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Observations on Massive Retrieved Human Allografts*

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ABSTRACT: Radiographic and histological studies of sixteen massive retrieved human allografts were carried out after the allografts had been in situ for four to sixty-five months. The studies demonstrated that union between the allograft and the host took place slowly at cortical-cortical junctions by the formation of an external callus derived from the cortex of the host, and it took place more rapidly at cancellous-cancellous junctions by internal callus advancing from the host into the allograft. Internal repair took place very slowly, was confined to the superficial surface and the ends of the graft, and had involved only 20 per cent of the graft by five years. The deep unrepaired portions of the graft retained their architecture, and where bone cement had been used to fix a prosthetic stem or an intramedullary rod to the allograft, there was no evidence of resorption of bone or loosening of the device. Soft tissues of the host became attached to the graft by deposition of a thin seam of new bone on the surface of the graft. A previous fracture of two grafts had healed before the time of retrieval. Analysis of the articular cartilage revealed no evidence that any chondrocytes had survived, even when the graft had been cryoprotected before it was preserved by freezing. The necrotic cartilage functioned well for as long as five years, and as it degenerated, it was covered by a pannus of fibrovascular reparative tissue. Two allografts that had been removed because of rejection were studied, and the observations were correlated with the clinical and radiographic data. After the initial dissection, radiographs and photographs of the specimens were made and then the specimens were bisected. The cut surfaces of the slabs were photographed under both incandescent and, when in vivo labeling with tetracycline had been done, ultraviolet light.

The slabs were fixed, decalcified, embedded in celloidin, cut into twenty-micrometer sections, and stained with hematoxylin and eosin. These preparations were studied with low-power microscopy (magnification, four to ten times), and spatial maps were prepared from tracings of the macrosections to determine the extent and distribution of revascularization and internal repair. Selected regions of the specimens were decalcified, embedded in paraffin, cut into five-micrometer sections, and stained with hematoxylin and eosin and, when articular cartilage was being examined, with toluidine blue and safranin O. These specimens were studied with high-power microscopy (magnification, forty-five to 450 times) for observation of the histological details of the reparative processes in the allograft and in the adjacent host bone, soft tissue, and articular cartilage.

Eight specimens were osteoarticular grafts. Three of them consisted of the proximal portion of the tibia; one, the distal part of the humerus; and one, the proximal part of the humerus. Two of the eight specimens had been preserved by freeze-drying before transplantation, and the other six specimens had been preserved by freezing at a temperature of -70 degrees Celsius after cryopreservation of the articular cartilage with glycerol.

Three of the specimens were intercalary grafts, and they included two femoral shafts and one proximal part of the humerus. All three had been preserved by freezing at -70 degrees Celsius. The remaining five specimens, four of the proximal part of the femur and one of the proximal part of the tibia, had been used as composites with prosthetic joints and had also been preserved by freezing at -70 degrees Celsius. None of the specimens had been sterilized secondarily with ethylene oxide or irradiation before transplantation.

The allografts ranged in length from seventeen to twenty-six centimeters (average, twenty-one centimeters).

Materials and Methods

The recipients of the allografts, nine of whom were female and seven of whom were male, ranged in age from thirteen to sixty-six years. Various combinations of radiographic, gross, macroscopic, and microscopic studies were done, and the observations were correlated with the clinical and radiographic data. After the initial dissection, radiographs and photographs of the specimens were made and then the specimens were bisected. The cut surfaces of the slabs were photographed under both incandescent and, when in vivo labeling with tetracycline had been done, ultraviolet light.

The slabs were fixed, decalcified, embedded in celloidin, cut into twenty-micrometer sections, and stained with hematoxylin and eosin. These preparations were studied with low-power microscopy (magnification, four to ten times), and spatial maps were prepared from tracings of the macrosections to determine the extent and distribution of revascularization and internal repair. Selected regions of the specimens were decalcified, embedded in paraffin, cut into five-micrometer sections, and stained with hematoxylin and eosin and, when articular cartilage was being examined, with toluidine blue and safranin O. These specimens were studied with high-power microscopy (magnification, forty-five to 450 times) for observation of the histological details of the reparative processes in the allograft and in the adjacent host bone, soft tissue, and articular cartilage.

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The allografts ranged in length from seventeen to twenty-six centimeters (average, twenty-one centimeters).
A plate and screws had been used to secure the allograft in eight patients; an intramedullary rod (two cemented and two uncemented), in four; and an intramedullary prosthetic stem, in four. Table I shows the age and sex of the patients, the diagnosis and stage of the lesions, the sites of the lesions, the types of resection or reconstruction, the lengths of the allografts, the types of preservation of the allografts, the types of fixation of the graft-host junction, whether there was a fracture or infection of the allografts, the clinical results, the reasons for retrieval, and the intervals that the allografts remained in situ. Nine specimens were retrieved because of complications with the graft, which included non-union (one graft), infection (two), rejection (two), and fracture (four), and seven specimens were retrieved after amputation or autopsy. The earliest that a specimen was retrieved was at four months and the latest was at sixty-five months. Most of the specimens were retrieved less than two years after implantation.

**Results**

Emphasis was placed on the rate and type of union at the graft-host junction, the amount and distribution of re-vascularization and internal repair, fracture of the allograft, the extent and type of attachment of soft tissue to the allograft, the extent of survival and subsequent changes in the articular cartilage of the osteoarticular specimens, and the fate of the interface between the allograft and the cement in the specimens in which bone cement had been used to
augment the fixation of the allograft.

**Union**

Two types of junctions were available for study. Most were cortical to cortical and a few were composed of abutting cancellous surfaces. At the cortical junctions, union took place by the gradual formation of an external callus that extended from the host bone onto the external surface of the allograft, where it became annealed to the surface of the allograft (Figs. 1-A, 1-B, and 1-C). In no specimen did callus appear to have extended from both sides of the junction and to have joined at the site of the osteotomy. The gap between the abutting cortices was filled with callus that flowed into it from the surrounding external callus. The external callus always appeared more mature than the reparative tissue in the gap. The callus joined the allograft at its original dimension without previous resorption. Histologically, the site of union was demarcated by a distinct cement line (Figs. 1-A, 1-B, and 1-C). There was a close correlation between radiographic evidence of union and the macroscopic and microscopic observations.

Union was usually evident externally before the radiolucent gap was obliterated. Junctions that were separated by a radiolucent gap were filled with immature reparative fibrovascular tissue or clefts. In two specimens, despite radiographic persistence of the radiolucent zone between abutting cortices, the macroscopic and microscopic findings demonstrated union by formation of immature cancellous callus (Figs. 2-A and 2-B). Junctions in which the gap had increased radiodensity, but was still visible, were filled with maturing cancellous callus, and those that had been obliterated were filled with mature haversian bone.
Almost without exception, when the tissue that filled the gap had matured into cortical bone, the haversian canals in the unifying seam were oriented perpendicular to the long axis of the bone rather than parallel to the canals in the cortex of the allograft or host. In no specimen did this orientation remodel — even at five years — to become indistinguishably intermixed with the allograft. The original contour of the allograft remained crisp and unchanged. Callus often extended along the periosteal surfaces of the allograft, one centimeter or more from the junction, and then tapered away to the original dimension of the allograft. In the one specimen that had been retrieved because of rejection alone, there was no union despite good fit and fixation. The junction gap was filled with chronic inflammatory cells, and a thick zone of inflammatory tissue about the external surface of the graft prevented union between the callus that extended from the host to the allograft.

Two specimens had been retrieved because of infection. In one, a composite specimen that had been retrieved at seven months, union was not affected by an intra-articular infection about the prosthesis at a distance from the junction. In the other specimen, an intercalary graft that consisted of the femoral shaft that had been retrieved at forty-one months, union was prevented at the proximal part of the junction by chronic granulation tissue that had filled the junction gap.
OBSERVATIONS ON MASSIVE RETRIEVED HUMAN ALLOGRAFTS

At the cancellous junctions, union took place more rapidly, without the formation of external callus. Fibrovascular reparative tissue from the abutting host invaded the marrow spaces of the allograft and differentiated into osteoblasts. These osteoblasts lined the surfaces of the trabeculae of the allograft with seams of reparative bone that established continuity with the trabeculae of the host (Figs. 3-A and 3-B). This reparative tissue penetrated only a few millimeters into the allograft and then stopped. With time, the tissue in the marrow spaces matured into hypocellular, dense, fibrous connective tissue that appeared to provide a barrier to further penetration of the allograft by reparative fibrovascular tissue. Even after several years, the cancellous area of the allograft deep to this barrier remained composed of the necrotic remnants of allograft marrow and intact but acellular trabeculae.

Radiographically, this mechanism was reflected by the presence of a narrow zone of increased radiodensity that had advanced into the allograft, which represented the deposition of new repair bone on the trabeculae of the allograft. As a radiolucent zone or gap was not present, this advancing zone of increased radiodensity was the clearest indication of union.

Revascularization and Internal Repair

The distribution and amount of revascularization and internal repair were closely associated. Wherever neovascularity had penetrated the allograft, the adjacent bone was undergoing repair, either osteoclastic resorption early or osteoblastic apposition later. The only consistent exception was in the medullary canal, where reparative fibrovascular tissue had entered the canal from the junction and had filled it for one or two centimeters. Here, the adjacent trabeculae or endosteal cortex often remained unrepaired, and when the fibrovascular tissue matured, these trabeculae became engulfed in dense, fibrous connective tissue. The pattern of revascularization and repair appeared quite consistent. On the external surface of the graft, a thin (one to two-millimeter-thick) layer of bone was laid down by mesenchymal proliferation that had been derived from adjacent host cells. This layer of bone was annealed to the necrotic cortex of the graft, the junction being marked by a distinct cement line. Where Volkmann canals had emptied onto the surface, buds of fibrovascular tissue had invaded and led to creeping repair.

Resorption often did not reach the peripheral cement line about the canal, and this in turn led to partial repair of osteons with an almost-complete absence of so-called cutting cones crossing the peripheral cement line and extending into the interstitial lamellae.

A similar pattern appeared at the graft-host junction. The repair bone that filled the gap was annealed to the surface of the graft by a distinct cement line. Intermittently, the cement line was perforated by vessels that had penetrated the haversian canals to initiate internal repair. Calculations from spatial maps prepared from the macrosections showed that, by one year, internal repair had extended no more than two millimeters deep from the surface of the graft and no more than three millimeters into the cortical ends. Less than 10 per cent of the entire graft was involved by this repair.
The amount of internal repair gradually increased so that, in the specimen that had been retrieved at sixty-five months, the peripheral repair had penetrated as much as four millimeters in some areas, the repair at the graft-host junction had penetrated twenty-five millimeters, and approximately 20 per cent of the graft had been repaired (Figs. 4-A through 4-D).

This pattern of repair was reflected in the radiographic contour and density of the cortices, which remained essentially unchanged and, as porosity increased in the adjacent host cortices, increased the disparity between the two. The isotope scans that were available for correlation also reflected this pattern closely—that is, the complete lack of uptake of isotope that was seen early was gradually replaced by a thin zone of increased uptake of isotope that outlined the periphery of the allograft, with well demarcated, more intense uptake at the graft-host junction.

In scattered areas, an even pattern of surface repair was not seen. In these specimens, the periosteal surface was pockmarked with unrepaired erosions that ranged in diameter from several micrometers to a few millimeters. These erosive cavities were filled with loosely arranged fibrovascular proliferations that were peppered with chronic inflammatory cells, and the edges often were lined by osteoclasts that were resorbing the underlying necrotic bone. Focal concentrations of inflammatory cells, foreign-body-type giant cells, or large histiocytes were only occasionally seen in the cavities. In some areas, these erosions were randomly interspersed between areas of surface repair. In other areas, the erosions were clumped closely together; in others, they were interspersed between unrepaired and unresorbed areas. Immediately beneath these areas of surface erosion, no re-
Figs. 3-A and 3-B: Cancellous union.
Fig. 3-A: An intercalary allograft that had been used to reconstruct the proximal part of the tibia was retrieved at six months. An anteroposterior radiograph shows the narrow zone of radiodensity just on the allograft side of the proximal cancellous junction (arrows). The distal cortical junction is marked by a radiolucent gap (arrows).

vascularization or internal repair was seen.

Fractures of the Allografts

Six specimens fractured. In four specimens that had been retrieved shortly after fracture, there was no evidence of internal repair along the fracture line and there was substantial erosion of the surface of the cortex at the site of the fracture. The remaining two specimens, which were retrieved at fourteen and fifteen months, had fractured at eight and three months before retrieval. In one, a proximal humeral osteoarticular allograft, the fracture was through the metaphysis, just proximal to the plate that had been used for fixation. The patient had been treated with a sling and swathe, and the fracture had healed with extensive callus (Figs. 5-A through 5-D). In the other specimen, an intercalary distal femoral allograft that had been used for arthrodesis of the knee, a fatigue fracture had occurred through the metaphysis, about an intramedullary rod. The patient had been treated with crutches and an orthosis, and this fracture also had healed.

The spatial maps from these two specimens showed extensive internal repair that extended well into the allograft along the fracture line, a great deal farther than would have been anticipated in this area had there not been a fracture. The histogenesis and amount of callus at the site of these fractures were consistent with their location and the way that they had been managed: there was a bulky external callus with a large cartilaginous component at the site of the humeral fracture and a smaller osseous callus about the healed fatigue fracture in the femur.

Soft-Tissue Attachment

On most surfaces, the adjacent soft tissue was firmly adherent to the underlying allograft. As the soft tissue was
A photomicrograph made at the site of cancellous-cancellous union in a specimen that had been retrieved fourteen months after allografting (× 90). The photomicrograph is divided into four zones. A = the cancellous zone of the host, which contains viable host trabeculae (HT) surrounded by hematopoietic marrow. B = the union zone, which is composed of host trabeculae (HT) that are united to graft trabeculae (GT) by new bone (NB). The combination results in thickened trabeculae at the site of union. This is reflected radiographically by the zone of radiodensity at the site of the union. C = the zone in the allograft just deep to the site of union, which is a necrotic unrepaired zone of graft trabeculae enmeshed in dense, hypocellular, fibrous tissue. This zone appears to block deeper revascularization of the allograft. D = the deeper unrepaired portion of the allograft, which is composed of necrotic unrepaired allograft trabeculae and the necrotic remnants of allograft marrow.

stripped away, slender tufts of fibrovascular tissue were seen entering the Volkmann or haversian canals that emptied onto the surfaces, and often a small drop of blood oozed from the canal.

Labeling with tetracycline and isotope scans indicated that a thin layer of new bone had been formed on the surface of the allograft. Macroscopic and microscopic examination of these areas showed a thin seam of appositional new bone on the surface of the allograft, beneath the soft tissue. On high-power microscopy, particularly in specimens that had been retrieved after twelve months, the mature bundles of eosinophilic collagen in the adjacent soft tissue were seen to be in continuity with the fibrillar lamellar collagen in the new bone on the surface of the allograft (Figs. 6-A and 6-B). This was clearly seen on polarized-light microscopy. The collagen that bound the soft tissue to the appositional new bone on the surface of the allograft did not cross the cement line that demarcated appositional living new bone from the underlying necrotic cortex of the allograft. Bonding of soft tissue was not seen at the exposed edges of erosion cavities or on the external surfaces on which no appositional bone had been laid.

In other areas, soft tissue could be stripped away easily by blunt dissection with a finger, and it appeared to be separated from the underlying cortex by soft, edematous, granulation-like tissue. On macroscopic and microscopic examination, the soft tissue in these areas was seen to be more reactive and less mature, and it was separated from the necrotic unrepaired cortex beneath it by a thin zone of chronic inflammatory tissue — the same type of tissue that filled the unrepaired resorption cavities. These stripped surfaces were non-fluorescent in the tetracycline-labeled specimens.

In the areas where the soft tissue was adjacent to exposed cancellous bone of the allograft, the soft tissue was firmly adherent. On macroscopic and microscopic examination, mature fibrous tissue was seen to extend from the overlying muscle into the marrow spaces between the trabeculae for a few millimeters, effectively attaching soft tissue to these areas of the allograft. The trabeculae in this narrow zone of fibrous invasion of the marrow spaces were occasionally lined by seams of new bone, but more often they remained as necrotic, unrepaired trabeculae with their original architecture.

In the specimens that had been retrieved because of rejection, and in the areas of active infection in the infected specimens, the adjacent soft tissue was separated from the underlying necrotic bone by a wide zone of inflammatory tissue. The soft tissue could be stripped away easily, and no appositional bone was evident on the surface of the allograft grossly or histologically.

In the osteoarticular specimens, various ligamentous and capsular structures between the allograft and the host had been repaired operatively. The sites of repair were often
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Easily identified by remaining suture material (Figs. 7-A, 7-B, and 7-C). There was solid healing in all specimens except those that had been obtained after rejection. Had suture material not marked the sites of repair, it would have been difficult to identify the sites of junction grossly because the tissues were so well integrated. However, on histological study, the sites were identified easily. A few millimeters from the junction in the allograft, the tissue was made up of acellular mature bundles of collagen that were arranged in an orderly architecture. Only an occasional macrophage or inflammatory cell was seen between the undisturbed bundles. At the junction, the tissue was composed of whirling, randomly oriented bundles of cellular connective tissue that were intimately admixed with fragments of acellular bundles of less intensely eosinophilic collagen. At the leading edge of the extension of this repair tissue into the allograft, narrow fingers of fibrovascular tissue penetrated between intact acellular collagen bundles, with no evidence of active inflammation or resorption. Polarized-light microscopy showed that, although the collagen was intimately admixed in the repaired areas, there was no continuity between allogeneic and host collagen. Where suture material was seen in host tissues, it was often fragmented and surrounded by inflammatory tissue that contained prominent foreign-body giant cells, but where suture was seen in unrepaired allogeneic tissue, it remained intact and there was no cellular response.

Two proximal tibial osteoarticular allografts included intact menisci. At ten and seventeen months after implantation, the menisci appeared grossly normal except at their periphery, where a rim of vascular granulation-like tissue was seen intermittently (Figs. 8-A and 8-B). Histologically, the menisci were acellular but had retained their normal architecture. The matrix of the fibrocartilage did not stain with either toluidine blue or safranin O. At the edges, where the vascular tissue was seen grossly, loose fibrovascular reparative tissue was intermixed with heavy bundles of acellular collagen.

Allograft-Cement Interface

In the specimens in which cement had been used either to fix a prosthetic stem to the allograft or to augment the
fixation of an intramedullary rod, there was no radiographic evidence of resorption of the allograft about the cement or clinical evidence of loosening for intervals ranging from eleven to forty-one months. The interface between the allograft and the cement was secure in all specimens, as seen both on gross inspection and on microscopy (Figs. 9-A, 9-B, and 9-C). Histological examination revealed no evidence of revascularization of the allograft adjacent to the cement and no evidence of ingrowth of tissue between the cement and the adjacent allograft. The interdigitation between the cement and the necrotic unrepaired allograft was intimate, and there was no evidence of fragmentation of the cement or of trabecular collapse.

Articular Cartilage

In all of the osteoarticular specimens, the remaining cartilage was acellular, and staining with toluidine blue or safranin O showed no histological evidence of metabolic activity in the matrices. No feature distinguished articular cartilage that had been preserved by freeze-drying without cryopreservation from articular cartilage that had been preserved frozen without lyophilization but with cryopreservation with glycerol. Despite the lack of survival of chondrocytes, the architecture of the matrix was well preserved in specimens that had been retrieved at six, ten, eleven, fourteen (two specimens), seventeen, or sixty-five months (Figs. 10-A, 10-B, and 10-C). However, a distal femoral specimen that had been retrieved at twenty-five months had moderately severe degenerative changes (Figs. 11-A through 11-D). Nothing distinguished this graft that had a more severely damaged site of articulation from the other grafts in terms of fit, radiographic changes, laxity of the joint, or clinical function of the joint. The only difference was that the patient who had moderately severe degenerative changes had been treated with a more prolonged period of non-weight-bearing and restricted motion due to a delay in union. Six months after implantation of the allograft, this patient was treated with a supplemental cancellous autogenous graft at the junction and an arthrotomy of the knee for the release of intra-articular adhesions. Subsequently, 85 degrees of flexion was obtained, ligamentous stability was achieved, unprotected weight-bearing was resumed, and there was a satisfactory functional result.

The better-preserved specimens had either minor fi-
brillar changes without erosion or had mild superficial erosive changes that were covered and the surface largely made congruous by a pannus of mature fibrovascular tissue. The extent of the surface that was covered by pannus was much smaller in the specimens that were retrieved less than one year after implantation than in the specimens that were retrieved after one year. None of these patients had important degenerative changes as seen radiographically or narrowing of the joint cartilage at the time of retrieval, nor did any of them have effusion, pain, or instability that necessitated external support.

Another interesting observation concerned the distal humeral osteoarticular allograft that was retrieved at sixty-five months. There was a congruous fit of the articulation between the allograft trochlea and the host olecranon, and the cartilage of the allograft trochlea had only minor superficial erosions that had been repaired by a thin, mature pannus. In contrast, the capitellum of the allograft was incongruous with the host radial head at the time of reconstruction, and the radiohumeral joint remained subluxated, albeit with satisfactory function. Compared with the congruent trochlea, the capitellar articular surface had much greater change, with major erosions that had been repaired incompletely by fibrocartilage.

The subchondral plate and adjacent trabeculae remained necrotic and unrepaired beneath all of the articulating surfaces of the allograft that had only minor changes. In the subchondral area, revascularization and trabecular

Figs. 5-A through 5-D: Repair of a fracture of an allograft.
Fig. 5-A: An anteroposterior radiograph of an osteoarticular allograft, made seven months after insertion, shows a one-month-old oblique undisplaced fracture just proximal to the fixation plate (white arrow). Immature callus formed on the medial aspect of the allografted humerus (black arrows).

Fig. 5-B: The patient was treated with a sling and swathe. An anteroposterior radiograph that was made at the time of retrieval, eight months after the fracture, shows mature union with a large external callus.
Fig. 5-C: A photomicrograph of the macrosection shows the amount of internal repair extending from the site of the fracture (arrows). This repair is in contrast to the unrepaired humeral head and the distal aspect of the shaft of the allograft (× 3).
A photomicrograph made at the site of the fracture shows the acellular abutting cortices of the allograft to be united by mature living bone (arrows) (× 45).

Figs. 6-A and 6-B: Soft-tissue healing to bone.
Fig. 6-A: An osteoarticular allograft, used to reconstruct the proximal part of the tibia, was retrieved at ten months. A photograph that was made during dissection shows the adherence of muscle (held in the forceps) to the cortex of the allograft.
Fig. 6-B: A photomicrograph shows the overlying soft tissues at the top joined by a zone of mesenchymal repair to the underlying necrotic allograft by a thin seam of appositional host bone. The junction between the seam of appositional host bone and the cortex of the allograft is marked by a distinct cement line (arrows) (× 30).
FIG. 7-A

Figs. 7-A, 7-B, and 7-C: Soft-tissue healing.

Fig. 7-A: An osteoarticular allograft consisting of the proximal part of the tibia was retrieved at six months, when an amputation was done because of a recurrent soft-tissue tumor in the thigh. A photograph of the medial aspect of the dissected specimen shows healing at the site where the medial collateral ligament of the allograft had been sutured to the medial collateral ligament of the host. The healed repair line was identified by the sutures (arrows).

Fig. 7-B: A photograph of the extensor mechanism shows the healing between the patellar tendon of the allograft (below) and the host tendon (above). The site of junction is indicated by the sutures (arrows).

resorption had occurred beneath the surfaces, with major architectural changes. In these specimens, there was clear radiographic evidence of subchondral revascularization, resorption, and repair — a distinct zone of decreased trabecular density, a few millimeters thick, immediately beneath the articular cartilage.

There was no evident relationship between the fate of the articular cartilage and the quality of the reparative response in the bone. In one specimen, the bone had been rejected with aggressive resorption and pathological fracture, and the articular cartilage was seen to have only mild changes at the time of retrieval. In another specimen, the cortices were coated with appositional repair tissue, there were no substantial areas of erosion, the soft tissue was firmly adherent, and union was mature, but the articular surfaces had major architectural changes.

Complications

Two specimens had been removed because of infection. One, a composite proximal tibial graft-knee prosthesis, was removed at seven months and the other, an intercalary femoral-shaft graft, was removed at forty-one months. In the composite specimen, the infection was confined to the area of the prosthesis and the immediately adjacent area of the allograft. It did not appear to have inhibited internal repair or attachment of soft tissue in the uninfected distal portion of the allograft. In the other specimen, the infection involved the proximal third of the intercalary femoral allograft. The proximal junction had not united, but the nonunion had not prevented satisfactory function for three years before the infection became apparent; stability of the reconstructed femur had been provided by a cemented intramedullary rod. The proximal infection had not inhibited union, soft-tissue adherence, or peripheral revascularization in the distal two-thirds of the specimen. It also had not caused resorption about the cement in the medullary canal of the proximal third of the specimen.

Discussion

The observations concerning these specimens are in accord with more extensive observations in animals\textsuperscript{1,3}, and they corroborate and substantially extend previous findings.
A photomacrograph of the site of union in Fig. 7-B shows the healing between the darker-stained cellular host tendon (above) and the lighter-stained allograft tendon (below). Suture material (S) is seen in the allograft tendon. The junction is marked by the arrows (× 5).

in human specimens that were obtained by biopsy or retrieval. While the small size of the sample limits the importance of the results, several of the implications are of interest. The security of fixation and the degree of contact between the cortices of the host and the allograft appear to influence the prevalence and quality of union at the junction. In these specimens, the time to union and maturation of the callus into haversian bone appeared to be shortened by intimate contact between the allograft and host. Fixation with either a plate or an intramedullary rod, if secure, seemed to be appropriate from the standpoint of union, and the use of cement for augmentation of intramedullary fixation did not appear to be deleterious biologically. The occasional finding that immature union had taken place despite the radiographic persistence of an intervening zone of relative radiolucency makes continued observation, rather than operative intervention, a prudent course for the management of a radiographic non-union that is not confirmed by other signs in this clinical setting.

The patterns of internal repair of most of the specimens in this study were remarkably similar. Peripheral repair penetrated only a few millimeters and then seemed to stop for as long as five years. Histologically, it was seen that the excavation of necrotic osteons about revascularized haversian canals often did not reach the outer aspects of the osteon, as it routinely does in the repair of cortical autogenous grafts. When filled in by repair bone, the excavation left an incompletely repaired osteon. The mechanism that...
governs the depth of vascular penetration and the extent of the reparative processes was not revealed by these observations. It may represent yet another subtle manifestation of histoincompatibility. The histological findings suggest that these allografts provided a passive physical substrate on which the reparative responses of the host were deposited (osteocoonduction) rather than an active ongoing stimulus to biological activity (osteoinduction).

Although one specimen showed no evidence of repair and had been rapidly resorbed, another demonstrated the opposite end of the spectrum: almost complete internal repair and rapid healing of a fatigue fracture. It appears that, although indolent incomplete repair is the norm, both rapid resorption and advanced repair are possible. The observations also suggest that the pattern of repair can be altered by other circumstances. In one of the allograft specimens in which a tumor had recurred on the surface, revascularization and repair were much more extensive in the adjacent underlying portion of the allograft than in the remainder of the specimen. Similarly, in the specimens in which a fracture had occurred months before retrieval, revascularization and internal repair were more extensive adjacent to the site of the fracture than they were in the remainder of the specimen.

The radiographs of these specimens accurately reflected the biological processes. The minimum penetration and incomplete repair of the osteons produced little or no increase in the porosity of the allograft, and this was clearly portrayed by the persistently homogeneous density of the graft. Only in the later (three and five-year) specimens did the density of the allograft begin to approach that of the adjacent host bone. While the radiographs could not detect early cortical erosion, larger cavities became evident and correlated well with the histological findings. Isotope scans showed increased uptake as the junctions healed, decreased uptake (compared with normal background uptake) in the unrepaired interior, and increased uptake along the repairing periphery.
The observations in these specimens support the clinical presumption that soft tissue can firmly attach to allografts. Soft tissue was bound to the external aspects of cortices through a seam of appositional bone that had been laid on the surface of the allograft. This seam was not the result of internal repair of the superficial osteons; rather, it was the result of host repair bone having been appositionally added to the surface of the allograft. This apposition did not continue to increase with time, did not produce hypertrophy of the allograft, did not seem to be influenced by stress, and remained for as long as five years with no substantial change. Circumstances that prevented or inhibited the deposition of this seam prevented firm adherence of soft tissue to cortical bone.

In none of the specimens in which bone cement had been used for fixation of the allograft to an intramedullary rod or to the stem of a prosthesis was there any clinical or gross evidence of loosening, radioluency about the cement, or revascularization or resorption in the allograft about the cement. There was also no hint, histologically, that this was likely in the future. This observation matches the experience of Rosenberg and Mankin, who reported the radiographic absence of radiolucent lines about prostheses that had been cemented into allografts. This suggests that the continuing absence of internal repair may be an advantage in this particular situation because, if revascularization leads to resorption, its absence may be beneficial.

The lack of survival of chondrocytes in the articular seam of bone precluded firm anchorage of soft tissue to cortical bone.

Figs. 10-A, 10-B, and 10-C: Articular cartilage.
Fig. 10-A: A photograph of the cut surface of a proximal tibial osteoarticular allograft, retrieved at ten months, shows well preserved articular cartilage without thinning degenerative changes or subchondral repair.
cartilage of the grafts that had been preserved by freezing and lyophilization was expected, but the observation that no chondrocytes survived in the grafts that had been pretreated with glycerol and not lyophilized was unexpected. This was particularly true in view of the preservation of the architecture of the cartilage, as seen radiographically, and the report of Mankin et al. that as much as 50 per cent of articular cartilage remains viable after freezing and thawing. The only viable cells were those of the pannus of fibrovascular repair tissue and, in one specimen, those in an area in which a portion of lyophilized articular cartilage had been replaced by reparative fibrocartilage.

Oakeshott et al. recently reported that twelve of eighteen fresh osteochondral allografts that had been retrieved at intervals of thirteen to ninety-two months contained viable transplanted articular cartilage. In that study, photomicrographs clearly showed the histological appearance of viable hyaline cartilage that was lacking in our sections that had been prepared with the same technique. This suggests that the freezing and storage rather than the operative transplantation is responsible for the necrosis. The other unexpected observation was that many areas of necrotic cartilage maintained surprisingly good architecture for periods of two and five years. Two clinical circumstances appeared to be associated with this phenomenon: a good anatomical fit of the graft and satisfactory stability of the joint that had been engendered by a good soft-tissue repair.

The histological circumstances that were associated with the preservation of the acellular matrix were a protective covering of fibrovascular pannus and an unrevascularized subchondral plate. The radiographic appearance of the joint did not reveal whether the joint surface of an allograft was composed of hyaline matrix or reparative fibrovascular pannus, or an admixture. In several of the specimens that had been retrieved after a longer period in situ, the preservation of a satisfactory cartilage space, as seen on radiographs, and the absence of the usual associated degenerative findings in the unrepaired allograft (osteophytes, subchondral cysts, subchondral hypertrophy, and fragmentation) was contravened by the gross and histological finding of an irregular joint surface covered by a fibrovascular pannus.

In the specimens in which the joint had fragmented, the fragmentation seemed to have been preceded by subchondral revascularization, which apparently is not necessarily a beneficial activity.

Two of the specimens in this study were retrieved because of what appeared to be outright rejection. Although,
Figs. 11-A through 11-D: Pannus over articular cartilage.

Fig. 11-A: A photograph of an osteoarticular allograft consisting of the distal part of the femur, made at the time of implantation, shows the appearance of the implanted articular cartilage.

Fig. 11-B

A photograph of the same specimen, retrieved at twenty-five months, shows the articulating surfaces to be covered with a pannus of reparative fibrovascular tissue.
as recently summarized by Goldberg et al., there has been voluminous evidence, in experimental animals, that histoincompatibility is detrimental to repair of allografts, continued destructive resorption is an unusual event in large cortical allografts in humans. There were no histological signs of acute inflammatory rejection (masses of admixed leukocytes, histiocytes, and multinucleated foreign-body giant cells) in any of the specimens that we analyzed. The observation that was most suggestive of an immunological phenomenon was the presence of a superficial area of resorption that was filled with a loose admixture of fibrovascular tissue and a few chronic inflammatory cells. It is possible that this is an expression of low-grade indolent rejection, although immunological confirmation is certainly lacking.

Another expression of rejection may be the unexplained, relatively high (about 10 to 15 per cent) rate of late infection that has been noted in many reports concerning large human allografts. Most of these infections are indolent, they are often preceded by erosion as seen on radiographs, and frequently the recovered bacteria are not common pathogens. This sequence has led to the speculation that this may represent contamination by means of sporadic bacteremia of an ongoing immunologically triggered rejection process that provides a ready bed for bacterial growth. Nothing was found in the two infected specimens in this group to contradict that speculation.

While the implications of these observations and speculations are interesting, it is clear that a much larger group of retrieved specimens must be studied in a more orderly and prospective fashion.

**Fig. 11-C**
A photomicrograph of the periphery of a distal femoral osteoarticular allograft, retrieved at fourteen months, shows the pattern of early pannus formation (arrows) extending over the acellular underlying articular cartilage (x 20).

**Fig. 11-D**
A photomicrograph of the central portion of a distal femoral allograft, retrieved at twenty-five months, shows that mature pannus has replaced the superficial half of the articular cartilage (arrows). The deep half of the articular cartilage and the underlying subchondral bone are necrotic and unrepaired.
References