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Morphological changes in bone following intramedullary implantation of methyl methacrylate

A morphometrical study of calcium deficiency

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Adult male Sprague–Dawley rats had a methyl methacrylate implant in their right femur. After 16 weeks a group of rats was given a calcium-deficient diet. The rats were followed for another 31 weeks. Due to calcium deficiency a loss of femoral bone mass occurred which was relatively greater in the non-operated femur, as compensatory periosteal bone apposition and remaining necrotic bone areas contributed to the bone mass in the operated femur. The calcium deficiency did not affect the interface between bone and implant, where a thin sleeve of new bone was formed. While the non-operated femur lost its bone through endosteal resorption, the loss of bone in the operated femur was due to intracortical resorption.

Key words: bone cement; bone remodelling; calcium deficiency; circulatory disturbance; diaphyseal bone; osteoporosis; rats.

Intramedullary implantation of methyl methacrylate causes bone necrosis which remains for a long period. Adjacent to the necrotic areas, cavities develop in the cortex. This process results in a bone loss resembling osteoporosis. It is therefore of great interest to evaluate the possible additional effects of calcium deficiency on the events following intramedullary implantation of methyl methacrylate.

Thus, the present investigation was undertaken with the aims of studying the long-term effects of methyl methacrylate implantation in the rat femur and the additional effect of long-term calcium deficiency. A computerized semi-automatic morphometrical system was used for the measurement of the morphological bone changes.

Material and methods

Forty-seven male Sprague-Dawley rats, with a mean weight of 397 ± 18 g at the start of the experiment were used. All animals received an intramedullary implant of methyl methacrylate (Palacos-R, Schering Corp, USA) in their right femur. The rats were divided into three groups:

Group I: 10 rats had a normal laboratory diet and were killed 16 weeks after operation.

Group II: 19 rats had a normal laboratory diet until 16 weeks postoperatively, when the diet was changed to a semisynthetic diet (Astra-Ewos, Södertälje, Sweden) containing 0.06% Ca and 0.84% P on a dry weight basis.

Group III: 18 rats had a normal laboratory diet until 16 weeks post-operatively, when the diet was changed to the same diet as in Group II, but containing 1.6% Ca and 0.84% P.

All rats had deionized water *ad libitum*. The rats in Groups II and III were killed 31 weeks after the change of diet. The observation period was chosen to ensure the development of calcium deficiency osteoporosis (Larsson 1969).

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Fluorochrome labelling. All rats received an intramuscular injection of oxytetracycline (Terramycin® with Xylocain®, Pfizer, Sweden) at a dose of 15 mg/kg body weight 48 h before sacrifice. The rats in Groups II and III received another injection 48 h before the change of diet.

Preparation of specimens. The rats were killed by an overdose of ether, and both femora were immediately freed from soft tissue. The length of the femora was measured with a caliper and a 2 mm thick section was sawn from the middle and used for microradiography. This section was embedded in Epon 812 (KEBO AB, Sweden) after fixation in 4% neutral formalin. It was ground to a thickness of about 80–100 μm. Microradiography was performed with a Siemens tube at 15 kV. The specimen was was placed in direct contact with Kodak spectroscopic plates 649–0.

Another section, about 5 mm thick, was sawn from the adjacent, distal part of the diaphysis and examined histologically. After fixation of the specimen in 4% neutral formalin the methyl methacrylate implant was dissolved with chloroform. Decalcification was performed with 30% formic acid, after which the specimen was embedded in paraffin. 5 μm thick sections were made and stained with haematoxylin-eosin.

The proximal part of the femur was used for fluorescence microscopy. The specimen was fixed and dehydrated in absolute ethanol and embedded in methyl methacrylate. With a rotating saw, a 1 mm section was sawn from the distal surface of the proximal part of the femur. This section was ground to a final thickness of 80–100 μm and examined in a Zeiss fluorescence microscope using a combination of exciter filters BE 38 and BG 3 and barrier filters 47 and 65.

Morphometry. Morphometric analysis of the microradiographs was performed with a Leitz ASM computerized, semi-automatic system. The areas measured are described in Figure 1.

Bacteriological examination. A bacteriological examination of the operated femur of eight randomly chosen rats from Groups II and III was carried out. At sacrifice, the skin over the right femur was shaved. After washing the skin with sterile 35 per cent hydrogen peroxide solution and 5 per cent tincture of iodine, a strictly aseptic approach was made through the skin, muscles and periosteum to the middle of the diaphysis of the right femur. With a dental bur a hole was made through the cortex under saline irrigation into the intramedullary implant region. A sample was taken with a sterile paper point, which was transferred to a bacteriological culture medium. A culture was made for both aerobic and anaerobic microorganisms.

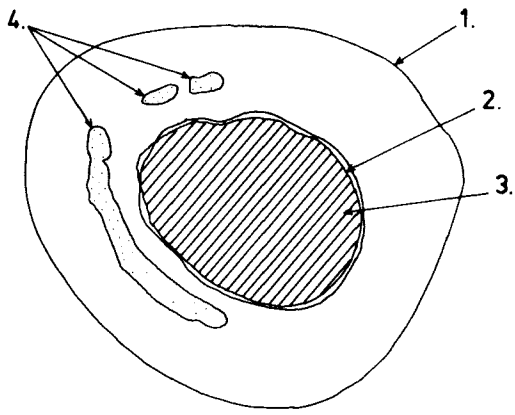

Figure 1. Areas measured by morphometry on microradiographs.
1. Total area (TA);
2. Central medullary area (CMA);
3. Implant area (IA);
4. Cortical cavities (CC). Total medullary area (TMA) = CMA + CC. Bone area (BA) = TA – TMA.

Statistics. Significance levels were tested with Student's *t*-test. The error of the morphometrical measuring method was calculated according to Eränkö (1955) and was found to be 1.29 per cent of the mean.

Results

Recovery after the operation was uneventful. All animals moved in a normal way and were able to load the operated leg normally. No clinical signs of local infection were seen. The mean body weight increased from 397 ± 18 g at the beginning of the experiment to 530 ± 17 g at 16 weeks postoperatively. A further increase was noted at 47 weeks postoperatively, in the low calcium group to 572 ± 31 g and in the normal calcium group to 542 ± 34 g.

The operated femurs in Groups II and III were respectively 0.4 mm and 0.6 mm longer than the corresponding control femurs. There was no difference in femur length between the low calcium group and the normal calcium group.

Microradiography. At 16 weeks postoperatively, there was a periosteal apposition of cor-

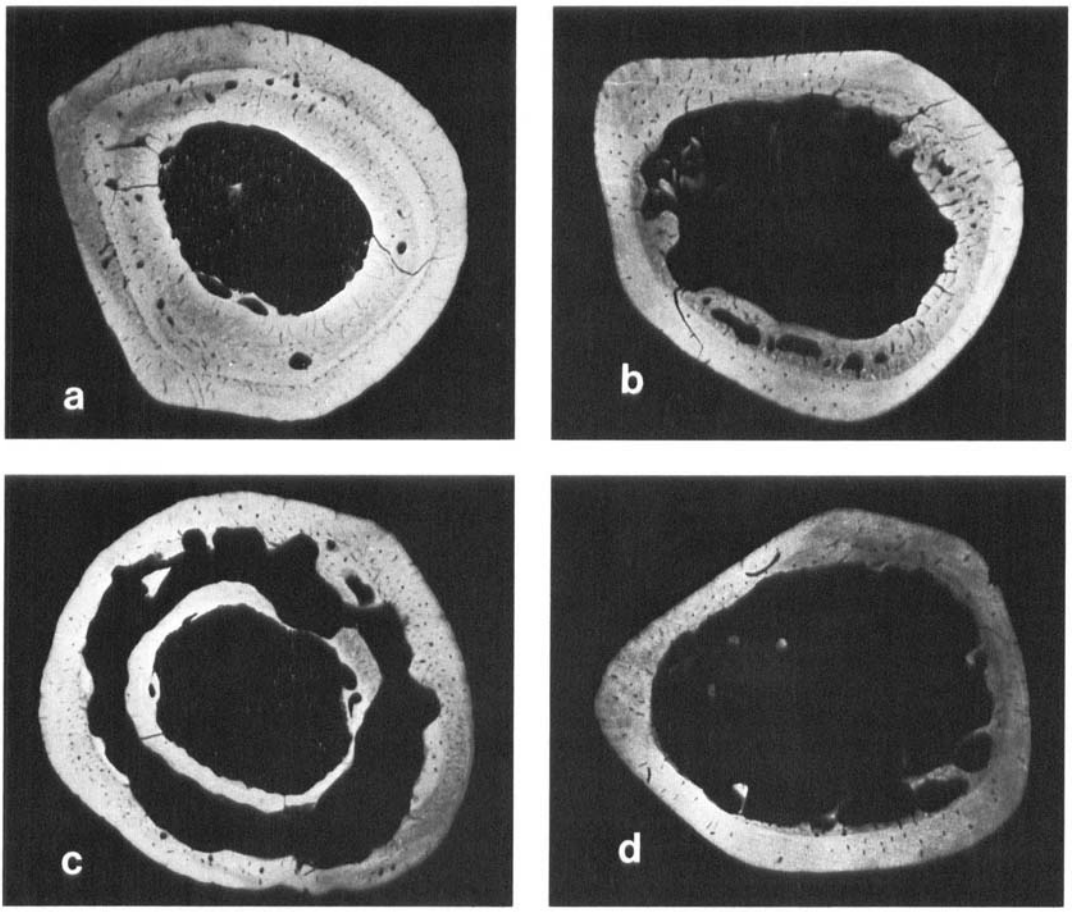

Figure 2. 47 weeks after blocking of the medullary blood supply by implantation of methyl methacrylate, the cross-section of the operated femur of the normal rats (a) was larger than that of the non-operated femur (b). The rats on the calcium-deficient diet for 31 weeks also had more bone in the operated femur in which bone was resorbed intracortically (c), as opposed to the endosteal resorption seen in the non-operated femur (d) ('10).

tical bone around the whole periphery of the operated femur. Cortical cavities were few and small and situated in the original cortex or at the border between old and new cortex. The bone surface in contact with the implant mostly presented a scalloped appearance.

At 47 weeks, the cortex of the operated femur generally contained more cavities than earlier. This was most evident in the low calcium diet group, where the cavities were larger and more confluent. The implants in both groups were covered with bone, although in many places only in the form of a thin sleeve, outside which there were large cavities. As in the 16 week group, the bone surface facing the implant showed a scalloped appearance.

Morphometry (Table 1). There was an increase in the total area (TA) in Groups II and III compared to Group I, and this increase was seen in both the operated and the non-operated femur. The central medullary area (CMA) of the non-operated femur in the low calcium group was much larger compared to that of the other two groups. Cortical cavities (CC) showed an increased area at 47 weeks postoperatively in the operated femur of both Groups II and III, while in the non-operated femur only Group III showed any significant increase compared to Group I.

The cortical bone area (BA) in Group II was smaller than that in Group I, while in Group III it was larger. The proportion of cortical

Table. 1. Morphometric analysis in mm² (mean ± S.D.) of transverse sections of the midpart of the femoral diaphysis at 16 and 47 weeks postoperatively

Group Diet	I Normal laboratory diet	II Experimental diet (low calcium)	III Experimental diet (normal calcium)	
Observation time (weeks)	16	47	47	Operated femur
Total area	23.4 ±0.96[a]	25.8 ±1.52	25.7 ±1.12[a]	
Central medullary area	6.38±0.76[a]	6.20±0.77[a]	6.20±0.36[a]	
Cortical cavities	0.53±0.62	4.06±1.54[a,b]	1.96±1.29[a]	
Total medullary area	6.91±0.57[a]	10.3 ±1.47[a,b]	8.16±1.18[a]	
Bone area	16.5 ±0.79[a]	15.5 ±1.44[a,c]	17.6 ±1.41[a]	
Implant area	5.83±0.52	5.63±0.62	5.92±0.33	
Total area	21.7 ±0.72	24.5 ±1.00	23.8 ±0.95	Control femur
Central medullary area	8.64±0.52	14.0 ±0.71[b]	9.79±0.67	
Cortical cavities	0.03±0.03	0.08±0.12[c]	0.53±0.25	
Total medullary area	0.67±0.52	14.1 ±0.54[b]	10.3 ±0.64	
Bone area	13.0 ±0.47	10.4 ±0.81[b]	13.5 ±0.86	

a. Difference from corresponding control value, $p < 0.001$.
b. Difference from corresponding values of Groups I and III, $p < 0.001$.
c. Difference from corresponding control of Group III, $p < 0.001$.

bone of the total transverse area also showed a significantly lower value in the low calcium group (Table 2). This loss of bone was most evident in the non-operated femur. The implant area (IA) showed only small variations between the three groups.

Histology. At 16 weeks postoperatively, the major part of the endosteal cortex of the operated femur contained empty osteocyte lacunae indicating bone necrosis. Bordering the implant a thin layer of bone containing osteocyte nuclei was seen. This layer of bone had a scalloped surface towards the implant. In the necrotic bone area formation of new bone was seen around blood vessels. Cavities were found in the endosteal third of the cortex or between the endosteal and the middle third of the cortex. The cortical cavities were not in direct contact with the implant area, but separated from it by the thin layer of vital bone bordering the implant. The cavities contained bone marrow. A thin fibrous membrane was seen between the implant area and the thin endosteal bone layer in a few specimens.

At 47 weeks postoperatively, the operated femur of the *low calcium group* had large cavities in the endosteal half of the cortex. These cavities were filled with bone marrow. The endosteal half of the cortex still contained large areas of necrotic bone which were being replaced by the cavities. A thin layer of bone containing osteocyte nuclei bordered the implant. The surface towards the implant was scalloped. A thin fibrous membrane between implant and bone was seen occasionally. The

Table 2. Percentage cortical bone of transverse sections from the femoral diaphysis at 16 and 47 weeks postoperatively

Group Diet	I Normal laboratory diet	II Experimental diet (low calcium)	III Experimental diet (normal calcium)
Observation time (weeks)	16	47	47
Operated femur	70.5±1.98	60.2±4.92[a]	68.3±4.33
Control femur	60.0±1.65	42.5±2.02[a]	56.6±2.55

a. Difference from corresponding values of Groups I and III, $p < 0.001$.

cavities in the cortex were sometimes divided into two layers, one layer just outside the thin endosteal bone sleeve and one layer in the midpart of the cortex at the border between necrotic and vital bone.

The operated femur of the normal calcium group showed the same thin scalloped layer of vital bone around the implant. Outside the thin vital bone sleeve there were often small or moderate sized cavities filled with bone marrow, followed by a layer of necrotic bone, occupying about one third of the cortex. New bone had formed around blood vessels in the necrotic areas. Between the necrotic bone and the periosteal vital bone, cavities of relatively large size containing bone marrow were seen.

Generally the appearance of the normal calcium group was that of a compact bone, often with large areas of necrosis and relatively few cavities of moderate size.

The control femur of the *low calcium group* contained some lacunae on the endosteal surface.

Fluorescence microscopy. At 16 weeks postoperatively some relatively large cavities in the endosteal third of the cortex of the operated femur were labelled on the outer surface while the inner surface of the cavities showed no labelling.

At 47 weeks postoperatively, the operated femur in both groups had two periosteal labels. The amount of bone between the labels did not differ between the groups. In both groups cortical cavities were seen with about 50 per cent of the surface labelled. In the low calcium group, the cavities were larger and more numerous than in the normal calcium group, but the amount and distribution of the label were the same. Both groups generally showed no labelling of the endosteal surface but the 16 week label could occasionally be seen at a small distance from the endosteal surface. The non-operated femur showed two periosteal labels and no difference could be seen between the groups regarding the amount of bone between these labels. About half of the surface of each cortical cavity was labelled with one label. This was also true for the endosteal surface where one scattered label was seen. The normal calcium group showed somewhat more labelling.

Discussion

In this study, Epon 812 was used as embedding medium for the undecalcified sections. Our intention was to avoid the disruption of the tissues caused by earlier mechanical or chemical methods for removal of the intramedullary implant. The implant was kept *in situ,* and thus it became possible to study the intact transverse area on both ground sections and corresponding microradiograms. By using Epon embedding, the interface between bone and implant was preserved and could be studied closely.

No effort has been made to evaluate the separate effects of polymerization heat and monomer toxicity in this study. However, other authors have found, in contradiction to what was earlier believed, that the effects of these traumata are of minor importance (Lundskog 1972, Lee et al. 1973, Biehl et al. 1974, Labitzke & Paulus 1974, Linder 1976, Rhinelander et al. 1979) and thus the surgical trauma, i.e. the permanent damage to the normal medullary vascular supply, remains the important factor to consider when studying the long-term effects of intramedullary implantation of methyl methacrylate.

The scalloped bone surface in contact with the methyl methacrylate implant was not the result of resorptive activity. The histological finding of a thin layer of vital bone bordering the implant indicated that this was instead a bone layer formed between 2 and 5 weeks after the implantation, and the scalloped surface was the result of bone adaptation to the polymer spheres described earlier by Willert et al. (1974). The bone sleeve had not been directly affected by the calcium deficiency at the termination of the experiment.

The total area (TA) was generally larger in the operated femur, because of the periosteal bone apposition which has been reported by many authors (Slooff 1971, Feith 1975, Lindwer & van den Hooff 1975). The calcium deficiency did not affect this apposition since no

significant difference was seen between the TA of Groups II and III.

The central medullary area (CMA) was influenced both by medullary occlusion and by calcium deficiency. The occlusion of the medullary canal apparently impaired endosteal resorption, as the CMA of the control femur was larger than the CMA of the operated femur. The largest difference regarding the CMA was seen in the low calcium diet rats due to the extensive endosteal resorption of the control femur.

The area of cortical cavities (CC) was larger in the operated femur of the low calcium group, indicating cortical osteoporosis. There was, however, also a difference between the CC of the operated femur in Groups I and III indicating an increase with time of the local osteoporosis induced by the medullary occlusion. In the control femur of the low calcium rats, CC had a low value due to the endosteal resorption removing areas where the medullary cavities would normally be situated.

The bone area (BA) was significantly smaller in the non-operated than in the operated femur. This difference depended both on periosteal bone apposition caused by medullary occlusion and on inhibited endosteal resorption in the operated femur. The percentage loss of bone in the calcium deficient animals was larger in the control femur despite the development of large cortical cavities in the operated femur. The formation of a connective tissue membrane between implant and bone was not as evident as in other experiments (Slooff 1971, Lindwer & van den Hooff 1975, Rhinelander et al. 1979). The difference could be due to the use of different animal species. Rhinelander et al. (1979) believe that the membrane is a way of bringing in regenerative blood supply to the diaphyseal cortex and that the membrane emanates from a thin layer of hemolyzed blood between the implant and bone, which becomes organized with time. However, the formation of the bone sheath in direct contact with the implant, which in the present investigation was seen at 16 weeks, does not support this idea.

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