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KIEL BONE IN THE TREATMENT OF PSEUDARTHROSIS

An Experimental Study

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In 1934 Orell published a comprehensive study on the applicability of macerated bone in surgery. He used bovine bone which he cleaned mechanically and treated with saline solutions, hot potassium hydroxide, and acetone. This material he called "os purum". When he implanted "os purum" subperiosteally, it became invaded by callus and was converted into an "os novum". He obtained good results by using os purum in the treatment of tuberculosis of the bones, cysts, and arthrodeses. For pseudarthroses, however, he preferred using os novum.

For some years, this method gained ground, but thereafter it was practically abandoned, although Goff (1944) submitted a fairly large clinical material successfully treated by os purum.

Incidentally, interest appeared to centre more on bone bank problems until Bauermeister (1958), on the basis of a comprehensive experimental study, laid down the principles for preparing the Kiel bone graft, viz. processing of young calf bone with H_2O_2 and extraction of the lipids by acetone.

He found this material to be equal to autologous bone, and this seems to have been confirmed by a number of experimental and clinical studies (Maatz & Bauermeister 1957, Koch 1957, Bauermeister 1961, Schilling & Nockemann 1962, Fuchs et al. 1963, Hallén 1966).

American workers too have displayed an interest in processed heterologous bone. In their experimental studies Basset & Creighton (1962) and Karges et al. (1963) arrived at the same result as Bauer-

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meister, and favourable results were obtained by Hurley et al. (1960) and Anderson et al. (1963) in large clinical materials.

However, negative reports have appeared also. In a large material of pseudarthroses Hopf (1957) found 44 per cent poor results with Kiel bone; and on the basis of an experimental study using Squibb Surgibone, Heiple et al. (1967) developed a highly negative attitude.

At the SICOT meeting in Paris 1966, Maatz (1967) recommended Kiel bone for the treatment of non-union and delayed union, finding 96 per cent good results, while Witt (1967) advised against using Kiel bone.

Accordingly, there is still disagreement concerning the indication for using processed heterologous bone.

MATERIAL AND METHOD

The experimental animals were 24 rabbits of mixed race, weighing between 2.5 and 4 kg and ranging in age from 6 to 12 months. By a previously described technique (Baadsgaard 1969) defect pseudarthroses were produced on the ulna. The defect measured 3/4 cm and was situated 3.5 cm distally to the tip of the olecranon process.

6–10 weeks later the rabbits were subjected to re-operation, the osteosynthesis material was removed, and grafting was done, on the right with Kiel bone and on the left with autologous cancellous bone. In two cases a Kiel bone graft was used for both sides. In 6 instances cortico-cancellous Kiel bone was used and in the others cancellous. The autologous graft was sawn out of the iliac bone immediately before the transplantation. The grafts, which measured about $3 \times 4 \times 25$ mm, were placed as on-lay grafts on that side of the pseudarthrosis which faced away from the radius (Figure 1) and were fixed to the bone ends with silk. Proximally as well as distally they reached to healthy bone. A polyethylene plate was placed between the radius and ulna, so that ingrowth of callus from the radius into the pseudarthrosis was prevented. In 3 cases infection occurred around the autologous graft. These specimens are not included in the material which thus comprises 26 Kiel bone grafts and 19 autologous controls.

The rabbits were killed at the time intervals stated in Table 1. Two days before they were killed, ^{45}Ca, 200 microcuries, and tetracycline, 100 mg, were injected intramuscularly. The specimen was assessed macroscopically by dissection and X-rays. Thereafter, it was divided transversely, and the proximal half of the graft with the proximal recipient site was used, following decalcification and embedding in paraffin, for conventional histological examination. The specimen was cut longitudinally, so that both the graft and recipient site were included, and stained with haematoxylin-eosin. Three sections from different depths were studied. The remaining part of the specimen was used for undecalcified ground sections, embedded in methyl metacrylate and sawn transversely. A cross-section of the graft on a level with the middle of the defect as well as a cross-section from a more distal site, containing graft as well as distal recipient site, were studied. The sec-

Table 1. Results

Age of graft (weeks)	Union proximally		Union distally		Callus in the graft on a level with the defect		Measurement of ^{45}Ca c/min	
	K	A	K	A	K	A	K	A
2	—	+	—	—	—	+	20	116
3	+	+	+	+	—	+	17	402
4	—	+	—	+	—	+	29	316
4	—	—	—	—	—	+	...	...
5	+	+	+	+	—	+	37	261
6	+	+	+	+	—	+	37	551
6	+	...	+	...	—	...	...	...
7	+	+	+	+	+	+	217	509
8	+	+	—	—	—	+	90	430
8	—	—	—	+	—	+	...	...
10	+	+	—	+	—	+	100	462
10	+	...	—	...	—	...	11	...
	—	...	—	...	—	...	7	...
11	+	+	—	+	+	+	48	98
11	—	—	—	—	—	+	...	...
12	—	...	—	...	—	...	29	...
	+	...	—	...	+	...	128	...
12	—	...	—	...	—	...	...	...
12	+	+	—	+	—	+	24	111
14	+	+	+	+	+	+	105	214
15	+	+	+	+	+	+	79	153
15	+	+	—	+	—	+	40	105
15	+	+	+	+	—	+	...	...
17	+	+	—	—	—	+	...	...
17	—	...	—	...	—	...	...	...
40	—	+	—	+	—	+	...	...
Total	16/26	16/19	8/26	14/19	5/26	19/19		
(%	62	84	31	74	19	100)		

Symbols: K = Kiel bone graft. A = Autologous graft.

tions were manually ground to a size of 100 μ and studied autoradiographically by the contact method, microradiographically, and by fluorescence microscopy. The activity in the sections was measured directly by a Geiger-Müller tube. Positives were made of the microradiographs, all of which were equally enlarged, and the area was measured by planimetry. The result of the activity measurements was corrected for background radiation and for any difference in the area of cross-section between the Kiel bone and the autologous graft.

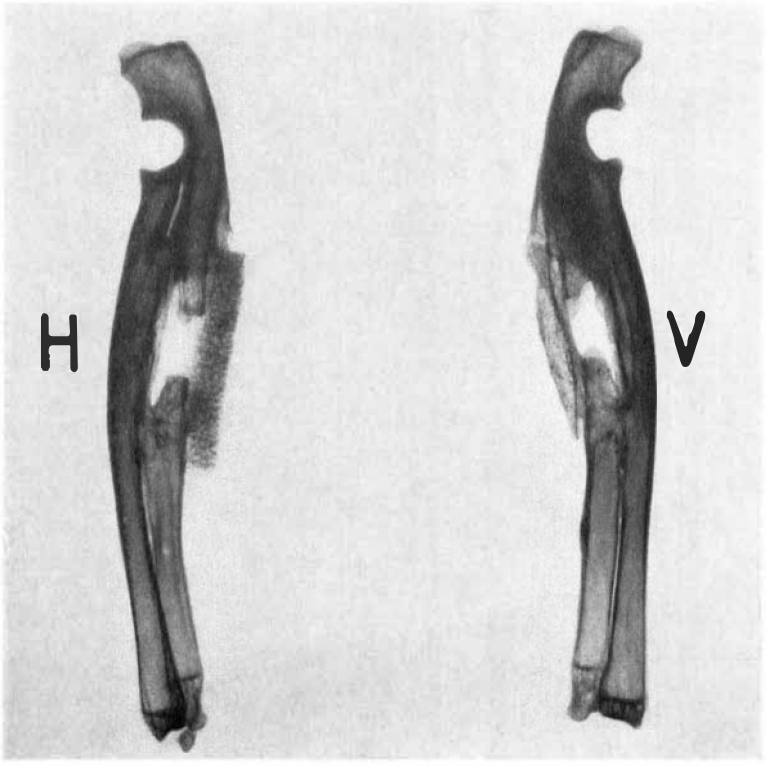

Figure 1. 5th week. Kiel bone graft marked H, autologous graft V.

RESULTS

The necrotic bone ends which delimit the defect in the ulna become revascularized and are gradually replaced by creeping substitution.

The Kiel bone graft is rapidly vascularized from the surrounding soft tissues. Two weeks after the transplantation it has been invaded by granulation tissue, and only small remnants of haematoma are left (Figure 2). Gradually the connective tissue assumes a firmer nature. At a late stage of the course, bone marrow may occur in the Kiel bone, but this marrow is of the active type only in the event of simultaneous regeneration of bone.

The Kiel bone graft gets bound to the recipient site by loose connective tissue. From the ulna osteoid tissue and callus grow towards the graft, reaching it by the third week and invading its profound part, new bone forming directly on the dead trabeculae (Figure 3). Gradually, the bone formation may spread further inwards in the

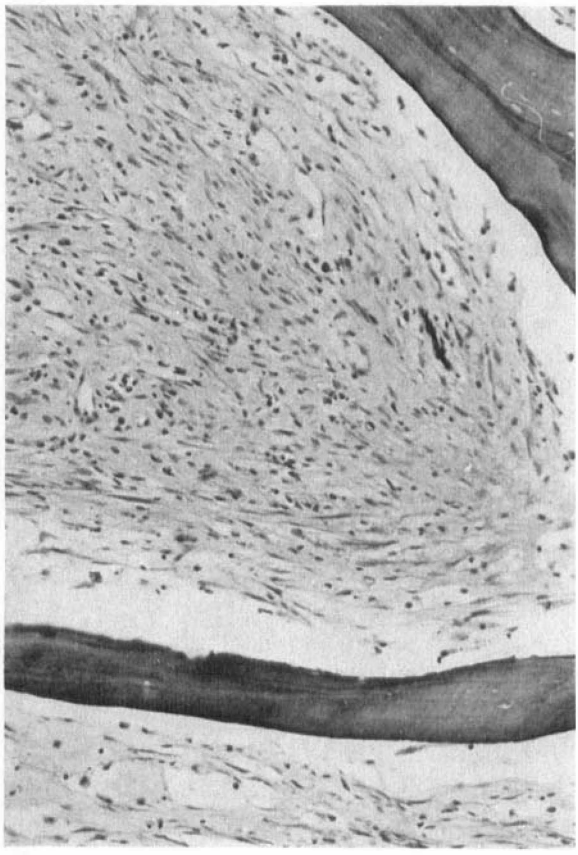

Figure 2. 2nd week. Central part of the Kiel bone graft invaded by amply vascularized connective tissue.

graft, but it seldom reaches its superficial part, and the callus very seldom traverses the Kiel bone graft on a level with the defect (Figures 4–5).

Throughout the course numerous osteoclasts are present, and clearly an increasing resorption is taking place (Figure 6). Thus, in the 40-week specimen the Kiel bone graft has been completely absorbed (Figure 7). The histological preparations did not show signs of immunological reaction.

The results for the 6 cortico-cancellous grafts do not differ from those for the cancellous Kiel bone grafts.

By comparison, the autologous grafts showed a more rapid and more reliable union. In all cases there was new formation of bone in the graft on a level with the defect, and substitution was far more

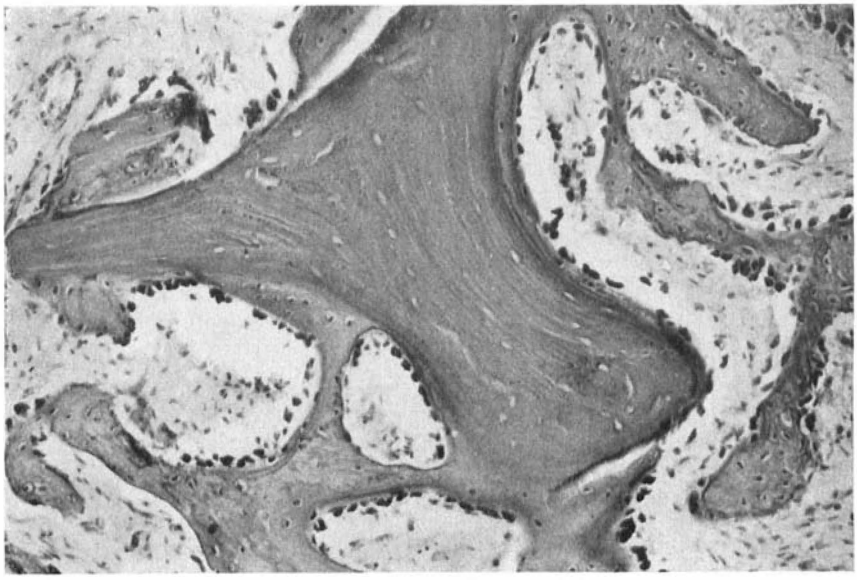

Figure 3. 3rd week. Ingrowth of callus into the Kiel bone graft from the proximal recipient site.

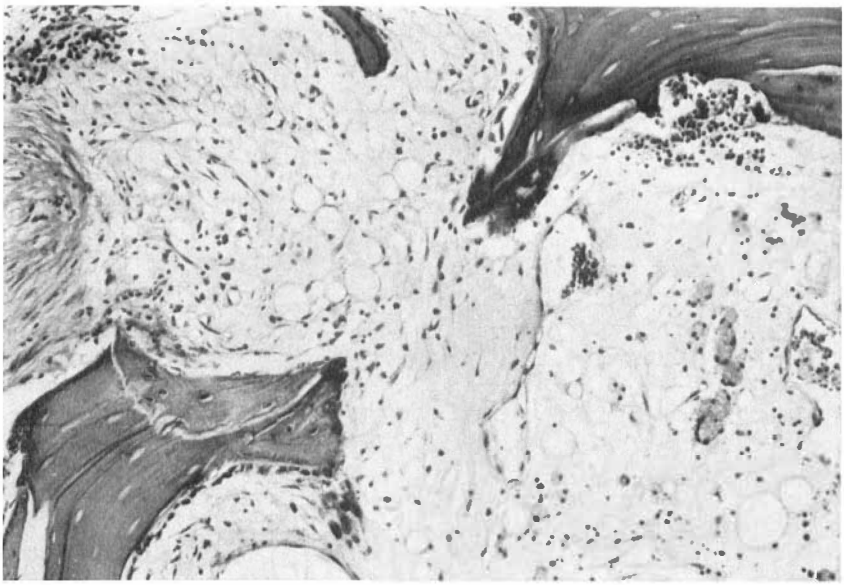

Figure 4. 7th week. From the Kiel bone graft on a level with the defect. The lacunae are empty. Some apposition of new-formed bone and osteoclastosis.

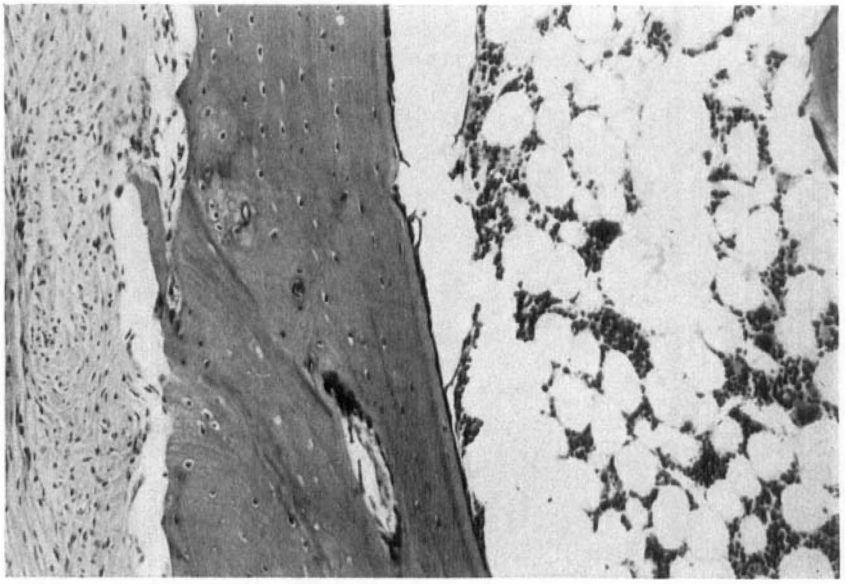

Figure 5. 7th week. From the autologous graft on a level with the defect. Ample new-formed bone and only sparse remnants of the necrotic graft.

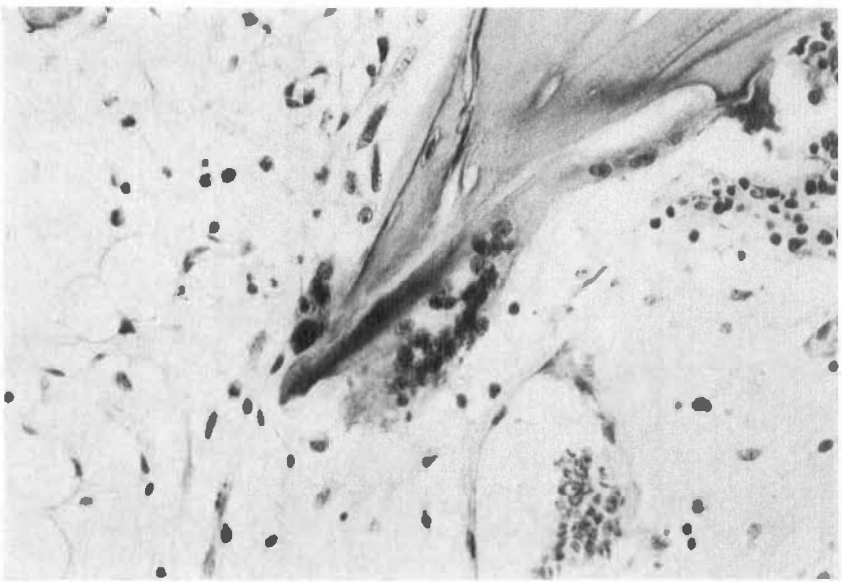

Figure 6. 7th week. High-power view of Figure 4. Kiel bone graft, new-formed bone, and osteoclasts.

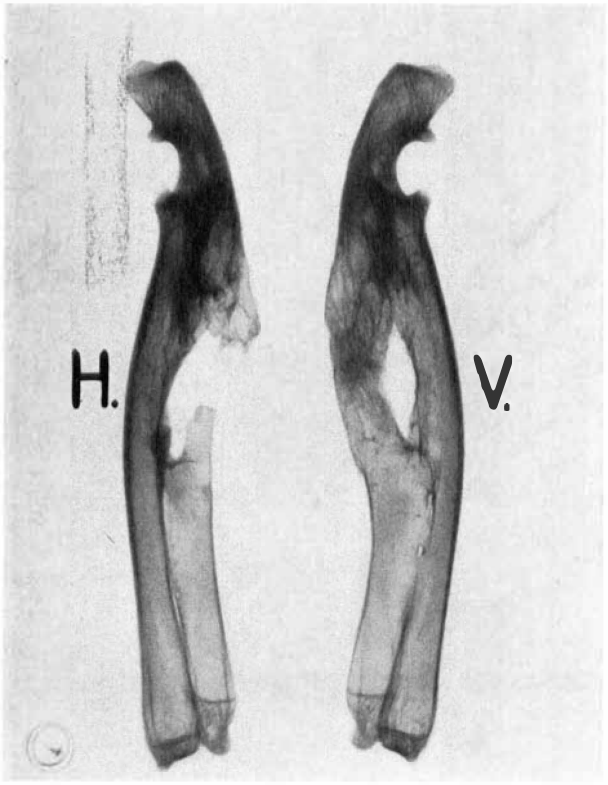

Figure 7. 40th week. The Kiel bone graft has been absorbed. The autologous graft shows solid union.

rapid, so that in the older specimens the graft had been converted into a long bone with a medullary cavity.

Autoradiography of the ground sections showed very pronounced blackening at the site of the new-formed bone, while the necrotic areas of the grafts showed only a faint, diffuse uptake, a little more pronounced on the surface of the trabeculae than inside (Figure 8). Clearly, the greater part of the activity is localized to areas where regeneration of bone has taken place.

Comparison of autoradiography, microradiography, and fluorescence microscopy with the results of Geiger-Müller counting showed a satis-

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Figure 8. 2nd week. Autoradiography. Cross-section of (a) Distal recipient site and Kiel bone graft. (b) Kiel bone graft on a level with the defect (outlines sketched). Only faint, diffuse uptake. No callus formation. (c) Distal recipient site and autologous graft. (d) Autologous graft on a level with the defect. The intensely blackened areas represent callus formation.

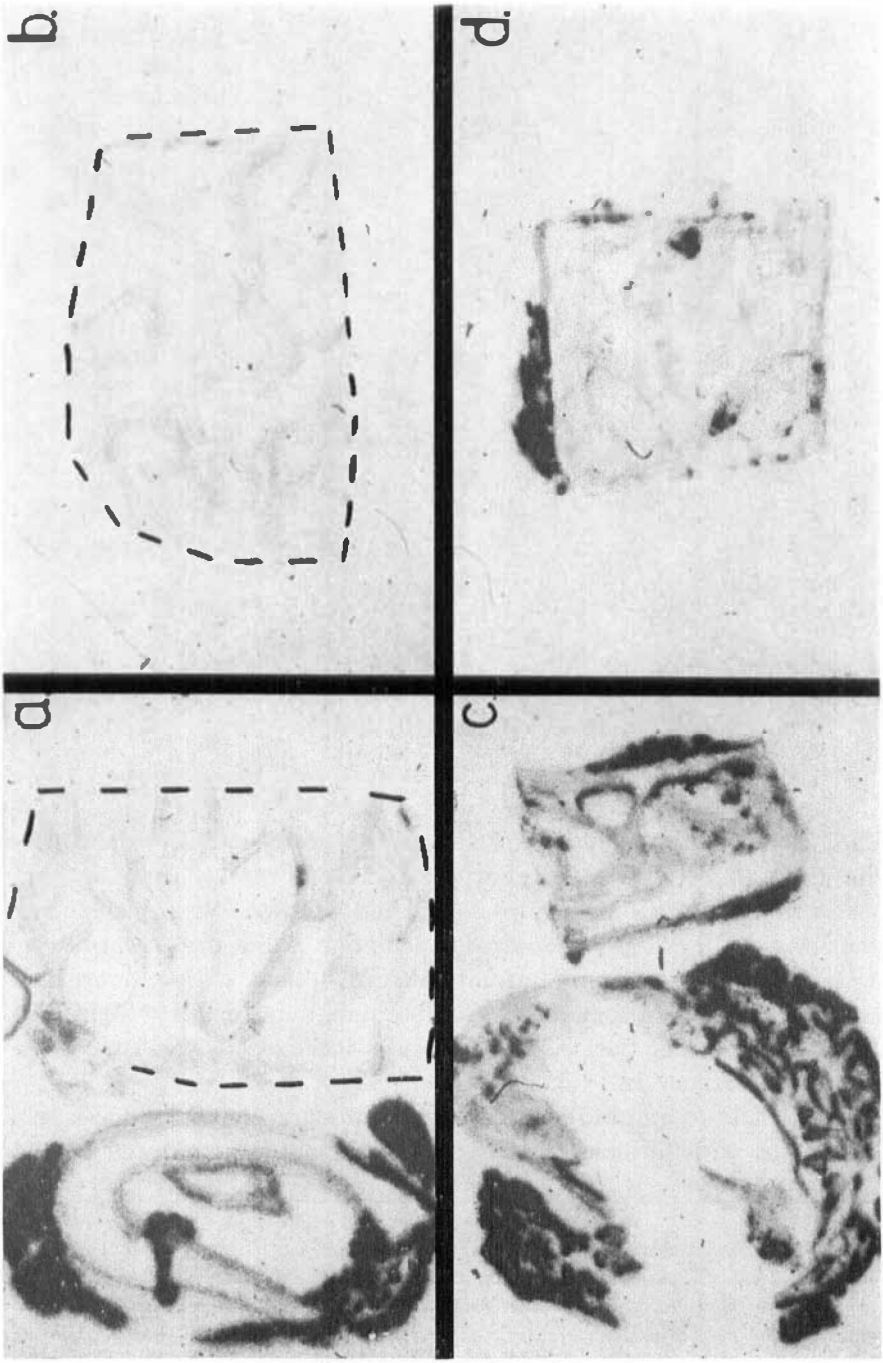

Figure 8.

factory agreement between the values found and the amount of new-formed bone in the same rabbit. The results are listed in Table 1. Individual variations are seen in the counts, presumably as a sign of a difference in age, calcium balance, and dosage. Only two cases, from the eighth to the tenth week, showed strikingly high values for the Kiel bone graft without showing regeneration of bone in the specimen. However, the corresponding values for the autologous graft were 4–5 times higher. A rabbit provided with a Kiel graft on both sides 12 weeks previously showed new bone formation in one graft, but not in the other. The count for diffuse labelling in the necrotic graft was 29; in that which also contained new-formed bone, 128.

DISCUSSION

On autoradiography of normal bone the uptake of ^{45}Ca was found to be partly faint and diffuse and partly more intense, viz. in not fully calcified Haversian systems, the so-called hot spots.

Cohen & Maletskos (1963) have performed quantitative determinations of the specific activity of the diffuse uptake, of hot spots, and of callus by measurements on autoradiographs and by direct measurement of samples of homologous grafts, callus, and recipient site. They found the diffuse uptake to be low and constant, proportional to the administered dose and independent of the time of administration as well as of the nature of the surgical procedure. For comparison, the specific activity of the hot spots was higher, on an average by a factor 20. By measurement of samples of the graft from animals injected two weeks after the transplantation, they found the specific activity to be higher than the diffuse uptake, by a factor 56–57, and in the callus by a factor 55–86.

In the present experiments all the grafts had become fully vascularized at the time of the injection. The autoradiographs showed a faint diffuse uptake, apparently equal in the Kiel bone and in the necrotic parts of the autologous graft. All preparations showing new bone formation showed several times higher counts. The share of passive, diffuse uptake by the necrotic bone in this process could only have been modest, and approximately the same is true for the Kiel bone graft and for the autologous graft.

The experimental conditions were not strictly standardized in respect to dosage, period from injection until sacrifice, the rabbits' calcium balance, and age. Moreover, for practical reasons the results are

not given as specific activity, but are related to the volume of the sample. Within the same rabbit, however, I obtained a quantitative expression of the biological activity of the studied grafts.

The experimental results differ from several of those mentioned in the introduction. One explanation is no doubt a difference in the experimental method. Maatz & Bauermeister, Fuchs et al., and Hallen used the Maatz spongiosa test, whereas Basset & Creighton and Karges et al. transplanted to small defects in cortical bone. The applicability of such experimental models in studying the clinical value of bone grafts has previously been discussed (Baadsgaard 1969). More agreement was found with the results of Heiple et al. who employed large total defects in long bones as recipient sites.

In comparing experimental results it must be realized that processed heterologous bone prepared by different methods may be of different composition. Gattow & Münzenberg (1963), for instance, found the Kiel bone to have an unchanged collagen content and an unchanged crystal arrangement, whereas Quigley & Hjørting-Hansen (1962), in ethylene diamine-prepared bone, found a nitrogen content of 0.4 per cent and an unchanged crystal arrangement. Fuchs et al. found in the Kiel bone a nitrogen content of 4.9 per cent, viz. close to normal for dried, defatted bone. They felt that they could demonstrate that the greater the protein content the greater was the osteogenetic effect of processed heterologous bone.

SUMMARY AND CONCLUSION

On defect pseudarthroses produced on rabbits, comparative studies of Kiel bone and fresh autologous cancellous bone, applied as on-lay grafts, were done. The specimens were studied radiologically, histologically, and by fluorescence microscopy, and quantitative determination of new bone formation was performed, using ^{45}Ca.

Union of the Kiel bone graft to the recipient site, proximally as well as distally in the pseudarthrosis, was slower and more uncommon than in the autologous control experiments. On a level with the defect new bone formation was found in 19 per cent of the Kiel bone grafts as compared with 100 per cent of the autologous grafts from the iliac crest.

On this basis it is concluded that the Kiel bone graft is less suited than fresh autologous bone grafts in the treatment of defect pseudarthroses.

REFERENCES

Anderson, K. J., LeCocq, J. F. & Mooney, J. G. (1963) Clinical evaluation of processed heterogenous bone transplants. *Clin. Orthop.* **29**, 248–263.

Baadsgaard, K. (1969) Defect pseudarthroses. *Acta orthop. scand.* in press.

Bassett, C. A. L. & Creighton, D. K. (1962) A comparison of host response to cortical autografts and processed calf heterografts. *J. Bone Jt Surg.* **44A**, 842–854.

Bauermeister, A. (1958) Experimentelle Grundlagen für den Aufbau einer neuen Knochenbank. *Hefte Unfallheilk.* **58**.

Bauermeister, A. (1961) Die Behandlung von Zysten, Tumoren und endzündlichen Prozessen des Knochens mit dem "Kieler Knochenspan". *Bruns Beitr. klin. Chir.* **203**, 287–310.

Cohen, J. & Maletskos, C. J. (1962) *Radioisotopes and Bone*, pp. 127–145. Blackwell, Oxford.

Fuchs, G., Stegemann, H. & Eger, W. (1963) Der transplantierte Knochenspan und seine Qualität nach partieller und vollständiger Enteiweissung bei erhaltener anorganischer Substanz. *Langenbecks Arch. klin. Chir.* **303**, 240–260.

Gattow, G. & Münzenberg, K. J. (1963) Die organische und anorganische Fraktion des Kieler Knochenspans im röntgenographischen Bild. *Arch. orthop. Unfallchir.* **55**, 453–461.

Goff, C. W. (1944) The os purum implant. *J. Bone Jt Surg.* **26**, 758–767.

Hallen, L. G. (1966) Heterologous transplantation with Kiel bone. *Acta orthop. scand.* **37**, 1–19.

Heiple, K., Kendrich, R. E., Herndon, C. H. & Chase, S. W. (1967) A critical evaluation of processed calf bone. *J. Bone Jt Surg.* **49A**, 1119–1127.

Hopf, A. (1957) *Verhandl. Dtsch. Gesell. Orthop.* Pp. 449–450.

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

Karges, D. E., Anderson, K. J., Dingwall, J. & Jowsey, J. (1963) Experimental evaluation of processed heterogenous bone transplants. *Clin. Orthop.* **29**, 230–247.

Koch, W. (1957) Über die Vitalisierung von implantierten Knochenspänen. *Verhandl. Dtsch. Gesell. Orthop.* Pp. 444–449.

Maatz, R. (1967) *Dixième Congrès International de Chirurgie Orthopédique et de Traumatologie.* Pp. 659–665. Les Publications Acta Medica Belgica, Bruxelles.

Maatz, R. & Bauermeister, A. (1957) A method of bone maceration. *J. Bone Jt Surg.* **39A**, 153–166.

Orell, S. (1934) Studien über Knochenimplantation und Knochenneubildung, Implantation von "os purum" sowie Transplantation von "os novum". *Acta chir. scand.* **74**, Suppl. 31.

Quigley, M. B. & Hjørting-Hansen, E. (1962) Crystal arrangement in anorganic bone. *Acta odont. scand.* **20**, 359–366.

Schilling, H. & Nockemann, P. F. (1962) Klinische Erfahrungen mit einem heteroplastischen Knochenmaterial. *Bruns Beitr. klin. Chir.* **205**, 89–98.

Witt, A. N. (1967) *Dixième Congrès International de Chirurgie Orthopédique et de Traumatologie.* Acta Medica Belgica, Bruxelles.