EXPERIENCE WITH THE USE OF REFRIGERATED HOMOGENOUS BONE*

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The history of the development of methods of bone transplantation is too long and the literature too voluminous to permit of any but the most superficial review in this paper. Ollier is credited with the first clinical experiment when in 1859 he transplanted a rabbit’s radius into an ununited fracture of the tibia. Macwen in 1881 first suggested the transplantation of bone from amputated limbs and Ponset in 1887 reported a clinical trial of this method. Bone transplantation was carried out sporadically by many surgeons but without much enthusiasm until Albee (1915) with his development of a sterile electrically driven motor saw popularised the method of using fresh autogenous bone grafts. During the following years this, with various technical modifications, became the standard surgical procedure.

Carrel (1912) reported his extensive work on preservation and transplantation of various types of human tissues after storing them in Ringer’s solution and plasma at temperatures of 1-2 degrees Centigrade. Gallie and Robertson in 1919 described their experiments with the use of boiled beef bone transplants and this method was given considerable clinical trial but soon abandoned. Transplants of ivory and cow horn were tried by other surgeons but without success. Orrell in 1938 reported on the use of os purum. This was bone from which the organic elements had been removed by chemical treatment, and the experiment was based on his studies of the healing of boiled bone which showed that the slow revitalisation was due to the difficulty of resorbing and removing the dried and coagulated cellular elements occupying the bony canals.

Inclan (1942) was the first to report the use of autogenous bone grafts which were removed at one operation, then stored in sterile jars containing citrated blood or Ringer’s solution at temperatures of 2-5 degrees Centigrade for varying periods, and then transplanted at a second operation. During the second world war it was a fairly frequent practice of orthopaedic surgeons who were working in amputation centres to make use of bone freshly obtained from amputated limbs for purposes of grafting in cases of ununited fractures. This bone caused no apparent reaction in the host and served its purpose well. The successful preservation of perishable foods by refrigeration at low temperatures and the commercial development of low temperature refrigerators led to the next step, the preservation of homogenous bone for surgical use.

In April 1946 a deep-freeze unit was installed at the Hospital for Special Surgery in New York and I began to use refrigerated homogenous bone as a substitute for fresh autogenous bone in surgical procedures. The early results appeared satisfactory and since then transplants of this type have been used with increasing frequency by myself and colleagues. In 1946 the number of such operations was nineteen; in 1947—forty-eight; in 1948—one hundred and six; and in 1949—one hundred and thirty-four. This was the beginning of what we now call the bone bank. Earlier papers described the technique employed and some of the results obtained. Other surgeons have also reported results and varying techniques, particularly the preservation of bone in merthiolate solution by Reynolds and Oliver (1949).

The experience with the use of refrigerated homogenous bone transplants at the Hospital for Special Surgery has now become so extensive, numbering 307 operations on 259 patients up to January 1, 1950, that it seems desirable to make a complete survey with the object of

evaluating the method, particularly with respect to the question of whether refrigerated homogenous bone may be used as a dependable substitute for fresh autogenous bone in surgery and of determining its potentialities and limitations. In order to do this I shall present evidence obtained from animal experiments, from histological study of bone specimens recovered from patients at varying periods after operation, and from an analysis of the clinical results.

COMPARISON OF HEALING OF DIFFERENT TYPES OF BONE TRANSPLANTS

Experimental evidence—A series of experiments was carried out on dogs by the author, assisted by Doctors H. L. Murray and H. S. Way. The experiments were of three different types:

1) Experiments to compare healing of a saw cut and of drill holes in homogenous bone specimens of uniform size but of different types implanted into the muscles of the back of dogs and not in contact with the host bone. The bone transplants included one fresh autogenous transplant, one that had been refrigerated for two weeks (short term) and one that had been refrigerated at greater length (long term). These experiments were intended to test the presence of any intrinsic factor (such as the presence of living cells) in the transplant that might contribute to different rates of healing.

2) Experiments to compare the healing of fresh autogenous cortical grafts with frozen homogenous cortical grafts of uniform size transplanted into gaps in the ulna after subperiosteal resection of segments of the bone. The healing of the transplants was observed by radiographic examination and by recovery of the specimens at different periods after operation.

3) Experiments to study the healing of frozen homogenous cancellous transplants. Here plugs of bone were removed with a trephine from the ilium and after refrigeration for one week were implanted into similar defects created in the iliac bones of other dogs. These specimens were recovered after different periods of time and studied.

DETAILS OF ANIMAL EXPERIMENTS

Experiment 1—Bone transplants into back muscles of dogs. Specimens were recovered at the end of one week, two weeks, three weeks, four weeks, six weeks, eight weeks, ten weeks and twelve weeks.

One week—There was considerable organising hematoma about all the transplants. In the autogenous transplant there was some evidence by microscope of early bone absorption.

Two weeks—All of the transplants were firmly adherent to the soft tissues. Microscopic examination showed no difference in the reactions around the different specimens. None showed any evidence of healing.

Three weeks—The autogenous fragment showed more fibrous tissue reaction than the short-term (two weeks) refrigerated homogenous transplant. The long term (thirteen weeks) homogenous transplant showed very little fibrous tissue reaction. In the autogenous piece microscopic examination showed new bone formation in the cancellous central part near the saw cut and there was no evidence of this in the refrigerated specimens.

Four weeks—Microscopic examination showed definite osteoblastic activity in the saw cut in the autogenous fragment. There were new bone formation and bone resorption along the border of the bone. The main mass of bone seemed dead. Both pieces of refrigerated bone were infected but there was some new bone formation in one drill hole in the long-term refrigerated bone (eleven weeks). In the short-term (two weeks) refrigerated specimen there was only evidence of bone resorption.

Six weeks (Figs. 1 and 2)—All three fragments were firmly adherent to soft structures. The autogenous piece appeared thinner and more osteoporotic than the others. Microscopic examination revealed bone resorption around the edges of the autogenous bone placed in muscle. There was a well-formed osteophyte filling the bottom of the saw cut. The short-term refrigerated transplant showed resorption along the edges and new bone formation in the bottom of the saw cut and in one drill hole. The long-term (six weeks) refrigerated transplant showed less resorption but there was a small amount of new bone in the saw cut.

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Figure 1—Autogenous cortical transplant six weeks after implantation in dog's muscle. Photomicrograph shows attempt at repair of saw cut, with new bone formation.

Figure 2—Refrigerated cortical transplant six weeks after implantation in dog's muscle. Note attempt at repair of saw cut with new bone formation.

Figure 3—Autogenous cortical transplant ten weeks after implantation in dog's muscle. Photomicrograph shows new bone formation at periphery but only slight revitalisation of the transplant.

Figure 4—Refrigerated cortical transplant ten weeks after implantation in dog's muscle. There is new bone formation at the periphery but the lacunar spaces in the transplant are empty.
Eight weeks—There was more reaction around the autogenous specimen, and microscopic examination showed resorption with new bone formation in the saw cut. There was evidence of new bone formation in one drill hole in the short-term (two weeks) refrigerated specimen. The long-term (four weeks) transplant was infected and showed no osteoblastic activity.

Ten weeks (Figs. 3 and 4)—There was more reaction grossly about the autogenous transplant than the other. Only one piece of refrigerated bone (three weeks) was used in this animal. On microscopic examination, there were many areas of bone resorption both peripherally and centrally in both specimens. In the autogenous piece there was new bone formation in the cut and drill hole and in the refrigerated piece a large osteophyte in the drill hole.

Twelve weeks—On gross examination the autogenous piece was paper-thin and very flexible, whereas the refrigerated bone (two weeks) showed some resorption but definitely less than the other. Microscopic examination showed marked osteoporosis of the autogenous fragment with new bone formation in the base of the saw cut and drill hole. There was no new bone formation in the refrigerated bone and much less osteoporosis.

In summary, these findings showed that the autogenous transplant aroused more soft tissue reaction than the refrigerated pieces and that this resulted in quicker and more rapid resorption. As for the refrigerated specimens, two were generally used, one refrigerated for two weeks (short term) and the other for increasingly longer periods (long term). There seemed to be no significant difference in the reaction of the two different pieces. Microscopically the autogenous pieces showed more reaction and a greater tendency towards healing than the refrigerated pieces. There was definite evidence of new bone formation in the autogenous fragment in three weeks whereas in the refrigerated specimen it appeared only at the end of six weeks. This may be interpreted as meaning that surviving cellular elements do play a part in the healing of an autogenous transplant, but it is noteworthy that in none of the fragments was complete healing of the saw cut or simulated fracture obtained, which seems to indicate that their role is minor and the difference not significant.

Experiment 2—Implantation of fresh autogenous and frozen homogenous cortical bone into operative defects in the ulna of both forelegs of dogs. A complete segment of the ulna measuring 1-5 centimetres in length was removed subperiosteally on both sides. The piece from the left ulna was immediately transferred to the gap in the right ulna while the gap in the left ulna was filled with a piece that had been removed previously from another dog and refrigerated for two weeks. The wounds were closed in layers and dressings applied but immobilisation was not employed because in the dog the ulna is not a weight-bearing bone. The experiment was performed in eight dogs and the specimens were recovered at weekly intervals until the end of four weeks and after that at bi-weekly intervals. In addition, radiographic examinations were made at various intervals.

One week—Radiographic examination showed very early callus formation in both specimens. On gross examination the autogenous transplant showed a good fibrous tissue sleeve around it with early callus proceeding from the host. The refrigerated transplant appeared dead but there was organising blood clot and some early callus formation from the host.

Two weeks—Radiographic examination showed that both transplants filled the ulnar gaps rather incompletely. There was slight early reaction evident in the form of rounding of the edges. Gross examination of the specimens confirmed the poor fit of the transplanted pieces. There was fibrous union of both, but the distal end of the autogenous transplant was more firmly adherent than the others. Microscopic examination showed fibrous union of the refrigerated transplant with callus being formed from the host. The autogenous transplant showed more evidence of healing with some callus along its sides, perhaps originating in the transplant.

Three weeks (Figs. 5 and 6)—Both radiographic and gross examination showed bony union of the autogenous transplant distally and of the refrigerated specimen proximally. There was fibrous union of the opposite ends. Microscopic examination demonstrated bony callus at the distal end of the autogenous transplant. The refrigerated transplant showed bony and cartilaginous callus proceeding from the host but the union was still fibrous. The substance of the bone appeared dead and resorbing. The healing of the autogenous piece appeared more advanced.

Four weeks—Nothing more than fibrous union could be demonstrated in either transplant. (This dog appeared older than the others and not in as good health.) Gross and microscopic examination of the autogenous transplant showed the substance of the bone to be dead and undergoing resorption with new callus from the host and fibrous union. There was slight infection of the refrigerated transplant but despite this there was a large amount of callus along both edges of the
Figure 5—Autogenous graft three weeks after implantation into a defect created in dog's ulna. External callus formation is well advanced. Figure 6—Refrigerated graft three weeks after implantation into a defect in dog's ulna. External callus is forming but is less advanced than in the autogenous graft.

Figure 7—Autogenous graft six weeks after implantation into a defect in dog's ulna. There is good external callus and the lower end of the graft is being revascularised. Figure 8—Refrigerated graft six weeks after implantation into defect in dog's ulna. Healing is more advanced than in the autogenous graft. There is heavy external callus and the interior of the graft is being replaced by new bone.
graft with fibrous union, and the transplant was undergoing resorption. The healing of the refrigerated specimen in this animal appeared more advanced than that of the autogenous graft. 

*Six weeks* (Figs. 7, 8, 11 and 12)—Radiographic examination showed a large amount of callus around the frozen transplant with only slight callus formation about the autogenous one. Gross and microscopic examination showed bony and cartilaginous callus proceeding from the host sides. There was evidence of greater reaction about the refrigerated specimen than the autogenous.

*Eight weeks*—The autogenous transplant was solid by radiographic examination at the distal end with fibrous union of the proximal end, whereas the refrigerated piece showed only fibrous union at both ends with marked resorption. Gross and microscopic examination of the autogenous transplant revealed bony union in one area and new bone formation in the substance of the transplant. There was slight inflammation around the refrigerated transplant but there was evidence of bony and cartilaginous callus from the host with slight new bone formation in the transplant. In this animal the healing of the autogenous piece appeared more advanced than that of the refrigerated bone.

*Ten weeks* (Figs. 9 and 10)—The proximal end of the autogenous transplant was united clinically, the distal end had fibrous union. The refrigerated transplant had moved after operation and had lost good position. Radiographic examination at the end of six weeks showed callus around both transplants with possible union of the autogenous transplant proximally. At the end of ten weeks it showed good bony union of the autogenous transplant proximally but not distally. There was callus about the refrigerated transplant but no solid union. Microscopic examination showed good bony union at one end of the autogenous transplant with fibrous union at the other but new bone was beginning to form. The refrigerated transplant showed fibrous union with cartilaginous callus and new bone formation in its substance. There was also central callus formation. Healing seemed further advanced and more mature in the autogenous than in the refrigerated transplant.

*Twelve weeks* (Figs. 13 and 14)—Radiographic examination showed solid union of both transplants distally but not proximally. There was evidence of more mature healing of the proximal end in the autogenous than in the refrigerated specimen. Microscopic examination confirmed the finding.
Radiographs of ulnar grafts twelve weeks after operation (dog). Figure 11—Autogenous graft. Figure 12—Refrigerated graft. The distal ends of both grafts are united and there is no real difference in the rate of healing.

Radiographs of ulnar grafts six weeks after operation (dog). Figure 13—Autogenous graft. Figure 14—Refrigerated graft. Healing of the refrigerated graft is more advanced than of the autogenous graft.
of bony union at the distal ends and of fibrous union at the other. In the refrigerated transplant
the bone was being revascularised very well and new bone which had been laid down earlier was
being remodelled. In the autogenous transplant there was good bony union at one end and fibrous
union at the other. There were a few areas of dead bone which were being replaced. There was
evidence of callus originating from the side of the transplant. In this animal there was about the
same amount of activity in both transplants.

It is difficult to summarise the observations from this experiment and to draw conclusions
because the results in the different animals were variable. They were doubtless influenced
to some degree by the age and health of the animals and also by technical imperfections in
some of the operations. In none of the transplants, whether autogenous or refrigerated, was
healing obtained at both ends of the bone in any of the animals. Bony callus formation
appeared earlier with the autogenous than with the refrigerated transplants, but at six weeks

![Figure 15](image1)
![Figure 16](image2)

Figure 15—Circular refrigerated cancellous (iliac) graft two weeks after implantation. Union is well advanced.
Figure 16—Circular refrigerated cancellous (iliac) graft three weeks after implantation. Peripheral healing
is complete and the central area is being revitalised.

the latter showed more healing than the former. At the end of twelve weeks the healing was
comparable on both sides. In both the substance of the bone underwent resorption and
replacement.

Experiment 3—Transplantation of a circular piece of refrigerated cancellous iliac bone into a
circular defect in the ilium. The bone was removed with a trephine and after refrigeration for one
week was transplanted into a defect in the ilium produced by the same instrument in another animal.
No autogenous control was employed in this series and the number of animals used was smaller.
Specimens were recovered at the end of one week, two weeks, three weeks, four weeks and six weeks.
One week—The iliac transplant showed organising blood clot and early callus from the host bone.
The transplant appeared to be dead and demonstrated halisteresis.

Two weeks (Fig. 15)—The iliac transplant appeared firmly adherent by fibrous tissue to the host
ilium. Microscopic examination showed dead bone with new osseous formation. There was bony
union in one area with callus arising not only from the host bone but also from the transplant.

Three weeks (Fig. 16)—On gross examination the iliac transplant was firmly adherent and seemed
to have developed bony union. Microscopically there was evidence of union of the graft with new
bone formation from both the graft and the host.
Four weeks—On gross examination there was no evidence of union of the transplant but microscopical examination showed callus formation from the host with some areas of living bone in the transplant. (This was the older dog who appeared in poor health.)

Six weeks—The iliac transplant showed solid fibrous union on gross examination. Microscopic examination showed callus from the host bone and osteoblastic activity in the transplant.

These experiments were carried out only on five dogs and were not controlled by the use of comparable autogenous transplants. Nevertheless, they demonstrated the earlier healing of cancellous bone compared with cortical bone. Evidence of bony callus formation was found beginning at the end of two weeks and this seemed to originate both from the host's tissues and from the transplant. On the whole, both union and replacement of the tissue of the transplant proceeded more rapidly than in the experiments with cortical bone.

GENERAL CONCLUSIONS FROM THE ANIMAL EXPERIMENTS

1) When transplants of autogenous and refrigerated homogenous bone are placed in the back muscles of dogs without contact with the host's skeletal tissues, resorption of the autogenous piece begins earlier and progresses more rapidly than in the refrigerated piece. In neither did healing of the saw cut or drill holes take place but definite evidence of osteoblastic activity and tendency towards repair was found in both—at the end of three weeks in the autogenous transplant and not until the end of six weeks in the refrigerated specimen.

2) When autogenous and refrigerated homogenous cortical transplants of uniform size were made into operative defects of the ulna in dogs, bony callus formation appeared earlier with the autogenous than with refrigerated bone, but at the end of twelve weeks the results with both were comparable. In none of the animals was healing obtained at both ends of the transplanted cortical segments.

3) Transplants of cancellous iliac bone which have been refrigerated for one week and are then replaced in operative defects in the ilium behave like autogenous transplants in human beings and show early bony callus formation beginning at the end of two weeks. This early healing is accompanied by rapid resorption and replacement of the bone in the transplant.

HISTOLOGICAL STUDY OF AUTOGENOUS AND REFRIGERATED BONE GRAFTS RECOVERED FROM PATIENTS

In a previous paper (Wilson 1947) I described histological studies of specimens of both autogenous and refrigerated homogenous bone transplants that had been recovered at secondary operations from patients in whom they had previously been implanted for arthrodesis or other purposes. The correction of scoliosis followed by extensive spinal fusion offered an excellent opportunity for study because it is frequently necessary to perform the fusion in two or three stages. Thus it was possible to use refrigerated bone chips on one side of the spinal column and autogenous bone chips on the other. Specimens of each were recovered at varying periods after implantation and compared under the microscope for healing (Figs. 17 to 19). I did not depend upon my own knowledge but called upon the expert advice of Dr Milton Helpern, Pathologist at the Hospital for Special Surgery and Assistant Medical Examiner for New York City, to whom I am glad to acknowledge my indebtedness and gratitude. Dr Helpern reported: "We found no evidence that the cells in the bone transplants survived in either case. The lacunar and interosseous spaces of the bone transplants, whether fresh or refrigerated, were uniformly empty of living cells in the early stages of healing but there soon occurred an invasion of fibroblasts and blood vessels followed by active absorption of the dead trabeculae with large numbers of osteoclasts present.
This process appeared to develop from the periphery of the transplant and then to penetrate into the interior. Three to four weeks after transplantation active new bone formation could be seen adjacent to the old trabeculae with both osteoid and osseous tissue present and many osteoblasts arranged about the latter. In other words the healing process seemed comparable in both the fresh autogenous transplants and the refrigerated transplants and whether the material was homogenous or autogenous seemed to make no difference.

Fig. 17  Refrigerated bone chips recovered at secondary operations in cases of spinal fusion. Figure 17—Girl aged thirteen years; eighteen days after operation. Revascularisation is occurring and nuclei may be seen in some of the lacunar spaces. Figure 18—Woman aged twenty-one years; thirty-five days after operation. Good evidence of revitalisation of the bone. Figure 19—Boy aged seventeen years; six months after operation. The bone is completely revitalised and has normal appearance.

CLINICAL RESULTS OF OPERATIONS IN WHICH REFRIGERATED HOMOGENOUS GRAFTS WERE USED

The clinical results from the use of refrigerated homogenous bone transplants in operations upon patients must be analysed both from the standpoint of wound healing and of ultimate bone healing.

Wound healing—In general, the wounds healed as well as if autogenous bone had been used. No unusual reactions were seen and there was no evidence of incompatibility with the host tissues. There were complications of wound healing in fourteen patients, with development of draining sinuses. Of these, six patients either were previously infected or were proved by positive cultures to be infected at the time of operation. Ten of the wounds with sinus formation subsequently healed without loss of the bone transplants. Of the remaining four the wound had to be opened and the grafts removed in two; and in the other two drainage still continues although healing of the underlying pathological condition has been obtained (including one cystic lesion of bone and one ununited fracture of the humerus). If we exclude the six previously infected cases, the rate of wound infection in the 307 operations was 2.6 per cent. In the whole series there was loss of bone in only two cases.

Bone healing—A follow-up study was made of all the patients upon whom operations had been performed to assess the healing of the bone and the final results. Since a period of at least six months must usually elapse before the result can be evaluated—and in some instances even longer—fifty-nine cases had to be excluded because of insufficient follow-up. Of the remainder, successful results were obtained in 85 per cent of 248 cases.

Analysis of the various operative procedures and results is presented in Table I. It will be seen that by far the largest number of operations were spinal fusions, of which 108 were for scoliosis, eighteen for tuberculous spondylitis and seventy-five for low-back
TABLE 1
Statistical Report on the Bone Bank to January 1, 1950

Total number of patients for whom bone bank bone was used—259. Total number of operations—307

<table>
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<tr>
<th>Analysis of operations:</th>
<th>Patients</th>
<th>Operations</th>
<th>Insufficient follow-up</th>
<th>Failures</th>
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<td>Totals</td>
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**Failures**—37 (15 per cent)

- Bone cysts
- Pseudarthrosis of spine. (Fusions for conditions other than tuberculosis)
- Pseudarthrosis of spine. (Fusions for tuberculosis)
- Osteomyelitic cavities
- Knee fusion
- Scaphoid peg
- Grafts for non-union

**Infections**—14 (6 were previously infected)

- Ten patients healed without loss of bone
- One osteomyelitic cavity necessitated removal of bone
- One case of non-union of humerus was operated upon through a wide adherent scar. The wound could not be closed, became infected, continues to drain, but union is solid
- One previously operated and infected bone cyst became re-infected, continues to drain, but cyst is completely filled in
- One bone cyst became infected and continues to drain, but the cyst is filled in

**Successes**—85 per cent
pain. In the whole group there were twenty-five instances of pseudarthrosis—a failure rate of 12.4 per cent. There were forty-one operations in which homogenous bone was used to fill cystic cavities or skeletal defects created by the excision of benign tumours and other pathological lesions. There was failure of healing in six or 14.5 per cent. Included in this group were ten cases of chronic osteomyelitis with bone cavities created by the excision of infected bone which were filled with cancellous bone chips. This is a type of case in which there will always be a considerable percentage of failure and the fact that healing was obtained in eight speaks well for the kind way in which the host tissues reacted to these transplants.

Arthrodesis of joints other than the spine in which homogenous bone grafts were used totalled twenty-five, of which eight were for tuberculous arthritis and seventeen for other conditions. There were two failures in this group (8 per cent). There were four failures among thirty-two operations for ununited fractures in different regions of the skeleton or a failure rate of 12.5 per cent.

In studying these results the same difference in rate of healing of cortical and cancellous bone was noted as was shown by Abbott et al. (1947) in autogenous grafts. Refrigerated cancellous bone quickly became incorporated in the host's skeleton, whereas cortical grafts often took a long time to become completely transformed although they united readily with the host’s bone. With respect to healing of refrigerated homogenous bone grafts in comparison with that of fresh autogenous grafts, as far as it could be determined by clinical evidence; we gained the impression that it was perhaps a little slower but that in the end the result was the same.

Another question we sought to answer was whether length of preservation made any difference in the healing of these transplants. The average length of refrigeration was fifty-four days. There were eight operations in which bone was used that had been preserved for more than one year. There were five failures in this group or 62 per cent. In seven of these eight cases the bone was obtained from the same source, the amputated limb of one individual. Healing occurred in three and failure in four. Among the successes were two in which the bone had been preserved for the longest period, namely 705 days (1-9 years), and 662 days. It is difficult to explain these contradictory results but a failure rate of 62 per cent compared with an overall rate of 15 per cent seems significant and suggests that a limit should be set on the time of preservation. I would suggest tentatively a period of one year. With long preservation there is danger of dehydration. Dried-out specimens should not be used.

DISCUSSION

The rationale for the use of different types of bone grafts in surgery depends upon an understanding of what happens when they are implanted in the human skeleton and the role they play in the healing of bone defects. Bone is a living tissue and therefore dependent upon nutrition which it receives through the circulation. When separated from its blood supply by transplantation it may exert an osteogenic stimulus in one of three ways:

1) Some of its osteoblastic cells may survive and cause new bone formation.
2) It may release a humeral factor which induces the formation of bone.
3) It may provide physical factors which stimulate or are made use of in the construction of new bone.

Carrel in his work on the preservation of different tissues outside the body found that bone and other connective tissue explants flourished in a plasma medium with cellular reproduction, but without formation of bone matrix or calcification. According to Abbott it is seldom possible to differentiate between living and dead parts of transplants until ten or fifteen days have passed and the effects of autolysis are evident. The survival of a living cell is marked by the absence of autolysis. He found that in an autogenous graft a good many of
the surface cells remained viable. In spongy bone this meant a good proportion of cells since this type of structure afforded large surfaces. In cortical bone it meant relatively few cells for here there was little surface. Urist (1942) noted, after study of the healing of fractures in young animals and the early evolution of the fracture callus in man, that ossification began from the cambium or deep layer of the periosteum, and from the endosteum. The fracture haematoma is replaced by granulation tissue which then differentiates into fibrocartilage but no portion calcifies or forms bone until it is reached by the penetrating osteogenic front, which develops from the endosteal and periosteal surfaces of the living fragments. It appears therefore that something is to be gained from the survival of cells in the transplant and that they will join the host cells in the production of calcifiable matrix which will lead to early union of a graft with the host. They will not speed significantly the resorption of large necrotic portions of the graft or hasten its transformation into living bone.

In the histological study of bone transplants, it may be observed that granulation tissue quickly envelops the major part of the graft, which appears dead, and leads to its resorption. As the dead bone is removed osteogenesis takes place. Some have postulated the presence of a humoral factor which stimulates the differentiation of young connective tissue into bone. Huggins (1931) has shown that in dogs bone will form with great regularity from certain fibrous connective tissues in the presence of growing bladder epithelium which contains large quantities of alkaline phosphatase. Levander (1938, 1946) postulated the presence of some extractable factor in a bone graft and succeeded in producing bone formation by the injection of alcoholic extracts of powdered bone in rabbits. Unfortunately, they found that injection of alcohol alone sometimes induced bone formation. Recent work reported by Berkley (1950) in which he transplanted fresh, frozen and boiled bone into the anterior chamber of the guinea pig's eye showed that both fresh homogenous and frozen homogenous transplants survived and induced new bone formation at some distance from the site of transfer. He suggested that this ability to initiate bone formation was due to many factors but was primarily dependent upon the presence of a biochemical substance liberated from the bone cells upon their disintegration. After consideration we must conclude however that although the existence of a humoral osteogenic factor is possible it has not yet been proved.

If then we find that cell survival plays but a small part in the healing of a graft except in the case perhaps of cancellous transplants, and if the presence of a humoral factor has not been demonstrated, how then are we to explain the undoubted beneficial effects of bone grafts in the healing of ununited fractures and other bone defects? It seems that we must fall back to the only other theory, which is that it exerts an osteogenic stimulus despite the fact that it may not be a living graft. The manifestation of this stimulus is the replacement of its dead components with new bone. How this stimulus is applied is not known. It may be through its supply of mineral elements or through its organic components or again that it affords a framework to guide ossification.

The process by which new bone is substituted for dead bone has been aptly termed "creeping replacement" by Phemister (1935). Nowhere can it be observed more readily than in the dead femoral head undergoing avascular necrosis after fracture of the neck of the femur with loss of blood supply. It is notable that the fracture may unite but the dead head must still undergo resorption and replacement. Microscopic study shows that there are three zones in this process. The first is narrow and formed of capillaries and supporting connective tissue. It invades all available spaces and erodes the bony structure. Immediately behind is the wider zone of transformation in which osteoblasts are seen to be forming new bone matrix. This gradually merges into the third zone or area of calcification. The new bone thus formed does not reduplicate the structure of the bone absorbed and is usually composed of fine trabeculae in cancellous arrangement. The final outcome depends upon how rapidly the process of ossification keeps up with the process of resorption, and this is also true of a graft. If the latter bridges a considerable bony defect then resorption generally gets ahead
and the graft fractures. If the graft is in contact with host bone throughout most or all of its length resorption and re-ossification can proceed from all surfaces rapidly and complete bony transformation is obtained with healing. In the last analysis therefore the result of the graft will depend to a large extent upon how it is implanted and used.

THE ROLE OF REFRIGERATED HOMOGENOUS GRAFTS

When it comes to a consideration of homogenous bone transplants which have been preserved by refrigeration for varying periods, there are no grounds for claiming that any living elements survive. It is true that certain bacteria and viruses have survived long periods of exposure to sub-zero temperatures, and Ehrlich reported in 1907 that malignant tumours could be transplanted successfully after being kept at freezing temperatures. The work of Gye et al. (1949) and of Mann (1949) indicates however that this was due to survival of virus and not of living cells. Gye pointed out that the freezing of a cell at -10 degrees Centigrade turns the water into ice but this does not carry with it the salt, which accumulates as brine and reaches a concentration of 15 to 20 per cent. Such a concentration would literally pickle any cell.

Notwithstanding the absence of living elements I believe that the evidence here presented indicates that the refrigerated homogenous transplant unites rapidly with the host's bone and that the results are comparable to those obtained with fresh autogenous grafts. All observers agree that a sterile autogenous graft is absolutely inert and gives rise to no inflammatory reaction. The same is true of the refrigerated homogenous bone graft. I attribute this to the fact that the cellular elements are preserved in a fresh state and can be quickly and easily removed by the host tissue and that resorption, revascularisation and re-ossification take place almost as rapidly as with a fresh autogenous graft in which at best only a few living elements survive.

CONCLUSIONS

1. Experimental evidence suggests that the autogenous graft exhibits some advantage over refrigerated homogenous grafts in that healing takes place more rapidly but that in the end the results are the same.
2. Histological study of fragments of healing grafts, both autogenous and refrigerated, that have been removed from human hosts shows no significant difference in the rate or method of repair.
3. Study of the clinical results of the use of homogenous transplants in 307 operations shows the bone to be well tolerated. The rate of infection in clean cases was 2-6 per cent; loss of bone occurred in only two cases.
4. Follow-up studies of 248 cases showed successful results obtained in 210, or 85 per cent. These are comparable with the results obtained with autogenous grafts. The healing of cancellous transplants takes place more rapidly than with cortical grafts. Transplants that have been preserved for more than one year do not heal as well as those that have been preserved for a shorter period and the failure rate is higher.
5. The operation of a bone bank is safe and practical. It offers great advantages to the patient and the surgeon from the standpoint of availability, abundance and the elimination of the necessity of secondary operations to obtain bone. When a bone bank is available the number of operations in which bone transplants are used will show a sharp increase.

REFERENCES


