THE CLINICAL USE OF COMBINED XENOGRAFTS OF BONE AND AUTOLOGOUS RED MARROW

A PRELIMINARY REPORT

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The beneficial effect upon osteogenesis of impregnating bone grafts with autologous red marrow is well documented. The experimental findings reported in a previous paper suggested that prepared xenograft bone might provide a good medium for osteogenesis by marrow cells. This paper is a preliminary report of the first clinical attempt to use xenografts of bone combined with autologous red marrow. Kiel bone, which was found the most suitable, was impregnated with marrow aspirated from the iliac crest and, apart from one case of infection, gave excellent results in twenty-eight patients under conditions covering a wide range of indications for bone grafting. Further trials should allow a more valid assessment.

Autologous marrow transplanted to heterotopic sites has been known to form new bone ever since the work of Goujon in 1869, but this occurs in only a proportion of experiments (Burwell 1964). Burwell (1961) was the first to record the high osteogenic potential of living autologous marrow cells in combination with foreign bone. Since then, the beneficial effect upon osteogenesis of impregnating various types of bone graft with autologous red marrow in experimental animals has been well documented for allograft bone (Burwell 1964, 1966; Mattes *et al.* 1968; Boyne 1970, 1973; Nade 1970) and more recently for xenograft bone (Plenk, Hollman and Wilfert 1972; Salama, Burwell and Dickson 1973).

The experimental findings reported in the last-named paper suggested that xenograft bone might be a good bone bank material provided it was deproteinised and either impregnated with living autologous red marrow as a composite graft or placed in a bed of bleeding cancellous bone, because xenograft did in some way facilitate osteogenesis by the red marrow. This is a preliminary report on the first attempt, to our knowledge, to use combined xenografts of bone and autologous marrow in clinical orthopaedic practice.

Although in our experiments fully deproteinised Oswestry bone gave the best results, we have used partly deproteinised Kiel bone because it is easily available, has better mechanical properties than Oswestry bone—which is very brittle and when mixed with marrow becomes like a paste—and because experimental results showed firstly, that in the long run Kiel bone seemed to favour osteogenesis more than Oswestry bone (which

was showing a decline by the twelfth week) and secondly, because it seemed to be less antigenic.

*Kiel bone**. Kiel bone is a commercial product consisting of partly deproteinised bone prepared from freshly killed calves. The bone is washed in water, extracted with hydrogen peroxide (which macerates through oxidation of the organic substances), treated with fat solvents and dried with acetone. It is then sterilised by gamma radiation. Modern immunological techniques, as used in recent experimental work (Elves and Salama 1974) and in clinical investigations to be presented in a separate report, suggest that it is very weakly antigenic both in rats and in the human host. On the other hand, it does not seem to possess active bone-inducing properties (Salama *et al.* 1973). The mechanism of the facilitating effects of Kiel bone upon osteogenesis by marrow cells seems to be only a passive one, firstly by osteoconduction and secondly by protection.

PREPARATION AND IMPLANTATION OF THE COMPOSITE XENOGRAFT-AUTOGRAFTS (CXA)

The recipient site is exposed and prepared according to general principles, with particular emphasis on vascularity of the graft bed, in the absence of which even fresh autologous bone will die and be resorbed. If internal fixation is being used, steps are taken to avoid any unnecessary exposure of the CXA to the device or any delay in closure of the wound. In cases of pseudarthrosis or bone defect, eburnated bone and scarred soft tissue are usually excised to provide a maximal bleeding surface for adhesion and vascularisation of the grafts. Whenever possible, osteoperiosteal flaps are elevated to allow the insertion of grafts beneath them. The wound is then packed while the CXA is prepared.

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Cancellous Kiel bone is used as a rule, but occasionally cortico-cancellous grafts with a thin backing of cortex for added rigidity. The cancellous bone blocks are cut into small slabs or chips not more than 5 millimetres thick so to provide a large surface area.

Autologous red marrow is now aspirated from the iliac crest by means of a wide-bore sternal puncture needle and an ordinary syringe. Large amounts of bone

to obtain as much marrow as necessary to ensure thorough impregnation of all the bone grafts; the largest amount obtained in this series was about 40 millilitres.

When a large amount of bone is to be used, it is divided into three or four parts which are placed in small sterile rectangular containers and each mixed with marrow separately. By tilting the container, elongated pieces of bone soak in the marrow aspirate, in which they are gently turned round until thoroughly impregnated. An almost unlimited amount of composite grafts can be prepared in this way.

Routine examination of a smear of the aspirate confirmed the presence of marrow cells in all of the first ten cases and now this precaution has been dispensed with. At first, heparin in very small amounts was used to prevent clotting of the marrow aspirate, but this was abandoned after the check smears showed changes in the staining properties of the marrow cells that suggested damage. When small amounts of aspirated marrow are mixed separately with small amounts of bone implants, clotting does not present a problem. The mixing is easy, and the eventual clotting is even useful in that it helps to retain the marrow within the intertrabecular spaces of the grafts.

As soon as the composite grafts are ready they are packed into the prepared graft bed and the wound is closed. Vacuum drainage can be used, but direct contact of the polyethylene tube with the grafts should be avoided.

CLINICAL MATERIAL

Since July 1973 composite grafts have been used in thirty-one bone-grafting procedures in twenty-eight patients (Table I). They have been used after excision of

Table I. Clinical material—Thirty-one operations in twenty-eight patients

Benign bone lesions	
Enchondromata	2
Unicameral bone cysts	5
Non-ossifying fibromata	5
Arthrodeses	2
Pseudarthroses	15 ^{††}
Traumatic bone defects	2 ^{**}

*Note that fresh autogenous iliac bone was added for pseudarthroses of humerus and ulna in one patient, and **for an extensive defect of the tibia in another patient. [†] As mentioned in the text, homologous bone was added in a child with a pseudarthrosis of the tibia.

marrow can be obtained from a single puncture. However, to ensure the greatest possible proportion of marrow to blood, and to avoid premature clotting, only 6 to 8 millilitres are aspirated at a time and the aspirate is rapidly mixed with the bone chips. Multiple punctures spaced at 1 to 2 centimetres along the iliac crest are used

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Figure 1—A non-ossifying fibroma of the lower end of the femur in a child aged ten, first treated by curettage and homogenous cancellous boiled bone grafts. Figure 2—The same case, showing the appearances after three years, before composite grafts were used. Figure 3—Five months later the density of the grafted area indicates that the rate of deposition of new bone exceeds that of resorption of the grafted bone.

Figure 4—At eighteen months remodelling is well advanced and there are no signs of recurrence.

Fig. 5

Fig. 6

Fig. 7

Figure 5—A unicameral bone cyst with pathological fracture in a girl aged nine, treated by curettage, internal fixation and composite grafts. Figure 6—The same case three months later. The limits of the cyst are quite indistinct and the fracture has united. Figure 7—By sixteen months there is obvious remodelling and commencing reformation of the medullary canal.

benign bone lesions, and for arthrodeses, pseudarthroses and traumatic bone defects. In one patient with pseudarthrosis of the humerus and ulna, and in another patient with a large traumatic defect of the tibia, a small amount of fresh autogenous iliac bone was added. These two patients were operated upon in the early days before there was sufficient clinical evidence of the reliability of the method.

Benign bone lesions. The cavities left after thorough curettage were packed with small slabs or chips of CXA, with care to ensure that the cavity was completely filled. In order to allow free access of blood, serum and newly formed capillaries, and to enhance the chances of

survival of marrow cells, the bony septa at both ends of the cavity were broken down, small perforations were made in the walls, and the cortical window which had been opened to gain access to the lesion was left open (Figs. 1 to 11).

Arthrodesis. Transarticular cortico-cancellous composite grafts were used twice. In one patient with a flexion adduction contracture and painful fibrous ankylosis of the hip due to septic arthritis, a corrective intertrochanteric osteotomy was performed, with transarticular nailing and composite cortico-cancellous grafting. In the second, a child with cerebral palsy, a "sandwich arthrodesis" of the wrist (Makin 1971) was performed. The distal part of the radius and the carpal bones were split into dorsal and volar sections and a flat plate of cortico-cancellous composite graft was laid between them. Plaster fixation was used in both patients and fusion was rapid.

Fig. 8

Fig. 9

Fig. 10

Fig. 11

Figure 8—A large non-ossifying fibroma in a girl aged thirteen, treated by curettage and composite grafts (60 millilitres). Figure 9—The same case showing the appearance after operation, when holes were made in the sclerotic wall of the cavity and grafts were inserted distally into the medulla. The outline of a thin cortico-cancellous graft placed medially can be clearly seen. Figure 10—Three months after operation, the increased density and fluffy appearance suggest that new bone formation is well advanced. Figure 11—At six months the appearances are even more favourable, with evidence of resorption of the bone grafts and commencing reformation of the medullary canal.

Pseudarthrosis. Composite grafts were used fifteen times in twelve patients. One patient had pseudarthroses of the humerus and of the ulna after a severe "side-swipe" injury which had initially been treated by internal fixation, anastomosis of the brachial artery and skin grafting. In this case, slivers of autogenous iliac bone were used together with the composite grafts at the second operation four months later.

Another patient had a pseudarthrosis of the radius and ulna six months after derotation osteotomy with internal fixation for a fixed pronation contracture due to Erb's palsy. One patient with severe initial multiple injuries had delayed union of the lowermost third of the tibia and a pseudarthrosis of the mid-shaft of femur nine months after open reduction and internal fixation. Four patients had a pseudarthrosis of the humerus, two in the uppermost third, one in the middle and one in the lowermost third; in this last case, internal fixation with autogenous bone grafts had already been used six months previously without success. Three patients had a pseudarthrosis of the femur—one in the lowermost third and two in the middle third.

One of these three patients had already been operated on twice before, using Kuntscher nails. At the time of composite bone grafting a plate was added to prevent rotation, but a few weeks later an infected haematoma had to be incised and drained. This was the probable cause of the only failure in our series. Histological examination of pieces of xenograft bone removed at that time showed infiltration with inflammatory cells and almost no new bone formation.

One patient had delayed union of the lowermost third of the tibia nine months after compression plating, with loosening of the screws and angulation into varus. A child with neurofibromatosis had initially been treated at the age of two for congenital pseudarthrosis due to a cyst of the uppermost third of the tibia by curettage and bone grafting, and osteotomy with intramedullary pinning to correct bowing of the lowermost third. At the age of four, there was an established pseudarthrosis at the site of osteotomy. After removal of the intramedullary pin and thorough excision of dense fibrous tissue from the region of the pseudarthrosis, the bone ends were inserted into a cuff-like graft of autoclaved cortical homologous bone and composite grafts were packed around. An intramedullary pin inserted from the heel was used for internal fixation. The child is still using a protective brace although the fracture is solidly united and the medullary canal clearly formed.

Bridging of bone defects. Composite grafts were used twice for defects resulting from war injuries associated with extensive soft-tissue damage. In one patient with a defect of about a third of the tibia for which a fibular by-pass was performed, slivers of autogenous iliac bone were added to the composite grafts. In another the composite grafts were used to bridge an irregular defect of about three centimetres at the junction of uppermost and middle thirds of the ulna. The grafts were placed around an intramedullary pin which had initially been used.

Twenty-six patients (twenty-eight operations) have already been followed for more than six months. The results have been most satisfactory except in the single case of infection.

DISCUSSION

The induction of osteogenesis is the main purpose of any bone-grafting procedure, and fresh autogenous iliac cancellous bone is considered to have the highest potential. It consists of a trabeculated bone substance filled with living red marrow. Experimentally it has been shown that after transplantation the mature bone largely dies, and that only the most superficial parts survive to be a source of osteogenesis (Abbott *et al.* 1947; Ham and Gordon 1952; Hutchison 1952; Siffert 1955; Burwell

1969). Host cells contribute to new bone formation by induction either by the bone matrix (obviously more in autologous bone) or by the marrow cells stimulating non-specific mesenchymal cells (Levander 1940; Friedenstein 1973). But most of the new bone formed by fresh iliac autografts is derived from two main sources: the relatively few surviving littoral osteoblasts of the grafted bone (Burwell 1964); and cells of the contained marrow (Burwell 1964), which are the main source of osteogenesis (Danis 1957).

The composite grafts used in this study consist of living autologous marrow and a foreign bone scaffolding which, although dead, has been shown in some way to facilitate osteogenesis by marrow cells (Salama *et al.* 1973). The new bone formed in these grafts is probably derived from the autologous marrow only.

The advantages of autogenous bone grafts lie therefore in the presence of a small number of surviving osteoblasts, in their possibly better bone-inducing properties, and in a lack of antigenic properties.

The reason why red marrow, after transplantation, is osteogenic is unknown. It has been explained theoretically on the grounds that necrosis of cells of the grafted marrow stimulates either its peripheral surviving cells (Burwell 1969) or non-specific mesenchymal cells of the host (Levander 1940; Friedenstein 1973); or that surviving young undifferentiated cells present in the marrow (Tavassoli and Crosby 1968) develop into osteogenic cells under the influence of adjacent host bone (Burwell 1965, 1966).

Therefore, whatever the mechanism of osteogenesis, the larger the amount of transplanted bone marrow used the greater should be the induction of new bone formation, and in this series success was probably favoured by the use of relatively large amounts of composite graft.

As regards antigenicity, our experimental study (Elves and Salama 1974) has shown that in rats Kiel bone has relatively low antigenic properties. These might have interfered with, but did not prevent, new bone formation in the added autogenous marrow (Salama *et al.* 1973). Preliminary immunological studies of our patients, including agglutination, complement fixation, lymphocytotoxicity tests and haematological studies, are well in accordance with these experimental findings and suggest that Kiel bone is very weakly antigenic in the human host, if at all (Salama and Gazit 1978). It is therefore not very surprising that such satisfactory results have been attained.

It may be argued that in some cases of non-union efficient internal fixation might have been sufficient. The radiographs, however, suggested that the grafts did play an active role in the consolidation of the fractures. In the treatment of enchondromata and bone cysts, satisfactory results have been achieved using various types of bone graft, but despite the limited number of patients included in our series, the consistently success-

ful obliteration of the cavities after curettage of the benign bone lesions cannot be ignored.

The advantages of the method are obvious. It is almost atraumatic. It is also time-saving, for it obviates the need for an additional operation to procure autografts and the laborious procurement and preparation of suitable allografts. It is easy, practical and safe, avoiding the numerous possible complications in the donor site. It is economical because it obviates the need for a sophisticated bone bank. Unlimited supplies of xenograft bone are available and, it would seem, can be rendered osteogenic by a simple procedure. This is clearly illustrated in our series, especially in the child with a defect of more than 60 millilitres shown in Figures 8 to 11.

It is quite possible that combining fresh autologous red marrow with other types of bank bone allograft or xenograft, or even with synthetic material that favours the induction of new bone, may provide better results.

At any rate, our clinical results seem to confirm our experimental findings and strongly suggest that direct contact between living cells with a known high potential for osteogenesis and such graft material has an undoubted advantage, because it places the appropriate receptive cells on the spot, allows the triggering of bone formation at the earliest possible time and enhances their bone-inducing properties.

It is true that from a statistical point of view this series does not provide proof of the infallibility of the method, but it is also true that it is practically impossible to make a statistically valid comparison with other methods of bone grafting owing to the large number of variables. It would seem, however, that the present technique deserves serious consideration. We think that such satisfactory results in disorders covering a wide range of indications for bone grafting provide enough evidence of reliability to warrant an extended trial and allow a more valid assessment.

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REFERENCES

- Abbott, L. C., Schottstaedt, E. R., Saunders, J. B. de C. M., and Bost, F. C. (1947) The evaluation of cortical and cancellous bone as grafting material. *Journal of Bone and Joint Surgery*, **29**, 381-414.
- Boyne, P. J. (1970) Autogenous cancellous bone and marrow transplants. *Clinical Orthopaedics and Related Research*, **73**, 199-209.
- Boyne, P. J. (1973) Induction of bone repair by various bone-grafting materials. In *Hard Tissue Growth, Repair and Remineralisation*, pp. 121-141. Edited by K. M. Elliott and D. W. Fitz-simons. Ciba Foundation Symposium 11 (New Series). Amsterdam, London, New York: Associated Scientific Publishers.
- Burwell, R. G. (1961) A study of homologous cancellous bone combined with autologous red marrow after transplantation to a muscular site. *Journal of Anatomy*, **95**, 613.
- Burwell, R. G. (1964) Studies in the transplantation of bone. VII. The fresh composite homograft-autograft of cancellous bone. An analysis of factors leading to osteogenesis in marrow transplants and in marrow-containing bone grafts. *Journal of Bone and Joint Surgery*, **46-B**, 110-140.
- Burwell, R. G. (1965) Osteogenesis in cancellous bone grafts: considered in terms of cellular changes, basic mechanisms and the perspective of growth-control and its possible aberrations. *Clinical Orthopaedics and Related Research*, **40**, 35-47.
- Burwell, R. G. (1966) Studies in the transplantation of bone. VIII. Treated composite homograft-autografts of cancellous bone. An analysis of inductive mechanisms in bone transplantation. *Journal of Bone and Joint Surgery*, **48-B**, 532-566.
- Burwell, R. G. (1969) The fate of bone grafts. In *Recent Advances in Orthopaedics*, pp. 115-207. Edited by A. G. Apley. London: J and A. Churchill Ltd.
- Danis, A. (1957) Etude de l'ossification dans les greffes de moelle osseuse. *Acta Chirurgica Belgica*, Supplement 3.
- Elves, M. W., and Salama, R. (1974) A study of the development of cytotoxic antibodies produced in recipients of xenografts (heterografts) of iliac bone. *Journal of Bone and Joint Surgery*, **56-B**, 331-339.
- Friedenstein, A. J. (1973) Determined and inducible osteogenic precursor cells. In *Hard Tissue Growth, Repair and Remineralisation*, pp. 169-185. Edited by K. M. Elliott and D. W. Fitz-simons. Ciba Foundation Symposium 11 (New Series). Amsterdam, London, New York: Associated Scientific Publishers.
- Goujon, E. (1869) Cited by Burwell, R. G. (1964).
- Ham, A., and Gordon, S. (1952) The origin of bone that forms in association with cancellous chips transplanted into muscle. *British Journal of Plastic Surgery*, **5**, 154-160.
- Hutchison, J. (1952) The fate of experimental bone autografts and homografts. *British Journal of Surgery*, **39**, 552-561.
- Levander, G. (1940) An experimental study of the role of the bone-marrow in bone regeneration. *Acta Chirurgica Scandinavica*, **83**, 545-560.
- Makin, M. (1971) Arthrodesis of the wrist in the child. *Journal of Bone and Joint Surgery*, **53-B**, 560.
- Mattes, T. von, Gibel, W., Wildner, G., and Marx, G. (1968) Experimentelle Untersuchungen zur Möglichkeit einer Rekonstruktion der Thoraxwand mit homologen Rippen nach dem Prinzip des Kieler Knockenspanns bei gleichzeitiger Spongiosa. *Zeitschrift für experimentelle Chirurgie und chirurgische Forschung*, **1**, 360.
- Nade, S. (1970) Bone-graft surgery reappraised: the contribution of the cell to ultimate success. *British Journal of Surgery*, **57**, 752-756.
- Plenk, H., Jun., Hollman, K., and Wilfert, K.-H. (1972) Experimental bridging of osseous defects in rats by the implantation of Kiel bone containing fresh autologous marrow. *Journal of Bone and Joint Surgery*, **54-B**, 735-743.
- Salama, R., Burwell, R. G., and Dickson, I. R. (1973) Recombined grafts of bone and marrow. *Journal of Bone and Joint Surgery*, **55-B**, 402-417.
- Salama, R., and Gazit, E. (1978) The antigenicity of Kiel bone in the human host. *Journal of Bone and Joint Surgery*, **60-B**, in press.
- Siffert, R. S. (1955) Experimental bone transplants. *Journal of Bone and Joint Surgery*, **37-A**, 742-758.
- Tavassoli, M., and Crosby, W. H. (1968) Transplantation of marrow to extramedullary sites. *Science*, **161**, 54-56.