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TRANSPLANTATION OF CALLUS-INVADED KIEL BONE TO DEFECT PSEUDARTHROSES

An Experimental Study

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In a previous study (Baadsgaard 1970) involving the transplantation of cancellous Kiel bone to experimental pseudarthroses on rabbits, bone formation was rarely found in the Kiel graft on a level with the defect pseudarthrosis, whereas fairly brisk ingrowth of newly formed bone was observed where the graft was in satisfactory contact with living bone of the recipient.

This finding has been reported several times before. For instance, Orell (1937), implanting "os purum", which accurately corresponds to a Kiel bone, obtained marked ingrowth of callus into this bone. He called a callus-traversed os purum an "os novum" and used this material for bone grafting on the same indications as fresh autologous bone grafts. Maatz & Bauermeister (1957), studying the Kiel bone by Maatz' spongiosa test, found that the cancellous Kiel grafts were completely traversed by callus just as quickly as were the fresh autologous grafts. The same finding was made by Karges et al. (1953), using another type of processed heterologous bone.

The purpose of the present study was to investigate to what extent cancellous Kiel bone, implanted subperiosteally, will be invaded by callus and to ascertain the properties of the os novum thus produced when grafted on defect pseudarthroses.

METHOD

The experimental animals were 21 rabbits of mixed race, full-grown or almost full-grown, aged 6–12 months. All the rabbits were subjected to two operations.

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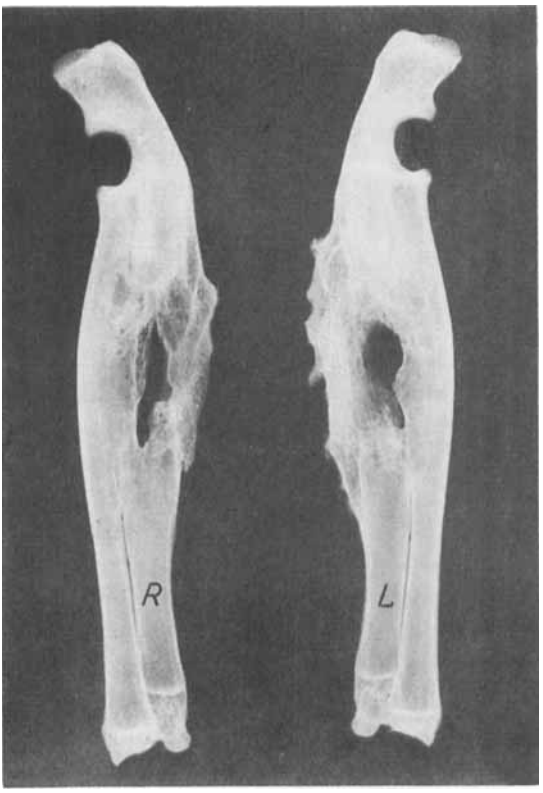

Figure 1. 6th week. X-ray of the specimens. L: os novum. R: iliac bone graft. Both grafts show solid union.

At the first operation a defect pseudarthrosis, 0.75 cm in length, was produced on both ulnae 3.5 cm distal to the tip of the olecranon process by a technique already described (Baadsgaard 1970). Moreover, a graft of cancellous Kiel bone, $4 \times 0.4 \times 0.3$ cm, was implanted subperiosteally on the left femur.

The second operation was performed 6–11 weeks later. The osteosynthesis material was removed from the pseudarthrosis on the left ulna. The Kiel bone was chiselled off the femur. A biopsy specimen was removed for histological examination, and the remaining 2.5 cm were placed as an on-lay graft on the side of the pseudarthrosis facing away from the radius, so that it just reached healthy bone at both ends. A thin polyethylene plate was inserted between the radius and ulna, so that ingrowth of callus from the radius was prevented. On the right, a control experiment was performed, using a graft 2.5×0.3 cm from the ala ossis ilii, full thickness of bone (Figure 1).

Infection occurred in 3 cases on the right. The autologous grafts from these cases were not included in the material which thus comprises 21 os novum grafts and 18 autologous cancellous grafts.

Two days before the rabbits were killed they were injected intravenously with ^{45}Ca 200 microcuries and intramuscularly with 100 mg tetracycline. The specimens were divided transversely. The proximal half was used for conventional histological

examination and the distal half for undecalcified ground sections, embedded in methyl metacrylate and sawn transversely. Three cross sections of the graft on a level with the defect and one from a more distal site, containing graft as well as the distal recipient site, were investigated. The sections were ground down to 80 μ and studied by autoradiography, microradiography, and fluorescence microscopy. Quantitative determination of the ^{45}Ca uptake was done as described previously (Baadsgaard 1970 a, b).

RESULTS

Biopsy from Os Novum Removed Prior to Grafting

Infection did not occur in any case around the implanted Kiel bone which was always found to have become fixed to the femur. In many instances there was bone growth around the graft so that it had to be chiselled or sawn off the femur. New bone formation was seen in exceptional cases on the superficial surface. A few grafts fractured during the removal, but the majority in a way so that it was possible to obtain a piece of sufficient length to cover the pseudarthrosis. On two pseudarthroses, however, the grafting had to be done with a fissured graft of os novum. At the histological examination of the novum biopsies all the specimens were found to be fully vascularized and invaded by connective tissue. Six implants had been completely traversed by callus, whereas in one case there was no ingrowth at all (Table 1). The implants had been *in situ* for periods between 6 and 11 weeks. There was no correlation between their age and the degree of callus invasion. Callus formation invariably appeared to issue from the femur, invading the Kiel bone by creeping apposition. The new bone was found both as a layer directly on the trabeculae and in the cavities within the cancellous Kiel bone (Figures 2 and 3).

Table 1. Ingrowth of callus into the implants on the femur.

	Callus invasion per cent	Average age days
10 implants	75–100	59
6 implants	0–20	60
Total average	60	58

Areas with new bone formation, incidentally, contained ample bone marrow, but the remaining part of the biopsy had been traversed

Figure 2. 9th week. Biopsy from the Kiel bone implant. Ample ingrowth of callus into the central part. × 56.

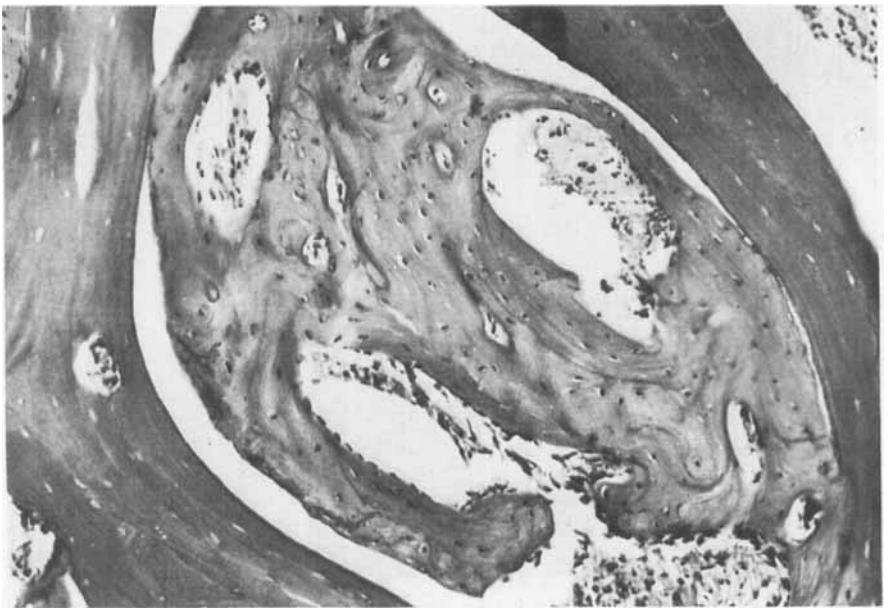

Figure 3. 9th week. Detail from same preparation as in Figure 2. × 140.

by well-vascularized connective tissue. Numerous osteoclasts and Howship lacunae were present. There were no histological signs of immunological processes.

Investigation of Grafts

The haematoma in the pseudarthrosis cavities will be displaced and absorbed in the course of the first two weeks after the operation by loose, well-vascularized connective tissue which fills the cavities, envelops the grafts, and ties them to the recipient sites. On the 8th day the first vessels are seen superficially in the iliac bone grafts as well as in the novum grafts, and from the 17th day all the grafts are fully vascularized. Both types of graft seem to undergo necrosis. The cell nuclei grow pyknotic, break down, and the cell limits become obliterated. In the novum grafts the included, necrotic callus is seen, sharply delimited from the live callus formed later (Figure 4). In the undecalcified ground sections fluorescence microscopy shows no uptake of tetracycline by the necrotic callus, whereas the newly formed one is highly fluorescent. In the same way, autoradiography shows but

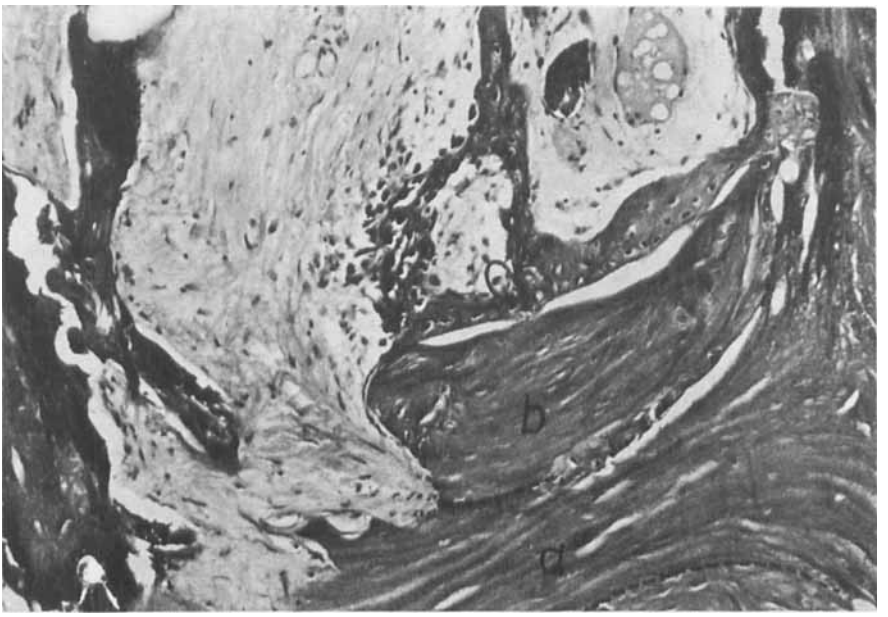

Figure 4. 5 weeks after transplantation. Central part of the os novum graft with a: Kiel bone, b: necrotic transplanted callus, c: live, new-formed callus. × 140.

Table 2. Results of quantitative determination of ^{45}Ca uptake by the grafts. N: os novum. C: iliac crest. +, ++, and +++ indicate callus formation in one-third, one-half, and the entire graft.

Age of grafts in days	Callus invasion into implant per cent	Callus formation in the grafts		Measurement of ^{45}Ca c/min	
		N	C	N	C
8	70	—	(+)	67	40
14	20	—	...	32	...
14	0	—	+	29	80
17	50	+++	++	282	135
19	75	+++	+++	262	137
21	70	+++	+++	244	242
22	75	+++	+++	256	227
26	10	+	+	72	47
28	50	+++	++	242	80
29	100	+++	...	180	...
31	10	+	+++	69	192
35	100	+++	+++	...	...
42	20	++	(+)	176	39
51	60	++	++	117	112
62	100	++	++	104	65
66	100	+++	+++	262	214
73	100	+++	+++	267	249
79	10	(+)	...	43	...
90	75	+++	+++	133	202
97	75	+	+	53	72
114	100	+++	+++	167	150

faint diffuse uptake of ^{45}Ca by the necrotic callus, corresponding in intensity only to the uptake by the Kiel bone itself and the necrotic parts of the iliac bone graft. From the 17th day there is ample callus peripherally on the novum graft, but callus is not seen in its interior until the end of the 3rd week. In the iliac bone graft the first traces of callus are found on the 8th day, arising from medullary tissue on the cut surface of the graft. In the youngest preparations, incidentally, callus formation appears to issue to the same extent from medullary tissue and from periosteum. On the 17th day incipient callus formation is found centrally in the iliac bone graft. The further course is very similar for the os novum and the iliac bone grafts. Union to the recipient sites occurs almost constantly on both sides from the 3rd week. Gradually, the grafts undergo a pronounced remodelling, and

after the 6th week there is a tendency for the formation of a medullary cavity, the grafts thus assuming the shape of long bones. At the same time, considerable osteoclastic resorption of the necrotic elements takes place, so that the original iliac bone graft can hardly be recognized. Resorption of the Kiel graft is somewhat slower, but in the oldest specimens it is difficult to discern the iliac bone from the novum graft.

The results of the quantitative determination of ^{45}Ca are shown in Table 2. The mean for the os novum grafts is 153 counts/min as compared with 134 counts/min for the autologous cancellous grafts. Four os novum grafts and two iliac bone grafts with no or negligible callus gave mean counts of 43 c/min and 40 c/min.

The quantity of callus included in the graft appears to determine the effect of the os novum grafts. Thus, the result for the six grafts in which the callus content of the Kiel graft was less than 20 per cent was 70 c/min, whereas the mean for grafts with a 50–100 per cent callus content was 188 c/min.

DISCUSSION

The explanation why in some cases the Kiel bone grafts on the femur showed a disappointingly slight invasion of callus is presumably to be found in the operative technique. For mechanical reasons the grafts had to be made rather large as compared with the dimensions of the femur, so that the periosteum had to be stretched tightly over them. This may have reduced bone formation from the periosteum. Furthermore, the grafts were not fixed to the femur. A greater and more constant ingrowth might presumably have been obtained by subcortical implantation in the iliac crest. However, this is difficult to practise on rabbits. Likewise, it would have been difficult to obtain pure os novum grafts without admixture of the iliac bone for the grafting experiment.

In assessing the results, it must be taken into consideration that the cross section of the Kiel bone was somewhat larger than that of the iliac bone graft, but in return only 60 per cent of the implant was invaded by callus. It is difficult to measure accurately the area of necrotic callus included in the graft on the undecalcified ground sections from the end of the experiment, since necrotic and live callus are so intimately mixed. Thus, the quantity of transplanted callus could not be determined on the sections used for the quantitative

determination of ^{45}Ca. Nevertheless, it is possible to estimate with reasonable certainty that the active part of the os novum graft was of very much the same size as the iliac bone graft. Thus, the two types of grafting material showed an equal osteogenetic effect.

Hellstadius (1942)), in os novum experiments on rabbits, found numerous surviving osteocytes in the grafts. Urist & McLean (1952) also found surviving cells on autologous grafting of fibrocartilaginous callus to the anterior chamber of rabbit eyes. These findings could not be confirmed in the present experimental series in which all osteocytes disappeared from the grafts. Possibly, osteoblasts and connective-tissue elements survive peripherally in the graft. This cannot be definitely decided in autografting.

SUMMARY AND CONCLUSION

Cancellous Kiel bone was grafted subperiosteally on rabbit femora. When removed 6–11 weeks later the implants showed 60 per cent ingrowth of callus.

The callus-invaded Kiel bone was transplanted as an on-lay graft on the defect pseudarthrosis on one ulna of the same rabbit, whereas the other ulna was grafted with fresh autologous cancellous bone.

The specimens were investigated radiologically, histologically, and by fluorescence microscopy, and quantitative determination of new bone formation was performed by ^{45}Ca.

The two types of grafting material behaved in the same way with respect to revascularization, union, and callus formation. Neither was found to contain surviving osteocytes.

It is concluded that the osteogenetic effect of callus-invaded cancellous Kiel bone and fresh autologous cancellous bone is the same.

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