochemistry and Physiology of Bone. Chapter 14. Edited by G. H. Bourne. Academic Press Inc., New York, 1956.

Frost, H. M., Villaeuva, A. R., Roth, H.: Tetracycline staining of newly forming bone and mineralizing cartilage *in vivo*. Stain Techn., *35:* 135, 1960.

Goldhaber, P.: Osteogenic induction across millipore filters *in vivo*. Science, *133:* 2065, 1961.

Guilleminet, M., Stagnara, P., Dulcost Perret, T.: Preparation and use of heterogenous bone grafts. J. Bone and Joint Surg., *35-B:* 561, 1953.

Hackethal, K. H.: Erfahrungen mit der Knochenbank. Beilageheft Z. Orthop., *87:* 59, 1956.

Ham, A. W., Harris, W. R.: Repair and transplantation of bone. *In* Biochemistry and Physiology of Bone. Chapter 16. Edited by G. H. Bourne. Academic Press Inc., New York, 1956.

Harris, W. H., Jackson, R. H., Jowsey, J.: The *in vivo* distribution of tetracyclines in canine bone. J. Bone and Joint Surg., *44-A:* 1308, 1962.

Hartley, J., Tanz, S. S., Schneider, M.: Osteogenesis produced by a chemical extract of bone. J. Mount. Sinai Hosp., *15:*385, 1949.

Heinen, J. H., Dabbs, G. H., Mason, H. A.: The experimental production of ectopic cartilage and bone in the muscles of rabbits. J. Bone and Joint Surg., *21-A:* 765, 1949.

Heiple, K. G., Chase, S. W., Herndon, C. H.: A comparative study of the healing process following different types of bone transplantation. J. Bone and Joint Surg., *45-A:* 1593, 1963.

Heslop, B. F., Zeiss, I. M., Nisbet, N. W.: Studies on transference of bone. I. A comparison of autologous and homologous bone implants with reference to osteocyte survival, osteogenesis and host reaction. Brit. J. Exp. Path., *41:* 269, 1960.

Hult, L.: Some experiences with a bone bank. Acta. Orthop. Scand., *19:*476, 1950.

Hulth, A., Olerud, S.: Tetracycline labelling of growing bone. Acta Soc. Med. Upsal., *67:* 219, 1962.

Hurley, L. A., Stinchfield, F. E., Bassett, A. L., Lyon, W. H.: The role of soft tissues in osteogenesis. An experimental study of canine spine fusions. J. Bone and Joint Surg., *41-A:*1245, 1959.

Idelberger, K.: Knochenkonservierung in Palavit und durch Gefriertrocknung. Beilageheft Z. Orthop., *87:* 69, 1956.

Inclán, A.: The use of preserved bone graft in orthopaedic surgery. J. Bone and Joint Surg., *24:*81, 1942.

Judet, R.: Hétérogreffes d'os frais. Mém. Acad. Chir., *75:* 524, 1949.

Kingma, M. J., Hampe, J. F.: The behaviour of blood vessels after experimental transplantation of bone. J. Bone and Joint Surg., *46-B:* 141, 1964.

Küppermann, W.: Knochen statt Metall bei der Osteosynthese. Beilageheft Z. Chir., *91:*511, 1959.

Lacroix, P.: Organizers and the growth of bone. J. Bone and Joint Surg., *29:*292, 1947.

Levander, G.: On the formation of new bone in bone transplantation. Acta Chir. Scand., *74:*425, 1934.

Levander, G.: A study of bone regeneration. Surg. Gynec. Obstet., *67:* 705, 1938.

Lexer, E.: Die freien Transplantationen. F. Enke. Stuttgart, 1924.

Lindahl, O., Orell, S.: Experiments with bone extracts. Acta Chir. Scand., *101:*136, 1951.

Maatz, R., Bauermeister, A.: Klinische Erfahrungen mit dem Kieler Span. Langenbecks Arch. Klin. Chir., *298:*259, 1961.

Magnolfi, R.: La terapia delle cisti ossee solitarie giovanili con innesti ossei. Arch. Ortop., *71:* 63, 1958.

Mawdsley, R., Harrison, G. A.: Fate of transplanted bone. Nature, *198:*495, 1963.

Meiss, W. C.: Transplantations avec de l'os conservé. Acta Orthop. Belg., *18:*19, 1952.

Milch, R. A., Rall, D. P., Tobie, J. E.: Bone localization of the tetracyclines. J. Nat. Cancer Inst., *19:* 87, 1957.

Milch, R. A., Rall, D. P., Tobie, J. E.: Fluorescence of tetracycline antibiotics in bone. J. Bone and Joint Surg., *40-A:*897, 1958.

Moe, J. H.: A critical analysis of methods of fusion for scoliosis. J. Bone and Joint Surg., *40-A:* 529, 1958.

Oberdalhoff, H.: Zur Frage der Knochenneubildung. Chirurg, *17:*123, 1947.

Ollier, L.: Traité expérimental et clinique de la régénération des os et de la production artificielle du tissu osseux. Masson et fils, Paris, 1867.

Orell, S.: Studien über Knochenimplantation und Knochenneubildung. Implantation von "Os Purum" sowie Transplantation von "Os Novum". Acta Chir. Scand., *74:* Suppl. 31, 1934.

Phemister, D. B.: Biologic principles in the healing of fractures and their bearing on treatment. Ann. Surg., *133:* 433, 1951.

Ray, R. D., Degge, J., Gloyd, P., Mooney, G.: Bone regeneration. An experimental study of bone-grafting materials. J. Bone and Joint Surg., *34-A:*638, 1952.

Ray, R. D., Sabet, T. Y.: Bone grafts: Cellular survival versus induction. An experimental study in mice. J. Bone and Joint Surg., *45-A:*337, 1963.

Rehn, J.: Indikationen zur Verwendung von Auto- und Homoiotransplantaten am Knochen. Langenbecks Arch. Klin. Chir., *308:*1021, 1964.

Report of the committee to study the preservation of bone. J. Bone and Joint Surg., *35-A:* 774, 1953.

Reynolds, F. C.: The fate of bone grafts (discussion). J. Bone and Joint Surg., *35-A:* 344, 1953.

Reynolds, F. C., Oliver, D. R.: Experimental evaluation of homogenous bone grafts. J. Bone and Joint Surg., *32-A:* 285, 1950.

Reynolds, F. C., Oliver, D. R., Ramsey, R.: Clinical evaluation of the merthiolate bone bank and homogenous bone grafts. J. Bone and Joint Surg., *33-A:*873, 1951.

Riska, E. B.: Spinal fusion in scoliosis. Acta Orthop. Scand., Suppl. 67, 1964.

Rokkanen, P., Slätis, P., Laine, H.: Oxytetracycline labelling of experimental aseptic necrosis of the femoral head. Ann. Chir. Gynaec. Fenn., *52:*695, 1963.

Roth, H.: Zur Frage der "Bone Bank". Schweiz. Med. Wschr., *80:* 345, 1950.

Roth, H.: Extraktversuche mit konserviertem Knochengewebe. Schweiz. Med. Wschr., *80:* 1051, 1950.

Roth, H.: Die Konservierung von Knochengewebe für Transplantationen. Springer, Wien, 1952.

Roth, H.: Spätresultate bei Pseudarthrosenoperationen mit konservierten Knochenspänen. Langenbecks Arch. Klin. Chir., *276:*183, 1953.

Sabet, T. Y., Hidvegi, E. B., Ray, R. D.: Bone immunology. II. Comparison of embryonic mouse isografts and homografts. J. Bone and Joint Surg., *43-A*:1007, 1961.

Saxen, L.: Tetracycline: Effects on osteogenesis *in vitro*. Science, *149*:870, 1965.

Saxen, L.: Personal communication to the author.

Schmid-Schmidsfelden, O.: Unsere Erfahrungen mit der Knochenbank. Wien. Klin. Wschr., *62*:977, 1950.

Schmid-Schmidsfelden, O.: Ein Weiterer Beitrag zur Transplantation von Kältekonserviertem Knochen (Knochenbank) mit Kritik der Spätergebnisse. Arch. Orthop. Unfallchir., *46*:515, 1954.

Schoch, J.: Kritisches zur Knochentransplantation. Z. Orthop., *88*: 69, 1956.

Schwemmer, G., Contzen, H.: Einheilungszeit bei auto-homoio- und heteroplastischen Knochenspänen. Langenbecks Arch. Klin. Chir., *308*:1031, 1964.

Sieber, E.: Ergebnisse mit kältekonservierten homoioplastischen Knochenspänen, speziell bei der Pseudarthrosenoperation nach Phemister. Langenbecks Arch. Klin. Chir., *279*:69, 1954.

Sieber, E.: Biochemische Untersuchungen an konserviertem Knochengewebe. Zbl. Chir., *81*: 538, 1956.

Sissons, H. A.: The growth of bone. *In* Biochemistry and Physiology of Bone. Chapter 15. Edited by G. H. Bourne. Academic Press Inc., New York, 1956.

Spemann, H.: Embryonic development and induction. Yale Univ. Press, New Haven, Conn., 1938.

Stringa, G.: Studies of vascularisation of bone grafts. J. Bone and Joint Surg., *39-B*: 595, 1957.

Tapp, E.: Tetracycline labelling methods of measuring the growth of bones in the rat. J. Bone and Joint Surg., *48-B*:517, 1966.

Trueta, J.: The role of the vessels in osteogenesis. J. Bone and Joint Surg., *45-B*: 402, 1963.

Urist, M. R.: Bone: Formation by autoinduction. Science, *150*:893, 1965.

Urist, M. R., Ibsen, K. H.: Chemical reactivity of mineralized tissue with oxytetracycline. Arch. Path., *76*:484, 1963.

Urist, M. R., McLean, F. C.: Osteogenetic potence and new-bone formation by induction in transplants to the anterior chamber of the eye. J. Bone and Joint Surg., *34-A*:443, 1952.

Vainio, S.: Luun autotransplantaatin regeneraatio. Kokeellinen tutkimus. Helsinki, 1948.

Vainio, S.: Observations on the regeneration of an autogenous transplant of the bone. An experimental investigation. Acta Chir. Scand., *100*: 86, 1950.

Vainio, S.: Transplantation of bone. An experimental study. Ann. Chir. Gynaec. Fenn., Suppl. 67, 1957.

Vainio, S., Solonen, K.: The regenerative ability of the autoplastic and deep-frozen homoplastic transplant of bone. A comparative experimental study. Ann. Chir. Gynaec. Fenn., *46*:222, 1957.

Vanderhoeft, P. J., Kelly, P. J., Peterson, L. F.: Determination of growth rates in canine bone by means of tetracycline-labelled patterns. Lab. Invest., *11*:714, 1962.

Weaver, J. B.: Experiences in the use of homogenous (bone bank) bone. J. Bone and Joint Surg., *31-A:* 778, 1949.

Willestaedt, H., Levander, G., Hult, L.: Studies in osteogenesis. Acta Orthop. Scand., *19:* 419, 1950.

Wilson, P. D.: Experience with a bone bank. Ann. Surg., *126:*932, 1947.

Wilson, P. D.: Follow-up study of the use of refrigerated homogenous-bone transplants in orthopaedic operations. J. Bone and Joint Surg., *33-A:*307, 1951.

Wilson, P. D.: Discussion. J. Bone and Joint Surg., *35-A:*345, 1953.

Zeiss, I. M., Nisbet, N. W., Hesslop, B. F.: Studies on transference of bone. II. Vascularization of autologous and homologous implants of cortical bone in rats. Brit. J. Exp. Path., *41:* 345, 1960.

FIGURES

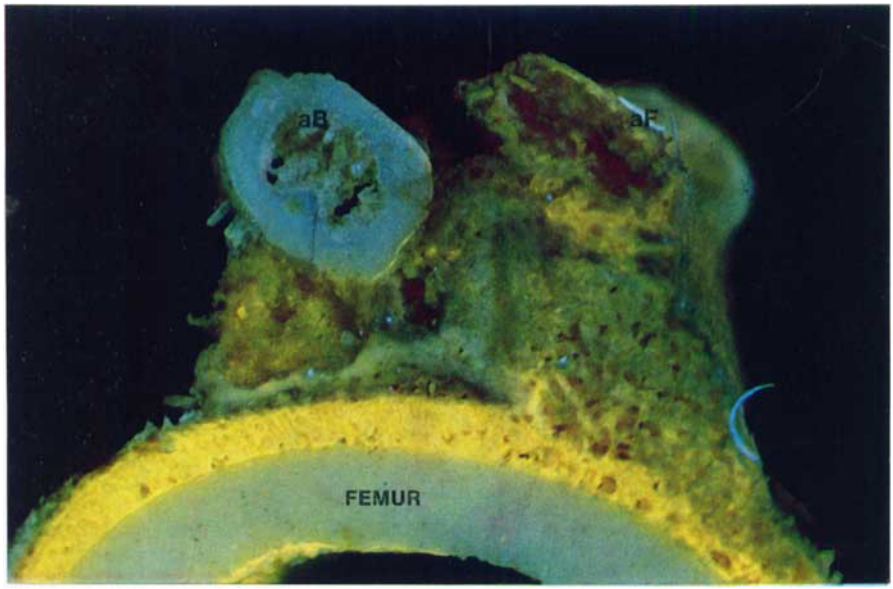


FIG. 2. — *(spec. 22). 10 days from grafting to last tetracycline injection. Cross section. The frozen autograft aB displays only bluish autofluorescence (no new-bone formation). The fresh autograft aF looks porous (resorption) and there is yellow tetracycline fluorescence (apposition). In the host bone there is lively trabeculation that is strongest at the site of the fresh transplant. The line of demarcation between the host bone and the fresh graft is not clear-cut.*

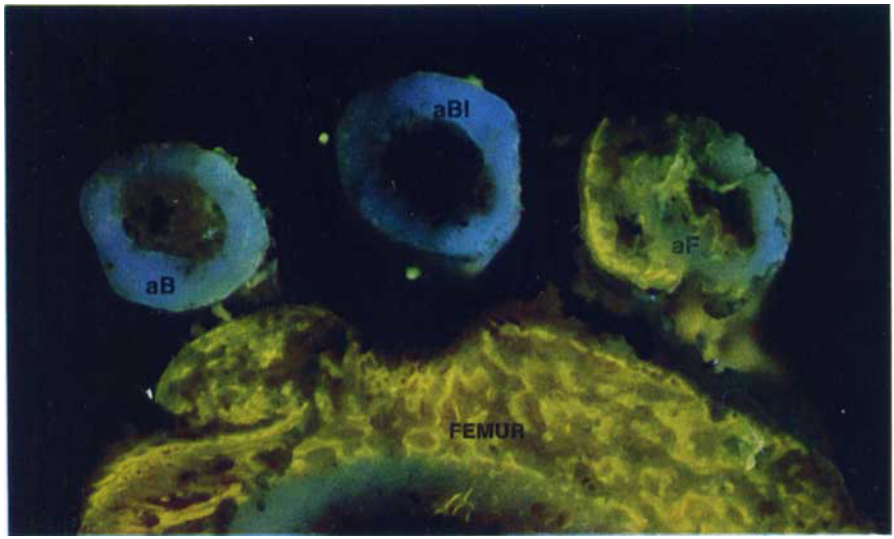


FIG. 3. — *(spec. 71). 14 days from grafting to last tetracycline injection. Cross section. In the fresh autograft aF yellow fluorescence indicates apposition. Note new-bone formation in bone marrow. The frozen autograft aB and the autogenous bone preserved in whole blood aBl are as if recently implanted.*

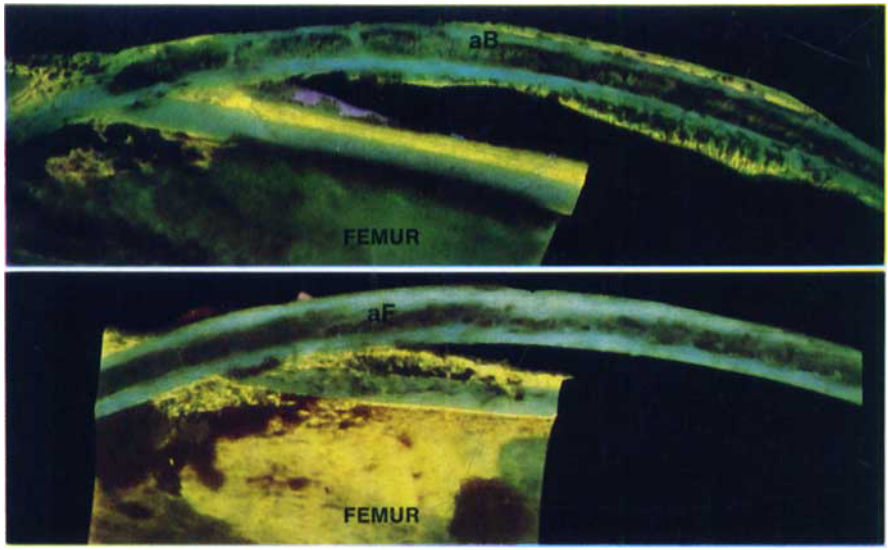


FIG. 4. — *(spec. 26). 10 days from grafting to last tetracycline injection. Longitudinal section. The frozen autograft aB displays bluish dense autofluorescence. In the fresh autograft aF there appears yellow fluorescence (apposition) mostly in that part which is not implanted in the host bone. Note that the new-bone formation seems to begin in the central part of the upper corticalis.*

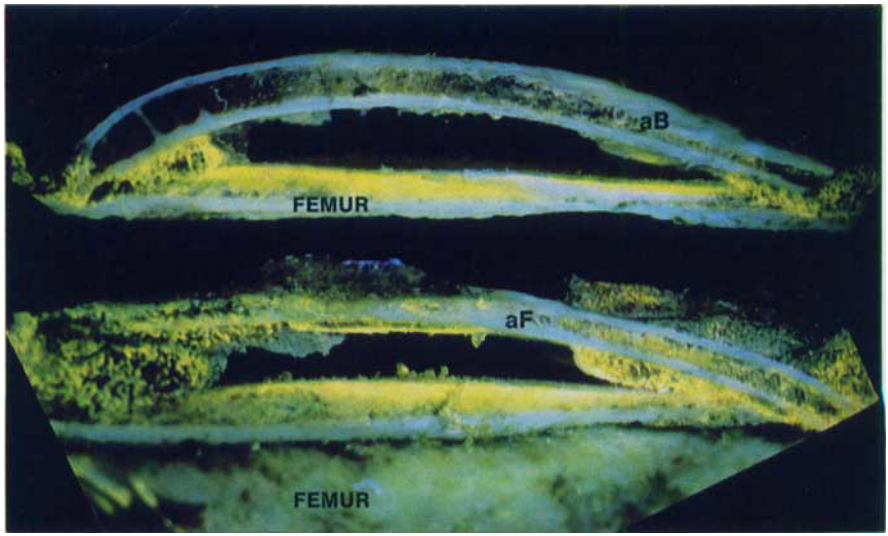


FIG. 5. — *(spec. 44). 15 days from grafting to last tetracycline injection. Longitudinal section. The autogenous frozen bone aB exhibits minimal tetracycline fluorescence outside the site of implantation. The fresh autograft aF is extensively reorganized especially in the distal part. At both ends of the transplant there is abundant new-bone formation derived from the host bone. This bone formation has distally resulted in fusion with the transplant.*

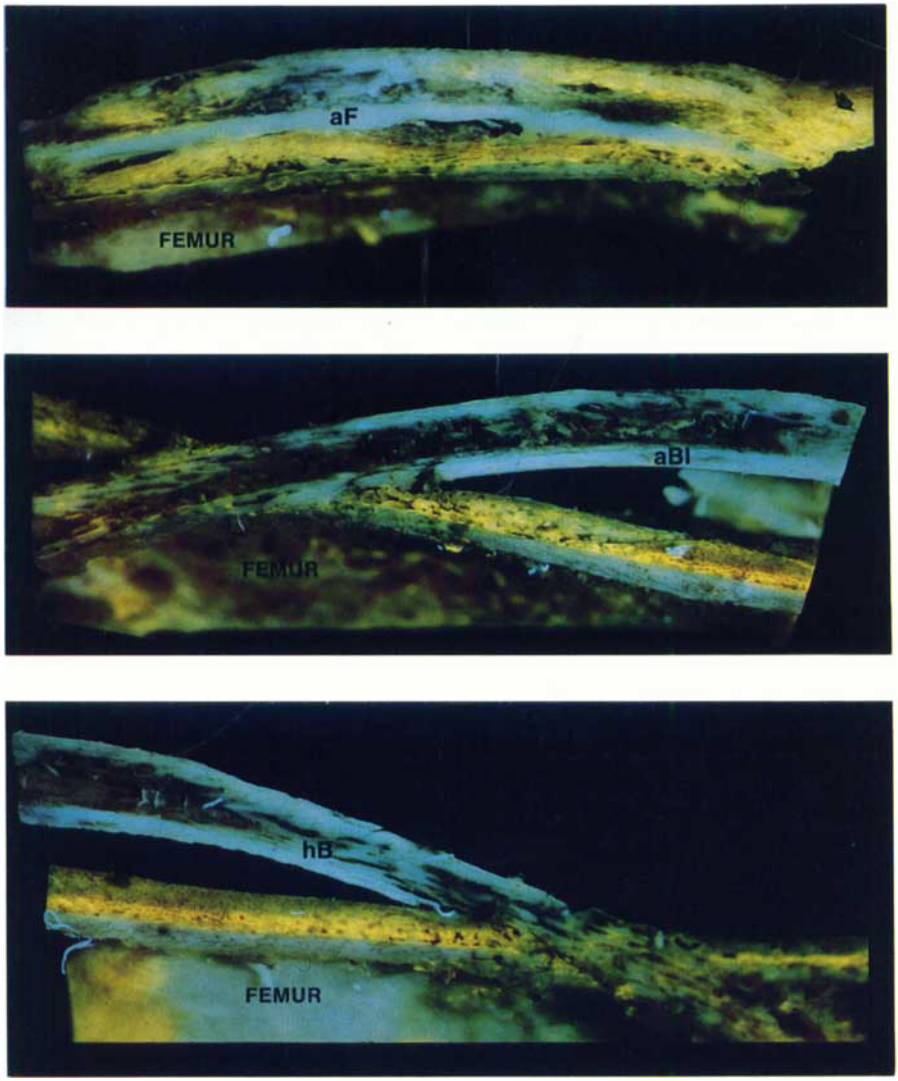


FIG. 6. — *(spec. 67). 3 weeks from grafting to last tetracycline injection. Longitudinal section. In the homograft hB and autograft preserved in blood aBl there is reorganization only at the site of implantation. The fresh autograft aF has yellow fluorescence in most parts of the transplant, especially in the upper corticalis. The bone formation derived from the host bone is almost non-existent in preserved bones, but in the fresh graft it has reached the surface of the transplant, and at the site of implantation the obtuse angle between the host bone and the graft is filled up with new bone (arrow).*

FIGS. 7. a, b, c, d and e. — *(spec. 57). 6 weeks from grafting to last tetracycline injection.*

— a. *Front view of the femur with the grafts. The frozen autograft aB and the autogenous bone aP preserved in Palavit are clearly seen. The fresh autograft aF between both preserved bones is hardly discernible from the host bone.*

— b. *Longitudinal section of the fresh autograft aF. This proximal part is almost completely incorporated into the host bone.*

— c and d. *Longitudinal section. In aB and in aP reorganization is found only at the site of implantation. Yellow colour indicated by the arrows is reflected from the surface of the fresh transplant. At the site of these transplants there is only very little new-bone formation derived from the host bone.*

— e. *Cross section along the dotted line of the distal part of the transplants (see fig. a.). The fresh autograft is found to be completely reorganized. Because of the new-bone forming, the transplant has lost its form to the extent that it is hard to say what part of it represents the original transplant. Tetracycline incorporation in the preserved bones is seen mainly in the part adjacent to the fresh transplant.*

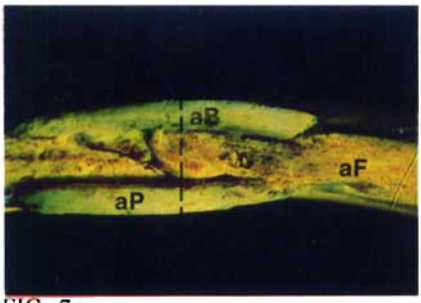


FIG. 7 a.

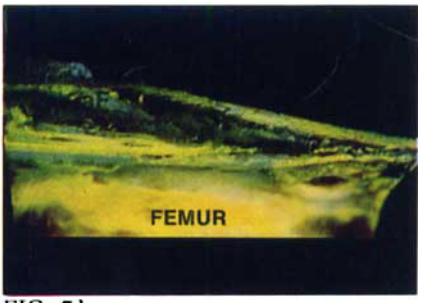


FIG. 7 b.

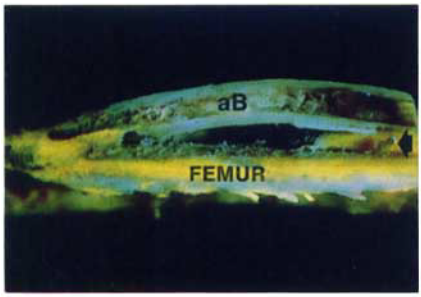


FIG. 7 c.

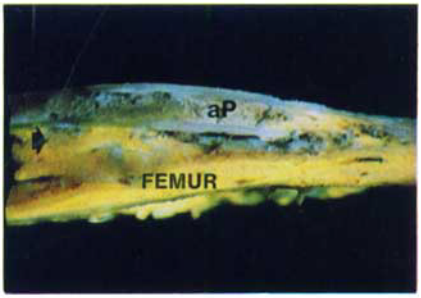


FIG. 7 d.

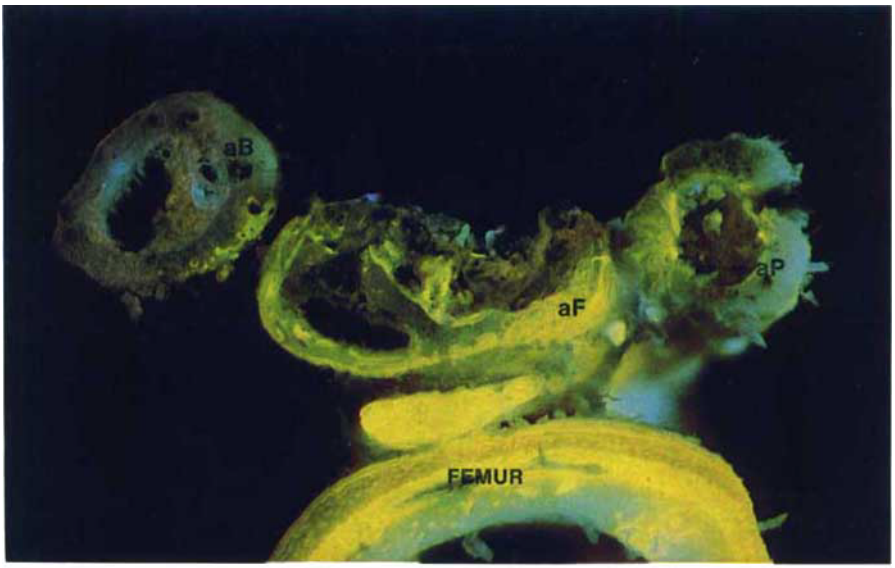


FIG. 7 e.

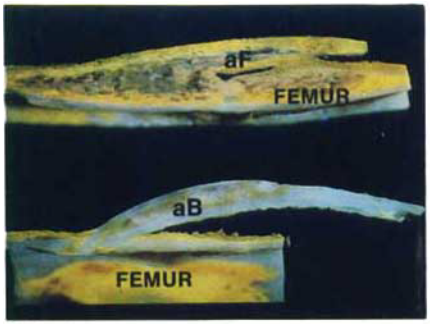


FIG. 8.

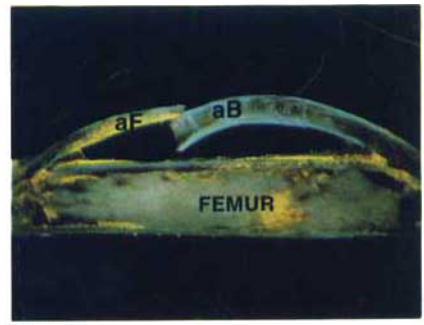


FIG. 9.

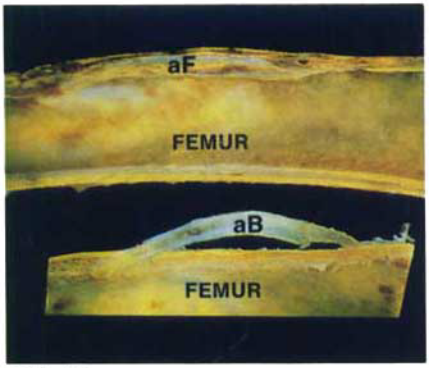


FIG. 10.

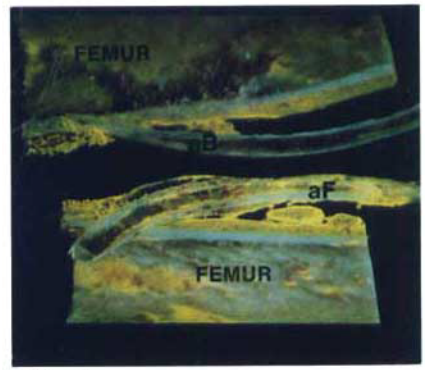


FIG. 11.

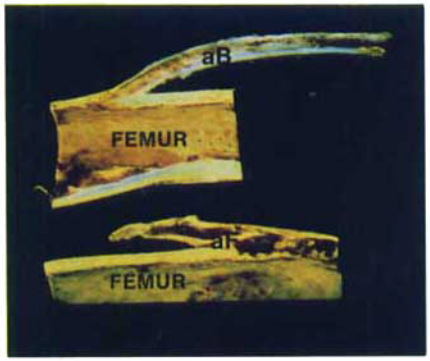


FIG. 12.

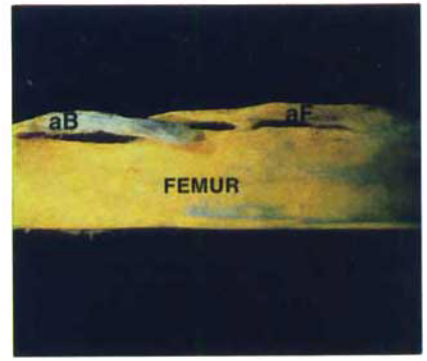


FIG. 13.

FIG. 8. – *(spec. 23). A month from grafting to last tetracycline injection. The fresh autograft aF is almost incorporated in the host bone. The autogenous frozen bone aB looks as if it had been recently grafted.*

FIG. 9. – *(spec. 17). 4 weeks from grafting to last tetracycline injection. The fresh autograft aF has started to reorganize and, peculiarly, new bone is present mostly in that part of the transplant which is not implanted in the host bone. The frozen autograft aB looks as if it had been recently transplanted.*

FIG. 10. — *(spec. 10). 5 weeks from grafting to last tetracycline injection. The fresh autograft aF is completely incorporated into the host bone. The frozen autograft aB exhibits scanty yellow fluorescence.*

FIG. 11. — *(spec. 32 s). 5 weeks from grafting to last tetracycline injection. The frozen autograft aB displays only bluish autofluorescence. In the fresh autograft aF there is lively resorption and apposition. The new bone derived from the host bone at the site of implantation has started to surround the transplant like a cuff.*

FIG. 12. — *(spec. 50). 2 months from grafting to last tetracycline injection. In the frozen autograft aB there is scanty new bone. The fresh autograft aF is fully reorganized and only the tip of it is not incorporated into the host bone.*

FIG. 13. — *(spec. 21). 3 months from grafting to last tetracycline injection. Side view. Both the autografts are clearly discernible. The fresh graft aF has lost its original shape. It exhibits abundant yellow fluorescence, which is almost lacking from the frozen bone aB. Only scanty new-bone formation at the transplants in the host bone.*

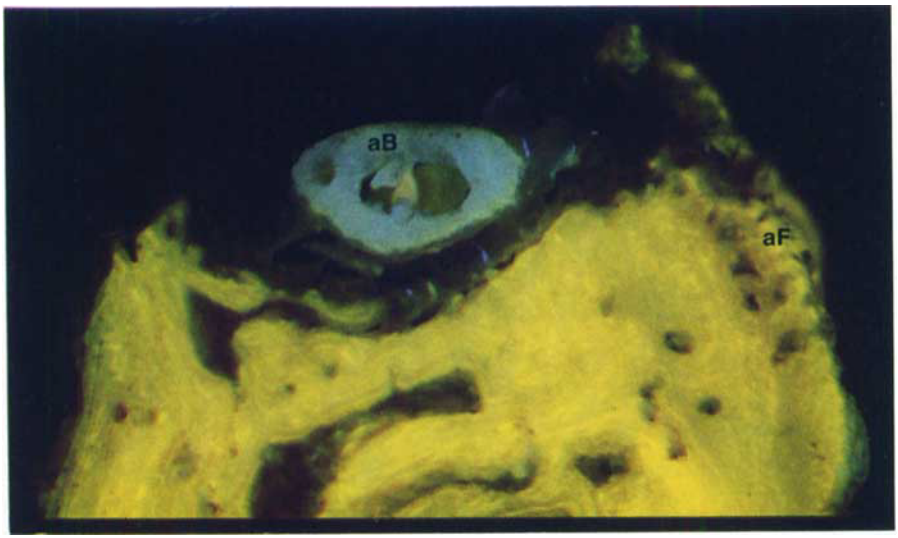


FIG. 14. — *(spec. 88). 3 months from grafting to last tetracycline injection. Gross section. The autogenous frozen graft aB has only scanty tetracycline incorporation and its contour is well defined. The fresh autograft aF has undergone complete lamellary reorganization and at this site is incorporated in the host bone. Note the strong new-bone formation in the host bone. The surface of the femur is not seen in the picture.*

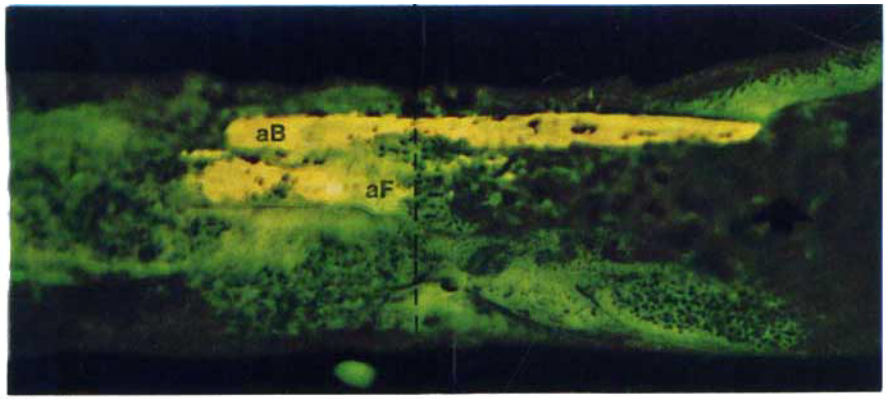


FIG. 15 a.

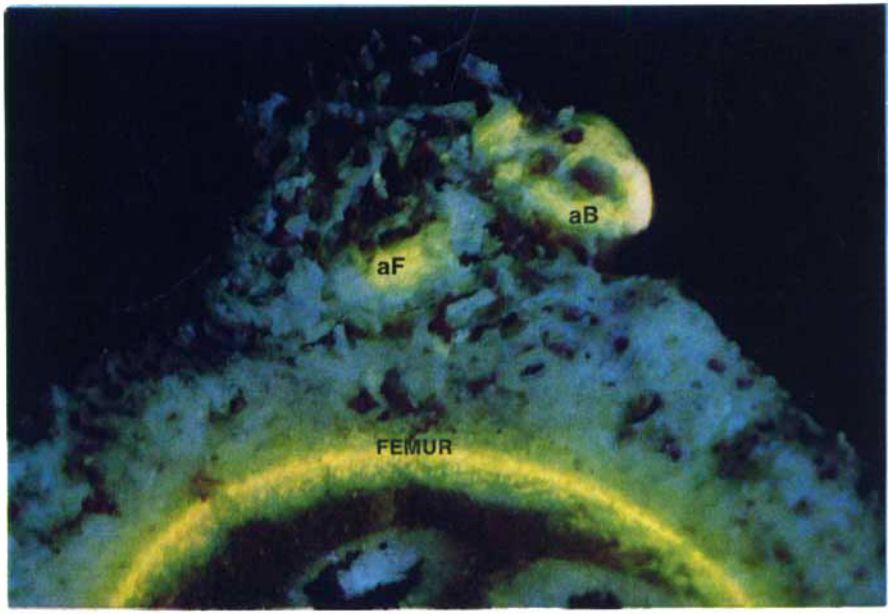
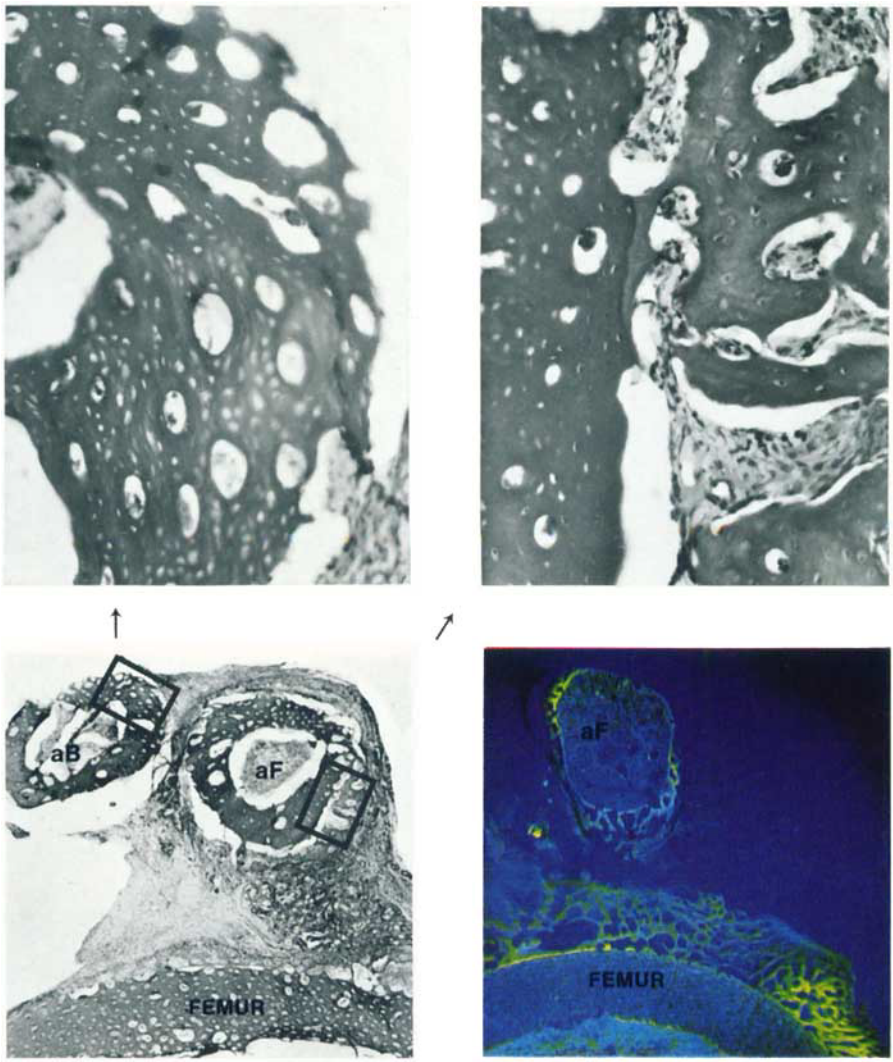


FIG. 15 b.

FIGS. 15. a and b — *(spec. 83) 3 weeks from grafting to killing. Tetracycline injection prior to grafting.*

— a. *Front view of the femur with the transplants. The frozen autograft aB has retained its yellow tetracycline fluorescence (minimal resorption). The fresh autograft aF excluding the distal part, has lost its yellow fluorescence through resorption. The arrow indicates the proximal end of the fresh autograft.*

— b. *Gross section along the dotted line (see fig. a). In aB the outline is only slightly broken and the loss of tetracycline has hardly started. In aF reorganization has progressed to a great extent and it is difficult to discern it from the bone formation derived from the host bone.*



The squared areas in the picture of the grafts are magnified in the figures above as pointed by the arrows.

FIG. 16. — *(spec. 135 s). 10 days from grafting to killing. Immature bone trabeculae are encountered on the external surface of the fresh autograft aF. Nucleus-containing lacunae of normal bone are seen, especially in the superficial parts of the graft. In tetracycline ground section (prepared from the site near histological section) there is yellow fluorescence in the area of immature trabeculae but not in that of normal bone tissue of the graft. The frozen autograft aB is necrotic all over. New-bone formation on the host bone.*

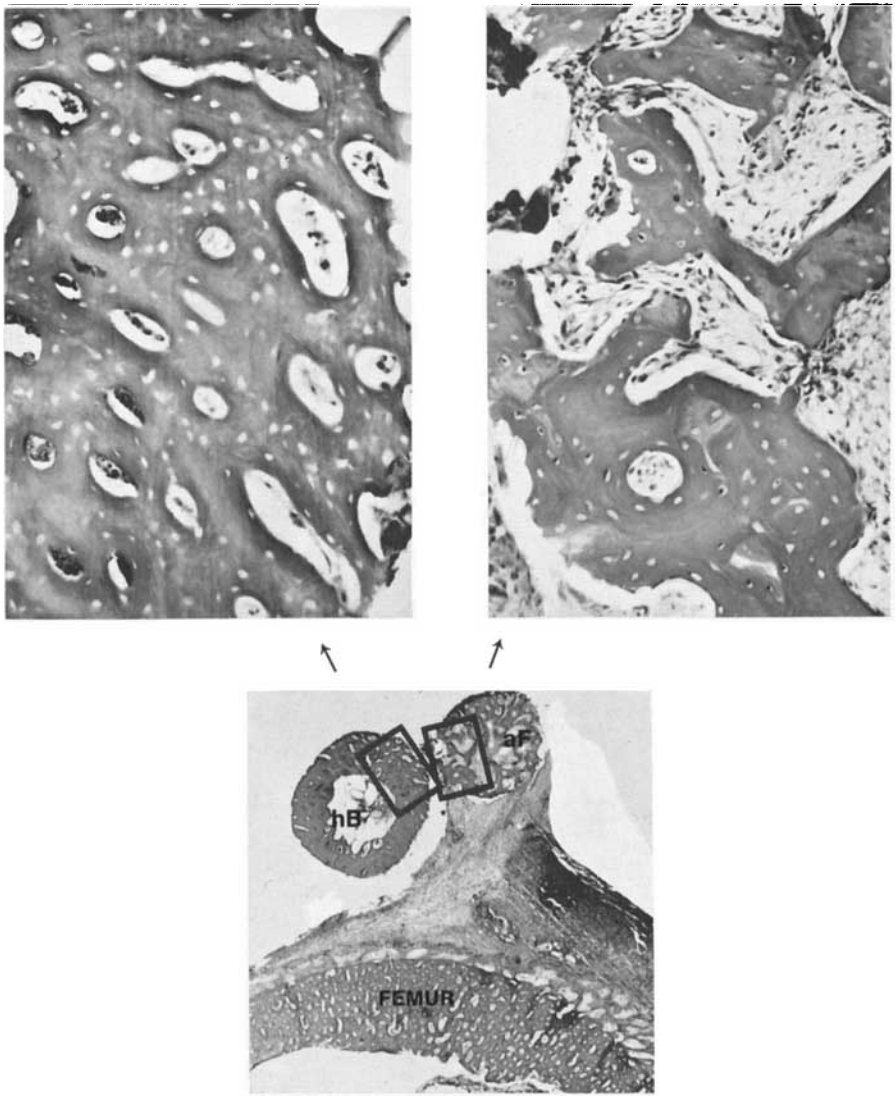


FIG. 17. — *(spec. 142 d). 8 days from grafting to killing. All lacunae of the homogenous frozen bone hB are empty. Islands resembling immature bone and nucleus-containing lacunae of normal bone are found in the fresh autograft aF. Abundant osteoblasts indicate active osteogenesis. Note the numerous cells in autogenous graft.*

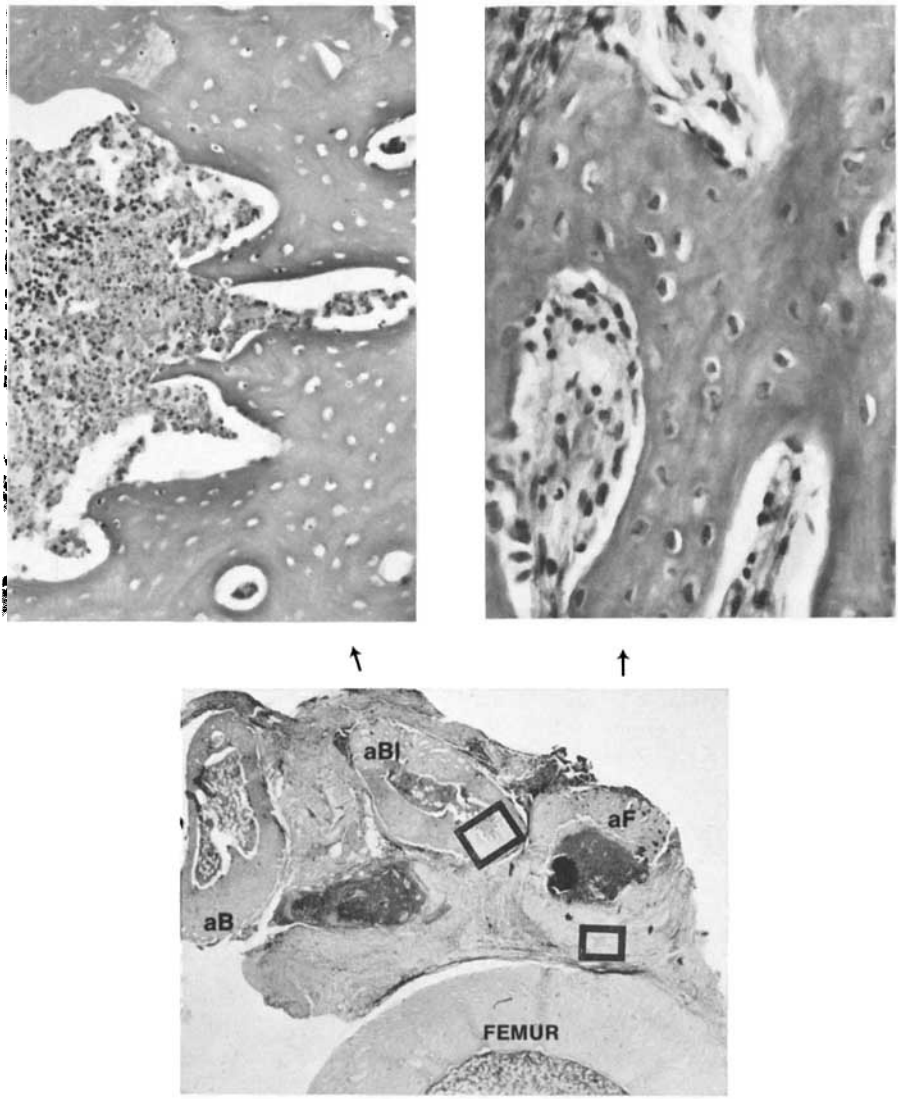


FIG. 18. – *(spec. 68). 16 days from grafting to killing. The autograft aBl preserved in blood and the frozen autograft aB are necrotic. Especially in that part of the fresh autograft aF which is close to the host bone the immature bone trabeculae and numerous osteoblasts indicate intensive reorganization. Cf. fig. 3.*

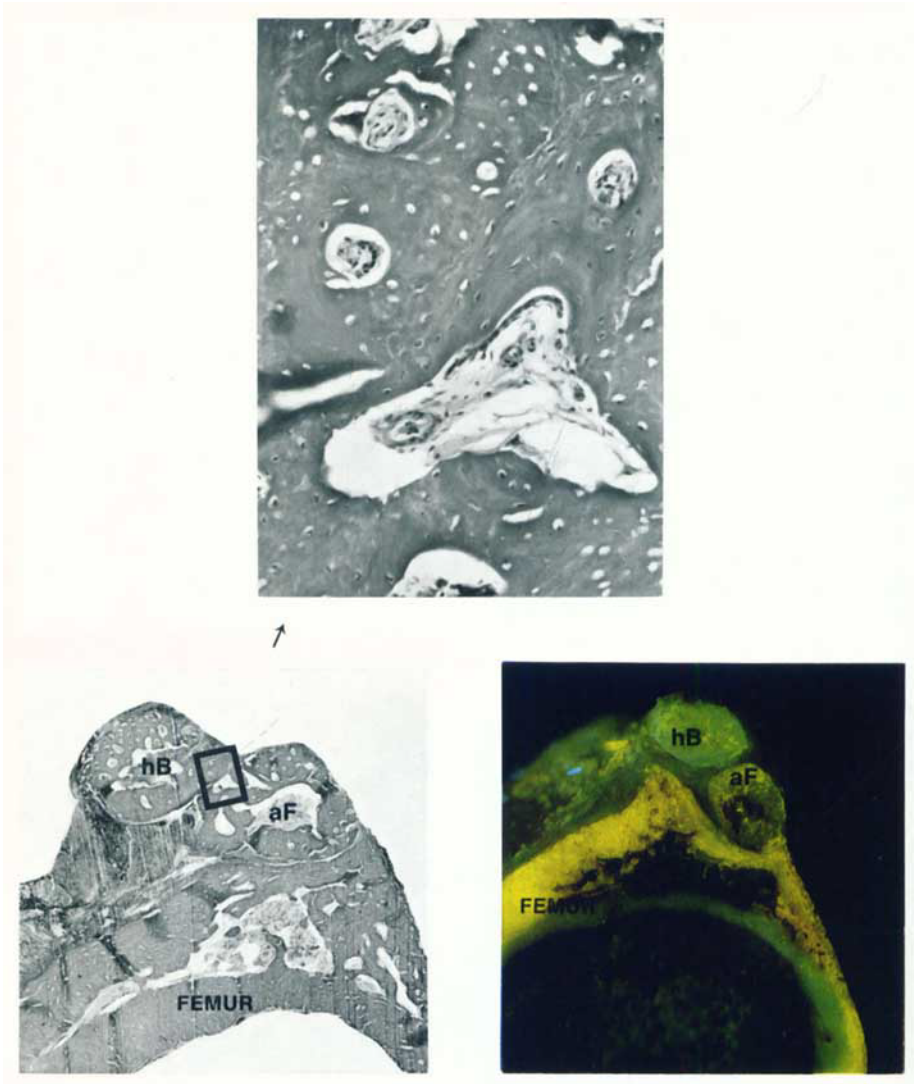


FIG. 19. — *(spec. 136 d). A month from grafting to killing. In histological section the transplants have become fused. The vital bone tissue of the fresh autograft aF ends with a sharp line of demarcation against the necrotic tissue of the frozen homograft hB. Some nucleus-containing lacunae in the frozen bone are seen mainly in the part adjacent to the fresh transplant. In tetracycline section (prepared from the site near the histological section) some spots of yellow fluorescence are found in the frozen graft. In the fresh graft the yellow fluorescence indicates considerable reorganization. New-bone formation from host bone has a drift towards the fresh autograft and is so abundant a separate cavity has developed.*

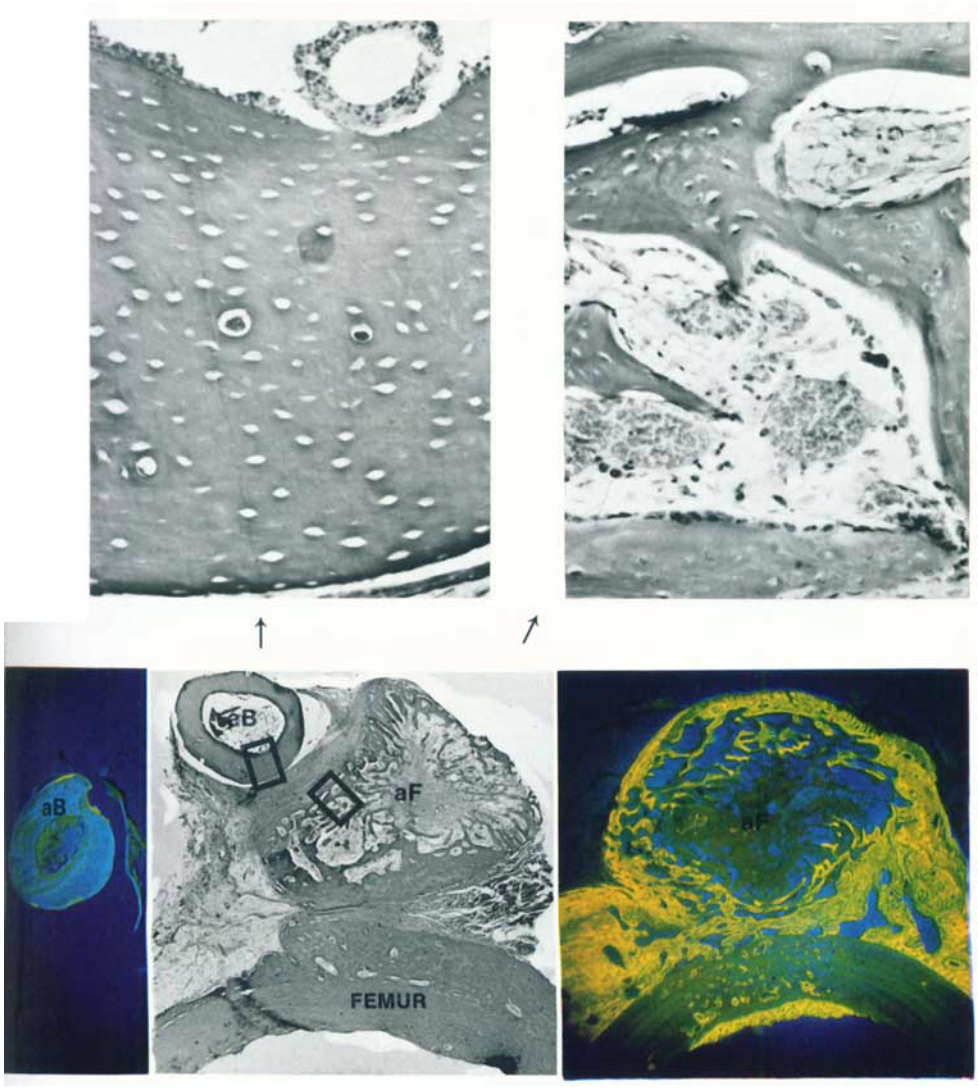


FIG. 20. — *(spec. 134). 51 days from grafting to killing. The fresh autograft aF has lost its shape and grown much larger than it was originally. Its bone tissue is vital all over and signs of active osteogenesis are seen at the bone margins. The frozen autograft aB is as if rejected alongside the fresh autograft. Its lacunae are empty. Trabeculation in the host bone is seen near the fresh autograft. In tetracycline ground section (prepared from a site near the histological section) yellow fluorescence indicates intense new-bone formation in the fresh graft aF. The strong trabeculae cement the transplant to the host bone. The frozen graft aB exhibits only minimal tetracycline fluorescence. Adult rabbit.*

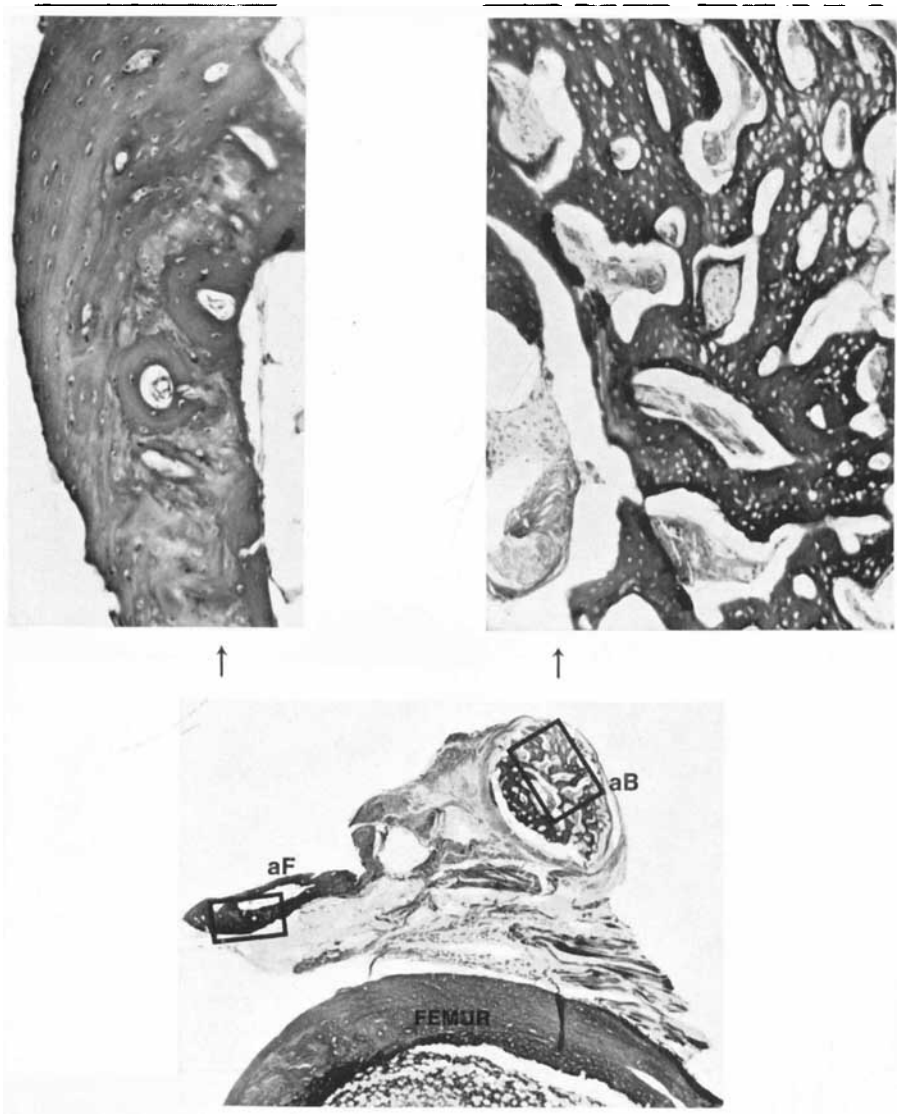


FIG. 21. — *(spec. 131 s). 3 months from grafting to killing. The fresh autograft aF has become denser and lost its original form and size. Its structure resembles the lamellary structure of the host bone. The frozen autograft aB is porous, but there are only scanty nucleus-containing lacunae.*

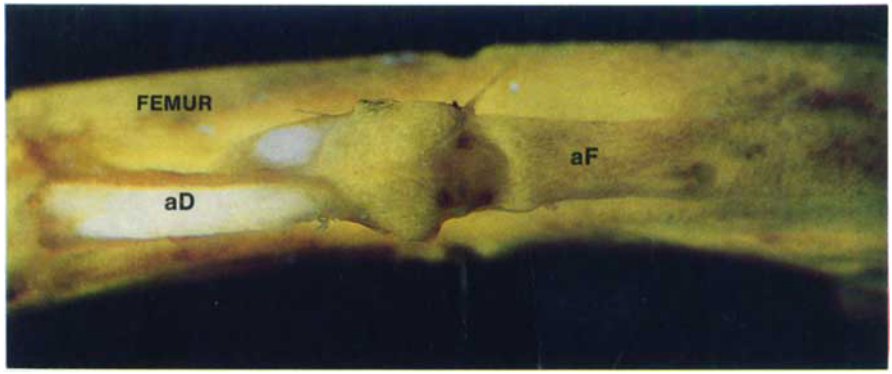


FIG. 22. – *(spec. 95). 5 weeks from grafting to last tetracycline injection. Front view of the femur with the transplants. The autograft aD preserved for one hour in the open air is as if recently implanted. The fresh autograft aF, excluding the distal end, is fully reorganized.*

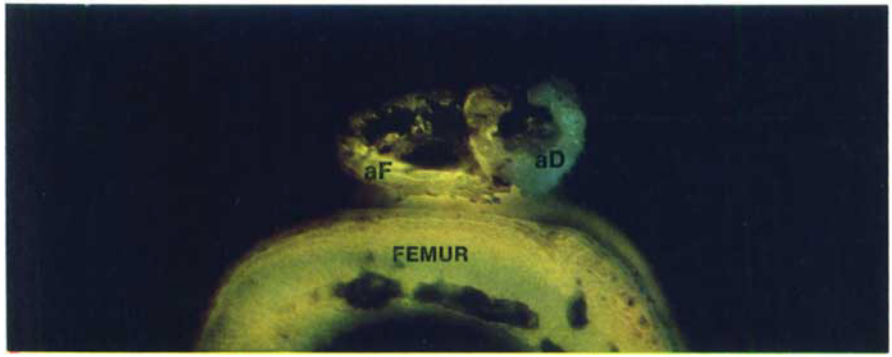


FIG. 23. – *(spec. 94). 5 weeks from grafting to last tetracycline injection. The fresh autograft aF has undergone lamellary reorganization. The autograft aD preserved for one hour in the open air displays tetracycline incorporation in the part adjacent to the fresh graft.*

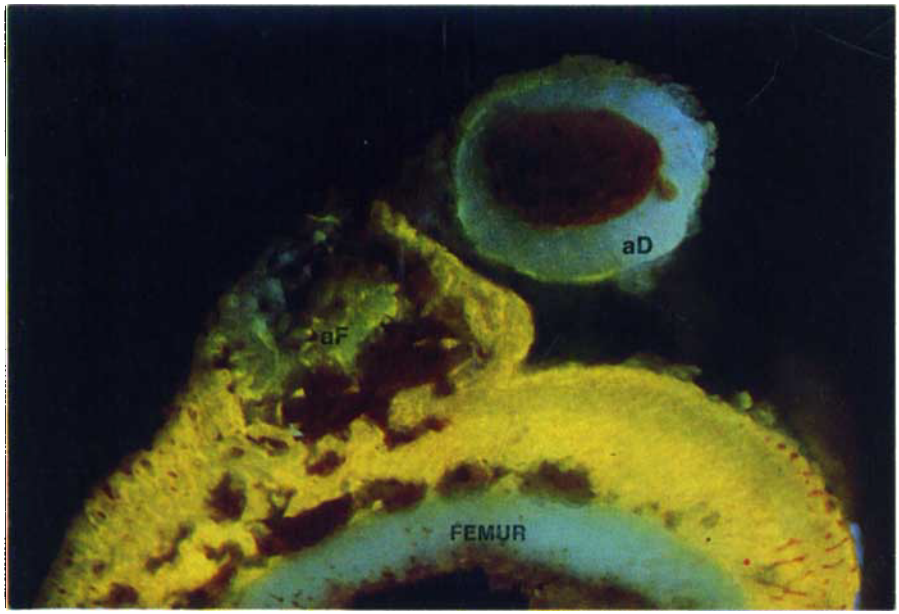


FIG. 24. — *(spec. 65). 15 days from grafting to last tetracycline injection. The autograft aD preserved for two hours in the open air displays bluish autofluorescence. In the fresh autograft aF there is strong reorganization indicated by yellow fluorescence. New-bone formation from the host bone has resulted in fusion with the fresh graft.*

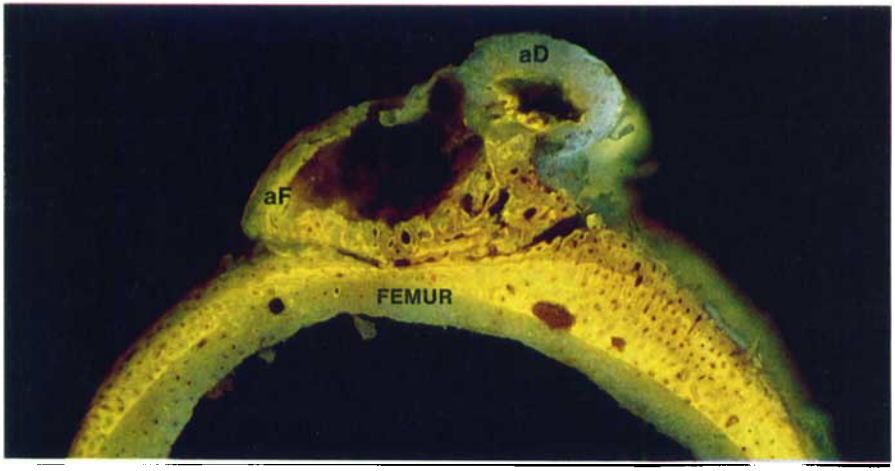


FIG. 25. — *(spec. 80). 3 weeks from grafting to last tetracycline injection. The autograft aD preserved for one hour in the open air displays only scanty yellow fluorescence in the part adjacent to the fresh autograft. The fresh autograft aF has undergone reorganization.*

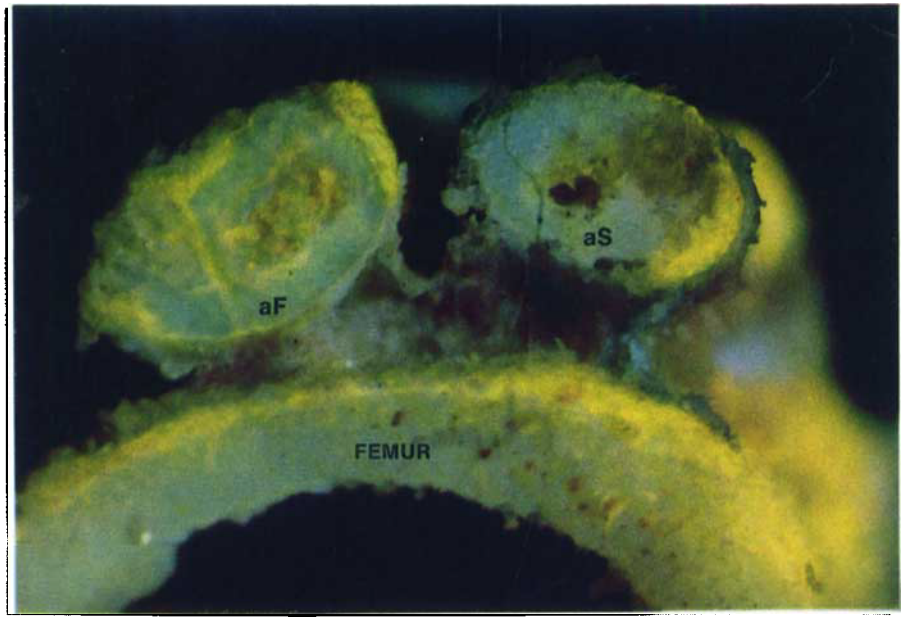


FIG. 26. — *(spec. 63). 15 days from grafting to last tetracycline injection. The fresh autograft aF and the autograft aS preserved for two hours in saline solution have no great differences although in the fresh graft reorganization has progressed somewhat further.*

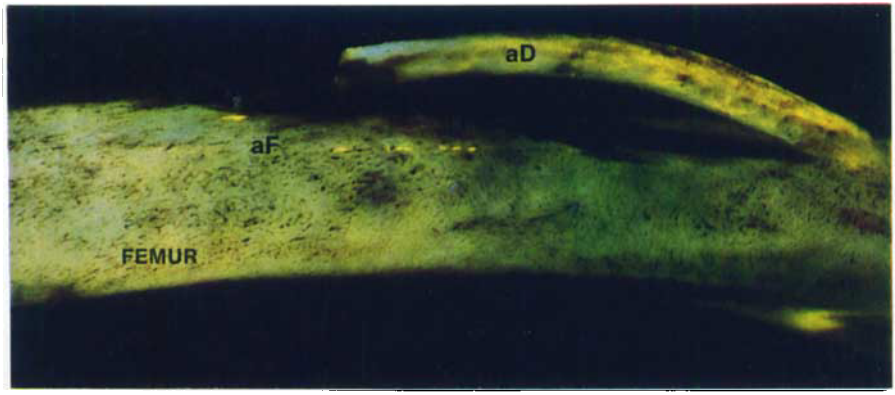


FIG. 27. — *(spec. 112). 6 weeks from grafting to killing. Tetracycline injection prior to grafting. Side view. The autograf aD preserved for one hour in the open air has retained considerable yellow tetracycline fluorescence (scanty resorption). The fresh autograft aF is completely incorporated in the host bone. Small yellow points on its surface indicate the site where the transplant is lying.*

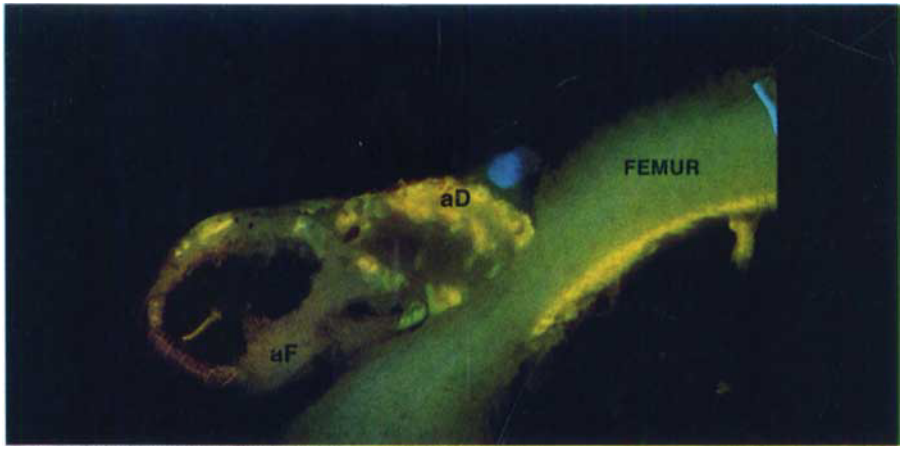


FIG. 28. — *(spec. 109). A month from grafting to killing. Tetracycline injection prior to grafting. Cross section. The autograft aD preserved for one hour in the open air has kept intense yellow tetracycline fluorescence (minimal reorganization). The fresh autograft shows only some spots of yellow fluorescence (considerable resorption of the original tetracycline-labelled bone).*

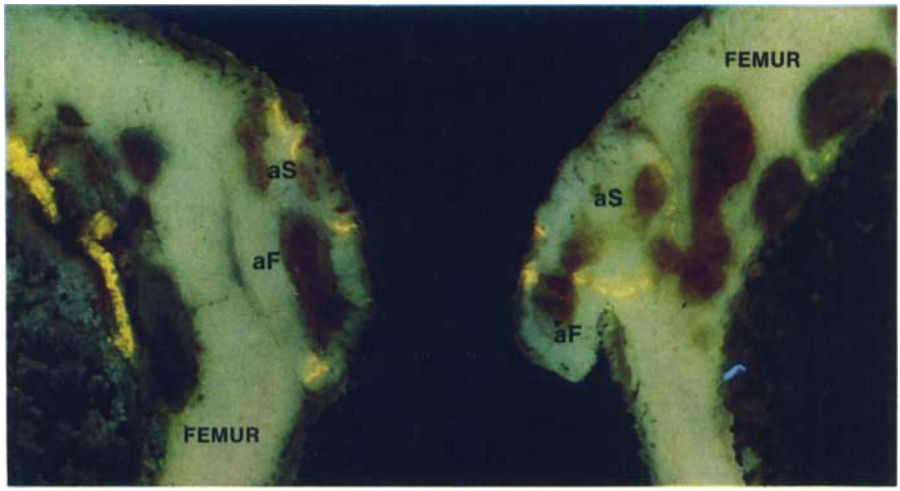


FIG. 29. — *(spec. 108). 6 weeks from grafting to killing. Tetracycline injection prior to grafting. Two cross sections. The fresh autograft aF and the autograft aS preserved for one hour in saline solution are incorporated in the host bone. Only some tetracycline spots left. No difference in reorganization.*

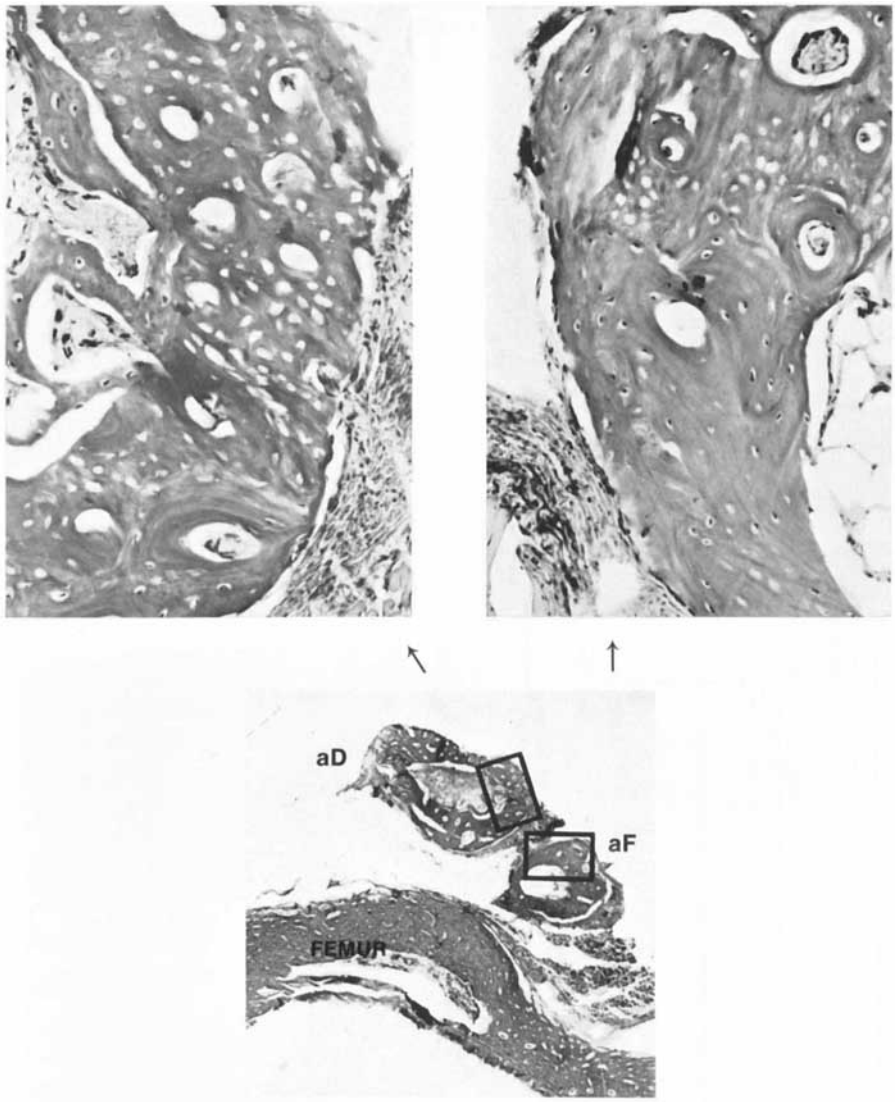


FIG. 30. — *(spec. 129). 43 days from grafting to killing. The fresh autograft has become denser and is definitely smaller in size than originally. Most of its lacunae contain a nucleus and its structure resembles the lamellary structure of the host bone. The autograft aD, preserved for one hour in the open air, is porous, but nucleus-containing lacunae are not seen anywhere else than in the part adjacent to the fresh graft and largely in the form of islands on the medullary aspect.*

CORRECTIONS

Page 14, line 7: read rounded instead of free

Page 15: line 4: read convex instead of concave

Page 22, line 27: read convex instead of concave

Page 27, line 4: read $\frac{\Delta y}{\Delta x}$

Page 35, line 9: Insert (Tylman and Ramotowski, 1961, Harrington, 1962)

Page 39: Test Number 22 was eliminated from the results because of the possibility of metastatic abscesses

Fig. 21: Strain gage A is in electrical series and no external connection should exist across the gage

Fig. 22: distance a is along the axis of the gages and must be joined to P by a perpendicular

Page 85: insert *Parker, H. E.:* Simplified Mechanics and Strength of Materials. 2ND Ed. John Wiley and Sons. New York, 1961