Second-Set Reaction Favouring Incorporation of Bone Allografts

The rejection of tissues and organ allografts is known to occur more rapidly in a second-set than in a first-set reaction[1]. Moreover, in orthotopic cortical bone grafts without rigid internal fixation a high rate of surface resorption was reported[2]. The pattern of bone healing seems markedly influenced by relative motion of the bone graft[3]. Therefore, the incorporation of cortical bone grafts was studied with rigid internal fixation under second-set conditions.

Fourteen home-bred mountain sheep were used; the observation time was 16 weeks. Donor and host were chosen for incompatibility with respect to antigens present on lymhocytes (Ly^+, Ly^-), to blood groups (R/–, r/r)[4], and to sex (male donors and female hosts)[5]. 3 to 4 weeks before transplantation, the recipient was presensitized[6] by an i.v. injection of $6-8\times10^9$ donor lymphocytes suspended in S 199 tissue culture medium.

Humoral antibodies were measured by cytotoxicity tests with trypan blue[7]. The rejection time of a Thiersch skin graft on the inside of the ear was determined to demonstrate cell-mediated immunity.

The graft consisted of a segment 1 cm in length with plane parallel surfaces and was taken from the middle of the metatarsal diaphysis of the donor using a two-blade saw. A corresponding segment was removed from the metatarsus of the host using the same saw. The defect was immediately replaced by the fresh allogeneic graft from which the periosteum had been stripped while the bone marrow remained untouched. Two DCP gauge plates[8] were used for rigid fixation of the graft by longitudinal compression. These gauge plates served to monitor changes in the initially applied longitudinal compression and thus to determine minimal amounts of bone surface resorption in areas of contact between host and allogeneic graft. To minimize functional load at the transplantation site an ankle arthrodesis in rectangular position was achieved by means of 2 screws.

The total amount of Haversian remodelling in the graft itself, in areas of contact and in the adjacent bone was quantitated by microradiography[9]. Fresh, undecalcified as well as methacrylate embedded histological sections were used.

On X-rays the osteotomy lines disappeared between the 6th and the 10th week. The radiological examination at weekly intervals did not reveal callus formation. Macroscopical observation at 16 weeks showed the fresh allogeneic graft to be united with the host corticalis (Figure 1). The plates were lined with a thin sheet of new bone especially at both ends, in the screw holes and on the part of the load cell facing the cortex. In microradiographies large quantities of new bone due to Haversian remodelling were observed (Figure 2).

Both the histological results and the pressure recordings showed no evidence of surface resorption in the contact areas between host cortex and graft. The initial compression of 73.3 kp ($\pm$ 4.8 SE) decreased gradually and very slowly reaching 29.2 kp ($\pm$ 4.5 SE) after 16 weeks (kp = kilogramme force = 9.81 Newton).

The cytotoxicity test indicated a low titer of antibodies in the host serum after sensitization with donor lymphocytes and the subsequent allogeneic bone grafting. Cell-mediated immunity (third-set response) was demonstrated by the shortened mean survival time of 6.9 days $\pm$ 2.13 SE of the Thiersch skin graft in the recipients as compared to 9.0 days $\pm$ 1.84 SE in non-sensitized control sheep ($P < 0.01$).

Our experiments have shown that under second-set conditions a cortical bone graft was found to be incorporated without bone surface resorption. The graft was incorporated by means of an intense remodelling of the Haversian system. Thus, in the graft itself a simultaneous process of bone resorption and bone deposition occurred, preserving at all times the shape and mechanical function of the grafted bone.

In order to obtain these results two important experimental conditions must be fulfilled: one is rigid fixation with interfragmentary compression and the other is grafting under condition of a second-set reaction. Rigid fixation of orthotopic cortical bone grafts has two effects: the first is graft stabilization preventing any relative motion between the contact surfaces. This is a prerequisite for direct invasion of the graft by Haversian system of the host. The second is earlier load transmission through the compressed cortical bone of donor and recipient. The latter is reduced to some extent by participation of the plate in stress transmission[10].

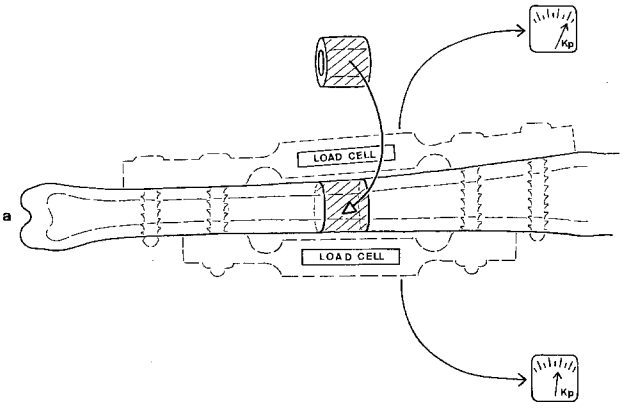
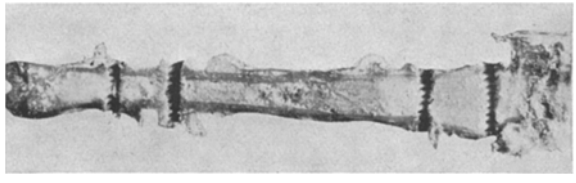


Fig. 1. a) Rigid fixation of the allogeneic cortical graft by two self-compressing plates (*DCP*'s). The load cells incorporated in the plates permit to monitor continuously the mechanical forces acting on the graft.



b) Microscopical appearance of the bone with the graft after sacrifice. The host had been presensitized by donor lymphocytes.

[1] G. D. Snell, A. Rev. Microbiol. *11*, 439 (1957).
[2] W. F. Enneking, J. Bone Jt. Surg. *39-A*, 597 (1957).
[3] H. B. Boyd, J. Bone Jt. Surg. *23-A*, 497 (1941).
[4] B. A. Rasmussen, Ann. N. Y. Acad. Sci. *97*, 306 (1962).
[5] E. J. Eichwald and G. R. Silmser, Transpl. Bull. *3*, 142 (1965).
[6] P. B. Medawar, J. Anat. *79*, 157 (1945).
[7] P. A. Gorer and P. O'Gorman, Transpl. Bull. *3*, 142 (1956).
[8] S. M. Perren, A. Huggler, M. Russenberger, F. Straumann, M. E. Mueller and M. Allgoewer, Acta orthopl. scand. Suppl. *125*, 7 (1969).
[9] K. V. Holmstrand, Acta orthop. scand. Suppl. *26* (1957).
[10] P. Matter, in preparation.

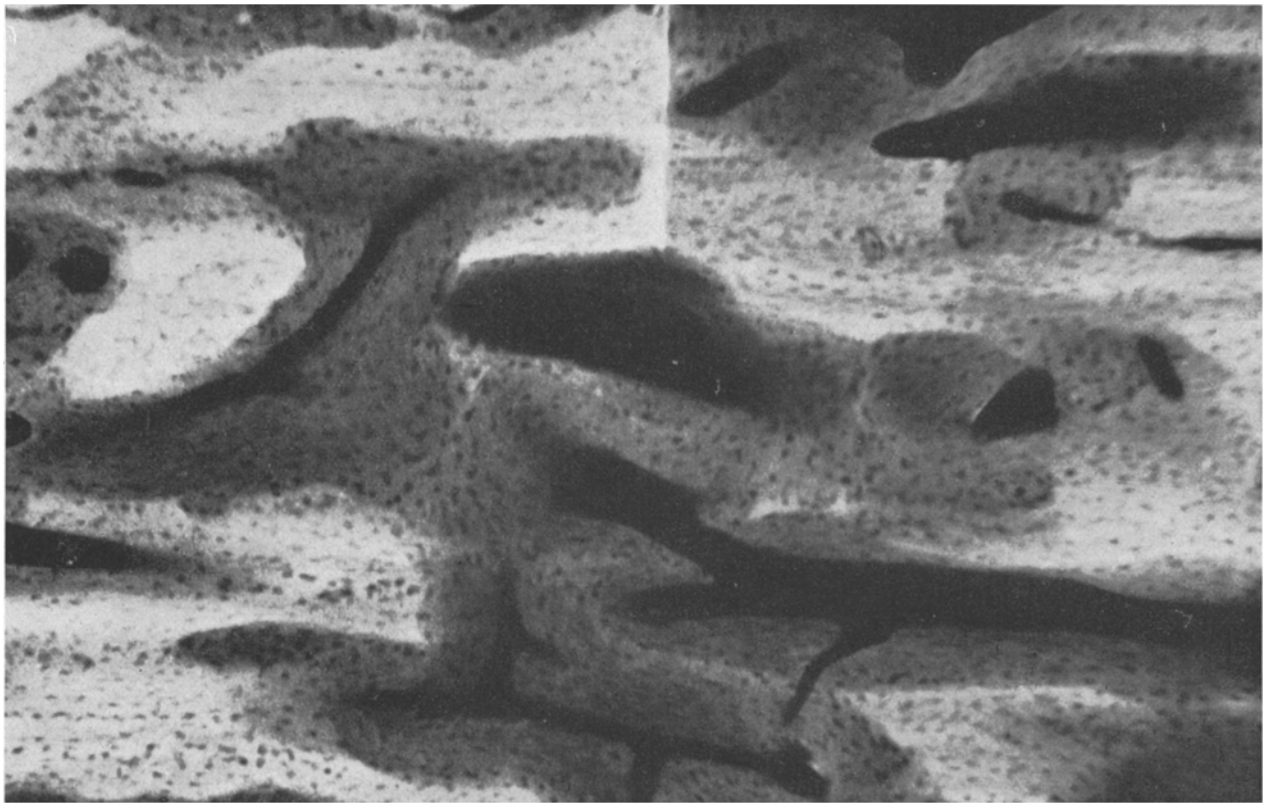


Fig. 2. Microradiographic appearance: The right side shows the graft and the left the donor corticalis. The osteotomy line can be seen in the upper third of the picture. The white areas indicate old bone and the grey areas new bone formation.

We wish to consider now the significance of the artificially induced second-set reaction for the higher remodelling rate in the transplanted bone graft. An earlier bone formation[11] and an intensified inflammatory response after presensitization with bone[2] have been described previously. In our experiments the allogeneic cortical graft with bone marrow was implanted into an animal which had been stimulated to produce cytotoxic antibodies. The higher titer of cytotoxic antibodies after implantation shows that a secondary immunological response is present. The observation of host cells such as lymphocytes, plasma cells, eosinophiles and histocytes in the graft has been explained as an earlier secondary immunological response[2,11,12]. It has been assumed that rapid destruction of the donor cells is obtained, the empty Haversian canals[13] being refilled by newly formed vessels. The latter process is bound to a mechanically stable supporting apatite structure.

The net effect of the mechanical stabilization seems to be the direct invasion of the graft by Haversian system of the host appearing as 'primary graft healing' similar to that of the primary bone healing[14]. The net effect of the second-set response is most likely a faster destruction of the bone allograft which on our experiment is paralleled by a simultaneous rapid bone deposition with host's own tissue. The fast rejection of the Thiersch grafts seems to exclude an alternative immunological explanation based on the enhancement phenomenon.

'Primary graft healing' in cortical bone under the condition of a second-set reaction has not been observed before. Our unexpected findings are most likely due in part to the application of the rigid fixation and in part to the more rapid destruction of the allograft and its rapid replacement by host's own elements. The second-set phenomenon in a cortical bone allograft contrasts with that of most other tissues because it leads to a 'beneficial' sequence of events. This fact could be of potential clinical value in bone and joint transplantation.

Zusammenfassung. Das durch Osteosynthese absolut stabilisierte allogene Kompakta-Transplantat zeigt bei dem durch Spenderlymphozyten sensibilisierten Empfänger wider Erwarten einen Knochenumbau, der wesentlich grösser ist als beim Ersttransplantat und dem eines Autotransplantates praktisch gleichkommt.

P. HUTZSCHENREUTER, M. ALLGÖWER, J. F. BOREL and S. M. PERREN

Swiss Research Institute, CH-7270 Davos (Switzerland),
19 July 1972.

[11] J. CHALMERS, J. Bone Jt. Surg. *41-B*, 160 (1959).
[12] R. G. BURWELL, G. GOWLAND and F. DEXTER, J. Bone Jt. Surg. *45-B*, 597 (1963).
[13] A. W. HAM, J. Bone Jt. Surg. *34-A*, 701 (1952).
[14] R. SCHENK und H. WILLENEGGER, Arch. klin. Chir. *19*, 593 (1963).