Acta orthop. scand. 53, 857–865, 1982

OSTEOINDUCTIVITY OF PARTIALLY DECALCIFIED ALLOIMPLANTS IN HEALING OF LARGE OSTEOPERIOSTEAL DEFECTS

DEVINDER GUPTA & S. M. TULI

Department of Orthopaedics, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India

About 3.5 cm osteoperiosteal circumferential gaps in rabbit ulnae were bridged by partially decalcified (by 0.6 M HCl) allogenic implants. 8–12 weeks after implantation complete bridging of the gap by new bone formation and union at host-graft junctions was observed in 97.2 per cent of the experiments. Instant permeability and the powerful osteo-inductive potential of the implant coupled with some degree of mechanical stability provided by partial decalcification ensured successful bone formation in almost all the experiments. It is suggested that partially decalcified allogenic bone matrix preserved in 70 per cent ethanol may be a reasonably good substitute for autologous bone graft.

Key words: allogenic transplantation; decalcified bone implants; osteogenesis

Accepted 8.v.82

It is universally accepted that autologous cancellous composite bone is the most effective osseous graft material (Wilson & Lance 1965, Parrish 1966, Enneking et al. 1980, Boyne 1973, Tuli 1972). However, it may not be practicable to procure the quantum, hence the need for the next best alternative. The ideal material would be one which retains mechanical advantage and inductivity and is bacteriologically sterile. Many sophisticated methods devised since that of Inclan (1942) are impracticable in economically under-developed countries because of perpetual power failures and lack of sophisticated facilities. Besides, even the best preserved bone (deep-frozen or lyophilised) has varying degrees of antigenicity (Burwell 1969, Brooks et al. 1963, Hurley et al. 1960, Ray & Holloway 1957, Musculo et al. 1976, Elves 1976, Friedlander et al. 1976).

Decalcified bovine bone chips were first used, with good results, by Senn (1889) for filling sterile osteomyelitic cavities. Interest in this material has recently been renewed (Urist et al. 1968).

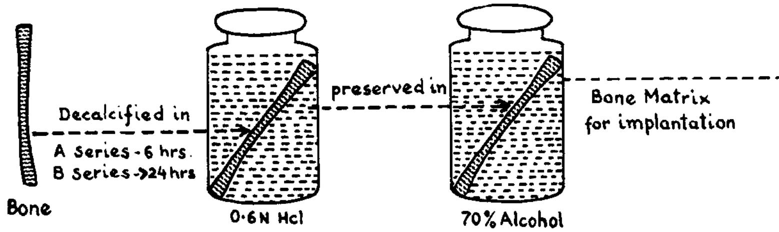
The aim of the present study was to evaluate the repairing capacity of partially decalcified preserved diaphyseal allogenic bone used to bridge large osteoperiosteal defects in rabbits. This was a part of on-going research on bone regeneration in the Bone Research Laboratory of the Department of Orthopaedics, Institute of Medical Sciences, Banaras Hindu University.

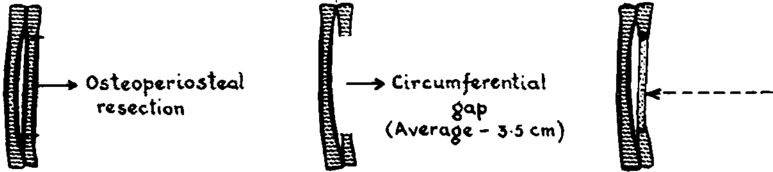
MATERIAL AND METHODS

Preparation and preservation of implants. The bones were obtained by sacrificing mature healthy rabbits and removing the major long bones aseptically. Soft tissue and periosteum were removed with a scalpel, and the bones were decalcified in 0.6 M HCl for 4–6 h at room temperature (15–20°C). The partially decalcified matrix was washed with distilled water thoroughly to re-

0001-6470/82/060857-09 $02.50/0

© 1982 Munksgaard, Copenhagen





DIAGRAMMATIC REPRESENTATION OF THE EXPERIMENTAL MODEL

Fig. 1. Diagrammatic representation of our experimental model.

move any traces of acid. The bone matrix was then sealed in 70 per cent ethanol at 4°C and stored for a maximum period of 30 days.

Operative procedure. Under general anaesthesia (Nembutal 30 mg/kg body weight, i.v.) a circumferential osteoperiosteal gap (average 3.5 cm) was created by sharp dissection in the diaphysis of mature rabbit ulna (Figure 1). Precautions were taken not to disturb periosteum of the ipsilateral radius. The preserved bone matrix was cut exactly the size of the gap, rinsed thoroughly with distilled water and fitted snugly into the gap. Approximation of muscles held the implant in place. The intact radius provides adequate splintage. Animals with technical failures like splintering of ulna, damage to the radius or gross infections were excluded from the final analysis; 36 animals were available for final analysis.

Follow-up. The parameters used for this study were periodical clinical and radiological examinations, macroscopic examination of the implant at the time of sacrifice, histologic studies of the reaped implants and tetracycline fluorescence studies of representative undecalcified sections. Animals were sacrificed at: 24 h (one animal), 8 days (one), 2 weeks (one), 3 weeks (two), 4 weeks (one), 5 weeks (one), 6 weeks (two), 9 weeks (two), and 12 weeks (36) post-implantation. The animal which did not show any bone radiologically by 12 weeks was considered a failure (1/36) and histological sections were prepared from adjoining soft tissues. Radiological examinations were carried out immediately post-operation and 4, 8 and 12 weeks post-implantation. For tetracycline fluorescence studies all the animals showing success were given 25 mg/kg body weight of oxytetracycline, i.m. for three consecutive days in the eighth post-implantation week. Two similar doses were further given with an interval of 10 days. The animals were sacrificed at least 72 h after the last dose. The part of the implant for fluorescence studies was stored in 5% neutral formalin (for maximum of 7 days). Transverse discs of 2–3 mm thickness and long segments of 5–7 mm were hand ground by the rapid manual grinding technique described by Frost (1966) for studies under ultraviolet fluorescent microscope (Fluoval – 2, Carl Zeiss, Zena, East Germany).

OBSERVATIONS

Clinically. There was no evidence of systemic or local immunogenicity.

Radiological studies. The immediate post-implantation X-rays revealed that the implant shadow was usually a little less dense than the host bone shadow. At 4 weeks an initial increase in density was seen in 11/36 animals (Figure 2) while decreased density or no change was seen in 25/36. One or both host-graft junctions showed evidence of union in 27/36. Peri-implant new bone formation was evident in 15/36. At 8 weeks, both host-graft junctions showed union in 35/36. The implants showed better remineralisation and remodelling. At 12 weeks, the overall

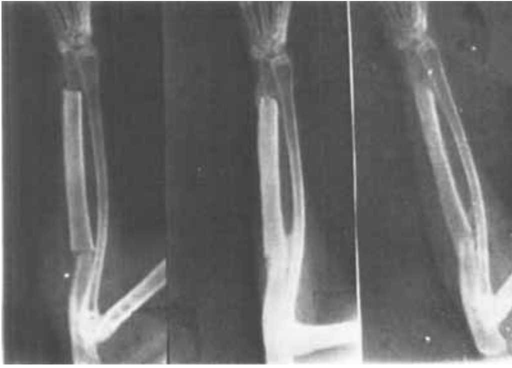


Fig. 2. Sequential skiagrams showing relatively increased density at 4 and 8 weeks post-implantation which is approaching near normal at 12 weeks (L to R).

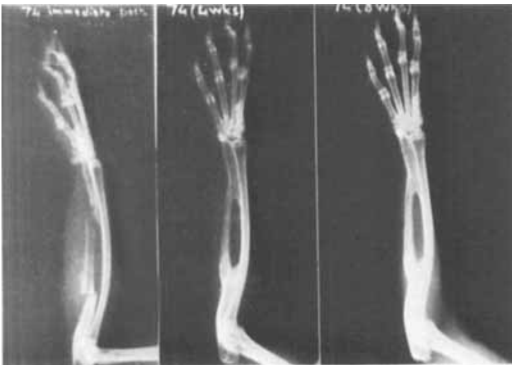


Fig. 3. Sequential skiagrams showing 'take up' and remodelling in a case of success (L to R) by 8 weeks post-implantation.

radiological success rate was 97.2 per cent. The density of implant returned to that of the host bone. Remodelling of the trabeculae, and the size and shape of the implant could be seen radiologically, with some variations in the bone mass in the implanted part (Figure 3). Finally, the peri-implant bone had also been incorporated into this new bone mass bridging the gap.

Macroscopic observations. With one exception the gap was filled with a bridge of bone, hard, pink and continuous with the host bone proximally and distally. In the one failure, the implant had apparently undergone resorption and there was a clear gap filled with scanty fibrous tissue, the bony ends smooth and tapering towards the ipsilateral radius. In the animals sacrificed early, at 8 days and 2 weeks, both host-graft junctions seemed to be fixed though still partially mobile; at 3–4 weeks, there was very little mobility of the implant; at 5–6 and 9 weeks the implants were firmly united at both host-graft junctions with no mobility at all. At 12 weeks the implanted part was almost indistinguishable except for the visible host-graft junctions. In general, from the first day the soft tissues showed increasing attachment to the implanted part until by 4 weeks they were firmly attached to the host bed.

Histological observations. Histological examinations of the implanted matrix throughout the observation periods revealed gradual, progressive cellularity, vascularity, and population by marrow cells. At 24 h post-implantation, the bone was in

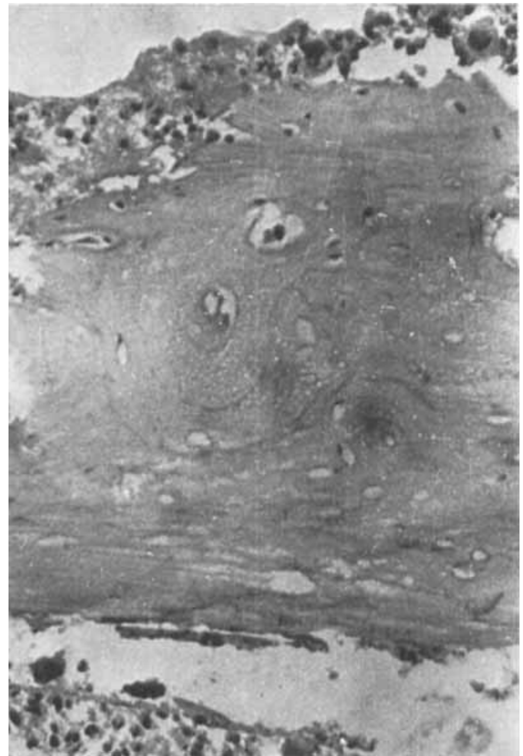


Fig. 4. Microphotograph, 24 hours post-implantation showing peri-implant cellular invasion, inflammatory cells and a few small giant cells. Only superficial lacunae show plump cells (Haematoxylin and Eosin ×150).

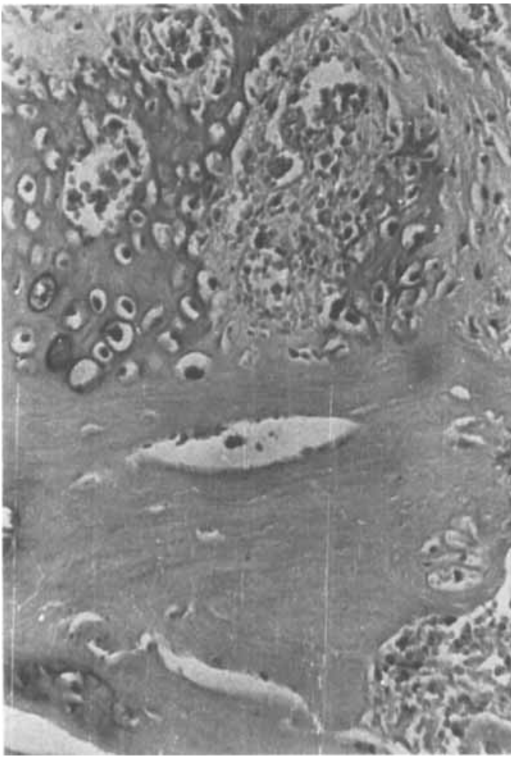


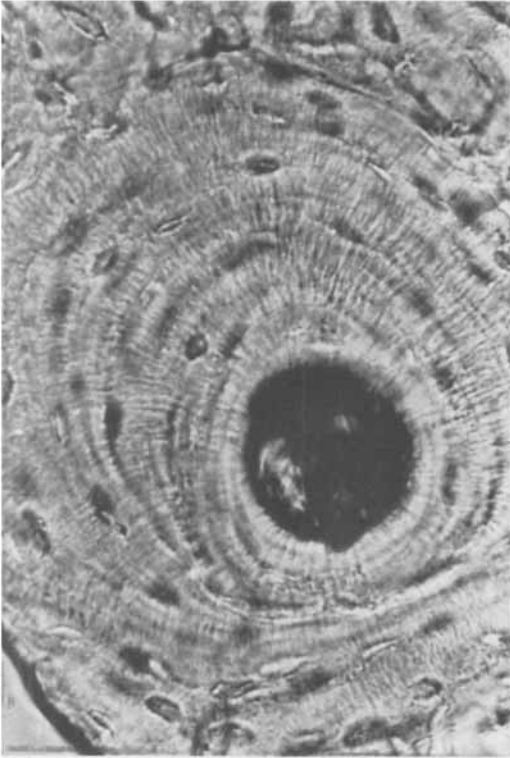
Fig. 5. Microphotograph 4 weeks post-implantation shows reparative mesenchymal cell invasion, in different stages of differentiation into bone cells. Old matrix, newly forming intramembranous and endochondral bone can be seen (Haematoxylin and Eosin ×150).

general acellular, except for the surface lacunae, some of which showed large, plump cells. There was an invasion in the matrix by reparative mesenchymal and some surgical trauma inflammatory cells and a very few giant cells (Figure 4).

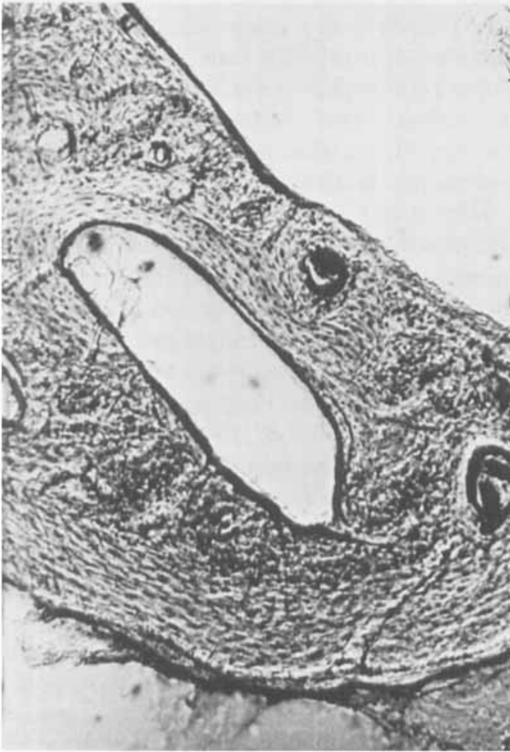
At 8 days post-implantation, most of the vascular spaces had been invaded by reparative mesenchymal cells, with a syncitium or mesh like extracellular substance secreted by these cells and surrounding these cells. Some surgical trauma inflammatory cells were seen, as well as macrophages and fibroblasts in different stages of differentiation to bone cells. A similar cellular reaction was apparent surrounding the implant and invading the cracks and old vascular channels. In the vascular spaces a number of immature vessels made of single layered endothelial cells were also seen. In some sections trabeculae were lined with a one-cell-thick "epithelium like" layer of osteoblasts. At places there were aggregations of marrow cells. Most of the lacunae in the matrix were populated by cells. At 3–4 weeks, most vascular spaces were filled with young connective tissue or reparative mesenchymal cells. The general picture showed plenty of woven bone with islands of old implants in-between. Almost all lacunae were populated by cells (Figure 5). Peri-implant cellular invasion laid down multilayered periosteum as well, the woven bone being replaced by more mature, circumferential lamellar and osteonal patterns of bone. At 12 weeks, the overall picture was of a reasonably mature tubular ulna. The periosteum was multilayered and new bone seemed to be enclosing the vascular grooves on the periosteal side in the form of intra-cortical vascular channels.

Microscopy of undecalcified sections and tetracycline fluorescence. Examination under incident light microscopy of the unstained hand ground sections revealed the general architecture of bone. Circumferential lamellae as well as tubular lamellae, lacunae and microcanaliculi were seen. In general, older, smaller, flatter lacunae occupied the periphery, while younger, larger and ovoid lacunae were at the centre of Haversian systems. The more mature the bone, the smaller and flatter were the lacunae. Microcanalicular anastomosis could be noted extending from the Haversian canal to endosteal and periosteal surfaces (Figures 6, 7).

Under ultraviolet light the hand ground sections showed irregular or woolly areas of intense yellow fluorescence at the sites of immature or woven bone. Lamellar bone revealed sharp, regular lines, bands, rings or ovals of fluorescence (Figure 8). In general, three bands were discernible on the periosteal and, to a lesser extent, the endosteal side. The width of the fluorescent bands varied depending upon the amount of new bone laid down during the 3 days of tetracycline administration. The thickness between the two bands obviously reflected the amount of new bone formed during the 10-day interval. Some vascular spaces showed one, two, and/or three bands or rings in the whole or part of either band, or none at all, demonstrating 'differential biologi-



6



7

Figs. 6 & 7. Microphotograph of unstained, hand-ground sections: Fig. 6 showing an enlarged osteon with ovoid and flattened lacunae, with intercommunicating canaliculi extending to the periphery and to central canal of osteon (×500). Fig. 7 shows the general architecture of a not fully mature tubular bone (×150).

cal activity of bone. In some preparations a number of osteons showed one common outer ring, i.e. one large vascular space at the time of first tetracycline dosage schedule, and within that space many divisions, produced during the second or third doses, which ultimately formed a number of osteons from the one original vascular space (Figure 9).

8

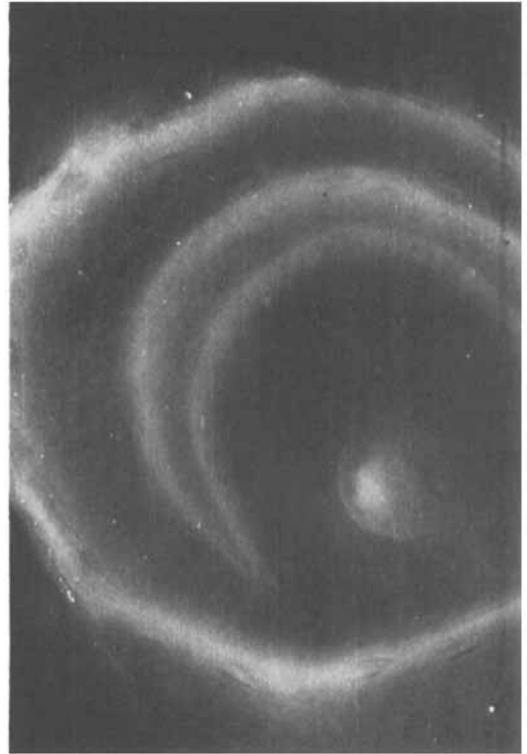


Fig. 8. Same as 6, under ultraviolet light. Shows all the three bands i.e., osteon formed and virtually matured within 1 month of tetracycline schedules. It also demonstrated 'differential biological activity' responsible for the shape osteon and overall bone is likely to take (×500).

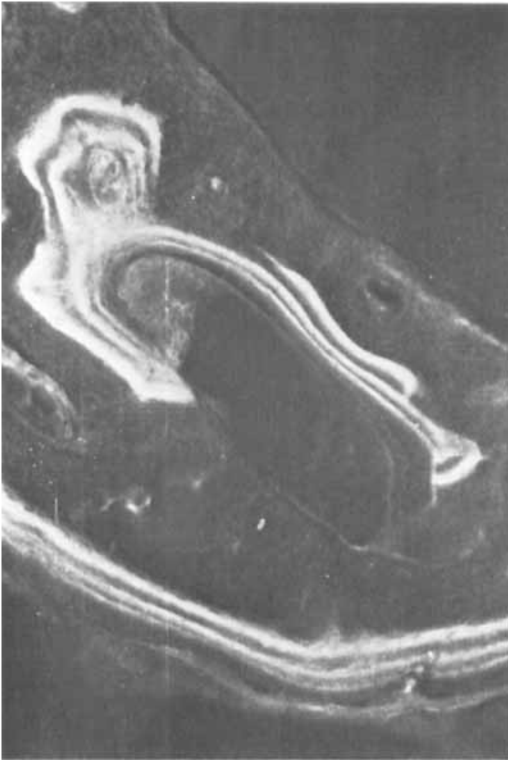


Fig. 9. Same as 7, under ultraviolet light. Shows three fluorescent bands on periosteal side, while minimal activity on endosteal side. Large vascular space beautifully demonstrates 'differential biological activity'. This space and the adjoining osteon were one large space which started dividing subsequently during second and/or third dosage schedule.

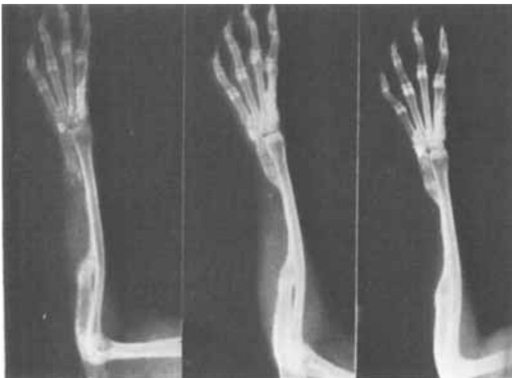


Fig. 10. Sequential skiagrams of a control showing smoothening of the ends of the gap, and their tapering towards ipsilateral radius.

Controls. The experimental models where the osteoperiosteal gaps were not bridged with any material or were bridged with a suitably sized spongostan (hemostatic gelatin sponge), served as controls for the present work. Eight such animals with spongostan and 15 with no material in the gap were followed up for a period of 12–16 weeks. None of these revealed any bone formation. By 12 weeks, the margins of the gap became smooth and tapered towards the ipsilateral radius (Figure 10). Macroscopic examination on sacrifice revealed a gap filled with scanty fibrous tissue with no attempt at bone formation.

DISCUSSION

Implants of allogenic decalcified bone-matrix have been reported to form bone under experimental conditions (Brooks et al. 1963, Boyne 1973, Chalmers et al. 1975, Gray & Speak 1979, Gupta & Tuli 1980, Koskinen et al. 1972, Nade & Burwell 1973, Oikarinen & Korhonen 1979, Tuli & Singh 1978, Tuli & Chaudhuri 1979, Urist 1965, 1976, Urist et al. 1970, 1975, Young 1964). Some clinical trials with fully decalcified and surface decalcified bone matrix have also shown encouraging results (Senn 1889, Sharrard & Collins 1961, Urist 1968) in sterile osteomyelitic cavities, posterior fusion of spine, and fracture healing.

Urist et al. (1967) postulated the bone inductive principle (BIP) produced by the interaction of extracellular substance secreted by reparative mesenchymal cells and the surfaces of decalcified bone matrix. Recently bone morphogenetic protein has been solubilized from sarcoma cells as well as demineralised bone matrix (Urist et al. 1979, Hanamura et al. 1980, Takaoka et al. 1980) and the active principle has been reported to be a complex hydrophobic glycoprotein (Urist et al. 1979).

Conflicting reports regarding the bone forming efficacy of allogenic bone-matrix may be traced to the chemical, physical and biological treatment of the bone-matrix before implantation. It is well established that decalcification using EDTA, nitric acid, and treatment with aminoacridine markedly reduces the bone forming potential of the

matrix (Urist 1965; Van de Putte & Urist 1968, Urist et al. 1968, Nade & Burwell 1977). As observed in the present work and the reports of others, 0.6 M HCl decalcified allogenic matrix provides the best internal milieu for the conversion of reparative mesenchymal cells into bone forming cells (Chalmers et al. 1975, Urist 1976, Nade & Burwell 1977, Tuli & Singh 1978, Tuli & Chaudhuri 1979). Inert porous implants made of acrylic polymer (Winter 1971) autoclaved bone (Tuli et al. 1977, Sijbrandij 1978), pyrolytic graphite composite (Rhinelander 1977) and carbonised wood (Colville 1980) placed under the physiological conditions of functional stress and strain have shown some degree of mineralization in the invading reparative mesenchymal cells. Tuli (1978) reported that *in vitro* incubation of the fresh bone matrix explants could adsorb up to 30–45 percent of the calcium in native bone by physico-chemical processes alone.

Allogenic bone grafts are well known to carry antigenicity when used as transplants. Freezing, storage, and processing of such material reportedly only reduces the immune response (Burwell 1969, Brooks et al. 1963, Hurley et al. 1960, Ray & Holloway 1957, Musculo et al. 1976, Elves 1976). 0.6 M HCl treated allografts in our series showed no appreciable local or systemic clinical evidence of immunogenicity, as also reported by other workers. In successful cases, the osteogenic turnover seemed to replace the original implant. Failure could still be presumed to be due to immunogenicity (Salama et al. 1973).

Radiological observations in our study showed an initial increase in density in 30.5% of the implants. More frequent radiological examination might have increased this number. Initial increased radiological density could be explained by "reverse creeping substitution" – as has been reported with autologous cancellous bone grafts (Enneking et al. 1975) where appositional bone formation is followed by resorption rather than resorption followed by accretion, as might be expected in the conventional process of creeping substitution. The matrix around the eroded chambers may also be recalcified as a result of adsorption of calcium (Tuli 1978) or diffusion of calcium ions from immature newly formed blood vessels (Linden 1975); though Firschein & Urist (1972) stated that recalcification of eroded matrix is brought about by osteoblasts and therefore recalcification is a consequence of and not a casual factor in bone induction.

Histological studies revealed new bone formation as early as the eighth post-implantation day which could be explained as appositional bone again suggesting 'reverse creeping substitution'. The surgical trauma inflammatory cells had disappeared by 3 weeks. The initial number of osteoclasts was relatively much less as compared to fibroblasts, the later could be seen in various stages of differentiation to bone forming cells from reparative mesenchymal cells. Later histological studies revealed firm incorporation and remodelling of new bone formed.

Tetracycline fluorescence in the new bone formed was much brighter than autofluorescence, diffuse fluorescence, and/or artifactual fluorescence (Treharne & Brighton 1979, Gupta & Tuli 1980). The immature or woven bone showed irregular or woolly fluorescence as compared to sharp bands seen in lamellar bone. The 'uptake' of fluorescence depended on the biological state of bone. Hence most of the areas showed 'differential biological activity' and this varied from area to area and unit to unit. The width of the fluorescent bands depended upon the amount of new bone laid down (during tetracycline dosage schedules) and this accounted for the shape osteon and overall bone was going to take. Many researchers have reported the inhibitory effect of tetracyclines on osteogenesis (Frost 1969, Treharne & Brighton 1979), the very investigative parameter for which it is being used. However, this effect has been shown to be dose dependent (Harris et al. 1968, Kruger 1975, Norton et al. 1968, Yen & Shaw 1972). In the dosage schedule used by us it did not seem to inhibit 'neoosteogenesis' (Gupta & Tuli 1980, Gupta et al. 1982). Some osteons showed all the three rings with very narrow central canal, showing that this osteon had formed and virtually matured during 1 month, the period of three dosages.

ACKNOWLEDGEMENTS

The authors are grateful to the Govt. of J & K (India) for grant of research fellowship to the first author. Also

to Prof. T. P. Srivastava, Dept. of Orthopaedics, I.M.S., B.H.U., Varanasi for providing all the facilities to carry out this work, Shri K. N. Singh and his team of the surgical experimental laboratory, Messers R. K. Srivastava & H. S. Phathak for histopathological work and Messers O. P. Gupta & G. C. Saxena for photography.

REFERENCES

Boyne, P. J. (1973) Induction of bone repair by various bone grafting materials. Hard Tissue Growth, Repair and Remineralization. Ciba Foundation Symposium. **11**, pp. 121–141, *Assoc. Scientific Publ.*, Amsterdam.

Brooks, D. B., Heiple, K. G., Herndon, C. H. & Powell, A. E. (1963) Immunological factors in homogenous bone transplantation. *J. Bone Jt. Surg.* **45**-A, 1617–1626.

Burwell, R. G. (1969) The fate of bone grafts. Recent advances in orthopaedics, A. G. Apley, VL 1 (ed.), pp. 115–207. J & A Churchill Ltd., London.

Chalmers, J., Gray, D. H. & Rush, J. (1975) Observations on the induction of bone in soft tissues. *J. Bone Jt. Surg.* **57**-B, 36–45.

Colville, J. (1980) Carbonised wood. An experimental bone implant. *J. Bone Joint Surg.* **62**-B, 259.

Elves, M. W. (1976) Newer knowledge of the immunology of bone and cartilage. *Clin. Orthop.* **120**, 232–259.

Enneking, W. F., Burchardt, H., Puhl, J. J. & Piotrowski, G. (1975) Physical and biological aspects of repair in dog cortical-bone transplants. *J. Bone Joint Surg.* **57**-A, 237–252.

Firschein, H. E. & Urist, M. R. (1972) Enzyme induction, accumulation of collagen and calcification in implants of bone matrix. *Clin. Orthop.* **84**, 263–275.

Friedlander, G. E., Strong, D. M. & Sell, K. W. (1976) Studies on antigenecity of bone. I. Freeze-dried and deep-frozen bone allografts in rabbits. *J. Bone Joint Surg.* **58**-A, 854–858.

Frost, H. M. (1966) Bone dynamics in metabolic bone disease. *J. Bone Joint Surg.* **48**-A, 1192–1203.

Frost, H. M. (1969) Tetracycline-based histological analysis of bone remodelling. *Calc. Tiss. Res.* **3**, 211.

Gray, D. H. & Speak, K. S. (1979) The control of bone induction in soft tissues. *Clin. Orthop.* **143**, 245–250.

Gupta, D. & Tuli, S. M. (1980) Tetracycline as vital stain in the study of bone biodynamics. *Ind. J. Orthop.*, **14**, 161–169.

Gupta, Devinder, Khanna, S. & Tuli, S. M. (1982) Bridging large bone defects with a xenograft composited with autologous bone marrow. An experimental study. Accepted for publication in Int. Orthop. (SICOT), Vol. 6.

Hanamura, J., Higuchi, Y., Nakagawa, M., Iwata, H., Nogami, H. & Urist, M. R. (1980) Solubilized bone morphogenetic protein (BMP) from mouse osteosarcoma and rat demineralized bone matrix. *Clin. Orthop.* **148**, 281–290.

Harris, W. H., Lavorgna, J., Hamblen, D. L. & Haywood, E. A. (1968) The inhibition of ossification in vivo. *Clin. Orthop.* **61**, 52–60.

Hurley, L. A., Zeier, F. G. & Stinchfield, F. (1960) Anorganic bone grafting. *Am. J. Surg.* **100**, 12–21.

Inclan, A. (1942) The use of preserved bone in orthopaedic surgery. *J. Bone Joint Surg.* **24**, 81.

Koskinen, W. V. S., Ryoppy, S. A. & Lindholm, T. S. (1972) Osteoinduction and osteogenesis in implants of allogenic bone matrix. *Clin. Orthop.* **87**, 116–131.

Kruger, B. J. (1975) Dose dependent ultrastructural changes induced by tetracycline in developing dental tissues of the rat. *J. Dent. Res.* **54**, 822.

Linden, G. J. (1975) Bone induction in implants of decalcified bone and dentin. *J. Anat.* **119**, 359–367.

Musculo, D. L., Kawai, S. & Ray, R. D. (1976) Cellular and humoral immune response analysis of bone allografted rats. *J. Bone Joint Surg.* **58**-A, 826–832.

Nade, S. & Burwell, R. G. (1977) Decalcified bone as a substrate for osteogenesis. *J. Bone Joint Surg.* **59**-B, 189–196.

Norton, L. A., Proffit, W. R. & Bennett, I. C. (1968) Effects of tetracycline on bone growth in organ culture. *Growth* **32**, 113.

Oikarinen, J. & Korhonen, L. K. (1979) The bone inductive capacity of various bone transplanting materials used for treatment of experimental bone defects. *Clin. Orthop.* **40**, 208–215.

Parrish, F. F. (1966) Treatment of bone tumours by total excision and replacement with massive autologous and homologous grafts. *J. Bone Joint Surg.* **48**-A, 968–990.

Ray, R. D. & Holloway, J. A. (1957) Bone implants preliminary report on experimental study. *J. Bone Joint Surg.* **39**-A, 1110–1128.

Rhinelander, F. W. (1977) A flexible composite as a coating for metallic implants-microvascular and histological studies. *Int. Orthop.* (SICOT) **I**, 77–86.

Salama, R., Burwell, R. G. & Dickson, I. R. (1973) Recombined grafts of bone and marrow. *J. Bone Joint Surg.* **55**-B, 402–417.

Senn, N. (1889) On the healing of aseptic bone cavities by implantation of antiseptic decalcified bone. *Am. J. Med. Sciences* **98**, 219–243.

Sijbrandij, S. (1978) Resection and reconstruction for bone tumours. *Acta Orthop. Scand.* **49**, 249–254.

Takaoka, K., Ono, K., Amitani, K., Kishmoto, R. & Nakata, Y. Y. (1980) Solubilization and concentration of a boneinducing substance from murine osteosarcoma. *Clin. Orthop.* **148**, 274–280.

Treharne, R. W. & Brighton, C. T. (1979) The use and possible misuse of tetracycline as a vital stain. *Clin. Orthop.* **140**, 240–246.

Tuli, S. M. (1972) Bridging of bone defects by massive bone grafts in tumorous conditions and osteomyelitis. *Clin. Orthop.* **87**, 60–73.

Tuli, S. M. & Singh, A. D. (1978) Osteoinductive property of decalcified bone matrix. An experimental study. *J. Bone Joint Surg.* **60**-B, 116–123.

Tuli, S. M. (1978) In vitro calcification of human and rabbit bone matrix by physical and chemical methods. *Ind. J. Med. Res.* **68**, 164–168.

Tuli, S. M., Chopra, S. C. & Chakravorty, B. (1977) Behaviour of autoclaved massive autologous bone grafts-an experimental study. *Ind. J. Orthop.* **11**, 1–19, 1977.

Tuli, S. M. & Chaudhuri, R. H. (1979) Effect of preimplantation treatment on the bone-forming potential of decalcified allogeneic and xenogeneic bone-matrix implants. *Arch. Orthop. Traumat. Surg.* **94**, 167–173.

Urist, M. R. (1965) Bone formation by autoinduction. *Science* **150**, 893–899.

Urist, M. R. (1976) Practical applications of bone research on bone grafts physiology. In instructional course lectures, the American Academy of Orthopaedics Surgery. Vol. XXV. St. Louis, C. V. Mosby Co. 1–26.

Urist, M. R., Silverman, B. F., Buring, K., Dubuc, F. L. & Rosenberg, J. M. (1967) The bone induction principle. *Clin. Orthop.* **53**, 243–283.

Urist, M. R., Jurist, J. M., Jr., Dubuc, F. L. & Strates, B. S. (1970) Quantitation of new bone formation in intramuscular implants of bone-matrix in rabbits. *Clin. Orthop.* **68**, 279–293.

Urist, M. R., Mijulski, A. & Boyd, S. D. (1975) A chemosterilized antigen-extracted autodigested alloimplant for bone banks. *Arch. Surg.* **110**, 416–428.

Urist, M. R., Mikulski, A. & Lietze, A. (1979) Solubilized and insolubilized bone morphogenetic protein. *Proc. Nat. Acad. Sci.* **76**, 1828–1832.

Urist, M. R. (1968) Surface- decalcified allogenic bone (SDAB) implants. *Clin. Orthop.* **56**, 37–50.

Urist, M. R., Lowell, T. A., Hay, P. H. & Strates, B. S. (1968) Inductive substrate for bone formation. *Clin. Orthop.* **59**, 59–96.

Van de Putte, K. A. & Urist, M. R. (1966) Osteogenesis in the interior of intramuscular implants of decalcified bone matrix. *Clin. Orthop.* **43**, 257–270.

Wilson, P. D. & Lance, E. M. (1965) Surgical reconstruction of skeleton following segmental resection for bone tumours. *J. Bone Joint Surg.* **47**-A, 1629–1656.

Winter, G. D. (1971) Heterotopic bone induced by synthetic sponge implants. *J. Bone Joint Surg.* **53**-B, 555.

Yen, P. K. J. & Shaw, J. H. (1972) Preliminary study of the inhibitory effects of tetracyclines on membraneous bone growth in rhesus monkeys. *J. Dent. Res.* **51**, 1651–1657.

Young, M. H. (1964) The repair of experimental defects in rabbit skulls observation after implantation with decalcified, deproteinised, and deep frozen homogenous whole bone. *J. Bone Joint Surg.* **46**-B, 329–335.

Correspondence to: Prof. S. M. Tuli, Department of Orthopaedics, Institute of Medical Sciences, Banaras Hindu University, Varanasi – 221005, India.