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# EFFECTS OF ALENDRONATE ON PARTICLE-INDUCED OSTEOLYSIS IN A RAT MODEL

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**Background:** Particle-induced osteolysis is currently a major problem affecting the long-term survivorship of total joint replacements. Alendronate is a third-generation bisphosphonate that blocks osteoclastic bone resorption. The objective of this study was to determine whether alendronate could prevent particle-induced osteolysis or restore (reverse) bone loss in established osteolysis.

**Methods:** A rat model of particle-induced osteolysis was used. A specially designed polyethylene implant was placed in the proximal part of the right tibia of seventy-two animals. Following four weeks of healing, the animals were randomized into control groups, a prevention group, or a treatment group. In the prevention group, animals received intra-articular injections of high-density polyethylene particles (mean size, 2  $\mu\text{m}$ ; all  $<10 \mu\text{m}$ ) at four, six, and eight weeks postoperatively. Alendronate (0.01 mg/kg/day) was administered concomitantly through an implantable pump from the fourth week through the tenth week. In the treatment group, animals were also exposed to polyethylene particles at four, six, and eight weeks, to establish bone loss, but they received alendronate subsequently, from the tenth week through the sixteenth week, to treat the bone loss. Positive (particle-only) and negative (saline-solution-only) control groups were assessed as well. Tissues were harvested at ten weeks in the prevention group and at sixteen weeks in the treatment group. Histological analyses and histomorphometric determinations of the periprosthetic bone volume were carried out.

**Results:** Histological examination showed a rim of new bone (neocortex) around the implant in the untreated and saline-solution-treated control animals (no polyethylene particles). Treatment with saline solution (no polyethylene particles) did not affect periprosthetic bone. Animals exposed to polyethylene particles had bone loss. In those that received alendronate, the bone loss was either prevented or reversed, and the quantity of neocortical and trabecular bone was increased compared with that of the controls. Alendronate effectively preserved periprosthetic bone in both the prevention and treatment groups.

In the prevention arm, the mean periprosthetic bone volume of the neocortex and the surrounding trabecular bone, as determined with histomorphometry, was  $21.5\% \pm 6.5\%$  in the saline-solution-treated controls (no particles),  $13.1\% \pm 5.9\%$  in the particle-treated animals, and  $32.6\% \pm 6.4\%$  in the alendronate-treated animals ( $p < 0.001$ ). In the treatment arm, the mean periprosthetic bone volume was  $27.2\% \pm 5.6\%$  in the saline-solution-treated controls,  $17.7\% \pm 6.2\%$  in the particle-treated animals, and  $30.2\% \pm 5.9\%$  in the alendronate-treated animals ( $p = 0.002$ ).

**Conclusions:** In our model, the intra-articular injection of polyethylene particles caused substantial bone loss around a loaded implant. Alendronate effectively prevented and treated the particle-induced periprosthetic bone loss.

**Clinical Relevance:** Alendronate may be useful in preventing particle-induced osteolysis around total joint implants. It may also elicit bone formation in established osteolytic lesions.

Currently, over 250,000 total joint replacements are performed annually in the United States<sup>1</sup>. While these procedures have revolutionized the treatment of arthritis, the implants have finite life spans and some eventually fail. In 1994, for example, over 40,000 revision arthroplasties were performed in the United States<sup>1</sup>. Revision procedures not

only are more challenging technically but also are associated with higher morbidity and cost and with less predictable long-term results<sup>1-4</sup>.

The leading cause for the late failure of joint replacements is aseptic loosening<sup>5-8</sup>. Particulate wear debris, particularly ultra-high molecular weight polyethylene particles from

TABLE I Experimental Groups

Group	Arm	Final No. of Animals	Saline Solution or Particles	Alendronate	End Point (wk)
A	Control	8	None	No	4
B	Control	7	None	No	10
C	Control	8	None	No	16
D	Prevention (negative control)	7	Saline solution at 4, 6, and 8 wk (no particles)	No	10
E	Prevention (positive control)	6	Particles at 4, 6, and 8 wk	No	10
F	Prevention (alendronate)	7	Particles at 4, 6, and 8 wk	At 4-10 wk	10
G	Treatment (negative control)	8	Saline solution at 4, 6, and 8 wk (no particles)	No	16
H	Treatment (positive control)	8	Particles at 4, 6, and 8 wk	No	16
I	Treatment (alendronate)	6	Particles at 4, 6, and 8 wk	At 10-16 wk	16

the bearing surfaces, causes osteolysis, an intense inflammatory foreign-body reaction that may ultimately result in massive bone loss and implant loosening<sup>8-14</sup>. Macrophages and foreign-body giant cells secrete potent mediators of bone resorption that result in the loss of bone<sup>15-20</sup>. There are currently no proven pharmacological measures for the prevention of osteolysis, and often the only treatment option is revision surgery. Many patients, particularly those who are poor operative risks, could benefit greatly from nonoperative treatment alternatives.

Alendronate, a third-generation bisphosphonate, works by blocking osteoclastic bone resorption and has been shown to prevent particle-induced osteolysis<sup>21,22</sup>. We hypothesized that alendronate might be useful not only in the prevention of particle-induced osteolysis but also in its treatment. These hypotheses were tested in a small-animal model of osteolysis<sup>23</sup>, in which the capacities of alendronate to prevent particle-induced osteolysis and to increase bone formation in established osteolysis were examined.

## Materials and Methods

### Study Design

This study, which was reviewed and approved by the Institutional Animal Care and Use Committee, was a randomized, prospective mixed-model experiment (Fig. 1). The Cambridge osteolysis model<sup>23,24</sup>, a simple and reproducible animal model for particle-induced osteolysis, was used. After a pre hoc power analysis was performed to determine the sample size, seventy-two rats underwent a hemiarthroplasty of the right knee with a specially fashioned polyethylene tibial implant. Healing was allowed to occur for four weeks. Untreated control animals (no saline solution or polyethylene particles) were killed at four, ten, and sixteen weeks (Groups A, B, and C, respectively; Table I). Experimental animals were then randomized to either a prevention arm or a treatment arm (Table I). In the prevention arm, there were three groups: (1) the saline-solution-treated group (Group D), which was not exposed to particles and which un-

derwent intra-articular injections of saline solution (negative control group); (2) the particle-treated group (Group E), which received polyethylene particles by means of three intra-articular injections (positive control group); and (3) the alendronate-treated group (Group F), which was exposed to particles and concurrently received alendronate. The animals in the prevention arm were killed at ten weeks. The treatment arm also included three groups: the saline-solution-treated group (Group G; negative control group); the particle-treated group (Group H; positive control group); and the alendronate-treated group (Group I), which was exposed to particles to produce bone loss and then subsequently received alendronate. These animals were killed at sixteen weeks. After the animals were killed, the specimens were examined histologically and periprosthetic bone volume was determined with histomorphometry.

### Animals

Seventy-two adult male Sprague-Dawley rats weighing 300 g were used. The rats were maintained on Laboratory Rodent Diet 5001 (PMI Feeds, St. Louis, Missouri) and water ad libitum and were caged individually. Unrestricted weight-bearing was allowed.

### Surgical Procedure

Anesthesia was obtained with intraperitoneal ketamine (Keta-set; 100 mg/mL) at a dose of 80 mg/kg and with xylazine (Rompun; 20 mg/mL) at a dose of 5 mg/kg. The right hindlimb was clipped free of hair and scrubbed with Betadine (povidone-iodine) soap and 70% isopropyl alcohol.

The right knee joint was approached through a medial parapatellar arthrotomy and with lateral dislocation of the patella. A bone tunnel 1 cm in length was prepared in the proximal part of the tibia with a custom-designed, handheld drill fitted with a 1.2-mm stainless-steel drill bit. Specially fabricated ultra-high molecular weight polyethylene implants (Fig. 2) were fashioned (Dana Biomedical Center, The Hospital for

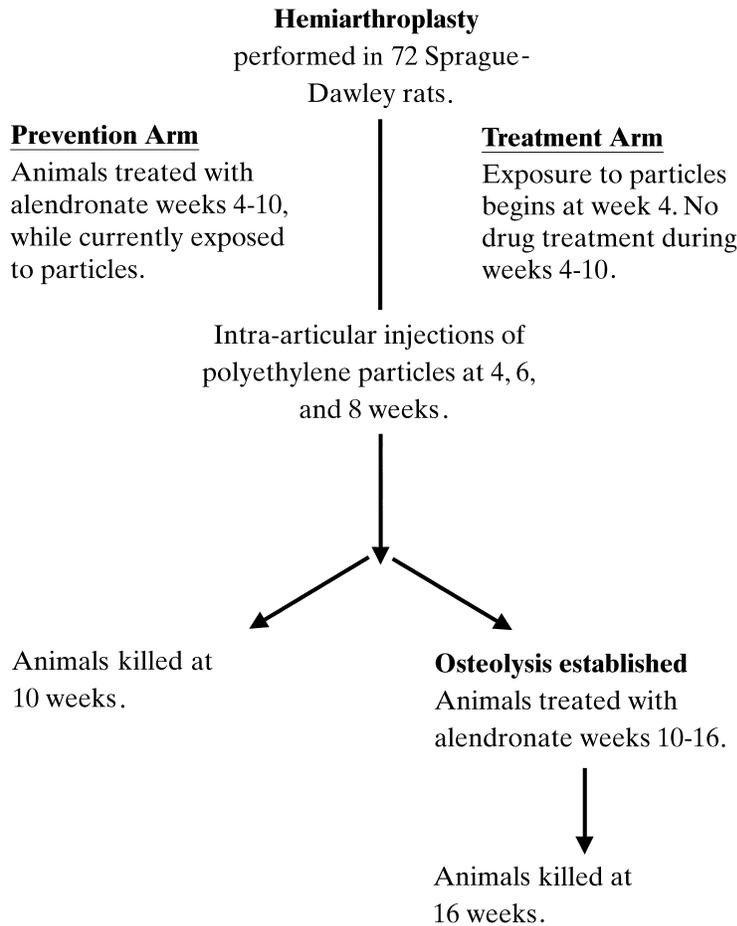


Fig. 1  
Experimental outline.

Special Surgery, New York, NY) and sterilized. The head of the implant was 2.5 mm in diameter and 1.5 mm in vertical height. The stem was 1.3 mm in diameter and 8.5 mm in length, and the total length of the implant was 1 cm. In order to allow the implant head to lie flush against the subchondral bone, a counterbore was used to create a 1.5-mm-diameter depression in the tibial plateau. Bone fragments were flushed from the joint by lavage with saline solution, and the implant was inserted in a press-fit fashion into the bone tunnel. The patella was replaced in the trochlear grooves, and the incision was closed in layers with simple interrupted sutures of monofilament nylon (Ethilon; Ethicon, Somerville, New Jersey).

Perioperative antibiotics were administered. The rats were monitored until they awoke and at least once daily thereafter by the veterinary staff. They were allowed unrestricted activity.

#### Particle Injections

High-molecular-weight polyethylene particles (generously donated by Mr. Neil Rushton, MD, FRCS, University of Cambridge, Cambridge, United Kingdom) were used in the study. Particle-size distribution was determined by a laser particle sizer. The mean particle size was 2

µm, and all particles were <10 µm; previous work showed that particles of this size produce osteolysis in this model<sup>23</sup>. The particles were sterilized prior to use. A suspension of particles (300 particles/mL) was prepared in a 1:50 solution of Sprague-Dawley rat serum and phosphate-buffered saline solution. Group-E and F animals in the prevention arm and Group-H and I animals in the treatment arm (Table I) received an intra-articular injection of 200 µL of the particle suspension into the operatively treated (right) knee at four, six, and eight weeks postoperatively (three injections per animal). At the same time-points, the saline-solution-treated control animals (Groups D and G) received intra-articular injections of the saline-solution vehicle only. All injections were made with a 25-gauge needle through the patellar tendon. Anesthesia was administered to reduce the stress of handling during these procedures.

#### Alendronate

Alendronate (MK-0217; 4-amino-1-hydroxybutylidene bisphosphonate sodium salt) is a bisphosphonate that was designed to inhibit bone resorption. Alendronate (Merck, West Chester, Pennsylvania) binds to apatite mineral and inhibits osteoclast-mediated bone resorption. Group-F animals in the prevention arm received alendronate for six weeks beginning four weeks postoperatively. Group-I animals in the treatment arm also received alendronate for six weeks, but beginning ten weeks postoperatively. The alendronate

**TABLE II Classification Scheme Used to Assign Histological Grades to Interfacial Membranes**

Grade	Severity	Histological Appearance
1	Benign	Organized lamellar fibrous membrane, acellular, no resorptive perforations of the neocortex
2	Mild	More cellular membrane, some osteoclasts, some scalloping/resorptive pits, evidence of resorption
3	Moderate	Aggressive cellular membrane, osteoclastic activity, foreign-body giant cells and neocortical perforations and resorption common
4	Severe	Membrane very aggressive or degraded, extensive neocortical destruction, many areas of resorption
5	None	Membrane completely absent, neocortex substantially eroded

was administered systemically at a dose of 0.01 mg/kg/day through an implanted mini-osmotic pump (Alzet, Palo Alto, California). The dose of alendronate (0.01 mg/kg/day) was based on effective dose ranges determined during preclinical studies of alendronate in rats<sup>25,26</sup> (personal communication; G. Seedor, Department of Bone Biology and Osteoporosis Research, Merck Sharp and Dohme Research Laboratories) and during previous studies at our institution<sup>27,28</sup>.

The mini-osmotic pumps that were used to deliver the alendronate were implanted subcutaneously posterior to the scapulae. A midscapular incision was made, and subcutaneous tissues were spread to create a pocket for the pump. The wound was closed with skin staples in standard fashion. Three biweekly implantations of a subcutaneous pump were required for each alendronate-treated animal. Procedures were synchronized with particle injections when possible.

#### Method and Time of Death

The rats were killed with CO<sub>2</sub> inhalation at appropriate end points, as shown in Table I, in compliance with the most recent recommendations of the American Veterinary Medical Association. The end points were based on those used in previously published reports by Howie et al.<sup>29</sup> and Allen et al.<sup>23</sup>.

#### Histological Analysis

The right hindlimb was removed en bloc, and the soft tissues were removed. The tibia was fixed in neutral buffered formaldehyde, dehydrated through a graded series of alcohols, and embedded in methylmethacrylate. Horizontal cross sections were cut, at a uniform depth of 2 mm distal to the articular surface of the tibia, distal to the head of the implant, around the proximal part of the stem. With use of a Reichert-Jung sliding microtome (Cambridge Instruments, Buffalo, New York) and a tungsten carbide knife, 58- $\mu$ m-thick sections of calcified tissue were collected. The sections were stained with hematoxylin and eosin, Masson trichrome, or von Kossa stain.

Two investigators blinded with regard to the study group examined each specimen independently with light microscopy and recorded a detailed description of the histological appearance of each. The neocortex and the trabecular bone of the proximal metaphysis were carefully examined for bone loss or evidence of resorption.

#### Histomorphometric Determination of Periprosthetic Bone Volume

Histomorphometry was performed with use of a semiautomated image analysis system. Von Kossa-stained sections were used to determine the percentage of mineralized periprosthetic bone. Bone volume in the periprosthetic tissues was determined as a percentage of total tissue volume, as described below.

For each specimen, the volume of the neocortex and the trabecular bone surrounding the neocortex (referred to in this article as the periprosthetic bone volume) was determined from a defined perimeter, of the same total area for all specimens, that included within it the neocortex and the trabecular

bone surrounding the neocortex. The cortical bone of the tibia was excluded because of artifacts from the sectioning process. Thus, the periprosthetic bone volume was determined from the ratio of neocortex plus trabecular bone area to total tissue area and is reported as a percentage.

Digitized images of the von Kossa-stained cross sections were used because they provided excellent discrimination between mineralized and unmineralized tissue. Data from the various groups were compared and analyzed statistically.

#### Membrane Grade

A five-part quantitative histological grading scheme was developed to classify the different types of interface membranes (Table II). Specimens were classified accordingly, and comparisons were made across groups.

#### Membrane Thickness

On representative cross-sectional images, the thickness of each periprosthetic membrane was measured directly at four different standardized regions. The mean thickness was then calculated for each specimen. Summary data were compiled and compared across groups.

#### Statistical Analysis

Outcome parameters included percent bone volume, membrane thickness, and membrane grade. Parametric data (bone

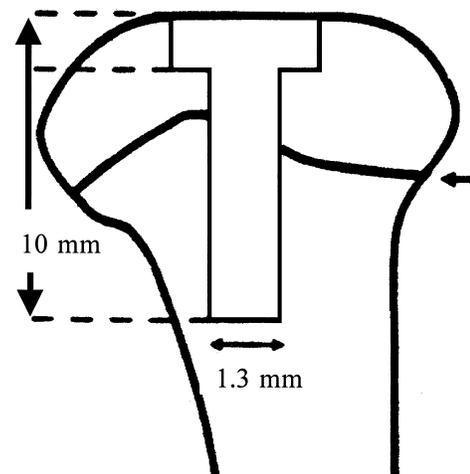


Fig. 2

Cambridge osteolysis model. An uncemented implant was placed surgically in the proximal part of the tibia so that the head of the implant was both loaded and directly exposed to the joint space. The implant was 10 mm in overall length and 1.3 mm in diameter distally. The head was 2.5 mm in diameter and 1.5 mm in length. Histological cross sections were taken 2 mm distal to the joint surface, distal to the head of the implant and through the narrow 1.3-mm-diameter stem. This location was just distal to the growth plate (small arrow to the right). (Reproduced, with modification, from: Allen M, Brett F, Millett P, Rushton N. The effects of particulate polyethylene at a weight-bearing bone-implant interface. A study in rats. *J Bone Joint Surg Br.* 1996;78:34. Reprinted with permission.)

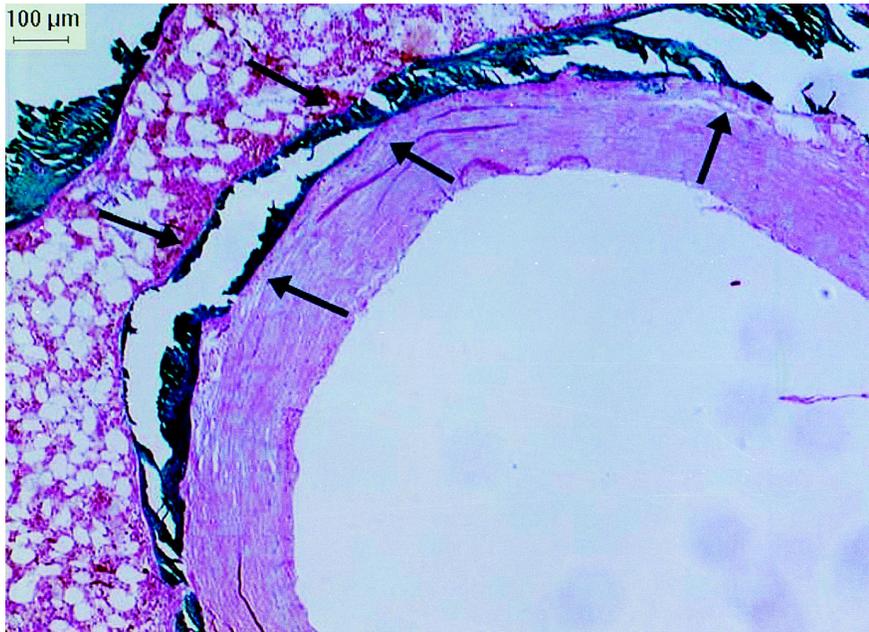


Fig. 3-A

**Figs. 3-A and 3-B** These photomicrographs of a saline-solution-treated animal in the ten-week prevention arm (Group D) are representative of the histological findings in the untreated and saline-solution-treated controls (Groups A, B, C, D, and G). There were no differences in the histological findings between the untreated and the saline-solution-treated animals. The horizontal cross sections were taken approximately 2 mm distal to the joint surface and stained with Masson trichrome stain, which stains mineralized tissue green. **Fig. 3-A** A low-power view ( $\times 40$ ) demonstrating the neocortex (arrows) that has formed around the implant. Note the artifact that is present from sectioning of these undecalcified specimens. The neocortex persisted in the untreated and saline-solution-treated animals for the entire duration of the study.

volume and membrane thickness) were statistically analyzed with two-way analysis of variance with post hoc Tukey multiple-comparisons tests. Nonparametric data (membrane grade) were compared with use of the Kruskal-Wallis nonparametric analysis of variance test with post hoc Dunn multiple-comparisons tests. P values of  $<0.05$  were considered significant.

## Results

Five animals died prematurely and were excluded from analysis. Two other specimens were damaged during histological processing and were also excluded. All groups had a

minimum of six specimens available for complete analysis (Table I).

## Histological Findings

### Untreated and Saline-Solution-Treated Controls

In the untreated controls (no polyethylene particles or saline solution; Groups A, B, and C), a rim of bone (neocortex) and a fibrous membrane formed around the stem of the implant by four weeks (Group A) and remained present throughout the ten weeks (Group B) or sixteen weeks (Group C) of the study. A similar neocortex and fibrous membrane formed around the stem of the implant in the saline-solution-treated

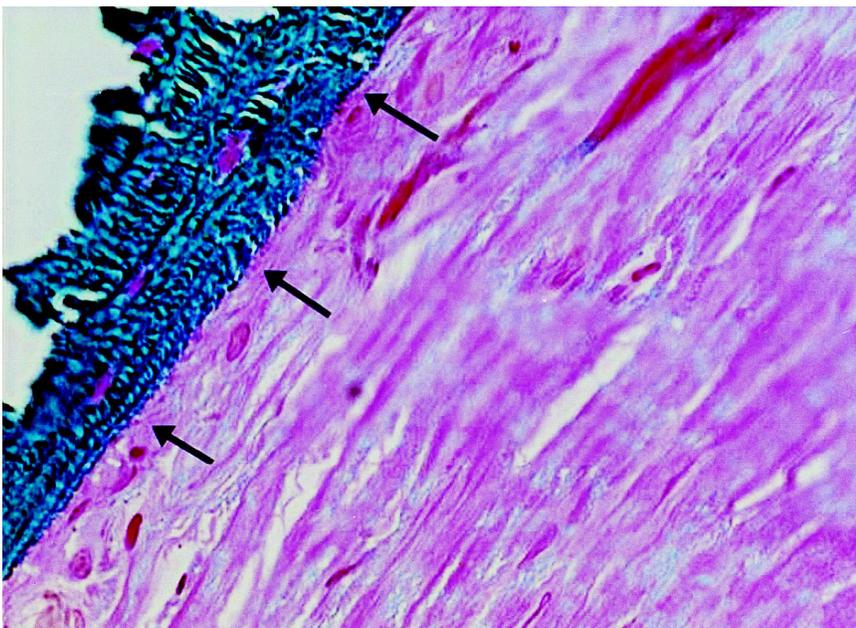


Fig. 3-B

A high-power view ( $\times 400$ ) at the membrane-neocortex interface (arrows). The periprosthetic fibrous membranes, which separate the implant from the neocortex, were acellular and benign in appearance, without infiltration into the surrounding bone.

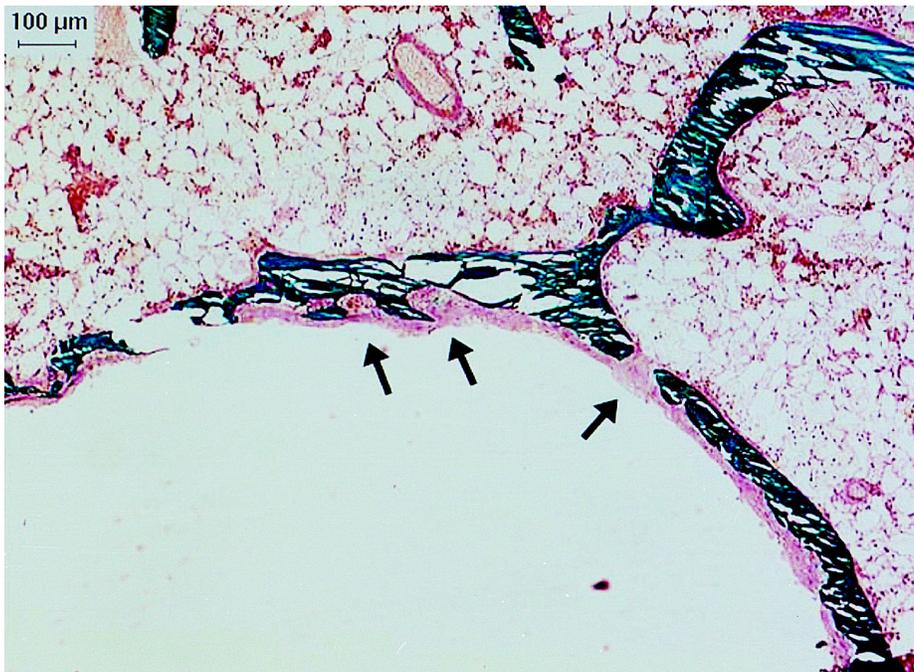


Fig. 4-A

**Figs. 4-A and 4-B** These photomicrographs of a particle-treated animal from the ten-week prevention arm (Group E) are representative of the periprosthetic bone loss that occurred when the animals were exposed to polyethylene particles. The particles were injected intra-articularly. This caused erosion of the neo-cortex and loss of trabecular and cortical bone. These horizontal cross sections were taken approximately 2 mm distal to the joint surface and were stained with Masson trichrome stain.

**Fig. 4-A** A low-power view ( $\times 40$ ) showing multiple perforations in the neo-cortex (arrows).

animals (no polyethylene particles; Groups D and G) (Figs. 3-A and 3-B). The fibrous membranes were thick and benign-appearing, and they did not infiltrate the neo-cortical bone. The fibrous membranes also were relatively acellular, were of variable thickness, and had linear arrays of fibroblasts with abundant, well-organized fibrous connective tissue. Osteoclasts and foreign-body giant cells were not seen. Intra-articular injections of saline solution (no polyethylene particles) had no effect on the histological findings; the untreated

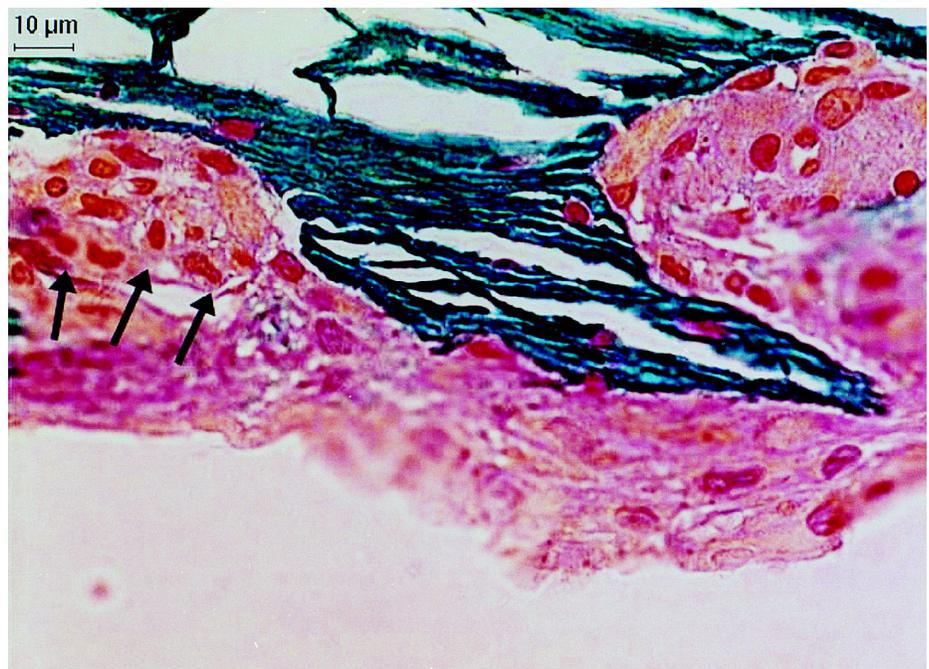
controls (Groups A, B, and C) and the saline-solution-treated controls (Groups D and G) had essentially identical histological appearances.

#### Particle-Treated Animals

In both arms of the study, periprosthetic bone loss occurred in animals treated with polyethylene particles but not with alendronate (Group E [prevention arm] and Group H [treatment arm]) (Table I). The histological findings in these positive

Fig. 4-B

A high-power view ( $\times 400$ ) demonstrating the periprosthetic membrane filled with inflammatory cells and invading the neo-cortical bone (arrows). Also note the less ordered fibrous structure of the membrane. These membranes were more cellular and aggressive than were those of the untreated and saline-solution-treated controls (Figs. 3-A and 3-B).



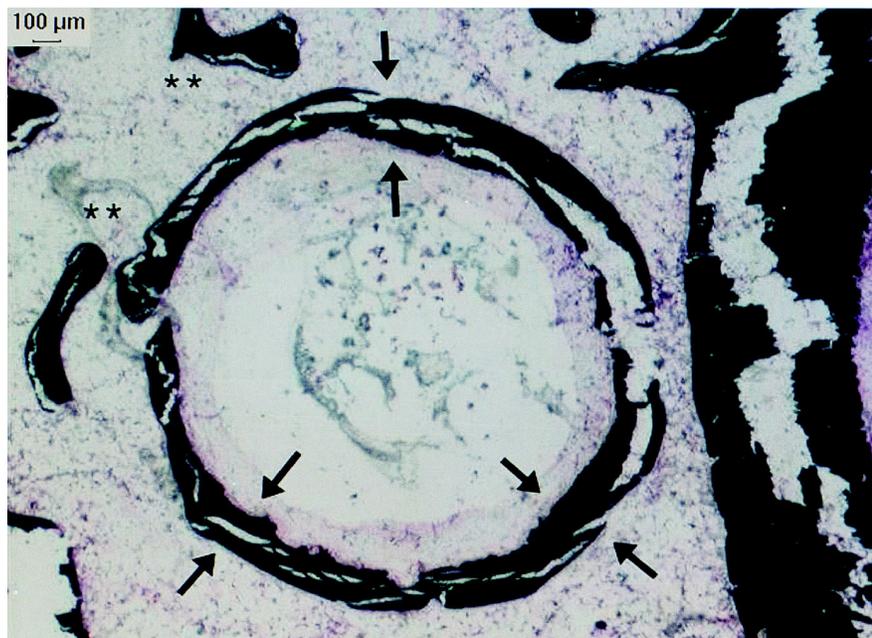


Fig. 5-A

**Figs. 5-A, 5-B, and 5-C** These photomicrographs were made of animals in the prevention arm of the study (×20). The horizontal sections were taken approximately 2 mm distal to the joint surface and were stained with the von Kossa technique, in which mineralized tissue stains black. Note the differences in neocortical bone (arrows) and trabecular bone (asterisks) among the three groups. **Fig. 5-A** Saline-solution-treated negative control (no particles) (Group D).

controls were characterized by thinning of the neocortex and osseous trabeculae, neocortical perforations, and an inflammatory response (Figs. 4-A and 4-B). The neocortex, trabecular bone, and cortical bone were decreased compared with those in the animals not treated with particles or saline solution (Groups B and C) and those in the animals treated with saline solution alone (groups D and G). In the particle-treated animals, osteoclasts and foreign-body giant cells penetrated the neocortical bone, and osteolysis occurred in the neocortex, trabecular bone, and cortical bone.

The interface membranes of the particle-treated animals

(Group E [prevention arm] and Group H [treatment arm]), were markedly different from those of the untreated controls (Groups B and C) and the saline-solution-treated controls (Groups D and G). Furthermore, membranes from the particle-treated animals were variable in thickness but more cellular, with less fibrous stroma. The membranes infiltrated and invaded the neocortices that surrounded the implants. Osteoclasts and foreign-body giant cells were seen in areas of neocortical perforation. The interfaces between neocortical bone and membranes were irregular and marked by areas of perforation. Polyethylene debris was localized to the membrane and

Fig. 5-B  
Animals exposed to polyethylene particles at four, six, and eight weeks (Group E).

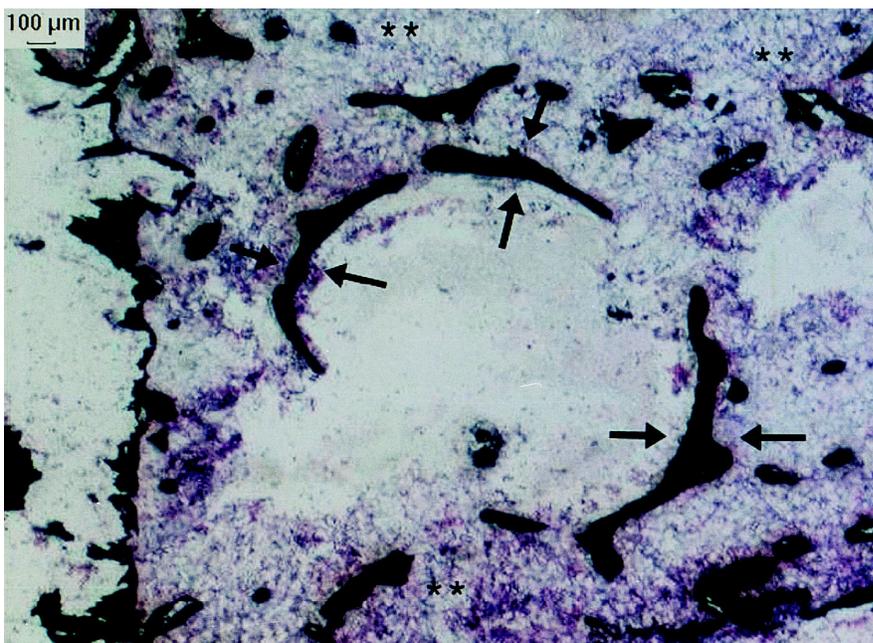
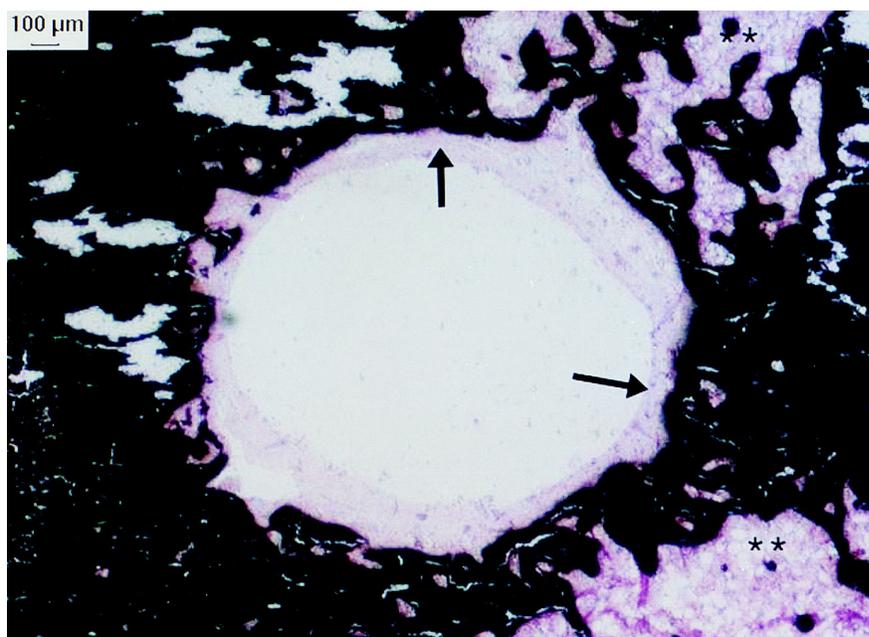


Fig. 5-C

Animals exposed to polyethylene particles at four, six, and eight weeks and concurrently treated with alendronate from the fourth through the tenth week (Group F). As can be seen in Figure 5-B, exposure to polyethylene particles caused bone loss in the neocortex, trabecular bone, and tibial cortex. Alendronate, when administered concurrently with the particles (Fig. 5-C), not only prevented particle-induced osteolysis with preservation of the neocortex but also resulted in huge increases in trabecular bone compared with that in the saline-solution-treated controls (Fig. 5-A).



could be seen under polarized light.

The process of particle-induced osteolysis was progressive between the tenth and sixteenth weeks (Group E compared with Group H). By sixteen weeks, three of the eight Group-H implants were surrounded by fluid and were no longer surrounded by a membrane, and four of the eight were grossly loose at necropsy.

#### Alendronate-Treated Animals

In comparison with the untreated (Group-B and C), saline-solution-treated (Group-D and G), and particle-treated (Group-E and H) animals, the alendronate-treated animals (Groups F and I) had distinct differences in both the peri-implant bone and the membrane. When administered either for prevention or for treatment, alendronate resulted in huge increases in trabecular bone volume compared with that seen in either the untreated or the saline-solution-treated controls. The neocortex was better preserved and there was more trabecular bone around the implants of the alendronate-treated animals (Figs. 5-A through 6-C). Alendronate, however, did not completely block the osteolytic process, as evidence of osteoclastic bone resorption was still present (Fig. 7).

The interface membranes in the alendronate-treated animals (Groups F and I) were less invasive than those in the respective particle-treated animals (Groups E and H). In the treatment arm, in which the alendronate was administered after the particles, the differences in the membranes between the particle-treated group (H) and the alendronate-treated group (I) were not as striking as were the differences in the prevention arm (Group E compared with Group F) (Figs. 4-A through 6-C). As was the case in the prevention arm (Group F), in the treatment arm the grades of the membranes from the alendronate-treated animals (Group I) were intermediate

between those of the saline-solution-treated (Group-G) and particle-treated (Group-H) animals.

#### Ten-Week Prevention Arm Compared with Sixteen-Week Treatment Arm

Bone quality appeared better in the alendronate-treated animals in the ten-week prevention arm (Group F) than in the alendronate-treated animals in the sixteen-week treatment arm (Group I). This was most apparent on examination of the von Kossa-stained specimens, where more mineralized bone was present not only in the neocortex but also peripherally in the cancellous bone closer to the endosteal surface. An interesting observation in the prevention arm was the presence of more polyethylene debris within the interface membranes of alendronate-treated animals (Group F) than in particle-treated animals (Group E). Alendronate seemed to have limited particle migration away from the implant. Under polarized light, we also observed more polyethylene particles in the periprosthetic tissues and less centripetally in the tibia in the prevention arm (Group F) than in the treatment arm (Group I).

#### Histomorphometric Periprosthetic Bone Volume

The effects of alendronate on periprosthetic bone volume (the combined volume of the neocortex and trabecular bone) are summarized in Table III. Because the histological findings in the untreated and saline-solution-treated animals were similar and because the results of our previous study were similar<sup>15</sup>, only saline-solution-treated specimens were analyzed with histomorphometry. In both the ten-week prevention arm (Groups D, E, and F) and the sixteen-week treatment arm (Groups G, H, and I), periprosthetic bone volume decreased after the injection of polyethylene particles and increased after administration of alendronate compared with those values in

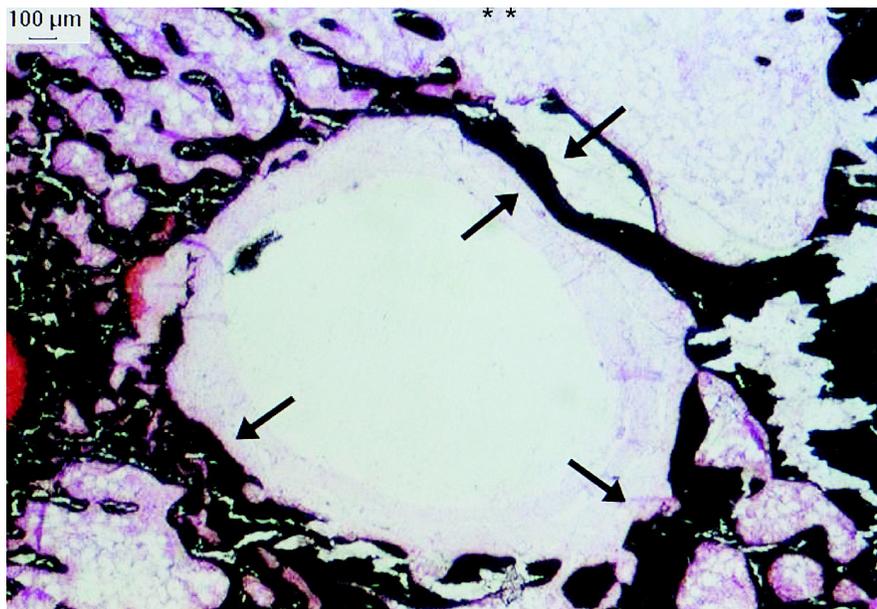


Fig. 6-A

**Figs. 6-A, 6-B, and 6-C** These photomicrographs were made of animals in the treatment arm of the study. The horizontal sections were taken approximately 2 mm distal to the joint surface and were stained with von Kossa stain. Note the differences in neocortical bone (arrows) and trabecular bone (asterisks) among the three groups. **Fig. 6-A** Saline-solution-treated negative control (no particles) (Group G).

the saline-solution-treated controls. These differences were all significant ( $p < 0.05$ ).

The model effectively demonstrated substantial bone loss, with significant differences between the periprosthetic bone volumes in the saline-solution-treated negative controls and the particle-treated positive controls. In the prevention arm Group D had significantly more bone than Group E ( $p = 0.048$ ), and in the treatment arm Group G had significantly more bone than Group H ( $p = 0.01$ ).

Alendronate treatment resulted in dramatic increases in periprosthetic bone volume compared with that of the saline-solution-treated negative controls (Table III). There were

highly significant differences between the particle-treated animals and the alendronate-treated animals in both the ten-week prevention arm (Group E compared with F) ( $p < 0.001$ ) and the sixteen-week treatment arm (Group H compared with I) ( $p = 0.002$ ).

In the ten-week prevention arm, the alendronate-treated (Group-F) animals had a highly significant increase ( $p = 0.005$ ) in bone volume when compared with the saline-solution-treated (Group-D) animals. In the sixteen-week treatment arm, there was no significant difference ( $p = 0.626$ ) between the saline-solution-treated controls (Group G) and the alendronate-treated animals (Group I), although the same trend was noted.

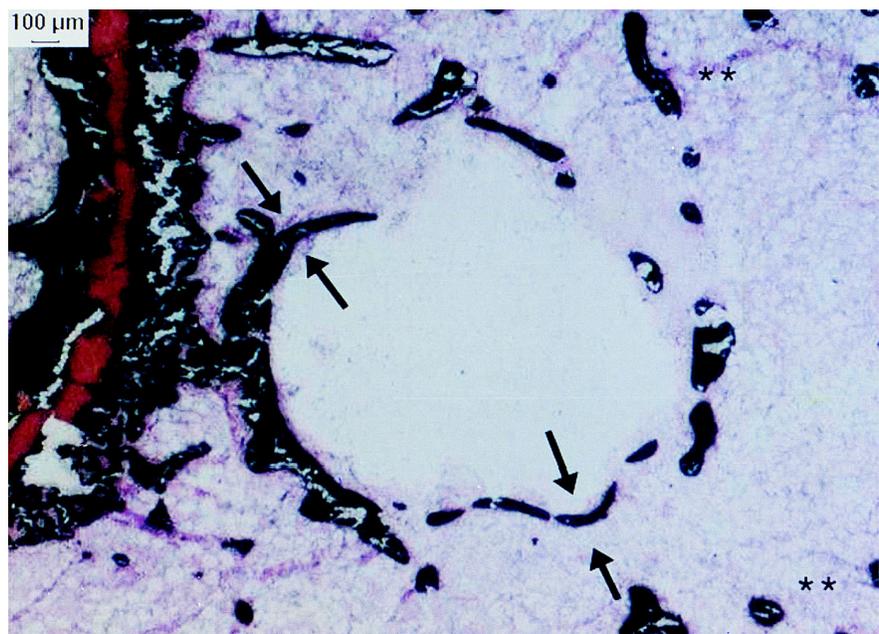
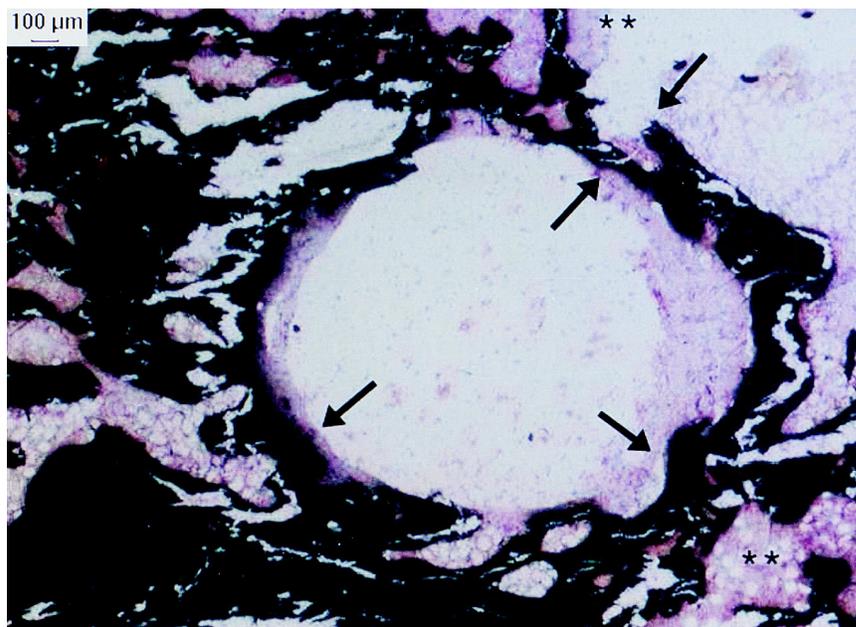


Fig. 6-B  
Animals exposed to polyethylene particles at four, six, and eight weeks (Group H).

Fig. 6-C

Animals exposed to polyethylene particles at four, six, and eight weeks and subsequently given alendronate from the tenth through the sixteenth week (Group I). Again, as is shown in Figure 6-B, polyethylene particles caused bone loss in the neocortex, trabecular bone, and tibial cortex. Figure 6-C demonstrates how administration of alendronate after particle-induced osteolysis had occurred increased trabecular bone and restored the neocortex to a level above that of the saline-solution-treated controls.



No significant differences were found when equivalent groups from the two arms of the study were compared (i.e., saline-solution-treated compared with saline-solution-treated, particle-treated compared with particle-treated, or alendronate-treated compared with alendronate-treated;  $p > 0.05$ ).

#### Membrane Grade

The membrane grades are summarized in Figure 8. They were generally lowest in the saline-solution-treated controls (Groups D and G) and highest in the particle-treated animals (Groups E and H). In both arms of the study, the alendronate-treated animals (Groups F and I) had lower membrane grades than the particle-treated animals (Groups E and H). Of all particle-treated animals, only one, an animal from the sixteen-week treatment arm (Group H), had a benign-appearing (grade-1) membrane.

In the prevention arm, the average score for the alendronate-treated animals (Group F) was 1.9 compared with 3.7 for the particle-treated animals (Group E). Statistical analysis with use of the Kruskal-Wallis nonparametric analysis of variance test revealed a  $p$  value of 0.0131, which is significant. Variation across groups was significantly greater than expected by chance. The Dunn multiple-comparisons test also revealed  $p$  values of  $<0.05$  for the differences between the particle-treated (Group-E) and saline-solution-treated (Group-D) controls and between the alendronate-treated (Group-F) and particle-treated (Group-E) animals.

In the treatment arm, the average score was 2.3 for the alendronate-treated animals (Group I) compared with 3.6 for the particle-treated animals (Group H). Although the trend was the same as that found in the prevention arm, Kruskal-Wallis nonparametric analysis of variance revealed a  $p$  value of 0.0537, which is considered not significant. The Dunn multiple-comparisons test did reveal  $p$  values of  $<0.05$  for the difference

between the particle-treated (Group-H) and saline-solution-treated (Group-G) controls.

#### Membrane Thickness

The membrane-thickness data are shown in Figure 9. Membrane thickness ranged from a mean of 252  $\mu\text{m}$  in the particle-treated animals (Group E) of the prevention arm to 76  $\mu\text{m}$  in the particle-treated animals (Group H) of the treatment arm. Comparisons across the three groups within each arm of the study did not reveal significant differences. However, comparison across the two arms of the study (Group E compared with H) did show a significant decrease in membrane thickness in the particle-treated animals over time ( $p = 0.009$ ), which most likely represents progressive osteolysis from a longer exposure to particles with implant loosening and membrane destruction. The membrane thicknesses of the two saline-solution-treated groups (Groups D and G) were not significantly different from those of the two alendronate-treated groups (Groups F and I) ( $p > 0.05$ ).

#### Discussion

The Cambridge osteolysis model used in this study is a small-animal model of particle-induced osteolysis<sup>23,24</sup>. It was developed to address some of the limitations inherent in previous small-animal models<sup>29,30</sup>. To simulate a cementless joint replacement, implants were inserted into the proximal parts of rat tibiae and were countersunk so that their heads rested flat against the subchondral bone, preventing distal migration and ensuring a connection with the joint surface. Thus, synovial fluid and wear debris had direct access to the bone-implant interface. Schmalzried et al. found this connection to be an important means of transit for wear debris in humans<sup>31</sup>. The Cambridge model also provided weight-bearing effects by achieving direct contact between the implant head and the

TABLE III Effects of Alendronate on Periprosthetic Bone Volume

	Bone Volume* (%)	
	Prevention Group	Treatment Group
Saline-solution-treated controls	21.5 ± 6.5	27.2 ± 5.6
Particle-treated controls	13.1 ± 5.9 (p = 0.048 vs. saline-solution-treated controls)	17.7 ± 6.2 (p = 0.01 vs. saline-solution-treated controls)
Alendronate-treated animals	32.6 ± 6.4 (p < 0.001 vs. particle-treated controls)	30.2 ± 5.9 (p = 0.002 vs. particle-treated controls)

\*Values are expressed as the mean and the standard deviation from a minimum of six specimens in each group.

femoral condyles. After a period of healing, a new rim of bone, the neocortex, was evident around all implants. We believe that the fibrous membrane that formed around the implants in the untreated and saline-solution-treated controls is indicative of loading and micromotion. The histological appearance was similar to that of fibrous membranes that form around porous ingrowth prostheses in humans.

Intra-articular injections of high-density polyethylene particles simulated implant wear and initiated the process of osteolysis<sup>12,23</sup>. The model is designed so that cyclical loading is transmitted to the bone-implant interface to potentiate the spread of wear debris. At harvest, animals that had received polyethylene particles displayed loss of bone and an intense foreign-body inflammatory response that mimicked that seen around loose implants in the clinical setting<sup>17,32,33</sup>. We found the Cambridge osteolysis model to be a simple and reproducible system in which to study the effects of particle-induced bone loss.

Alendronate, a third-generation bisphosphonate, is a potent inhibitor of bone resorption<sup>25,34</sup> and has been shown to be effective in the treatment of several diseases characterized by increased bone resorption<sup>6</sup>.

Alendronate has less effect on osteoblasts, and in turn bone formation, than earlier-generation bisphosphonates

do<sup>35</sup>. The drug binds tightly to apatite and is subsequently released around the osteoclasts, interfering with bone resorption and ruffled border formation<sup>25,36,37</sup>. Although the exact molecular mechanism of alendronate remains unclear, the overall effect is the inhibition of osteoclastic bone resorption. As particle-induced osteolysis is a problem of excessive bone resorption, we believe that alendronate may prevent and possibly reverse this type of bone loss.

Shanbhag et al. recently reported on the use of alendronate in the treatment of wear-debris-mediated osteolysis in a cementless canine total hip arthroplasty model<sup>22</sup>. They found that, during the twenty-four-week study, treatment with alendronate inhibited particle-induced osteolysis around the implants but had no effect on macrophages or inflammatory mediators. Their findings are consistent with the pharmacodynamics of alendronate, which blocks resorption by inhibiting osteoclasts, and they support our findings as well. In the present study, osteoclasts were present in alendronate-treated animals. Because of their study design and the model that they used, Shanbhag et al. could not answer questions about the use of alendronate to reverse or prevent particle-induced osteolysis.

The current study was undertaken to test the hypothesis that alendronate could be used to prevent and treat particle-induced osteolysis. Our histological data lend support to this

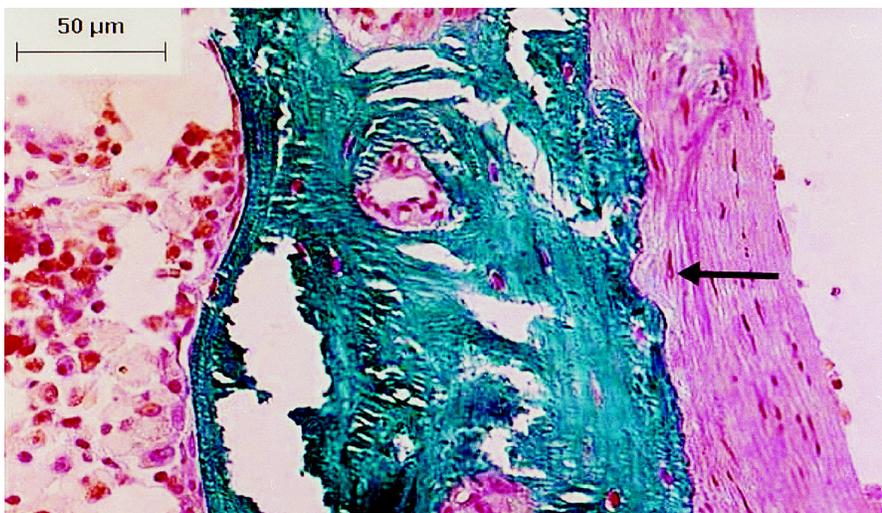


Fig. 7

Alendronate acts by blocking osteoclastic activity, although it did not completely eliminate it. Scalloped bone surfaces (arrow) mark sites of osteoclastic bone resorption in this view (Masson trichrome, ×200). This animal is from the ten-week prevention arm and received alendronate from the fourth through the tenth week (Group F).

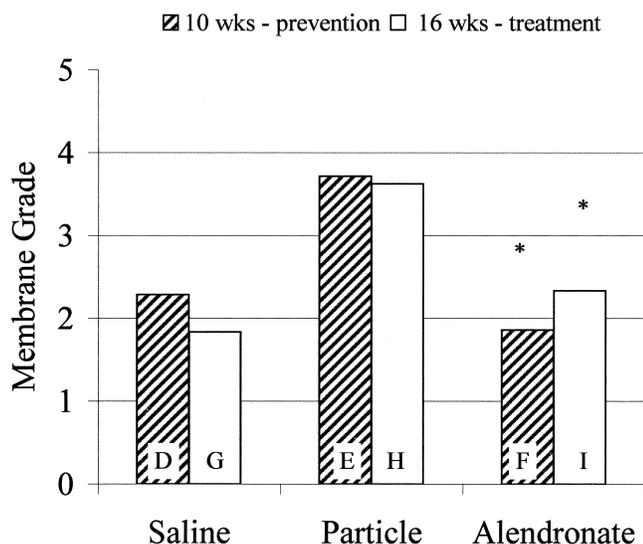


Fig. 8  
Membrane grades. Based on our qualitative five-part grading scheme (Table II), animals that received alendronate had significantly lower membrane grades than did particle-treated animals (asterisk denotes  $p < 0.05$ ).

hypothesis. A six-week course of alendronate at a dose of 0.01 mg/kg/day decreased particle-induced periprosthetic bone loss when administered in preventative and therapeutic modes. Polyethylene particles reproducibly caused a histological response that resulted in periprosthetic bone loss and mimicked an aseptically loose prosthesis. The high-density polyethylene particles used in our study were of the small size that seems to be of greatest concern clinically<sup>12,15,38</sup>, and the histological response was typical of that seen in association with failed total joint replacements<sup>17</sup>. Inflammatory responses at the interface membranes and periprosthetic bone loss were caused by the polyethylene particles. Alendronate prevented or at least retarded the process of particle-induced bone loss. Bone mass in the proximal part of the tibia was increased by the administration of alendronate in both arms of the study.

In the current study and in the study by Shanbhag et al.<sup>22</sup>, alendronate was shown to prevent bone loss when administered concurrently with particles. Sabokbar et al. recently showed that bisphosphonates are capable of inhibiting particle-induced bone resorption *in vitro*<sup>21</sup>. To our knowledge, however, the current study is the first to show that alendronate has beneficial effects on periprosthetic bone when administered therapeutically, after particle-induced bone loss has occurred. Bone volume was increased significantly after the administration of alendronate.

Membrane thickness appeared to be inversely proportional to membrane grade, at least in the prevention arm. Although statistical analysis was hampered by widespread scatter in the data, there was a strong trend for the more benign-appearing membranes to be thicker and the more inflamed membranes to be thinner. Similar findings were reported in a previous experiment in which polymethylmethacrylate pins

were used in the Cambridge osteolysis model<sup>24</sup>. Our explanation for this observation is that more aggressive membranes cause more bone loss and more implant loosening, eventually resulting in mechanical destruction of the membranes and therefore thinner membranes. It is unclear whether alendronate protects the periprosthetic membrane from mechanical destruction because of greater bone volume and less loosening of the implants or whether alendronate has effects on fibroblasts in the membrane.

The distribution of polyethylene debris within the proximal part of the tibia varied across groups. Polyethylene debris was much more confined to the interface membranes in the alendronate-treated animals, particularly in the ten-week prevention arm. While the mechanism whereby alendronate inhibits osteolysis is presumed to be the inhibition of osteoclasts, a secondary effect of alendronate may be the containment of the inciting particulate debris in the interface membranes, preventing spread to adjacent bone. If the neocortex that forms around the implant is preserved, there is no channel for the migration of particles. Thus, periprosthetic bone could be protected by alendronate in two ways: first, osteoclasts, inhibited by alendronate, would not resorb bone, and second, polyethylene debris, confined to the interface membrane, would not have access to the remaining periprosthetic bone.

In our study, alendronate did not completely eliminate either the presence or the activity of osteoclasts, as there was evidence of both in some of the alendronate-treated animals. The data from the current study and that by Shanbhag et al.<sup>22</sup>

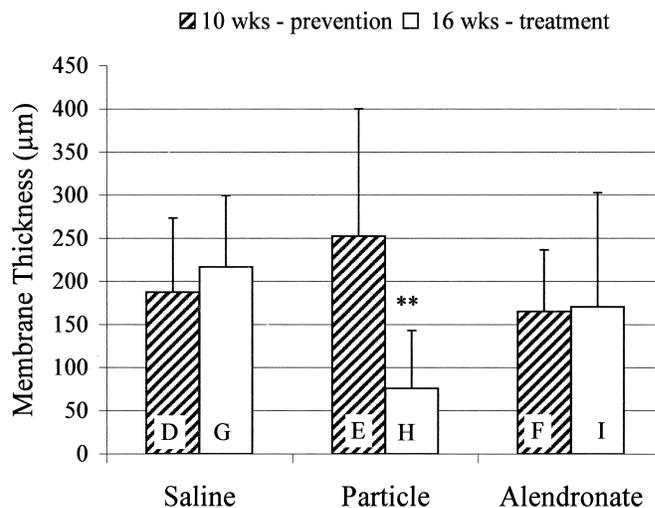


Fig. 9  
Membrane thickness. Animals from the sixteen-week treatment arm who received polyethylene particles only (Group H) had the thinnest interface membranes. Animals who received alendronate (Groups F and I) had thicker membranes that were more similar to those of the saline-solution-treated animals (Groups D and G). Over time, membranes in the saline-solution-treated animals became thicker (Group D compared with G), whereas those in the particle-treated animals became thinner (Group E compared with H) (double asterisk denotes  $p < 0.01$  compared with the value at ten weeks).

show that alendronate does decrease particle-induced bone loss, presumably through reduced osteoclastic activity. In a recent study by Astrand and Aspenberg, instability-induced bone loss was not affected by alendronate<sup>39</sup>. At a dose of 0.063 mg/kg/day, alendronate was unable to inhibit instability-induced bone loss, although it did affect bone-remodeling. We believe that the data from these three studies support the contention that particle-induced bone resorption and instability-induced bone resorption occur by different mechanisms<sup>22-24,39</sup>.

In summary, particle-induced osteolysis is the major problem affecting the long-term survival of total joint prostheses. The strategy outlined in the current study is a biological approach that involves blocking the final common pathway for particle-induced bone resorption. While such a scheme shows great promise, many questions, such as long-term effects of alendronate on periprosthetic bone, appropriate dosing regimens, and relevant monitoring methods, remain. ■

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