HEALING OF NON-VASCULARISED DIAPHYSEAL BONE TRANSPLANTS

AN EXPERIMENTAL STUDY

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Four different experiments were performed to study the healing of a large, non-vascularised, diaphyseal, bone segment in adult cats. In the first experiment, a 4 cm segment of tibia with its periosteum was excised and replaced in its bed. The other experiments were similar, except that in the second, the periosteum of the segment was removed, in the third its medullary canal was blocked with a Silastic rod, and in the last group the segment was isolated from its muscle bed by a Silastic sheet. The reparative processes were quantified by estimating the resorption index, the cortical new bone formation index, the callus encasement index, and the osteocyte count. Bone resorption and apposition occurred in the segment even when the periosteum was absent or the medullary canal was blocked, with osseous union at both ends by eight to 12 weeks, provided the segment was not isolated from its muscle bed. Thus, the muscle bed played a significant role in these reparative processes.

A large diaphyseal, segmental bone defect is not an uncommon problem in orthopaedic practice and several surgical methods are available for bridging such defects. Though free vascularised bone grafts have been advocated recently (Weiland, Daniel and Riley 1977), the older methods of bridging these gaps with autogenous non-vascularised bone grafts are still successful (Enneking, Eady and Burchardt 1980). There is, however, little experimental evidence on the healing processes of such a large non-vascularised cortical bone segment (Phemister 1914; Enneking et al 1975). We report a study of the healing of large avascular segments of the diaphysis in cats.

MATERIALS AND METHODS

Adult cats whose epiphyses were seen radiographically to be closed, were anaesthetised with intraperitoneal pentobarbitone. Four different experiments were conducted, using 28 cats in each.

In the first experiment, the control group, a large segment of the tibial diaphysis 4 cm long (two-thirds of the diaphysis) was removed using an oscillating saw (Fig. 1a). The devascularised segment was replaced immediately into its original bed and the soft tissues were closed with 3-0 chromic catgut sutures. The limb was then immobilised in an above-knee plaster cast with the knee in 90° of flexion and the foot plantigrade. In the other three experiments, an identical procedure was performed except that in the second experiment, the periosteum was removed from the segment (Fig. 1b); in the third, the medullary canal of the segment was occluded (Fig. 1c) with a tight-fitting Silastic rod; whilst in the last group, the segment was isolated from its muscle bed by a tube of Silastic sheet (Fig. 1d).

The healing processes were studied at 1, 2, 4, 6, 8, 12 and 16 weeks using four cats for each period of observation. In all experiments, radiographs were taken immediately after operation and when the animal was killed; at that time the mobility of the proximal and distal fracture sites was also studied. The union at both ends of the diaphyseal segment was assessed clinically, radiologically and histologically. Longitudinal sections of decalcified specimens were cut 10 μm thick and stained with haematoxylin and eosin for histological examination. Mid-sagittal sections of the tibia of each group were taken to quantify healing.

Bone resorption, new bone formation and 'callus encasement' of the segment were quantified using the following indices. The resorption index was defined as the total area of all resorption cavities present in both
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Fig. 1

The four experiments. (a) First experiment: a large segment of the tibial diaphysis has been removed with its periosteum intact, and is being replaced in its original muscle bed. (b) Second experiment: the periosteum (P) has been completely removed; the segment, now devoid of its periosteum is likewise replaced in its bed. (c) Third experiment: the medullary canal of the segment has been occluded by a tight-fitting Silastic rod; the inset shows complete occlusion of the medullary cavity. (d) Fourth experiment: an identical segment has been isolated from the surrounding muscle bed by a tube of Silastic sheet; the tube was formed by wrapping the sheet around the segment and the adjacent bone ends and stitching the overlapping edges of the sheet with 4-0 nylon sutures.

Histological section of control segment at eight weeks magnified 12 times, showing clearly resorption cavities in both cortices (cortical margins outlined) and also the area of callus formation around the segment (margins also outlined). The callus was not confined to region of fracture sites but surrounded the whole segment (callus encasement).

cortices expressed as a percentage of the total area of both cortices in that segment. A 12 times magnified, photographic print of the histological section was made, and the central 2 cm portion of the segment (24 cm on the magnified print) was measured (Fig. 2). The areas of the cortices and the resorption cavities were mapped out on graph paper whose smallest square was 1 mm² and the areas were counted. The areas measured were accurate to the nearest square millimetre.

The cortical new bone formation index was defined as the total area of new bone formed within the cortex of the diaphyseal segment expressed as a percentage of the total area of cortex. New bone formation was studied microscopically at a magnification of 60. Ten microscope fields, five in each cortex, were chosen at regular intervals within the central 2 cm portion of the segment and used for measurement. Photographs were taken and, from each print, the total area of new bone present was
measured graphically as a percentage of the total area of that field (Fig. 3). The average of 10 values measured was taken as the cortical new bone formation index for that specimen.

In many specimens, callus was not confined to the fracture sites, but it was frequently seen encasing the whole diaphyseal segment. The callus encasement index measured the area of callus present around the central 2 cm portion of the diaphyseal segment expressed as a percentage of the total area of both cortices, using the same graphical method measured on the same 12 times magnified print used for calculating the resorption index (see Fig. 2).

The osteocytes of the diaphyseal segment were also counted and the percentage of lacunae occupied by osteocytes was taken as an indicator of the viability of a bone segment. The counting was performed under the microscope at a magnification of 200. Ten microscope fields, five in each cortex, at regular intervals within the central 2 cm portion of the segment were selected for counting. The total number of lacunae occupied by osteocytes was expressed as a percentage of the total number of lacunae present in that field. The average of 10 counts was taken as the osteocyte count for that segment.

F test was used to evaluate the difference between the variance of two groups and if a significant difference was found, a t approximation formula (Chase 1985) was used. The p value was then obtained.

RESULTS

In the control group, fracture union (bridging by bone) occurred by about eight weeks (Fig. 4a). However, in the groups where the periosteum was removed or the medullary canal blocked, healing was slightly delayed and occurred only by 12 weeks (Figs 4b and 4c). In contrast, in the group where the segment was isolated from its muscle bed, fracture union did not occur even at 16 weeks (Fig. 4d).

Figure 5 shows the resorption index for the four

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**Fig. 3**

Histological section of control segment at 12 weeks (x 60). The field shows appositional new bone formation (NB) taking place on the surface of resorption cavities (RC).

**Fig. 4**

The healing process. (a) Naked-eye view of histological section of a specimen in the control group at eight weeks, showing union by bony callus. (b) Similar view of a specimen in the group where the periosteum was removed at 16 weeks, with osseous union at both fracture sites. (c) Naked-eye view of a specimen in the group where the medullary canal was blocked; at 12 weeks there is bony callus at both fracture sites with small residual areas of cartilage. (d) Similar view of a specimen in the group where the segment was isolated from its muscle bed; even at 16 weeks no fracture callus was seen bridging the proximal fracture gap on the left. The distal fracture site was bridged by fibrovascular tissue with areas of cartilage. A thin layer of fibrovascular tissue (F) can be seen around the segment appearing to be continuous with the fibrovascular tissue of the distal callus.
groups. In the control group, resorption reached its peak (13%) at 12 weeks and dropped to 6.5% at 16 weeks. In the groups where the periosteum was removed or the medulla was blocked, no statistical difference was observed compared with the controls. In contrast, when the muscle bed was isolated by a Silastic sheet, resorption activity was markedly impaired (about 2% even at 12 weeks) and this was statistically significant.

The cortical new bone formation index in the first three groups gradually increased with time up to 16 weeks (Fig. 6), but no statistical difference was observed. In contrast, in the fourth group where the muscle bed was isolated, cortical new bone formation was markedly impaired even at 16 weeks and this was statistically significant.

Callus encasement of the diaphyseal segment occurred from the second week and there was no statistical difference in the first three groups. However, in the last group no callus encasement of the segment was seen (Fig. 7).

Figure 8 shows the osteocyte counts for all four experimental groups. The counts dropped to about 15% at four weeks in all groups. It then remained at this level in the first three groups, whereas in the fourth group, where the
muscle bed was isolated, it continued to drop further to about 7% and this difference was statistically significant.

**DISCUSSION**

In this study, quantities were estimated on standardised longitudinal sections taken from the mid-sagittal plane of each specimen. By standardising the sections, valid comparisons could be made between all four groups. The whole of each section including the bone segment and the fractures at both ends were studied.

In many studies comparing non-vascularised with vascularised grafts, union of the fractures in non-vascularised grafts was found to be a major problem (Haw, O'Brien and Kurata 1978; Bell et al 1985). However, in our study, bony union of a large non-vascularised segment occurred by eight weeks. Even when the periosteum of the segment was absent or the medullary canal was blocked, bony union still occurred, though it was slightly delayed, provided the segment was not isolated from its surrounding muscle bed. In marked contrast, no fracture healing occurred when the segment was isolated from its muscle bed. This shows that the muscle bed made a significant contribution to the fracture healing process. An important feature on examination of the entire segment was 'callus encasement' of the segment as a part of the healing process (Dell, Burchardt and Glowczewskie 1985).

Histology provided a vital method of assessing bone viability. Indeed, the demonstration of repair activities occurring in the bone segments signified that dead bone was undergoing healing and becoming viable. However, the traditional method of assessing bone viability by osteocyte count alone remains controversial (Catto 1965; Kenzora et al 1978). Therefore great caution must be exercised when assessing bone viability by osteocyte count alone.

Our studies showed that when the periosteum of the segment was removed or the medullary canal of the segment was blocked, healing of the bone segment still occurred as shown quantitatively by the resorption index, the cortical bone formation index and the callus encasement index. There was no significant difference in the healing processes in these two groups compared with the control segments. In contrast, if the segment was isolated from its muscle bed, all three reparative processes were impaired. Therefore, the muscle bed played a significant role in the healing of such large, diaphyseal bone segments.

Brookes et al 1961, showed that the flow of blood in the cortex is centrifugal. In our experiments, revascularisation of the segments was studied using microangiography and this work will be presented in a separate paper.

**Conclusions.** In the cat's tibia, fracture healing of a large avascular segment of diaphysis occurred without much difficulty. The segment healed by reparative processes of bone resorption, new bone formation and 'callus encasement' of the segment. This occurred even when the periosteum was removed from the segment or the medullary canal was blocked, provided the segment was not isolated from its surrounding muscle bed. Therefore, the muscle bed played a significant role in the healing of such a large diaphyseal segment.

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**REFERENCES**


