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TRANSPLANTATION OF PEDICLE BONE GRAFTS TO FRESH SKELETAL DEFECTS AND DEFECT PSEUDARTHROSES

An Experimental Study

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In a previous paper Baadsgaard & Medgyesi (1965) reported on the survival of pedicled cancellous and cortical bone grafts. With the technique used, pedicled cancellous grafts were found to survive the detachment from the donor site. In the pedicled cortical grafts most of the osteocytes disappeared, while the blood supply, and thereby the osteogenetic effect, was preserved. The literature on the subject was reviewed.

Later Medgyesi (1965) studied the chances that pedicled cancellous bone grafts had of healing in a necrotic recipient site. He demonstrated that some revascularization and invasion of new-formed bone may occur by way of the pedicled bone graft. Furthermore, Medgyesi (1968), investigating the function of the muscle pedicle, found that the most detrimental factor to the blood supply of a bone graft through a muscle pedicle is torsion of the pedicle, while flexion and tension affect the nutrition of the graft less.

The object of the present study was to investigate the ability of pedicled cortical bone grafts to heal a total defect in a long bone.

MATERIAL AND METHOD

The experiments were performed on 43 rabbits of mixed stock, mature or nearly mature, in the age range 6 to 12 months.

The rabbits were anaesthetized by intravenous injection of Nembutal. The

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operative sites were shaved and disinfected with iodine. Penicillin was administered prophylactically, both locally and intramuscularly, total dose 300,000 units.

(1) 16 rabbits were used for transplantation to a fresh ulnar defect. A 0.75 cm long total defect was sawn into both ulnae, 3.5 cm distally to the tip of the olecranon process. The calcified interosseous membrane was preserved intact, so that both ulnar fragments were stable in relation to the radius. From the dorsal aspect of the proximal end of the ulna a bone graft, approx. 20 × 4 × 2 mm, was sawn out, throughout the thickness of the corticalis. On the right side, a muscle pedicle, consisting mainly of the m. flexor carpi ulnaris, was preserved. The muscle pedicle covered more than half the periosteal surface. It was dissected to a length so that by simple flexion of the pedicle the graft could cover the ulnar defect, the proximal end of the graft against the distal fragment. The graft was fixed on the side of the defect facing away from the radius as an on-lay graft by two silk ligatures applied through burr holes in the ulna. This fixation was entirely sufficient. On the left side the operation was carried out in the same way as on the right, but with a free bone graft.

(2) 27 rabbits were used for transplantation to defect pseudarthroses on the ulna. It has previously been demonstrated (Baadsgaard 1969a) that in rabbits, defect pseudarthroses may be produced on the ulna and that these defects do not heal spontaneously. By the same technique a 0.75 cm long defect pseudarthrosis was produced on the ulna 3.5 cm distally to the tip of the olecranon process. At re-operation 6–10 weeks later, the osteosynthesis material was removed and grafting was done, as described under (1), on the right using a pedicled bone graft and on the left using a free bone graft. A thin plate of polyethylene was inserted between the radius and ulna to prevent invasion of callus from the radius. On both fragments the grafts just reached healthy bone.

The rabbits tolerated the operations well. Two cases of infection occurred and two of stress fracture of the radius with displacement. These cases were excluded.

The specimens were assessed grossly by dissection and X-ray examination. After decalcification, the specimens listed in Tables 1 and 3 were embedded in paraffin and cut longitudinally, the graft as well as the proximal and distal recipient site being included in the section. The specimens were stained with haematoxylin-eosin and with van Gieson-Hansen's connective-tissue staining.

5 rabbits (Table 2) were labelled with tetracycline, 50 mg i.m., at one week's interval, receiving reverin (pyrrolidine methyl tetracycline) and terramycin (oxytetracycline). The specimens were embedded in methyl metacrylate, sawn transversely, and ground to a thickness of 80 μ. Several sections containing the graft alone taken on a level with the defect as well as sections containing graft and proximal or distal recipient site were studied by fluorescence microscopy.

11 rabbits (Table 4) received ^{45}Ca, 200 microcuries i.v., and tetracycline, 100 mg i.m., two days before being killed. Undecalcified ground sections were prepared as described above. The ground sections were studied by fluorescence microscopy, microradiography, and quantitative determination of the ^{45}Ca uptake as described in a previous paper (Baadsgaard 1969b). In some cases there was a difference in the thickness of the ground sections on the right and left. Correction was done on the basis of a self-absorption curve (Figure 1). This curve was plotted on the basis of 60 measuring results from 12 different sections, further ground after each measurement.

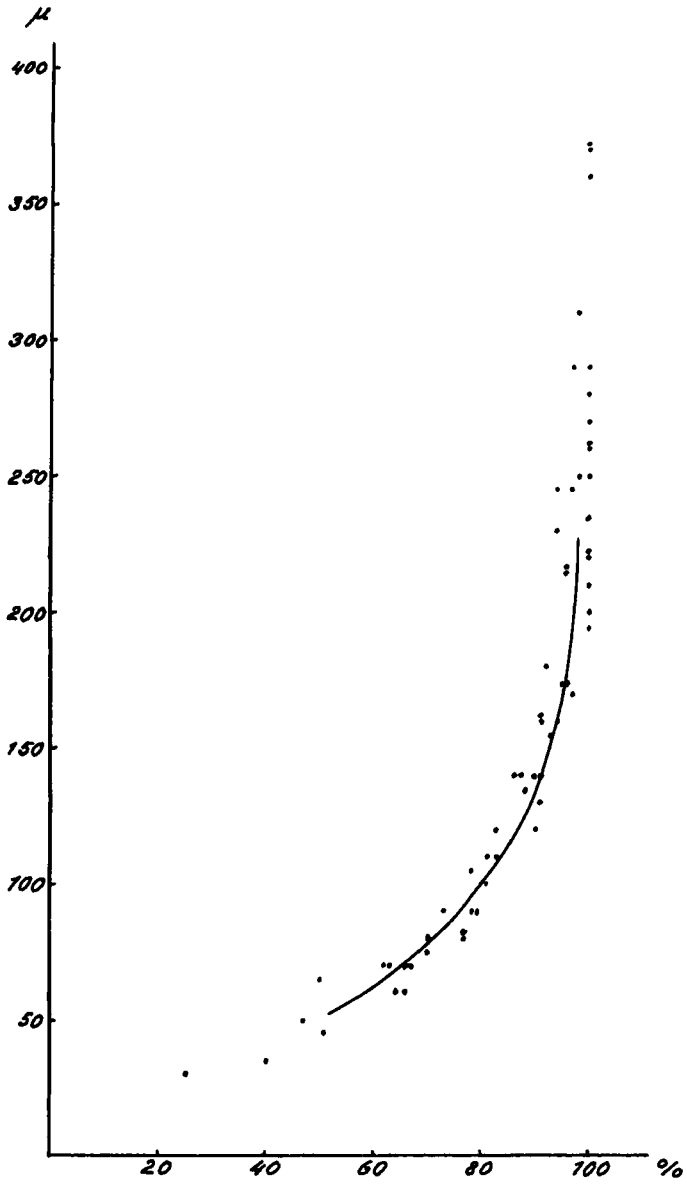

Figure 1. Self-absorption curve for ^{45}Ca-labelled bony tissue embedded in methyl metacrylate. The curve approaches asymptotically the lines $\times = 100$ per cent and $x = ky$.

Table 1. Transplantation to a fresh defect in the ulna. Results of histological examination.

Age of graft in days	Healing				Callus formation				Vascularization of graft	
	proximally		distally		periosteally		endosteally			
	P	F	P	F	P	F	P	F	P	F
16	+	—	+	—	+++	++	+	—	+++	++
18	+	+	+	+	+++	+++	(+)	—	++	++
20	+	—	+	—	++	+	+++	—	+++	++
22	+	+	+	+	++	+	++	+	+++	+++
24	+	+	+	+	+++	(+)	+++	—	+++	+++
25	+	+	+	+	++	++	+++	++	+++	+++
29	+	+	+	+	++	+	+++	++	+++	+++
32	+	+	+	+	++	+	++	+	+++	+++
35	+	+	+	—	+++	—	+++	—	+++	++
40	+	+	+	+	++	+	+++	+	+++	+++
41	+	+	+	—	++	+	+++	+	+++	+++

P: Pedicled bone graft. F: Free bone graft.

+, ++, and +++ indicate callus formation in a layer corresponding to ⅓, ½, and the entire thickness of the graft and vascularization corresponding to ⅓, ½, and the entire graft.

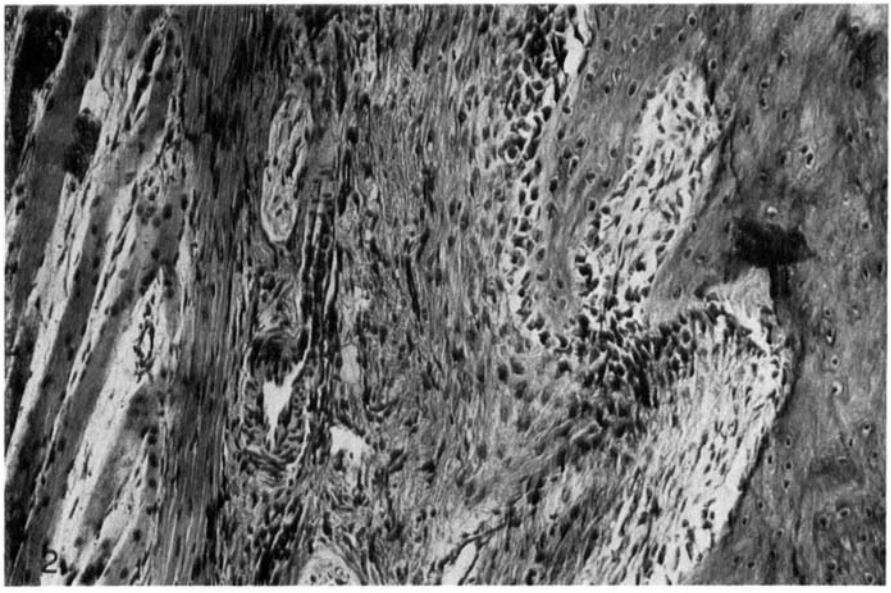

Figure 2. 3rd week. Pedicled bone graft to a fresh defect. Muscle pedicle and subperiosteal bone formation.

RESULTS

Transplantation to Fresh Ulnar Defect (Tables 1 and 2)

Dissection and X-ray examination showed all pedicled grafts to have healed at both ends, while the free grafts appeared to heal somewhat later and somewhat less constantly.

Histological examination showed the muscle pedicle to consist of normal-looking striated muscle with normal vessels. At the attachment of the muscle to the graft the collagenous fibrils continued from the pedicle into the hypertrophic periosteum, and the periosteal callus was oriented in the same direction as the muscle fibres.

Periosteal hypertrophy and periosteal callus formation were most marked deep to the attachment of the muscle pedicle, and almost consistently there was more periosteal callus on the pedicled than on the free grafts. Occasionally, there was actually an exostosis at the attachment of the pedicle. An even more striking difference was found in respect to endosteal callus which started earlier and was more marked on the pedicled grafts. Callus formation appeared to start at the site of the open medullary cavity on the cut surface, spreading thence and growing towards the defect. Older specimens often showed formation

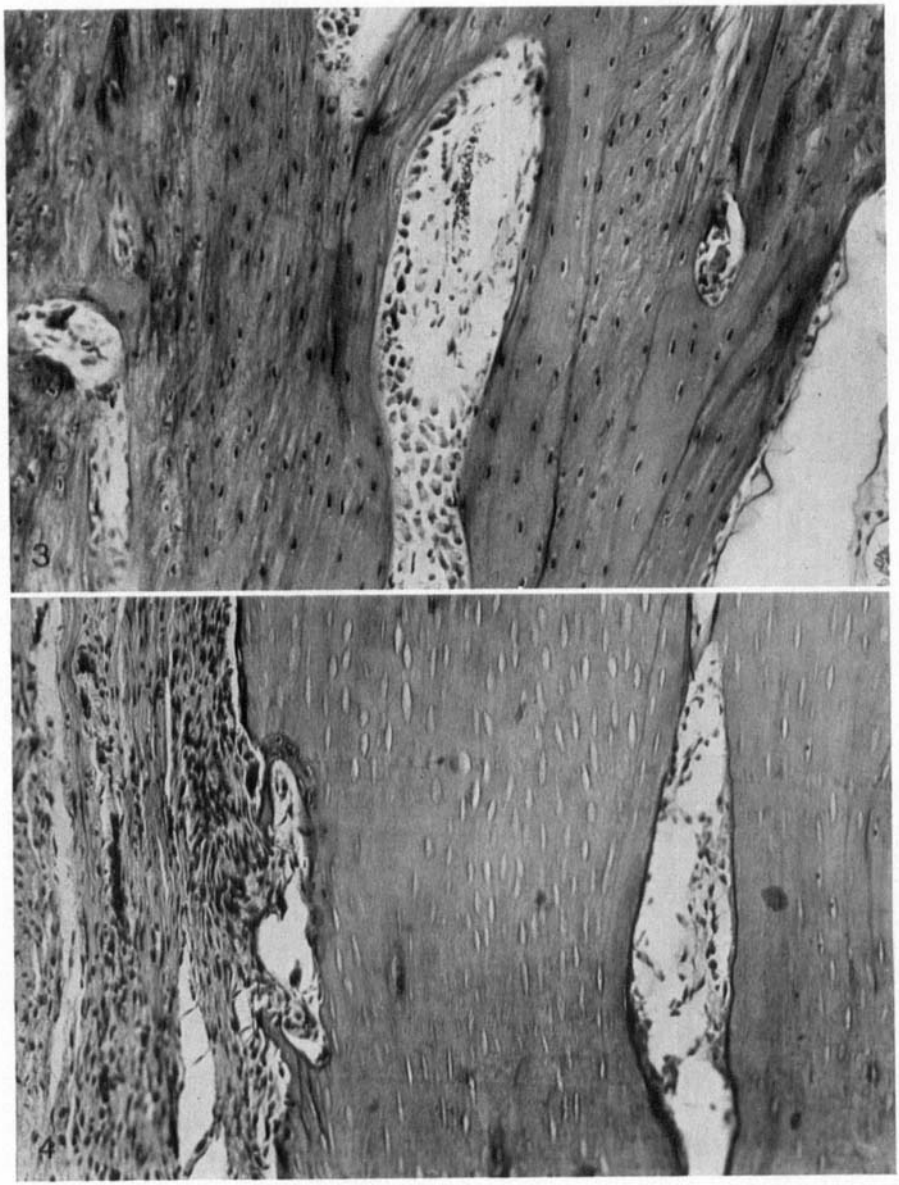

Figure 3. Same specimen as in Figure 2. Vascularized cavity lined with osteoblasts. Surviving osteocytes within the graft.

Figure 4. Free bone graft from the same rabbit as shown in Figures 2 and 3. Only incipient periosteal bone formation. Lacunae empty.

Table 2. Transplantation to a fresh defect in the ulna. Results of double-labelling with tetracycline.

Age of graft in days	Administration of tetracycline on day	Number of labellings	
		P	F
16	R 9 + R 14	1	0
22	R 7 + T 14	2	1
28	T 6 + R 13 + T 20	3	2
36	T 9 + R 14 + T 20 + R 27	4	3
43	R 7 + T 16 + R 21 + T 28	3	2

R: Reverin. T: Terramycin.

of a medullary cavity in the endosteal callus, the graft assuming the nature of a long bone.

The pedicled grafts contained major or minor strands of cortical bone with normal-looking osteocytes, especially towards the periosteal surface. In the free grafts, on the other hand, all the osteocytes disappeared. Vascularization and creeping substitution, it would appear, start somewhat earlier in pedicled than in free grafts.

Tetracycline was administered to 5 rabbits of this group, and the undecalcified ground sections were studied by fluorescence microscopy. The results are shown in Table 2.

Administration of tetracycline on the 6th–9th day labelled 3 out of 5 pedicled grafts, but none of the free grafts. After the second administration, on the 13th–16th day, all pedicled and 3 of the free grafts were labelled. It may be deduced, then, that the pedicled grafts have contained functioning vessels, with incipient creeping substitution from about one week after the transplantation, while corresponding processes did not start in the free grafts until about 2 weeks after the transplantation.

Transplantation to Ulnar Pseudarthroses (Tables 3 and 4)

In principle, the grafts undergo the same changes that were found after transplantation to fresh defects. However, certain quantitative changes are caused by the fibrous capsule of the pseudarthrosis and the poor blood supply in the region.

Periosteal callus formation was slight, and it was only during the first 2 weeks that pedicled grafts had the lead. Endosteal callus was well-marked in the pedicled grafts from the 4th week, and throughout the experimental period it was clearly more advanced in the pedicled

Table 3. Transplantation to defect pseudarthroses in the ulna. Results of histological examination.

Age of graft in days	Healing				Callus formation				Vascularization of graft	
	proximally		distally		periosteally		endosteally			
	P	F	P	F	P	F	P	F	P	F
7	—	—	—	—	+	—	+	—	+++	(+)
7	—	—	—	—	++	+	+	—	++	—
8	—	—	—	—	+	—	+	—	+++	+
10	—	—	—	—	+	+	+	+	++	+
12	+	+	+	+	+	+	++	(+)	+++	+
13	—	—	—	—	++	(+)	+	(+)	+++	+
18	+	+	+	+	+	+	++	++	+++	+
23	+	+	+	+	+	+	+	+	+++	++
32	+	—	+	—	+	+	+++	+	+++	++
34	+	+	+	—	+	+	+++	+	+++	++
40	+	+	+	+	+	+	+++	+++	+++	+++
41	—	—	+	—	++	++	++	++	+++	+++
42	+	+	+	+	+	+	+++	+	+++	+++
57	+	—	+	—	++	+	+++	+	+++	+++
57	+	+	+	+	+	+	++	++	+++	+++
62	+	+	+	—	+	+	—	—	+++	+++

Table 4. Transplantation to defect pseudarthroses in the ulna. Results of quantitative study using ^{45}Ca.

Age graft in days	Callus formation periosteally		Callus formation endosteally		Vascularization of graft		Measurement of ^{45}Ca. c/min.	
	P	F	P	F	P	F	P	F
4	—	—	—	—	—	—	22	24
5	+	(+)	—	—	+	+	164	76
7	(+)	(+)	—	—	—	—	3	9
8	+	(+)	+	(+)	+++	+	307	112
20	++	+	++	—	+++	+	280	78
28	++	+	+	+	+++	++	363	172
40	+	+	+++	+++	+++	+++	156	153
57	+	+	+++	++	+++	+++	138	102
69	+	+	++	++	+++	+++	21	39
84	+	+	+++	++	+++	+++	161	100
96	+	+	++	++	+++	+++	61	61

than in the corresponding free grafts. However, the difference was most distinct in respect to vascularization, the pedicled grafts showing almost complete vascularization as early as one week after the transplantation, while this was not attained by the free grafts until after the 6th week.

11 of the rabbits in the pseudarthrosis group were given ^{45}Ca. The mean activity in 3 cross-sections of the graft was measured. Callus formation and vascularization in the same sections were assessed by fluorescence microscopy and autoradiography. The results are listed in Table 4. On the whole, they correspond to the results of investigation by traditional histological technique (Table 3).

DISCUSSION

As is apparent from Tables 1–4, the pedicled grafts showed a distinct advantage over the free grafts in respect to vascularization and callus formation during the first 6 weeks, but thereafter the difference decreased. From these findings it may presumably be deduced that the pedicled bone grafts also possess a more reliable healing capacity. However, this cannot be proved, as the experimental rabbits were killed, after varying periods, from 1–14 weeks after the transplantation, and there were but a few rabbits in each group and only 9 after

the 6th week. However, there appears to be a tendency to earlier healing of pedicled bone grafts.

Baadsgaard & Medgyesi (1965) have previously used a muscle pedicle 1 cm in length. At that time, it was pointed out that the blood supply to cortical bone is rather sparse, and indeed it was observed that most osteocytes gradually disappeared from the pedicled cortical grafts. Nevertheless, it could be concluded that the muscle pedicle was able to preserve an intact vascular net in the periosteum and bone. In the present study the muscle pedicle was invariably at least 2 cm in length and flexed 180°. Zucmann (1961) demonstrated that a muscle detached from the surrounding tissues will very soon become revascularized and Medgyesi (1968) reported that flexion of the muscle pedicle has little influence upon the blood supply to the graft. And yet, it must be assumed that the longer the muscle pedicle the poorer the blood supply, at least temporarily, especially when the base of the pedicle is distally as in the present experiments. Indeed, the difference between pedicled and free grafts was somewhat less marked in the present experiments than in the previous ones.

In homotransplantation to animals pre-sensitized to the donor, Chalmers (1959) demonstrated that callus formation in and around the bone grafts will issue partly from surviving cells in the grafts and partly from the recipient's surrounding soft tissues by induction. This observation was later confirmed by Goldhaber (1961) in experiments using bone grafts wrapped in millipore, by Ray & Sabet (1963) using isotransplantation of bone treated with tritium-labelled thymidine, and by Arora & Laskin (1964) in a sex chromatin study of isografts. Thus, it may be considered an established fact that in free, autologous bone grafting the graft contains surviving cells, but the quantitative ratio between the osteogenetic action by surviving cells and by induction is not known in further detail. Since comparison of pedicled with free bone grafts, where inductive action is presumed to be the same, shows a more favourable effect of the pedicled graft, the explanation must be that larger parts of the pedicled graft survive the transplantation.

SUMMARY

In experiments on rabbits the survival and healing capacity of pedicled and free bone grafts, placed as on-lay grafts on fresh defects and defect pseudarthroses in the ulna, were studied. The specimens were assessed

by X-ray examination, histological examination, fluorescence microscopy, and quantitative determination of the ^{45}Ca uptake.

Vascularization and callus formation proved to start earlier and be more marked in pedicled than in free bone grafts. The difference was most marked during the first 6 weeks.

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