

## LONG-TERM TREATMENT OF OSTEOPETROSIS WITH RECOMBINANT HUMAN INTERFERON GAMMA

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**Abstract Background.** Congenital osteopetrosis is a rare osteosclerotic bone disease characterized by both a defect in osteoclastic function and reduced generation of superoxide by leukocytes. The disease is frequently fatal during the first decade of life. A six-month trial of therapy with recombinant human interferon gamma-1b in eight patients with osteopetrosis provided evidence of benefit, prompting this study of more prolonged therapy.

**Methods.** We studied 14 patients with severe osteopetrosis treated with subcutaneous injections of recombinant human interferon gamma-1b (1.5  $\mu$ g per kilogram of body weight per dose) three times per week for at least 6 months; 11 patients were treated for 18 months. We assessed the effect of therapy by evaluating the patients' clinical status, measuring blood counts and biochemical markers of bone turnover, and performing bone marrow imaging and bone biopsies.

**Results.** After 6 months of therapy, all 14 patients had

decreases in trabecular-bone area (determined by histomorphometric analysis of bone-biopsy specimens) and increases in bone marrow space (determined by marrow imaging), and the improvement was sustained in the 11 patients treated for 18 months. The mean ( $\pm$ SD) hemoglobin concentration increased from  $7.5\pm 2.9$  to  $10.5\pm 0.3$  g per deciliter ( $P=0.05$ ), and superoxide generation by granulocyte-macrophage colonies increased ( $P<0.001$ ) after 18 months of therapy. In six patients for whom pretreatment data were available, there was a 96 percent decrease in the frequency of infections requiring antibiotic therapy during interferon treatment. There were no side effects necessitating the discontinuation of therapy.

**Conclusions.** Long-term therapy with interferon gamma in patients with osteopetrosis increases bone resorption and hematopoiesis and improves leukocyte function. (N Engl J Med 1995;332:1594-9.)

CONGENITAL osteopetrosis is a group of disorders resulting in decreased osteoclastic function and hence decreased bone resorption. The accumulation of sclerotic bone compromises marrow space and cranial-nerve foramina and predisposes patients to pathologic fractures. Most patients become blind or anemic before six months of age and die because of poor resistance to infection, neurologic deficits, or bone marrow failure during the first decade — often the first two years — of life.<sup>1-4</sup> Bone marrow transplantation is curative therapy,<sup>5</sup> but an acceptable donor can be found for only about 40 percent of patients. Overall survival 23 months after transplantation was 47 percent in a European study, and 62 percent of the survivors were considered to have been cured.<sup>4</sup> High-dose calcitriol therapy has been reported to ameliorate osteopetrosis in 25 percent of patients.<sup>6</sup>

In addition to bone resorption, the generation of superoxide by peripheral-blood leukocytes is defective in patients with osteopetrosis.<sup>3,7,8</sup> Because patients with chronic granulomatous disease, a rare genetic disorder of superoxide generation, respond to therapy with recombinant human interferon gamma-1b with increased superoxide generation and have fewer infections,<sup>9</sup> and because interferon gamma-1b increased marrow space

in mice with osteopetrosis and microphthalmos,<sup>10</sup> we treated eight patients who had osteopetrosis with interferon gamma-1b for six months.<sup>3</sup> During treatment, all the patients had increases in the production of superoxide by cultured leukocytes, decreases in the number of severe infections, and increases in bone resorption. These encouraging results led to the prolongation of therapy in these patients and the treatment of additional patients. This paper describes the results of interferon gamma-1b therapy in 14 patients with osteopetrosis who were treated for at least 6 months; 11 of these patients were treated for 18 months.

## METHODS

### Patients and Treatment

We evaluated 20 patients with osteopetrosis, diagnosed on the basis of the presence of dense bones in radiographs of the axial and appendicular skeleton, an excess of trabecular bone, and the presence of cartilaginous remnants in bone specimens obtained from the iliac crest, for possible inclusion in the study. Eight of these patients were included in our earlier report.<sup>3</sup> Patients with anemia, thrombocytopenia, or cranial-nerve dysfunction were classified as having severe disease. Peripheral-blood mononuclear cells containing granulocyte-macrophage progenitors were obtained from these patients, cultured, and tested to determine whether the addition of interferon gamma-1b to the incubation medium increased the production of oxygen radicals by the cells.<sup>3</sup> There was a statistically significant increase in the cells from 17 of the 20 patients, which qualified them for inclusion in the study. Patients who had received therapy with calcitriol, prednisone, or both or who were receiving one or both of these medications when first evaluated by us were not excluded. Fourteen patients were eventually enrolled in the study, which was approved by the Food and Drug Administration and by the institutional review board at the Medical University of South Carolina; all the patients or their parents gave informed consent.

The clinical characteristics of the patients at the time of entry into the study are shown in Table 1. Thirteen of the 14 patients had a history of infections requiring intravenous antibiotic therapy in the hos-

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**Table 1. Characteristics of the 14 Patients with Osteopetrosis before Therapy with Interferon Gamma-1b.**

CHARACTERISTIC	NO. OF PATIENTS
<b>Age group</b>	
Children — mean $\pm$ SD, 4 $\pm$ 4 yr	12
Adults — 25 and 30 yr	2
<b>Clinical feature</b>	
Blindness	9
Optic atrophy	3
Audiologic compromise (hearing loss >20 dB)	3
Splenomegaly	14
Anemia and thrombocytopenia	14
Transfusion-dependent	5
Previous infections	
Requiring intravenous antibiotic therapy	13
Chronic osteomyelitis	3
<b>Previous treatment</b>	
Calcitriol (1.5 $\mu$ g/kg/day)	6
Prednisone (1 mg/kg/day)	5
Low-calcium diet (400 mg/day)	5
Bone marrow transplantation (with failed engraftment)*	3
<b>Treatment while receiving interferon gamma-1b</b>	
Calcitriol	6
Low-calcium diet	14

\*Bone marrow transplantations were performed 1, 12, and 13 years before the initiation of interferon gamma-1b therapy.

pital or at home. Six patients had been followed in our clinic while receiving calcitriol therapy, during which all had temporary improvements in bone turnover, growth, and hematopoiesis. All six, however, were having progressive loss of bone marrow function at the time of enrollment in this study. Two adult patients and one child with severe osteopetrosis had undergone bone marrow transplantation, with initial improvement followed by relapse when the engraftment failed.

Before the initiation of therapy with interferon gamma-1b, all the patients were admitted to the General Clinical Research Center. The six patients receiving calcitriol continued that therapy. Five patients were receiving prednisone, which was discontinued. Dietary calcium intake was limited to 300 to 400 mg per day to prevent hypercalciuria and to facilitate detection of the mobilization of bone mineral. If the serum calcium concentration during treatment with interferon gamma-1b fell below 7.5 mg per deciliter (1.87 mmol per liter), dietary calcium intake was increased by 25 percent.

The 14 patients were treated with interferon gamma-1b (Actimmune, Genentech, South San Francisco) at a dose of 1.5  $\mu$ g per kilogram of body weight if the patient weighed less than 10 kg, or 50  $\mu$ g per square meter of body-surface area per dose if the weight was above 10 kg. (The cost of interferon gamma-1b purchased from a pharmacy would be approximately \$15,500 per year for a patient weighing from 1 to 10 kg or with a body-surface area of less than 2 m<sup>2</sup> who used single-dose vials containing 100  $\mu$ g of interferon gamma-1b.) The drug was self-administered or administered by a parent (except when the patients were hospitalized) by subcutaneous injection three times weekly (usually on Monday, Wednesday, and Friday). Both children and adults were treated with acetaminophen (15 mg per kilogram per dose, with a maximal dose of 650 mg) before each dose of interferon gamma-1b and every 4 to 6 hours for 24 hours afterward to limit hyperpyrexia.

#### Assessment of Efficacy

The effects of interferon gamma-1b were assessed after 1, 6, and 18 months of therapy (data from the 1-month assessment are not given in this report). These assessments included audiologic and ophthalmologic examinations. Developmental milestones and intelligence were evaluated in children under the age of 10 years. Bone turnover was assessed by measuring serum calcium, phosphorus, and alkaline phosphatase; 24-hour urinary excretion of calcium, hydroxyproline, and *N*-telopeptide, a specific marker of the degradation of bone collagen; and the amount of trabecular bone in biopsy speci-

mens from the anterior iliac crest. In the last six patients enrolled, the bone density of the lumbar spine and the trochanteric region of the femur was measured by dual-energy x-ray absorptiometry (DEXA scan, Hologic, Waltham, Mass.). The effect of bone resorption induced by interferon gamma-1b on bone marrow space was assessed by technetium-99m sulfur colloid scanning of the bone marrow.<sup>11</sup> Computerized tomography of the skull was used to measure the area of the cranial-nerve foramina.

Changes in hematopoiesis were quantified by complete blood counts, including platelet and reticulocyte counts. White-cell phagocyte function was assessed by nitroblue tetrazolium staining of granulocyte-macrophage colonies derived from cultures of peripheral blood.<sup>3</sup> To provide a control group of patients with osteopetrosis who had not been treated with interferon gamma-1b, the records of six patients treated with calcitriol in our clinic for 18 months before entering this study were analyzed to determine the frequency of serious infections. (The frequency of infection in patients referred to us before they had received any therapy was higher, but this sample was biased, since the majority of these patients were referred partly because of their propensity to have infections.) We assessed the incidence of serious infections prospectively in these six patients during interferon gamma-1b therapy. Bacterial infections confirmed by culture and bacterial pneumonia diagnosed radiographically were considered serious infections if the patient was hospitalized or required intravenous antibiotic therapy for five days or more.

#### Analytic Methods

The cores from iliac-crest biopsies were processed as described previously.<sup>3,6</sup> The number of osteoclasts per square millimeter of trabecular-bone-surface area was quantified. The trabecular-bone area (the percentage of the biopsy field occupied by bone) was measured by histomorphometric methods and computerized video analysis (Cue 3, Cue Squared, Tamarac, Fla.).

The granulocyte-macrophage colonies present in vitro after five days of culture were stained with nitroblue tetrazolium.<sup>3,7</sup> The amount of staining was quantified by microdensitometry, with the use of an Olympus IMT inverted microscope and the Cue 2d software and analysis system (Cue Squared).

The serum concentrations of alkaline phosphatase, calcium, and phosphorus were measured with a Technicon AutoAnalyzer (Tarrytown, N.Y.). Urinary calcium, hydroxyproline, and type I collagen cross-linked *N*-telopeptide were measured in aliquots of 24-hour urine samples with the use of a Technicon AutoAnalyzer, radioimmunoassay (Nichols Institute, San Juan Capistrano, Calif.), and enzyme-linked immunosorbent assay,<sup>12</sup> respectively.

#### Statistical Analysis

The results are expressed as means  $\pm$ SD for each analysis period. The Mann-Whitney nonparametric rank test was used to compare results. Correlations between variables were determined with the Pearson product-moment correlation coefficient ( $r_p$ ). All statistical tests were two-tailed.

## RESULTS

During 18 months of therapy, three sets of parents withdrew their children from the study. Two children were withdrawn after 6 and 12 months of therapy because there was no regression of their neurologic disease (blindness), although both had increased bone resorption, an increase in the hemoglobin concentration of more than 1.0 g per deciliter, and an increase in white-cell superoxide production. The third patient, a child who had undergone bone marrow transplantation, was withdrawn from the study after one year of therapy because of lack of improvement.

The 11 patients treated with interferon gamma-1b for 18 months had increases in bone resorption, bone marrow space, and the diameter of optic-nerve foramina.

Urinary excretion of hydroxyproline and *N*-telopeptide increased significantly (Table 2), and at 18 months urinary *N*-telopeptide excretion was correlated with the urinary excretion of calcium ( $r=0.38$ ,  $P=0.001$ ) and hydroxyproline ( $r=0.37$ ,  $P=0.002$ ) and with trabecular-bone area ( $r=-0.28$ ,  $P=0.05$ ). Serum concentrations of calcium, phosphorus, and alkaline phosphatase did not change during therapy.

The net effect of the increase in bone resorption was a significant decrease in trabecular-bone area after 6 and 18 months of therapy (Table 3). The number of osteoclasts did not change, suggesting an increase in the activity of each individual osteoclast. The bone-biopsy specimens obtained before therapy (Fig. 1A and 1C) showed a reduced number of osteocytes, unmineralized cartilage, and poorly mineralized bone. During therapy, the cartilaginous islands decreased in size or disappeared and the bone spicules were more uniformly stained, suggesting more normal mineralization (Fig. 1B and 1D). Bone density, measured in the last six patients enrolled, decreased in all areas studied (Table 3), although the decrease was statistically significant only at the lumbar spine ( $P=0.05$ ).

Computed tomography revealed a significant increase in the cross-sectional area of the optic foramina and auditory canals ( $P=0.05$ ) during therapy (Table 3). No patient recovered vision, but one patient had an increase in hearing of more than 40 dB. No patient had a reduction in hearing, vision, or facial-nerve function during therapy.

The sequential bone marrow scans showed an increase in the uptake of radionuclide indicative of an in-

Table 3. Evidence of Bone Resorption in Patients with Osteopetrosis Treated with Interferon Gamma-1b for 18 Months.\*

INDEX	BEFORE TREATMENT	AFTER 6 MONTHS OF TREATMENT	AFTER 18 MONTHS OF TREATMENT
Analysis of bone biopsy			
Trabecular-bone area (% of total biopsy specimen)	55.3±15.0 (12)	33.2±18.0 (12)†	34.5±3.5 (6)‡
No. of osteoclasts/mm <sup>2</sup> of bone-surface area	0.18±0.10 (12)	0.20±0.38 (12)	0.14±0.20 (6)
Bone mineral density measurements			
Right trochanter (g/cm <sup>2</sup> )	1.93±1.85 (6)	0.87±2.33 (6)	1.52±0.73 (6)
Lumbar vertebrae, L1-L4 (g/cm <sup>2</sup> )	1.26±0.95 (6)	1.08±0.39 (6)	1.05±0.17 (6)§
Cranial-nerve foramina			
Area of optic foramen	7.3±5.9 (14)	9.0±3.4 (14)	14.2±2.9 (11)§
Area of auditory canal	9.4±1.7 (14)	10.3±2.2 (14)	14.6±5.9 (11)§

\*Data are expressed as means ±SD. Values in parentheses indicate the number of patients for whom results were available. Only 6 of 14 patients had measurements of bone density at all scheduled times (at base line and at 6 and 18 months). P values given below are for the comparison with the corresponding base-line value (before interferon gamma-1b treatment) and were calculated with the use of the Mann-Whitney nonparametric rank test.

† $P=0.002$ .

‡ $P=0.02$ .

§ $P=0.05$ .

crease in the volume of bone marrow in each patient. Representative radionuclide scans of the bone marrow from two patients are shown in Figure 2. Along with the increase in marrow space, the mean hemoglobin concentration increased from 7.5 to 10.5 g per deciliter ( $P=0.05$ ) (Table 2), and the mean white-cell count increased from 7800 to 13,500 per cubic millimeter. Nine of the 11 patients treated for 18 months had increases in their platelet counts of more than 50,000 per cubic millimeter; the 2 patients with no increase in the platelet count had massive splenomegaly. These two patients underwent splenectomy, after which their platelet counts rose threefold (the final counts were 300,000 and 150,000 per cubic millimeter). Of the five patients who were dependent on transfusions at the beginning of therapy, only one was still transfusion-dependent after 18 months, and in this patient the frequency of transfusions was reduced from once every week to less than once every 6 months.

The difference between the height of the nine children in the study and that of normal children of the same age (expressed as the number of SDs below [-] or above [+] the normal value) decreased after therapy with interferon gamma-1b (from  $-3.2±1.7$  SD to  $-2.7±0.9$  SD;  $P=0.43$ ). In untreated children, an increase in the difference from normal children (i.e., a greater negative value of the standard-deviation score) would be expected.

The mean production of oxygen-derived free radicals by granulocyte-macrophage colonies increased during therapy ( $P<0.001$ ) (Table 2). Before therapy, the mean ( $±$ SD) number of serious infections was  $5.5±4.9$  per patient per year. These infections included 9 episodes of sepsis, 28 episodes of pneumonia (diag-

Table 2. Laboratory Studies in Patients with Osteopetrosis Treated with Interferon Gamma-1b for 18 Months.\*

TEST	BEFORE TREATMENT	AFTER 6 MONTHS OF TREATMENT	AFTER 18 MONTHS OF TREATMENT
Markers of bone resorption			
Serum calcium (mg/dl)	9.0±0.8 (14)	9.4±0.7 (14)	9.1±0.3 (11)
Serum alkaline phosphatase (IU/liter)	366±501 (14)	282±339 (14)	431±719 (11)
Urinary calcium (g/g of creatinine)	0.19±0.26 (14)	0.71±0.79 (14)†	0.50±0.60 (11)‡
Urinary hydroxyproline (mg/g of creatinine)	0.18±0.15 (14)	0.51±0.33 (11)§	0.31±0.16 (11)†
Urinary <i>N</i> -telopeptide ( $μ$ mol/mol of creatinine)	305±486 (11)	1209±914 (11)§	773±571 (11)†
Hematologic values			
Hemoglobin (g/dl)	7.5±2.9 (14)	9.4±0.4 (14)	10.5±0.3 (11)†
Platelets ( $×10^{-3}/mm^3$ )	63±54 (14)	135±97 (14)¶	187±127 (11)†
White cells ( $×10^{-3}/mm^3$ )	7.8±4.9 (14)	11.7±10.9 (14)	13.5±13.9 (11)
Nitroblue tetrazolium reduction (optical-density units)	0.26±0.07 (14)	0.59±0.34 (14)¶	0.41±0.03 (11)¶

\*Data are expressed as means ±SD. Values in parentheses indicate the number of patients for whom results were available. All P values given below are for comparisons with base-line values (before interferon gamma-1b treatment) and were calculated with the use of the Mann-Whitney nonparametric rank test. To convert serum calcium values to millimoles per liter, multiply by 0.2495.

† $P=0.05$ .

‡ $P=0.065$ .

§ $P=0.01$ .

¶ $P=0.02$ .

|| $P<0.001$ .

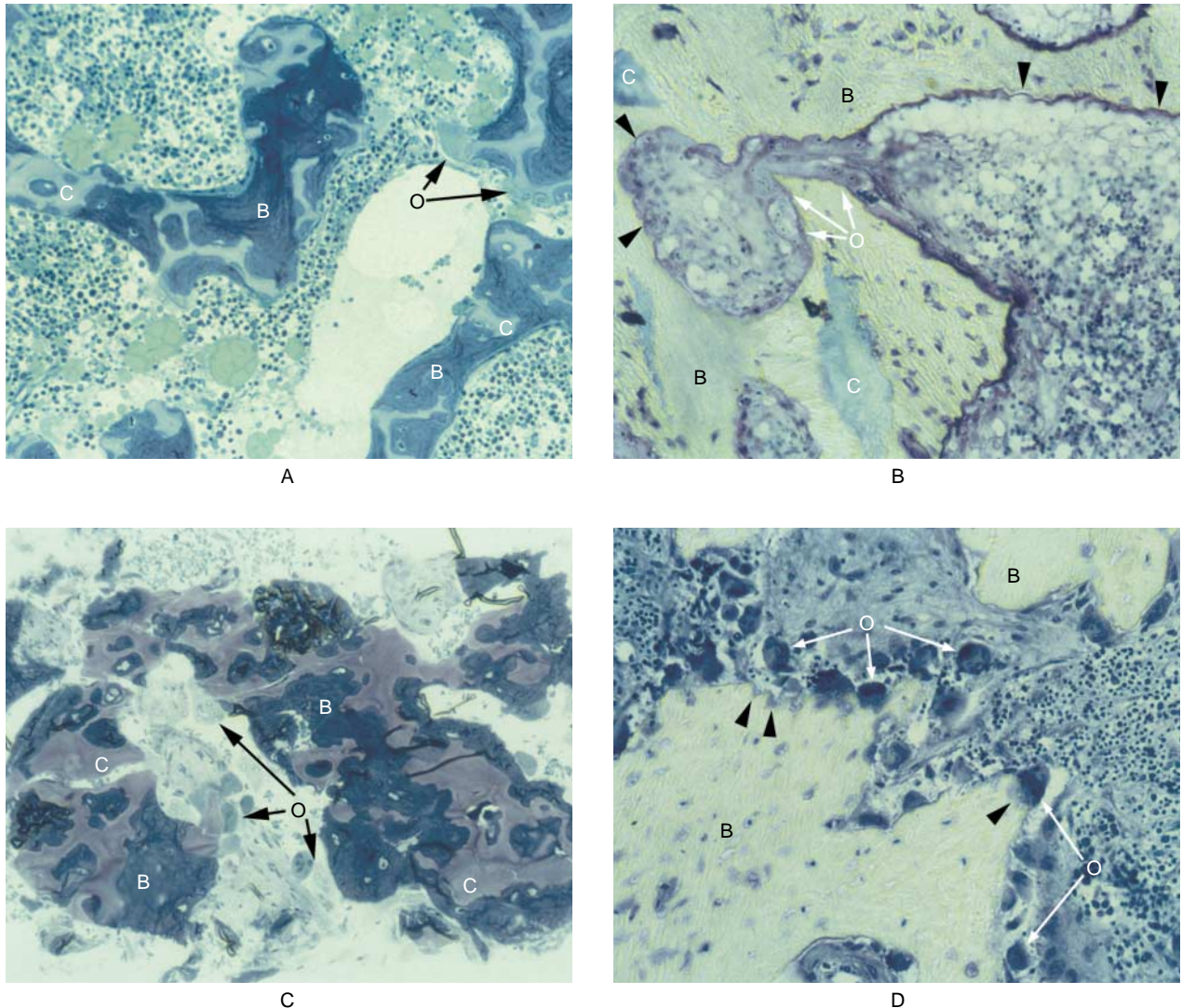


Figure 1. Bone-Biopsy Specimens of the Anterior Iliac Crest from Two Patients with Osteopetrosis before and after 18 Months of Treatment with Interferon Gamma-1b (Toluidine Blue Stain,  $\times 140$ ).

Panel A shows a 1- $\mu\text{m}$ , Epon-embedded section of a pretreatment biopsy specimen from a seven-month-old boy. Intensely stained, mineralized trabecular bone (B) and streaks of lightly stained cartilage (C) are visible. A few osteoclasts (O) line the bone surface, with no apparent formation of Howship's lacunae. Panel B shows a 5- $\mu\text{m}$ , methyl-methacrylate-embedded section of a specimen obtained from the same patient after 18 months of therapy. A combination of coarse trabecular bone and porous cortical bone (B), lightly stained and mineralized, and islands of cartilage (C) are visible. Osteoclasts (O) have formed numerous lacunae (arrowheads), giving the bone surface a scalloped appearance. Note that the scalloped bony margins of the marrow space are intensely stained, whereas osteoblasts and osteoid deposition are absent. Panel C shows a 1- $\mu\text{m}$ , Epon-embedded section of a pretreatment biopsy specimen from an 11-month-old boy. Coarse trabecular bone (B) with cartilaginous streaks (C), similar to those in Panel A, is evident. Lacunae are absent, even though osteoclasts (O) adjoin the bone surface. Bone marrow cells are absent in this section. Panel D shows a 5- $\mu\text{m}$ , methyl-methacrylate-embedded section obtained by biopsy from the same patient after 18 months of therapy. A combination of mineralized coarse trabecular bone and porous cortical bone (B), lined with numerous osteoclasts (O) with brush borders and adjoining lacunae (arrowheads), is visible. Islands and streaks of cartilaginous tissue are absent.

nosed radiographically), 3 cases of osteomyelitis, 8 urinary tract infections, and 1 case of cellulitis at the entry site of a central venous catheter. During the 18-month treatment period, the mean frequency of infections decreased to  $0.2 \pm 0.4$  per patient per year ( $P = 0.02$ ). No patient had septicemia during therapy. Three patients had chronic mandibular osteomyelitis on entry into the study. During therapy, one of the three patients had a

complete remission, one had no progression of osteomyelitis, and the third had evidence of revascularization of areas of necrotic bone.

All the patients had at least one episode of an elevation in temperature above  $38^\circ\text{C}$  and of mild, transient diarrhea during the first six months of therapy. Two patients had severe hyperpyrexia (temperatures higher than  $40^\circ\text{C}$ ), and one patient had severe di-

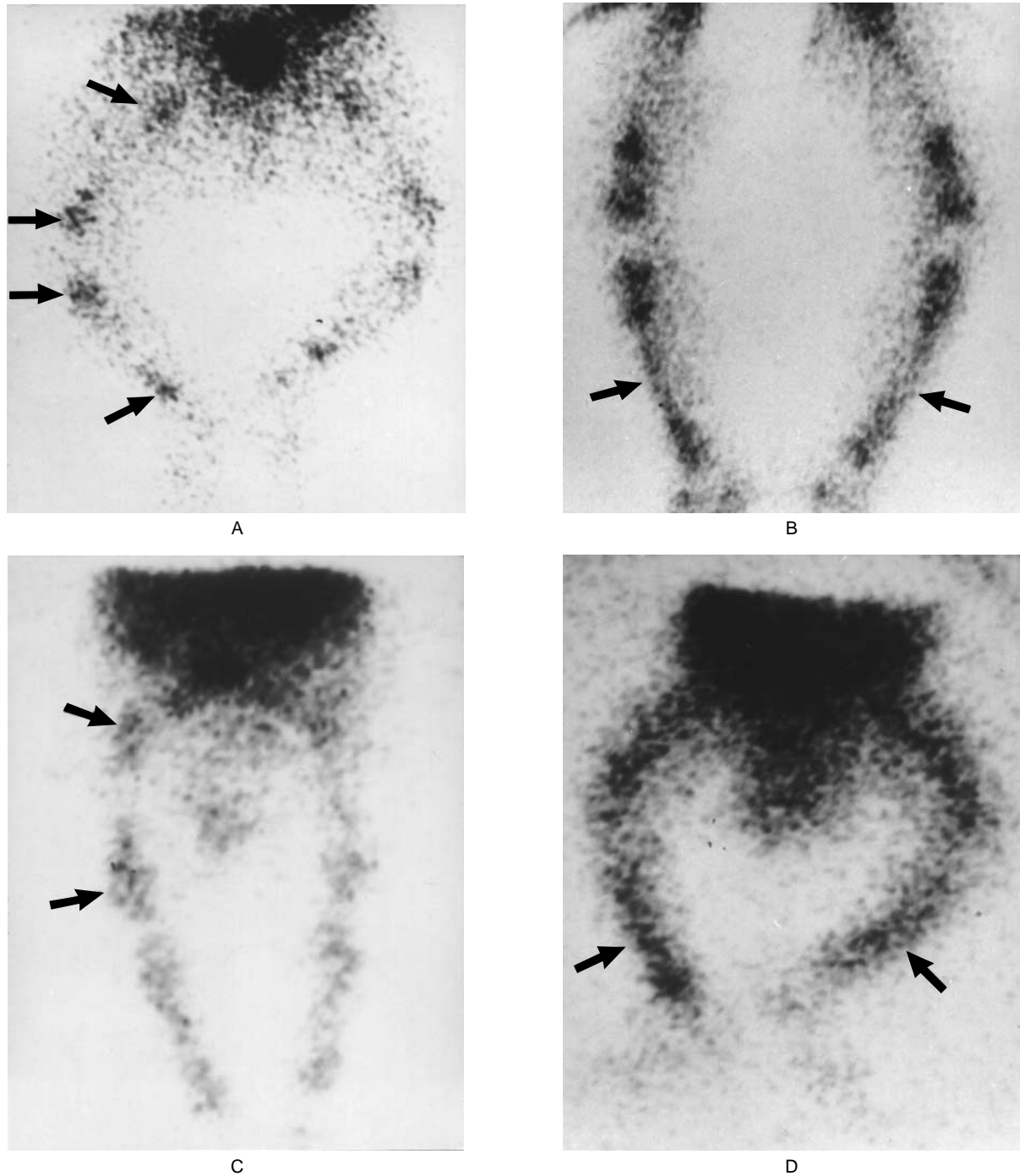


Figure 2. Technetium Tc 99m Sulfur Colloid Scans of the Bone Marrow in Two Patients with Osteopetrosis before and after 18 Months of Therapy with Interferon Gamma-1b.

Panels A and C, pretreatment scans of the patients whose biopsy specimens were shown in Figure 1, show uptake of radionuclide in the bone marrow of the long bones of the lower extremities. In both patients, radionuclide uptake is confined to the subepiphyseal regions (arrows). Panels B and D, obtained from the same patients after 18 months of therapy with interferon gamma-1b, show an increase in radionuclide uptake in the bone marrow. Note especially the increase in labeling of the diaphyseal region of the tibia in both patients (arrows).

arrhea; both side effects disappeared after a 50 percent reduction in the dose of interferon gamma-1b. Two patients had hypocalcemic tetany; each responded to a 25 percent increase in calcium intake with a rise in serum calcium concentrations to more than 8.0 mg per deciliter (2.0 mmol per liter). No patient re-

ported any major side effects after the first six months of therapy.

#### DISCUSSION

This study confirms and extends our earlier report of the efficacy of interferon gamma-1b in patients with

osteopetrosis.<sup>3</sup> Treatment with interferon gamma-1b for 18 months stabilized or improved the conditions of all 11 patients treated for the full 18 months, and none of the 14 patients treated for at least 6 months died.

In osteopetrosis, the ability of osteoclasts to resorb bone is defective. Despite data suggesting that interferon gamma-1b reduces bone resorption *in vitro* and when given to animals with hypercalcemia of malignancy,<sup>13-15</sup> all our patients with osteopetrosis had sustained biochemical evidence of increased bone resorption. Bone biopsies (in 12 patients) showed decreases in the area of trabecular bone. These results are consistent with the increase in bone resorption reported in normal rats given interferon gamma-1b.<sup>16</sup> We hypothesize that interferon gamma-1b stimulated the production of osteoclastic superoxide, which increased the degradation of bone matrix by osteoclasts.<sup>17-20</sup>

The decrease in the number of infections during therapy with interferon gamma-1b, in association with the increase in superoxide production by granulocyte-macrophage colonies, provides evidence that patients with osteopetrosis have a correctable defect in white-cell phagocyte function. The patients' improvement, along with their improved bone resorption, appears to be responsible for the stabilization of the osteopetrotic condition. Although patients had minor side effects, they were treatable, transient, and tolerable, and it is possible that lower doses of interferon gamma-1b would be equally effective. Thus, treatment with interferon gamma-1b provides both a reasonable therapeutic option for patients with osteopetrosis who are not candidates for bone marrow transplantation and an opportunity to stabilize the clinical condition of patients awaiting transplantation.

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