PREPARATION AND USE OF HETEROGENOUS BONE GRAFTS

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The successful use of frozen homogenous bone has made the bone bank an essential part of the equipment of the orthopaedic surgeon. The chief difficulty associated with such banks, however, is that of acquiring human bone in sufficient quantity and quality. Most banks have more demands than they can satisfy. Moreover, the necessity for taking bone as soon as a suitable occasion arises means that staff, time and premises have to be constantly available—a set of circumstances that does not always apply.

The use of animal bone would solve these problems, but past reports indicate that such heterogenous bone grafts have seldom been successfully incorporated in the host bone. We were, however, informed by Messrs J. Judet and R. Judet, of Paris, that they had had considerable success with heterogenous grafts prepared by a special technique. We therefore decided that the problem deserved further investigation. This paper reports our results.

OBTAINING THE BONE

We have been using bone taken from calves between the ages of six months and eight months. We selected the calf as a source because it is easy for us to get calves and to select suitable ones. Between the ages of six and eight months the epiphysial cartilages are not too large and the bone is not too dense. Bone taken from older animals was incorporated in human hosts much more slowly.

Each animal is tested with tuberculin before slaughter and a careful necropsy always follows the removal of the bone so that we are satisfied that the beast is healthy.

The calf is slaughtered separately from other animals in an empty abattoir. The carcass is slung up in hooks; the hind legs are shaved, scalded with boiling water, painted with 10 per cent formalin and amputated through the hip joints by veterinary surgeons, who use the same aseptic technique that would be observed in an operating theatre. The amputated limbs, wrapped in sterile sheets, are taken into the graft-preparation room. We have chosen hind limbs in preference to fore limbs because they are larger and because the bone is more easily prepared and is generally of a better quality. Aseptic removal of bone from the hind limbs is obviously a little more difficult, but the problem can be overcome if the technique is really scrupulous. A longitudinal incision is made from one end of the limb to the other. The incision is towelled and the metatarsus, tibia and femur are exposed and removed through it. The removed bones, held in forceps, are cut into rods of compact bone 15 to 25 centimetres long, and into grafts of cancellous bone in cubes of 15 millimetres × 15 millimetres. Strong instruments are necessary for the removal and dissection of the bones. An electric circular saw 15 centimetres in diameter, driven by a 5 h.p. motor, is used to cut up the bone. The saw and motor are sterilised at 180 degrees Centigrade. All preparation of the bone is done inside an air-conditioned room of about thirty cubic metres. Ultra-violet light is switched on twenty-four hours before and is maintained during the whole procedure. Petri dishes are exposed the whole time for bacteriological control. Communication with the exterior is by a port-hole. The operator and his staff (one assistant and a specially trained nurse) are dressed in sterile gowns and wear sterile gloves. Special spectacles are worn to protect their eyes from the ultra-violet rays.

The bone fragments are placed in a sterile bottle, which is immediately closed by a sterile stopper. The bottle is then placed inside a second tube, which is also sterile. The

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whole is then numbered and labelled. Since the bottles are transparent the nature of each
graft can be seen, so that the statement on the label can be checked. Small fragments of the
grafts are cultured for both aerobic and anaerobic organisms and the double bottles are
placed in the refrigerator and stored at \(-35\) degrees Centigrade until they are ready for use.

No bone is used earlier than one week after freezing. This allows time for the bacteriological
report and for us to be certain that the graft is sterile. Sterility of the graft has been difficult
to obtain. At first many grafts had to be rejected but the various sources of contamination
were traced and eliminated and sterility is now the rule. We do not consider that any of our
precautions are excessive. Many of the failures in the grafting of compact bone appeared to
be due to contamination during the preparation of the grafts.

When the grafts are to be used the outside bottle is opened and the inside bottle is dropped
on to the surgeon’s table so that its opening can be delayed until the last moment.

**PREVIOUS WORK ON HETEROGENOUS GRAFTING**

Heterogenous bone grafting has a long history. In 1885 Ollier published the results of
sixty heterogenous grafts, most of which were failures. He concluded, nevertheless, after
successfully transplanting rabbit bone into cat, that one of the chief factors determining
success was the size of the fragments, which must be small. He used refrigeration to store
homogenous and heterogenous grafts and claimed one success in grafting a fragment from a
rabbit which had been dead for twenty-four hours and kept at a temperature of 2 degrees
Centigrade. Since Ollier’s report, Schmitt (1893), Barth (1895), Axhausen (1909), Baschkirzew
and Petrov (1912), Calvé (1935) and Orell (1937) have published experimental work on
heterogenous grafting, but have not obtained uniformly satisfactory results. Indeed it was
not until 1949, when the Judet brothers used calf bone refrigerated at low temperatures, that
incorporation of such grafts in human bone was shown to occur with any regularity.

**PRELIMINARY EXPERIMENTS**

Our own experiments began in 1949. At first we used transplants of comparatively
small size—10 millimetres \(\times\) 4 millimetres \(\times\) 4 millimetres. We tried many varieties of graft:
refrigerated, non-refrigerated and freeze-dried, compact and spongy, onlay and inlay. In onlay
grafting we have placed the graft subperiosteally without erasing the host cortex. In inlay
grafting we have cut trenches 5 millimetres \(\times\) 25 millimetres with an oscillating saw, and we
have been able to place calf bone and autogenous bone in the same trenches, thus providing
a control.

We have now performed thirty transplants of calf bone into nineteen dogs and eleven
guinea pigs. We used the radius as the host bone in the dog. In the guinea pig we used the
femur, nibbling the lower metaphysis with a rongeur, holding the graft in place on the sawed
bone by suturing muscle over it.

**Material studied**—We made complete histological studies of fifteen calf-dog transplants
(Figs. 1 to 5).

- **Non-refrigerated calf-dog transplants**—Six transplants were studied. Two were inlay transplants
  of cancellous bone; they were examined on the fortieth and fiftieth days. Four were onlay
  transplants of compact bone; they were examined on the twenty-first, forty-second and
  seventy-seventh days.

- **Frozen calf-dog transplants**—Nine transplants were studied. Three were cancellous transplants,
  of which two (inlay transplants) were examined on the sixty-second and sixty-fourth days
  and one (onlay) was examined on the sixtieth day. Six were compact transplants, of which
  five (inlay) were examined on the eighteenth, thirty-sixth, forty-second, sixty-eighth, and
  sixty-eighth days, and one (onlay) was examined on the sixty-second day.

**Observations**—Our observations from these experiments were as follows. 1) Cancellous
bone was not as satisfactory as compact bone for experimental study because its wide texture
made examination difficult. 2) Onlay transplants placed under the periosteum without
An implant of refrigerated calf bone in the shaft of the radius of a dog. The implant was fixed into the host bone by intramedullary impaction. Figure 1—Transverse section of a specimen obtained eighteen days after operation. Note the endosteal reaction. Figure 2—Higher magnification. The graft and the host bone are united by bone trabeculae and by osteoid tissue. (Preparation by Dr Feroldi.)

Graft of refrigerated calf bone into dog radial shaft. Figure 3—Transverse section on sixty-eighth day. Excellent incorporation of the graft. The enlargement of the Haversian canals is evidence that remodelling of the bone is still taking place. Figure 4—Same preparation in higher magnification.
opening up the host cortex and with no other fixation than muscle-suture did not "take" as regularly and as certainly as did the control homo-transplants. It should be emphasised that the onlay technique was not merely subperiosteal implantation of the graft, for the graft bed was very vascular and had been stripped of some surface bone in the process of periosteal elevation. 3) In all cases of inlay grafting of refrigerated calf bone incorporation of the graft was complete microscopically and macroscopically. 4) In most of the cases when grafts were of non-refrigerated calf bone the "take" was uncertain.

Histologically, successful grafts showed a progressive remodelling in the transplanted bone exactly comparable to that in autogenous grafts. Generally speaking we have obtained results similar to those of Reynolds and Oliver (1950).

**LATER EXPERIMENTS**

The second series of experiments was begun in 1950. It included a study of heterogenous transplants of larger pieces of compact bone. In most cases the experiments consisted of

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**Fig. 5**

Transplant of non-refrigerated compact calf bone into the femoral shaft. Specimen obtained four days after operation. Longitudinal section. The graft is united to the host bone by several fine lamellae, the bone reaction resembling throughout an early creeping substitution. Elsewhere the graft is separated from the host bone and fibrous tissue has been deposited between them.

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resection of the shaft of the radius in dogs and bridging the defect by a heterogenous graft (Figs. 6 to 8). The graft was cut to a half-lap joint at each end and fixed by two screws. The limb was then fixed in plaster-of-paris, but because of rapid soiling and destruction of the cast by the animal external fixation seldom exceeded three to eight days.

We have also performed epiphysial resection with replacement of the articular ends of the bones. This experiment was carried out on the humerus of the dog. The grafts were taken from sheep of the same size as the dog. They were preserved by freezing. Fixation was by a half-lap joint by the technique of Merle d'Aubigné.

**Material studied.** *Shaft resections*—Eight transplants were observed. Three were grafts of
non-refrigerated calf bone bridging gaps. Of these dogs one died from pneumonia five days after operation; the other two are still alive. Five transplants were of refrigerated calf bone bridging gaps. Of these five dogs one died in a kennel epidemic six months after operation. In one the graft was extruded without suppuration six months after operation; its fixation had been inadequate. Two dogs are still alive with the graft in situ. The fifth dog was killed twelve months after operation.

Epiphysial articular resections—Two transplants were observed. One was a graft of sheep bone replacing the uppermost quarter of the humerus. The dog is still alive and the graft is well incorporated. The other was a graft of refrigerated sheep bone replacing the lowest quarter of the humerus.

Observations and conclusions—Our conclusions from the second series of experiments are as follows. 1) It is necessary that the grafts should be fixed firmly. Extrusion of the transplant has occurred when screw fixation was inadequate. 2) After grafting, the limb must be immobilised. The difficulty of immobilisation in animals has caused some pseudarthroses to develop in the middle of the grafts during the remoulding process. 3) Heterogenous grafts of non-refrigerated compact bone are unsatisfactory for the bridging of large defects. The grafts gradually disappear or simply sequestrate. The part of the graft immediately adjacent to the host bone can be partly incorporated. 4) Pseudarthrosis in the centre of the graft or at its end has a tendency to spontaneous union after about one year. 5) Heterogenous transplants are not satisfactory for replacement after epiphysial resection. The joint in the neighbourhood of the graft becomes stiff and crushing of the epiphysis occurs, producing changes similar to those seen in osteochondritis.

We considered that these results justified a trial of refrigerated calf bone in human surgery.
THE USE OF REFRIGERATED CALF BONE IN HUMAN SURGERY

We have used refrigerated calf bone in eighty patients. The operations in which grafts were used included arthrodesis of the hip, spinal fusion for Pott's disease, spondylolisthesis and sciatica, and filling of bone cavities (Figs. 9 and 10).

In general the results are comparable with those obtained from autogenous grafts. In eight cases unsatisfactory results were obtained. In six of these failure was due to infection: three infections occurred when the operating theatre was under repair and the operating conditions under suspicion. In one case calf bone was used to replace a human lunate bone; the graft was not incorporated and was partly extruded. In one other failure contact with the host bed was unsatisfactory. Altogether in eight out of eighty patients failure was associated with sepsis, a similar incidence to that occurring in autogenous grafting operations.

We have noticed that when large amounts of refrigerated bone are used, as in operations for spinal fusion in idiopathic scoliosis, a collection of serous fluid may form around the graft. This may discharge during the next few days or it may be aspirated. The presence of this fluid does not appear to prevent the incorporation of the graft.

In some of our earlier operations we used long lengths of bone obtained from older animals. This more massive bone took much longer to incorporate. Exploration of such a graft six months and one year after the original operation showed non-union.

DISCUSSION

We believe that many of the failures in heterogenous and in autogenous grafting may be explained by mistakes in the preparation of the grafts or of their beds. A particularly rigorous aseptic technique is necessary, for the procedures necessary in the preparation of the grafts allow many opportunities for contamination. The preparation of the bed is of
great importance. It must be healthy and vascular, it must allow firm fixation of the graft, and the fragments to which the graft is to be fixed are not to be regarded as if they were inert prostheses. They must not be cut with a circular saw because this will produce heat necrosis of the surface of the bone.

When all these conditions are fulfilled the incorporation of grafts of heterogenous bone is satisfactory and in all ways comparable with the results obtained from autogenous grafts. We have demonstrated this in operating on idiopathic scoliosis by grafting the upper part of the spine with heterogenous bone and the lower part with autogenous bone. Biopsy later showed no difference between the two in either the naked-eye appearance or histology, although it must be admitted that in some cases, especially those in which grafts had been taken from older animals, incorporation does appear to be a little slower if heterogenous bone is used.

SUMMARY

1. The preparation of heterogenous grafts of calf bone is described.
2. The results of experimental application of such grafts in dogs and guinea pigs are recorded.
3. The results of eighty heterogenous grafts in humans are reported.
4. It is concluded that if the conditions are carefully controlled the results of heterogenous grafting are satisfactory.

REFERENCES