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A CLINICAL ASSAY OF CATHODE RAY STERILIZED CADAVER BONE GRAFTS

By

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INTRODUCTION

Fresh cadavers may be used to provide adequate supplies of bone for Bone Banks. However, despite the wide availability of cadaver bone, relatively little is used because of the difficulties imposed by aseptic procurement techniques. Development of sterile room facilities for graft collection is rarely practical. Bone removed aseptically in the autopsy room is usually contaminated by air-borne bacteria. Therefore, to make the cadaver an effective source of bone, methods for dealing with contamination have been applied. In the past, autoclave or merthiolate sterilized bone has often proved inferior as a graft (1, 2). Accordingly, the search has continued for a technique which will sterilize without altering the graft's biological effectiveness. Several promising experimental methods have been developed in the past five years. Cathode radiation (3, 4, 5), radioactive cobalt (6), ethylene oxide (7), and beta propiolactone (8) have been used to sterilize bone and other tissues.

In 1956, the preliminary results of the clinical use of cathode radiated cadaver bone were reported from the New York Orthopaedic Hospital (9). The study showed that cathode radiation effectively sterilized cadaver bone contaminated with a wide spectrum of bacterial organisms. However, because of the relatively small number of cases and short follow-up, it was impossible to assess the clinical adequacy of the radiated bone grafts. This report extends the study to show that the grafts behave satisfactorily in patients.

METHOD

The method is relatively unchanged from that published in 1956 (9). If the next of kin signs a specific permit allowing retention of tissues for therapeutic purposes, suitable refrigerated cadavers are accepted up to 24 hours after death. We exclude cases with malignancy, viral diseases, bacterial or metabolic diseases involving local donor sites. Bone is collected in the autopsy room from iliac crests, alternate ribs, and in males from fibulae and antero-medial tibial regions. Attempts are made to exclude foreign bodies, such as hair, from donor areas, but no aseptic technique is used. Grafts are cut to appropriate lengths and widths, but are allowed to be *no thicker than 1.5 cm.* The grafts are then packaged in 3 heat sealed plastic bags and stored in a deep freeze at —35° C. Bacterial control samples taken from each graft are cultured before and after radiation. Deposits are shipped in dry ice to the Massachusetts Institute of Technology, where they are radiated with the 3 mev Van de Graaff generator at a dosage of 2,000,000 roentgen equivalent physicals (rep). The grafts are returned in dry ice after radiation. Since the original method was published, a few changes have been made.

For added strength, we now use a heat sealed outer bag of 0.5 mil mylar—2.5 mil polyethylene laminate instead of the plain polyethylene. A $^1/_{16}$ inch diameter pyrex glass bead is sealed in the outer envelope to serve as a dosimeter for each deposit. This bead turns brown after adequate radiation has been delivered to the deposit unit. The fragile thermos shipping containers have been replaced by a fiber board box lined with styrofoam insulation.[1] It should be emphasized that transportation and delivery of the shipping units is expedited with Air Express so that delays in route are avoided. If the bone is allowed to thaw at any time after it is frozen and before use, it must be discarded. The bags are checked for integrity of the seal by squeezing under water just prior to initial freezing and again immediately before use.

RESULTS

In the past three years 2,144 bone grafts have been collected from 74 autopsies. There has been a wide pre-radiation bacterial spectrum (Table I). Staphylococcus aureus, coagulase positive and streptococcus non-hemolytic have been isolated from 40 % of all autopsies. Post-

[1] Developed by Mr. K. A. Wright, MIT, Cambridge, Massachusetts.

TABLE I

Bacteria Isolated from Pre-Radiation Bone Samples.

Organism	% Autopsies present
Staphylococcus aureus, coagulase positive	40
Streptococcus, Non-hemolytic	38
Escherichia coli	28
Bacillus subtilis	25
Clostridium welchii	20
Streptococcus hemolyticus	15
Proteus vulgaris	15
Staphylococcus aureus, coagulase negative	12
Aerobacter aerogenes	11
Enterococcus	11
Paracolon bacillus	9
Alcaligenes	1.5
Diphtheroids	1.5
Micrococci	1.5

radiation bacteriologic control bone samples have been routinely negative after one week of aerobic and anaerobic culture.

385 bone deposits were discarded for technical reasons (thawing, etc.). The remaining 1,759 radiated grafts were used on 1,037 patients. In this group, 9 cases have developed major wound infections (i.e beneath the deep fascia or involving the graft area) for a rate of 0.9 %. Only 2 of the patients in this group have had organisms isolated from their wounds similar to those identified in the pre-radiation bone samples. In both instances, the organism was staphylococcus aureus, coagulase positive.

Biopsies were obtained from 38 cases ranging from 16 days to 504 days after the grafting procedure. The quality and rate of bone graft healing progress is comparable to the findings of experimental studies. There is an initial deposition of reactive bone on the surface of the graft by the host (Fig. 1). This phase is rapidly followed by a wave of revascularization during which the pre-existing vascular channels of the graft are invaded and markedly enlarged by the host tissues. As the bulk of the graft matrix is removed during revascularization, it is rapidly replaced by osseous tissue developing from host cells (Fig. 2). Ultimately, the small islands of dead bone graft matrix left behind during revascularization are removed by a process of appositional bone growth. There has been no evidence of an acute inflammatory cellular reaction indicative of a homograft response during the 2 to 6 weeks postoperative period. The invasion, removal, and replacement of graft matrix

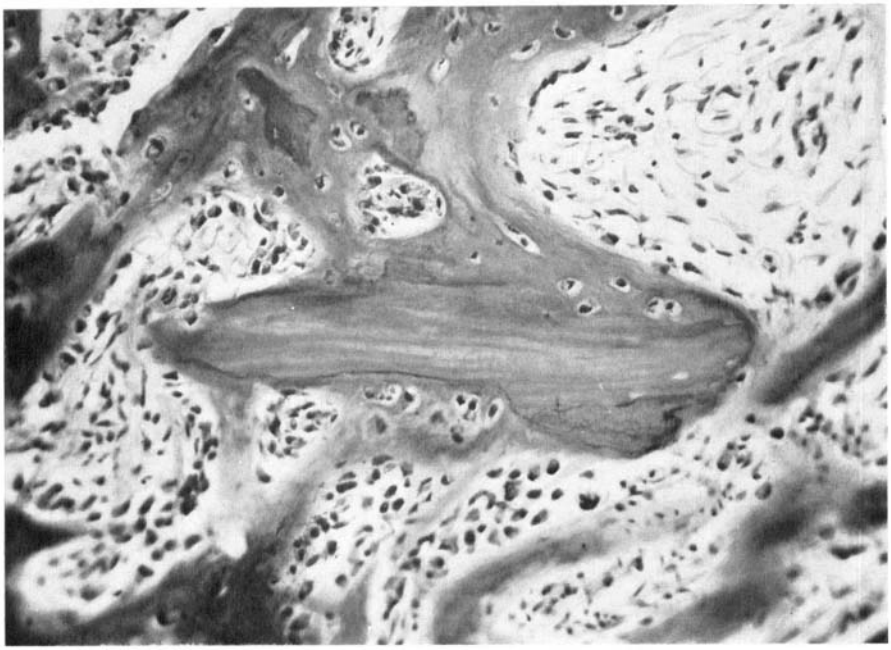

Fig. 1.
Biopsy of spine fusion at 20 days, H and E × 240. Reactive new bone surrounding a fragment of dead, radiated graft.

has progressed rapidly enough to preclude positive identification of residual material in sections of a massive onlay graft of adult tibial bone after 12 to 18 months.

For an analysis of the clinical results, patients were divided into 2 categories. The first was limited to those cases showing unequivocal biopsy and/or roentgenographic evidence of the progress of radiated graft incorporation. 51 patients, followed-up a minimum of 16 months, were in the group. There were nearly equal numbers of arthrodeses, long bone reconstructions, and cavity packings. Cortical, cortical-cancellous and cancellous radiated grafts were used exclusively in 44 cases. Radiographically, host incorporation and elimination of cancellous and cortical-cancellous grafts was rapid, being quite complete in 4 to 8 months, depending upon the mass and size of the grafted bone. Cortical grafts were remodeled at a somewhat slower rate, taking up to 16 months in cases where a large, thick piece of cortex was grafted. In the 7 cases receiving both autogenous and radiated homogenous bone, the two types were placed in identifiable positions. There were 8 clinical

TABLE II

Clinical Failures in which Radiated Bone was Used.

Case	Result	Fate of graft	Reason for Failure
1. Arthrodesis ankle	Pseudarthrosis	Incorporated by host (milled bone)	Neuropathic joint (compression arthrodesis not attempted)
2. Arthrodesis ankle	Pseudarthrosis	Incorporated by host	Neuropathic joint (compression arthrodesis not attempted)
3. Lengthening, congenitally short tibia	Pseudarthrosis	Absorbed after union to host at proximal end	Loss of fixation between graft and distal tibial segment with angulation, graft not mechanically functional
4. Reconstruction of femoral neck for congenital pseudarthrosis	Persistent pseudarthrosis	Graft united to femoral shaft, but not to ossification center of head	Poor mechanical fixation, possible deficiency of host osteogenic cells.
5. Curettage infected Giant cell tumor, radius	Chips of grafted bone became infected and were removed	Milled bone chips infected	Dead bone used in an infected area
6. Non-union, humerus	Continuing pseudarthrosis after this third attempt to gain union	Grafts united with host and showed x-ray evidence of incorporation, but graft fractured.	Inadequate postoperative fixation
7. Non-union of tibia with chronic osteomyelitis	Continued pseudarthrosis	Good graft incorporation where in contact with host bone, absorption in tibial gap.	Inadequate fixation, antecedent *infection, cicatrix with poor* blood supply.
8. Intra-articular arthrodesis, hip	Pseudarthrosis	Incorporated by host proximal and distal to pseudarthrosis.	Inadequate postoperative fixation

Fig. 2.

Biopsy of spine fusion at 80 days, H and E × 140. Active osteolysis and osteoclasis at left of field. Perivascular new bone formation in center of graft. Reactive new bone at periphery of graft appears at top of field.

failures who developed pseudarthrosis or partial absorption of the graft. This gives a success rate of 85 %. However, it must be pointed out that there was evidence that each of the 8 clinical failures was attributable to either host or surgical deficiencies (Table II). There was only one case which demonstrated graft failure. In spite of this, the patient had a satisfactory clinical result (Fig. 3).

The second category of cases contained the first 100 consecutive spine fusions in which cathode radiated bone was used. This group was followed a minimum of two years before evaluation. Since the patients received mixtures of autogenous and radiated bone, it was impossible to attribute succes or failure to either type. In this group 81 patients underwent lumbosacral fusions (1 to 3 levels) and 15 of these individuals developed pseudarthrosis confirmed by operation and/or "bend" x-rays. 14 patients undergoing thoracolumbar (scoliosis) fusions did well except for one patient who developed a pseudarthrosis. 3 patients with thoracic fusions went on to complete bony union, while 1 patient

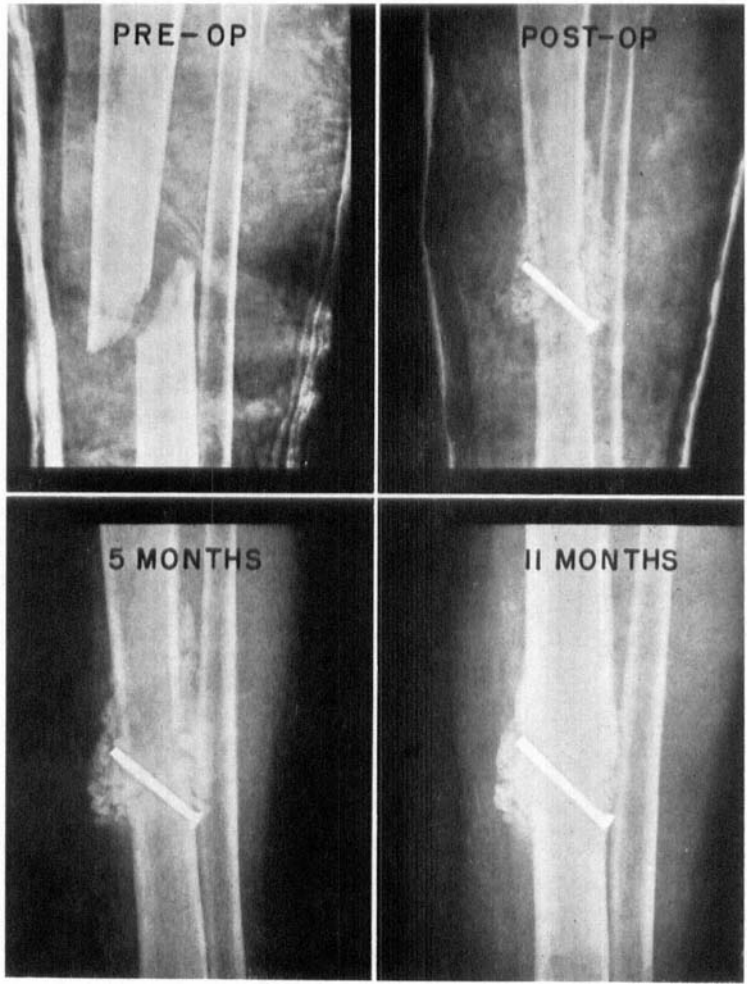

Fig. 3.
"Internal suture" of fractured tibia. Milled, radiated chips packed at fracture site. Discreet chips—unincorporated at 11 months. This is a grafting failure, though a clinical success with the fracture healed.

of 2 undergoing cervical fusions developed graft absorption. In the total group of spine fusions, 17 patients developed pseudarthrosis. The clinical success rate was therefore 83 %. In the study a total of 271 joints of the spine were fused, with pseudarthrosis developing subsequently at 24 of these levels. The success rate per joint would be 91.1 %.

DISCUSSION

There is little doubt that fresh autogenous cancellous bone is the prime choice for grafting in any case where a massive osteogenic response is desirable. The surfaces of spongy (particularly marrow) bone trabeculae are coated with a layer of endosteal cells possessing a potent bone forming capacity when activated. In addition, autogenous cells can survive permanently when transplanted and may begin functioning shortly after operation. However, it should be emphasized that to obtain the osteogenic advantages offered by fresh autogenous cancellous bone, certain rules must be observed. Cells of the graft must not be killed by procurement or subsequent surgical handling. Graft particle size should be limited to optimal dimensions (circa 5 mm) (10) to promote initial cell nutrition by diffusion and to abet rapid, effective revascularization. The graft bed should offer adequate opportunity for intimate contact between the graft and its source of nutrition.

Most of the osteogenic advantage of autogenous bone is lost when one substitutes cortical bone, unless the blood supply of the grafted segment is maintained during and following transplantation. Nutrition of cells by diffusion is effective in mature bone over a distance of no more than 0.1 mm (11). Therefore, since active circulation is not rapidly restored in a cortical graft the vast majority, if not all, of cells die. Experimental studies, utilizing inlay *cortical* bone grafts, have shown that the ultimate fate of both fresh autogenous and preserved homogenous bone is identical (12). In the very early phases of graft incorporation, up to 1 month, the autogenous bone seemed to be sligthly more efficiently invaded by host tissues. For these reasons, we rarely feel justified in procuring a fresh autogenous cortical graft from a second operative site.

It is therefore our belief that properly preserved bone homografts may serve a useful function. Often sufficient supplies of autogenous cancellous bone may be unavailable to fill large cyst cavities or to augment fusion sites in scoliotics. In emergencies, banked grafts can save blood and time. We use the term "graft" rather then "implant" when describing the preserved bone deposits, since by definition a graft is "anything inserted into something else so as to become an integral part of the latter" (13).

In the present study we have clinical evidence to support the experimental findings that the sterilizing dose of cathode radiation (2,000,000 rep) is below the critical matrix damaging dose (5). This is not the case with merthiolate stored and autoclaved bone which give inferior results

on grafting (1.2). Cobalt-60 radiation is an effective method for sterilizing and gives a satisfactory graft (6). Though penetration is essentially unlimited, the method is currently not practical for obtaining large quantities of radiated bone. With the largest cobalt-60 "pot" in the country, it takes several hours to sterilize enough bone to do one spine fusion. The one technical disadvantage to cathode radiation is its limited penetration. While not limited to essentially surface sterilization, as are ethylene oxide and beta propiolactone, cathode rays will not effectively penetrate more than 1.5 cm of cortical bone. Therefore, bone samples must be limited to a maximum thickness of 1.5 cm. From a practical standpoint this has not been a serious factor in our experience.

We must emphasize that cathode radiation is only a method for sterilization at the present—a technique of tissue preservation must be used in addition. We have kept the bone deposites frozen prior to, during, and after radiation. The inexorable growth of tissue ice crystals at temperatures as warm as —35° C produces a gradual degenerative change in bone. Therefore, grafts are kept arbitrarily a maximum of 6 months. Freeze-drying would make possible an unlimited storage time at room temperature, but has not been employed because of technical problems of the method.

The effectiveness of cathode radiation in producing a sterile graft is attested to by the lack of any positive post-radiation cultures and the low wound infection rate in the patients in this study. The rate of 0.9 % is lower than many previously reported rates of 4 to 9 % (14, 15). Random patients received prophylactic postoperative antibiotics, but we doubt if this factor has a bearing on our results.

The survey of case results was divided into 2 categories. This was done because it was felt that data derived from the spine fusion cases was only satisfactory for amplification of other more definitive results. Since spine fusions done in our institution follow a modified Hibbs technique, autogenous bone chips are used in all cases. Therefore, in these cases, it can be stated only that the addition of radiated bone did not impede the progress of the fusion. The rate of 83 % success is comparable to the rates reported in studies of spine fusions of comparable scope.

It is interesting to note that all 8 failures responsible for a 15 % failure rate, in the definitive group, seemed to be caused by host and surgical deficiencies. For example, we wonder if it is not asking a little too much of banked bone to effect a bony union between a femoral

shaft and a poorly developed femoral head ossification center, where previously there had been a congenital pseudarthrosis? The bone homograft relies upon the host for the local cellular impetus to osteogenesis. Therefore, if there is a flaw in the bone forming capacity of cells in an area of congenital pseudarthrosis one might expect graft failure.

Orthopaedic literature is cluttered with thousands of reports on substitutes for autogenous bone (16, 17, 18). These range from accounts of experiences with ivory and plaster to pleas for the use of fresh heterogeneous bone. From a wealth of experimental and clinical experience several points should be emphasized.

The cells of fresh homografts and heterografts do not permanently survive transplantation. In addition, these grafts evoke a host immune-inflammatory response which may adversely affect the results of bone grafting. The wider the genetic gap between host and donor, the more intense the rejection phenomenon. Therefore, the results of implanting cow bone in humans will be poorer than those of planting human bone in humans, in adequately controlled studies. There is indication that both the homograft and heterograft responses are modified by killing and certain types of preservation. Recent attempts to ablate the antigenicity of heterogenous bone (19, 20) have had partial success.

The primary function of the dead bone graft (autogenous, homogenous or heterogenous) is to serve as a scaffold or histologic template for osteogenesis, and on occasions, as an absorbable means of fixation. As yet, it is impossible to unequivocally assign an inductive role to the matrix of the graft. However, it is reasonably safe to postulate that induction of undifferentiated connective tissue elements is going on in the region of a graft. It would be indeed unfortunate if pleuri-potential cells in the reparative area were influenced by properties of the graft to differentiate into fibrous tissue.

To be successful, a dead bone graft must be invaded and replaced by host osseous tissue. A piece of bone walled off within a fibrous, foreign body capsule and never revascularized, fails in its intended function. Unfortunately, many chemicals (i.e. merthiolate, alcohol, formalin, etc.) and physical forces (i.e. heat, ice crystals, etc.) so severely alter bone matrix that invading host cells may be provoked to deviate from an osteogenic to a fibrogenic pattern. The same undesired sequence of cellular events can be obtained from most bone heterografts through intense immunologic phenomena. It is therefore essential that punctilious standards for graft procurement and storage be established and maintained. Can bone which is highly antigenic or chemically altered

by pathologic physiology be accepted for deposit in a bone bank? The answer would seem to be an emphatic no. Once a piece of bone has been deposited, it is mandatory that storage techniques be applied which will limit matrix change to a level consistent with rapid host acceptance and replacement.

Finally we feel that, graded on the basis of inherent osteogenic capacity and host acceptance, there are first, second, and third rate bone grafts. Fresh autogenous cancellous bone and fresh autogenous cortical-cancellous bone, with its blood supply largely intact, are first rate grafts in both respects if their cells are not killed by the surgeon. Fresh autogenous cortical bone is readily accepted by the host, but has poor inherent osteogenic potential. Properly frozen and freeze-dried, radiated and non-radiated, cortical or cancellous bone is a second rate grafting material. Third rate grafts have little or no osteogenic potential, may be poorly tolerated by the host and can, in some individuals, adversely affect the outcome of the grafting procedure. We include in the last class merthiolate stored and autoclaved homogenous or autogenous bone and fresh, preserved or altered (Anorganic, ethylenediamine extracted, macerated and plasma stored) heterogeneous bone. We wonder if the clinical use of third rate grafts can be justified at the present providing first or second rate grafts can be made available.

SUMMARY

1. Properly preserved bone homografts may serve a useful function.
2. Cathode radiation is a satisfactory method for sterilizing frozen cadaver bone.
3. The wound infection rate in a group of 1,037 patients grafted with 1,759 radiated bone grafts was 0.9 %.
4. Biopsy and radiographic evidence of radiated bone graft incorporation reveals a pattern and rate comparable to those observed in experimental studies.
5. The sterilizing dose of cathode radiation is below the critical matrix damaging dose from a clinical standpoint.
6. The clinical success rate of 85 % is lower than the graft success rate (1 failure in 51 cases). The results compare favorably with those reported by others with non-radiated frozen and freeze-dried homogenous bone.

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RESUME

1. Des greffes humaines d'os bien conservés peuvent avoir une fonction utile.
2. Les rayons cathodiques sont une méthode satisfaisante pour stériliser les os gelés de cadavres.
3. Le taux d'infection de la plaie dans un groupe de 1037 malades chez lesquels il avait été pratiqué 1759 greffes osseuses exposées aux rayons a été de 0,9 %.
4. La biopsie et la radiographie de greffer osseuses exposées aux rayons cathodiques montrent un dessin et un taux comparables à ceux observés au cours des études expérimentales.
5. D'un point de vue clinique, la dose stérilisante des rayons cathodiques est inférieure à la dose critique nuisible à la moelle.
6. Le succès clinique s'établit au taux de 85 %, qui est inférieur à celui du succès de la greffe (1 échec dans 51 cas). Ces résultats sont favorables comparés à ceux rapportés par d'autres ayant utilisé des os gelés n'ayant pas été soumis aux rayons cathodiques ou des os homogènes séchés et gelés.

ZUSAMMENFASSUNG

1. Richtig aufbewahrte homologe Knochenspähne können eine nützliche Funktion haben.
2. Kathodenbestrahlung ist eine zufriedenstellende Methode der Sterilisierung von gefrossenem Kadaverknochen.
3. Die Häufigkeit der Wundinfektion in einer Gruppe von 1037 Patienten, die mit 1759 bestrahlten Knochenspähnen behandelt wurden, war 0,9 %.
4. Die Biopsie und der röntgenologische Beweis an einverleibten, bestrahlten Knochentransplantaten zeigt eine Anordnung und ein Verhalten auf, die jenen in experimentellen Versuchen ähnlich sind.

5. Die Sterilisationsdosis der Kathodenbestrahlung liegt unter der kritischen gewebsschädigenden Dosis, von einem klinischen Gesichtspunkte gesehen.
6. Die klinische Erfolgsrate von 85 % ist niedriger als die Erfolgsrate der Verpflanzung (1 Misserfolg in 51 Fällen). Die Ergebnisse erlauben einen günstigen Vergleich mit jenen zu, die von anderen bei der Verwendung von nichtbestrahltem gefrossenem und frostgetrocknetem, homologen Knochen berichtet werden.

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