Induction of De Novo Bone Formation in the Beagle

A Novel Effect of Aluminum

L. D. Quarles, H. J. Gitelman,* and M. K. Drezner

*Departments of Medicine, Physiology, and Pathology, Duke University Medical Center, Durham, North Carolina 27710; and *Department of Medicine, University of North Carolina, Chapel Hill, North Carolina 27514*

Abstract

To define the primary effects of aluminum on bone in the mammalian species, we examined the dose/time-dependent actions of aluminum in normal beagles. Administration of low dose aluminum (0.75 mg/kg) significantly elevated the serum aluminum (151.7±19.9 μg/liter) compared with that in controls (4.2±1.35 μg/liter) but did not alter the calcium, creatinine, or parathyroid hormone. After 8 wk of therapy, bone biopsies displayed reduced bone resorption (2.6±0.63 vs. 4.5±0.39%) and osteoblast covered bone surfaces (2.02±0.51 vs. 7.64±1.86%), which was indicative of low turnover. In contrast, prolonged treatment resulted in increased bone volume and trabecular number (38.9±1.35 vs. 25.2±2.56% and 3.56±0.23 vs. 2.88±0.11/mm) which was consistent with uncoupled bone formation.

Administration of higher doses of aluminum (1.20 mg/kg) increased the serum aluminum further (1242.3±259.8 μg/liter) but did not affect calcium, creatinine, or parathyroid hormone. However, after 8 wk of treatment, bone biopsies displayed changes similar to those after long-term, low-dose therapy. In this regard, an increased trabecular number (3.41±0.18/mm) and bone volume (36.5±2.38%) again provided evidence of uncoupled bone formation. In contrast, in this instance poorly mineralized woven bone contributed to the enhanced bone volume. High-dose treatment for 16 wk further enhanced bone volume (50.4±4.61%) and trabecular number (3.90±0.5/mm).

These observations illustrate that aluminum may stimulate uncoupled bone formation and induce a positive bone balance. This enhancement of bone histogenesis contrasts with the effects of pharmacologic agents that alter the function of existing bone remodeling units.

Introduction

Recent studies have identified aluminum as an agent which can profoundly influence bone turnover (1–13). However, a variety of discordant observations, as well as the complex clinical circumstances in which aluminum effects have been observed (10, 12, 14), have precluded establishing with certainty the primary effects of aluminum on bone. We designed the present study in order to examine the dose/time-dependent effects of aluminum administration on bone histology in the normal beagle, an animal model which displays dynamic bone remodeling similar to that in man. Our data indicate that aluminum treatment induces de novo bone histogenesis, an unprecedented pharmacologic event.

Methods

Study protocol. 18 2-yr-old male beagles, weighing ~ 15 kg, were randomly divided into three groups. During the study all animals were housed under identical conditions in standard kennel runs and were fed a normal diet containing 1.2% calcium, 0.6% phosphorus, and 2,200 U/kg (of diet) vitamin D_3 (Purina Lab Chow, Ralston-Purina Co., St. Louis, MO). The vitamin D supplementation maintained normal 25 hydroxyvitamin D levels in each group throughout the study (69.1±4.5, 65.2±4.7, and 57.7±19.2 ng/ml, respectively). The group 1 dogs received 0.75 mg elemental aluminum/kg as aluminum chloride intravenously three times per week for 16 wk while we administered a higher dose of aluminum, 1.20 mg/kg, to group 2 beagles over a similar period. At the end of 8 and 16 wk of study, we biopsied the bones of dogs from both groups 1 and 2. In addition, at similar time intervals we obtained plasma for measurement of total and ionized calcium, phosphorus, creatinine, PTH, alkaline phosphatase, and aluminum. We employed bone biopsies and plasma measurements from age-matched beagles treated with pH-matched sodium chloride (as vehicle) (group 3) as normative values for comparison in these studies.

Biochemical studies. Plasma calcium, phosphorus, creatinine, and alkaline phosphatase were measured by colorimetric techniques using an autoanalyzer (Technicon Instruments Corp., Tarrytown, NY). The ionized calcium concentration was determined using an ICA1 analyzer (Radiometer Inc., Copenhagen, Denmark), and values were corrected to a pH of 7.4.

We measured the plasma PTH concentration using a radioimmunoassay kit (Nichols Laboratory, San Juan Capistrano, CA) designed to measure the amino-terminal portion of the molecule according to previously described methods (15). We validated assay sensitivity by demonstrating that the PTH concentration in beagles (24.2±2.2 ng/ml) decreased significantly after parathyroidectomy (12±2.0 ng/ml), and increased upon induction of vitamin D deficiency (71±2.0 ng/ml).

We assayed the plasma aluminum concentration according to previously described methods (16) using a flameless atomic absorption spectrophotometer (Perkin-Elmer Corp., Instrument Div., Norwalk, CT) with a graphite furnace. Interference was minimized by using a diluent containing ammonium hydroxide. During periods of aluminum chloride treatment, plasma was obtained for assay 24 h after intravenous administration of the drug.

Bone studies. After 8 and 16 wk of study transcortical bone biopsies were obtained alternately from the left and right anterior iliac crest of group 1 and 2 dogs under general anesthesia. In order to minimize variability due to variation of the biopsy site, we reproducibly procured the bone samples from an area ~ 8 mm from the anterior iliac crest. We administered tetracycline HCL (15 mg/kg i.v.) to each dog over a 48 h period from day 21 to day 19 before biopsy and Calcein (15 mg/kg

Part of this work has appeared in abstract form in *Kidney Int.* 29:169:1986.

Address all correspondence to Dr. L. Darryl Quarles, Box 3285, Duke University Medical Center, Durham, NC 27710.

Received for publication 21 April 1987 and in revised form 2 November 1987.

J. Clin. Invest.

0021-9738/88/04/1056/11 $2.00
Volume 81, April 1988, 1056–1066

Table I. Plasma Biochemistries in Control and Aluminum-treated Beagles

	Control	Low dose aluminum		High dose aluminum	
		8 wk	16 wk	8 wk	16 wk
Ionized calcium (*mmol/liter*)	1.31±0.02*[a]	1.28±0.02[a]	1.28±0.01[a]	1.29±0.03[a]	1.30±0.01[a]
Total calcium (*mmol/liter*)	2.5±0.05[a]	2.4±0.01[a]	2.4±0.05[a]	2.5±0.04[a]	2.3±0.10[a]
Phosphorus (*mmol/liter*)	1.52±0.09[c]	1.32±0.08[b]	1.20±0.13[ab]	1.25±0.05[b]	1.1±0.06[a]
Creatinine (*mg/dl*)	0.7±0.08[a]	0.7±0.06[a]	0.7±0.02[a]	0.8±0.04[a]	1.0±0.04[b]
Alkaline phosphatase (*IU/liter*)	30.8±3.54[a]	36.8±5.84[a]	24.0±3.42[a]	48.1±7.59[a]	38.8±8.0[a]
Parathyroid hormone (*pg/ml*)	27.1±3.6[a]	20.3±1.1[a]	22.7±3.0[a]	17.4±3.8[a]	16.1±2.2[a]
Calcitriol (*pg/ml*)	37.2±5.0[a]	61.2±7.2[a]	70.6±9.4[a]	63.0±15.4[a]	31.1±5.6[a]

Data were compared by one-way ANOVA and Tukey's multiple range test. Values that do not share a common superscript are significantly different at $P < 0.05$. * Values represent mean±SEM.

i.v.) from day 6 to day 4 before biopsy. Bone specimens were fixed in ethanol and embedded in methyl methacrylate, unstained or prestained by the methods of Villanueva (17). 20- and 5-μm sections from the iliac crest specimens were prepared for histomorphological examination according to previously published methods (14). Staining for aluminum was performed on 5-μm prestained and unstained sections by a modification of methods reported by Maloney et al. (18). Bone aluminum content was measured as previously described (14).

Histomorphometric analysis of both static and dynamic parameters of trabecular bone remodeling was performed utilizing a semiautomated system (Osteoplan, Carl Zeiss Inc., Thornwood, NY) as detailed elsewhere (19). Supplemental analysis was accomplished as necessary by using an integrated reticle (Merz-Schenk, Wild, Heerbrugg, Switzerland) (20). The following histologic functions were quantitated: (*a*) trabecular bone volume (Tb.BV/TV), the percentage of the sample composed of trabecular bone; (*b*) relative lamellar osteoid volume (LOV/BV), the percentage of trabecular bone volume composed of unmineralized lamellar bone; (*c*) woven osteoid volume (WOV/BV), the percentage of the trabecular bone volume consisting of woven osteoid; (*d*) fractional osteoid surface (OS/BS), the percentage of the trabecular bone surface covered by osteoid; (*e*) mean osteoid seam width (MOSW); (*f*) resorptive surface (RS/BS), the percentage of nonosteoid covered trabecular bone surface on which Howship's lacunae are present; (*g*) osteoblastic surface (Ob.S/BS), the percentage of trabecular bone surface on which osteoblast-covered osteoid is present; (*h*) osteoblastic index (OBI), the number of osteoblasts per 10 cm of bone surface; (*i*) volume of marrow fibrosis (Fib/TV), the percentage of fibrosis per total tissue volume; (*j*) mineral apposition rate (MAR)[1], the average interlabel distance divided by the days elapsed between the administration of the fluorescent labels; (*k*) labeled fraction of osteoid (MS/OS), referred to as mineralization front and defined as the percentage of osteoid-covered trabecular bone surface exhibiting a fluorescent second label with a minimum width of 3 μm; (*l*) labeled fraction of trabecular bone (MS/BS), referred to as mineralizing surface and defined as the percentage of mineralized bone surface exhibiting a fluorescent second label with a minimum width of 3 μm; and (*m*) aluminum surface (Al/BS), the percentage of trabecular bone surface bearing aluminum stain. In addition, we calculated the following functions according to previously published methods: (*a*) corrected mineral apposition rate (cMAR), the rate of mineral apposition averaged throughout the lifespan of the osteoid seams (14); (*b*) mineralization lag time (MLT), a measure of the time (in days) during the lifespan of an osteoid moiety when mineralization is not occurring (14); (*c*) tissue based mineralized bone formation rate (BFR/TV), the volume of mineralized bone formed per year (14); and (*d*) the mean trabecular number (Tb.N), a derived index related to bone volume by the following formula: bone volume = mean trabecular thickness × mean trabecular number (21).

Statistical analysis. Results are expressed as the mean±SEM. Statistical evaluation for differences between groups was done using one-way analysis of variance with *a posteriori* testing by the Tukey multiple comparison procedure. We chose this approach after preliminary evaluation of the data employing two-factor analysis of variance (aluminum dose and duration of therapy) revealed important interactions between the factors for many of the dependent variables studied. Since the presence of such significant interaction prevents meaningful interpretation about the independent factors and allows conclusions about combinations of dose and duration only, use of one-way analysis of variance with each combination of dose and duration classified as a separate treatment results in statistical data identical to those obtained after two-way analysis of variance with *a posteriori* testing of important interactions (22). All computations were performed with the Statgraphics software package (Statistical Graphics Corp., Inc., Princeton, NJ) employing an IBM PC-AT computer.

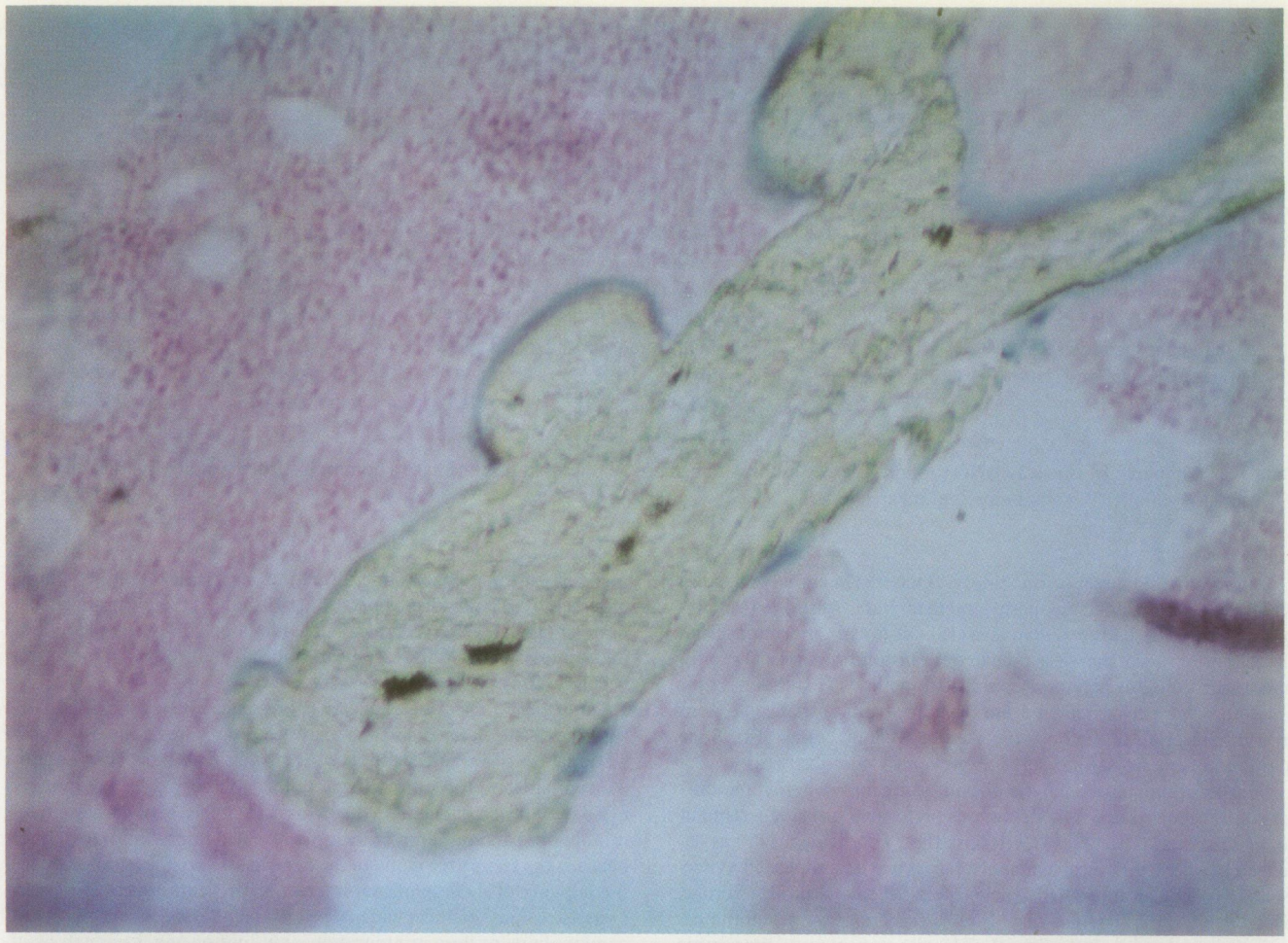
Materials. The authentic 1,25 dihydroxyvitamin D_3 (1,25$[OH]_2D_3$) used in the assay for this metabolite was a gift from Dr. Milan Uskokovic, Hoffmann-La Roche, Inc. (Nutley, NJ). [^{3}H]1,25$(OH)_2D_3$ (90 Ci/mmol) was purchased from Amersham Corp. (Arlington Heights, IL). Adult beagles were obtained from Hazelton Research Animals Corp. (Cumberland, VA).

Results

Low dose aluminum. Administration of aluminum at a relatively low dose resulted in a significant elevation ($P < 0.001$) of the serum aluminum concentration in treated beagles (151.7±19.9 μg/liter) compared with that in controls (4.2±1.35 μg/liter), but did not significantly alter the serum total and ionized calcium, creatinine, immunoactive PTH, or alkaline phosphatase after 8 or 16 wk of treatment (Table I). However, the serum phosphorus concentration declined significantly at each interval. The serum calcitriol levels tended to increase but the levels were not significantly different from those of controls (Table I).

Quantitative histomorphologic analysis of bone biopsies revealed unique abnormalities after both 8 and 16 wk of treatment. At the earlier time, representative sections from the biopsies of treated dogs (Tables II and III) displayed a reduction of bone resorption and osteoblast-covered bone surfaces, changes characteristic of a low turnover state. In addition,

1. *Abbreviations used in this paper:* BFR/TV, tissue based mineralized bone formation rate; MAR, mineral apposition rate; cMAR, corrected MAR; MLT, mineralization lag time; MS/OS, labeled fraction of osteoid.



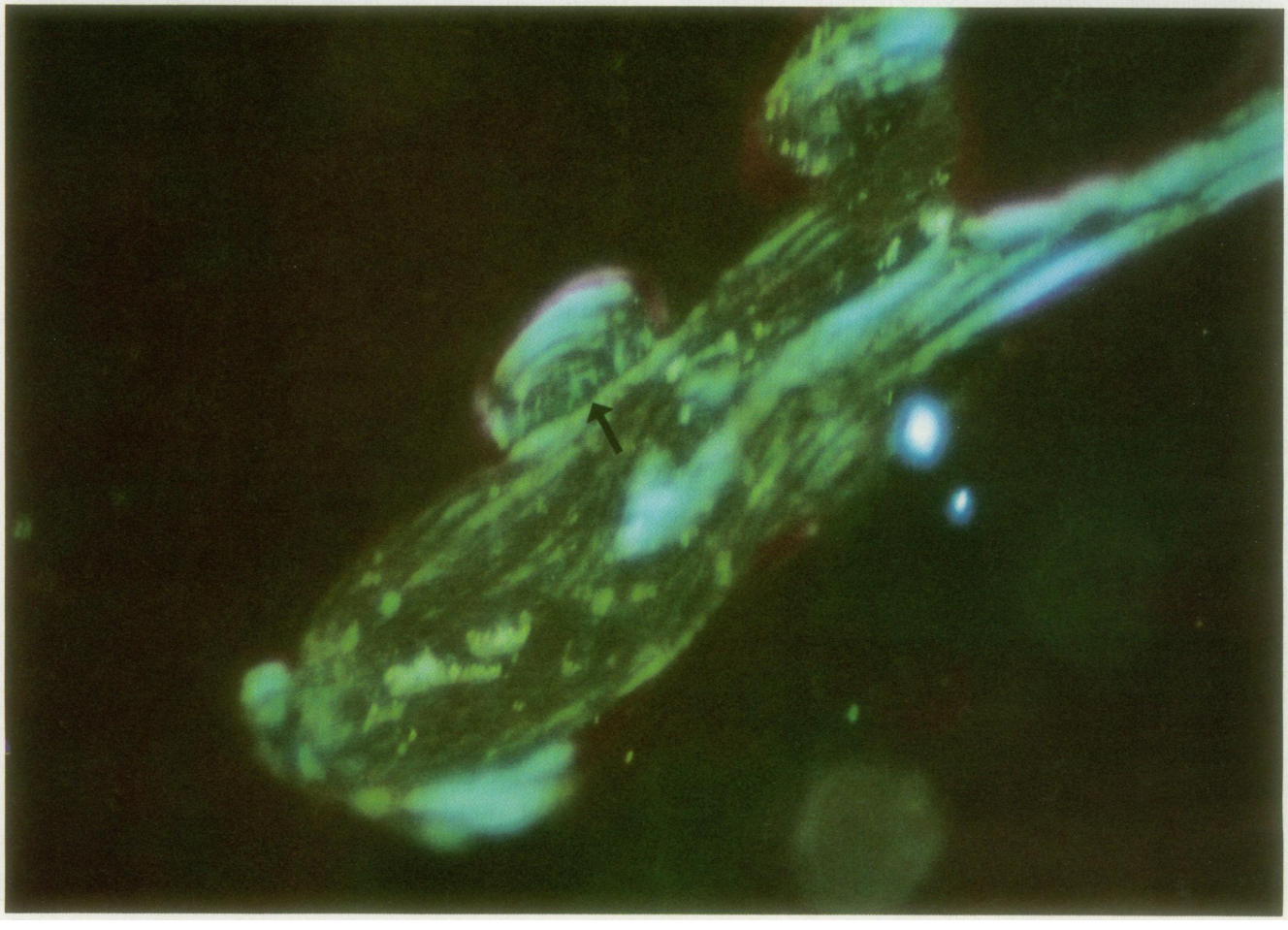


Table II. Static Indices of Trabecular Bone in Control and Aluminum-treated Beagles

	Control	Low dose aluminum		High dose aluminum	
		8 wk	16 wk	8 wk	16 wk
Trabecular bone volume (Tb.BV/TV) (%)	25.2±2.56*[a]	32.3±1.35[ab]	38.9±5.14[b]	36.5±2.38[b]	50.4±4.61[c]
Trabecular number (TB.N/*mm*)	2.88±0.11[a]	2.72±0.10[a]	3.56±0.23[bc]	3.41±0.18[b]	3.90±0.50[c]
Lamellar osteoid volume (LOV/BV) (%)	3.4±0.68[a]	1.8±0.28[a]	3.68±0.48[a]	3.89±0.95[a]	5.26±0.80[a]
Woven osteoid volume (WOV/BV) (%)	0[a]	0[a]	0[a]	11.5±4.58[b]	2.43±0.96[a]
Osteoid surface (OS/BS) (%)	16.9±2.50[a]	12.2±1.07[a]	18.9±2.54[a]	37.2±6.71[b]	35.9±3.16[b]
Mean osteoid seam width (MOSW) (*μm*)	9.92±0.842[a]	10.3±0.391[a]	12.6±0.74[a]	22.2±2.91[b]	18.1±1.76[b]
Osteoblastic surface (Ob.S/BS) (%)	7.64±1.86[c]	2.02±0.51[a]	4.38±0.75[b]	7.09±1.74[bc]	6.05±1.53[bc]
Resorptive surface (RS/BS) (%)	4.5±0.39[c]	2.6±0.63[b]	3.4±0.76[bc]	1.1±0.41[a]	0.6±0.19[a]
Osteoblast index (OBI) (No./10 *cm*)	240.8±52.5[b]	69.7±22.8[a]	174.1±33.3[b]	807.7±150.6[c]	918.3±313.1[c]
Aluminum surface (AL/BS) (%)	0[a]	8.9±2.2[b]	23.8±1.7[c]	25.4±2.8[c]	33.9±2.4[d]
Bone aluminum content (*μg/g*)	2.2±0.4[a]	65.8±7.7[b]	161.7±25.2[d]	125.2±14.9[c]	152.2±7.62[cd]
Marrow fibrosis (Fib/TV) (%)	0[a]	0[a]	0.63±0.43[a]	30.1±11.3[b]	31.3±13.9[b]

Data were compared by one-way ANOVA and Tukey's multiple range test. Values that do not share a common superscript are significantly different at $P < 0.05$. * Values represent mean±SEM.

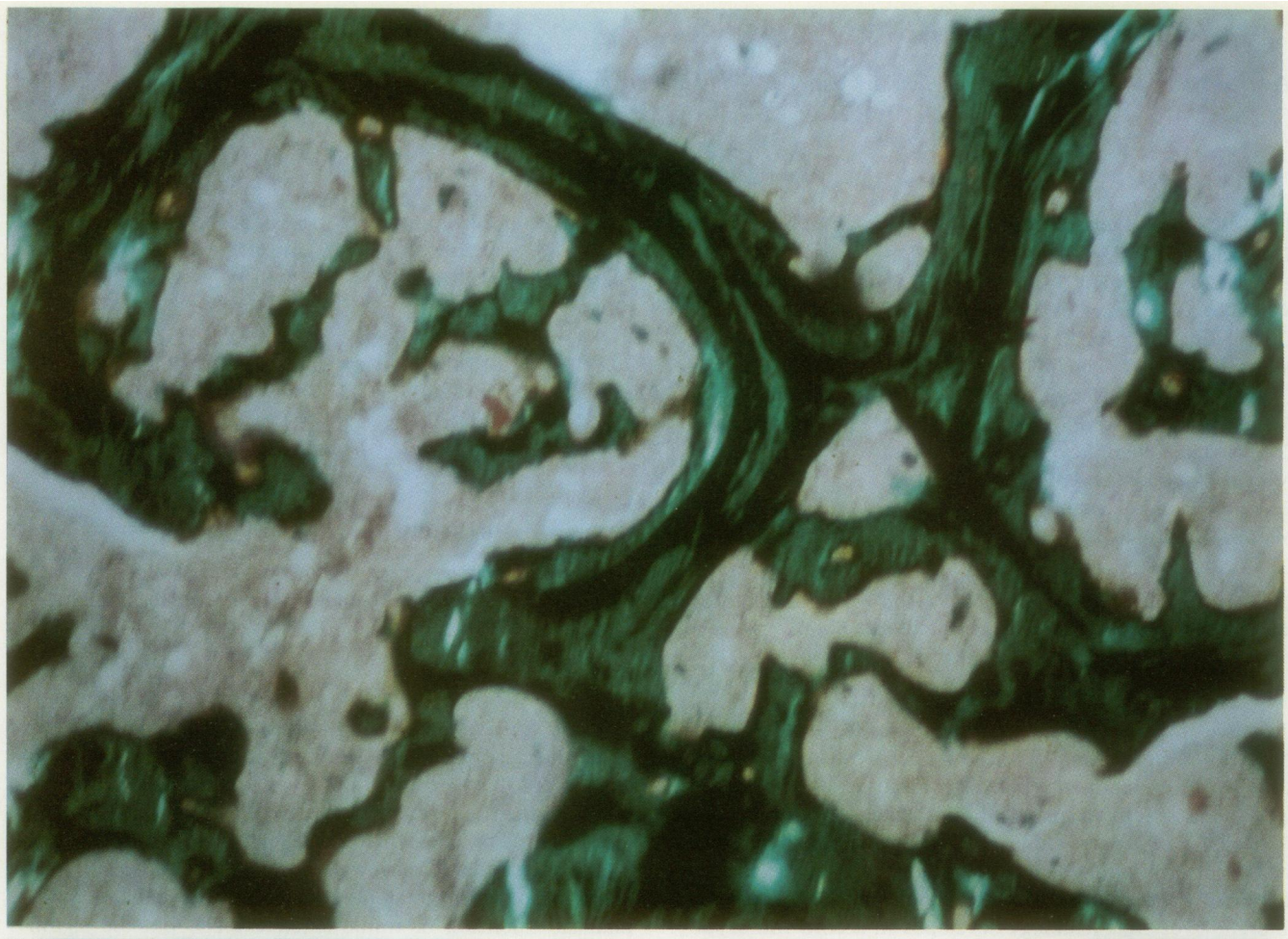
defective mineralization, evidenced by a decreased MS/OS, occurred in association with the reduced osteoblast population. However, maintenance of osteoid surface and volume, as well as seam width, in spite of diminished tetracycline labeling, indicated that a proportional inhibition of osteoid synthesis had occurred. Commensurate with these changes was that the mineralized bone formation rate significantly decreased. The alterations of bone histology developed in accord with a substantial increment of the bone aluminum content compared with that of controls (Table II). Moreover, histological staining revealed that the diminished bone turnover developed in association with aluminum covering 9.3±2.8% of the bone surfaces.

Surprisingly, the continued administration of aluminum for up to 16 wk produced alterations of bone histology which sharply contrasted with those evidenced at 8 wk. Biopsies from treated dogs displayed evidence of de novo bone formation marked by buds of new lamellar bone appearing at previously inactive surfaces (Fig. 1) and arborization of trabeculae (Fig. 2). Consistent with these observations was a significant increase of bone volume and trabecular number compared with that in biopsies of controls. These changes occurred in concert with restoration of the osteoblast population, improvement of bone resorptive activity, and early evidence of peritrabecular fibrosis (Table II). Similar to specimens from 8 wk, however, 16-wk biopsies displayed a persistence of excessive inactive osteoid, maintaining a diminished MS/OS. However, BFR/TV was not decreased (Table III) since the total bone surface exhibiting mineralization remained normal. These histological changes were associated with a further increment of bone aluminum content and bone surface aluminum compared with those in 8-wk-treated animals and controls (Table II).

High dose aluminum. Administration of the higher dose of aluminum increased the serum aluminum concentration (1,242.3±259.8 μg/liter) to a level significantly greater ($P < 0.001$) than that in controls, as well as dogs treated with a lower dose ($P < 0.001$). However, high dose treatment for 8 and 16 wk, similar to the low dose regimen, did not result in an alteration of serum total and ionized calcium, alkaline phosphatase, calcitriol, or immunoactive PTH, but did significantly decrease the serum phosphorus. In addition, beagles receiving high dose therapy for 16 wk experienced a modest, but significant, elevation of serum creatinine levels (Table I).

After 8 wk of treatment bone biopsies displayed changes similar to those evidenced after 16 wk of low dose therapy. In this regard, a more abundant arborization of trabecular bone and consequent increased trabecular number and bone volume again provided evidence of enhanced de novo bone formation (Fig. 3, Table II). In this instance, however, poorly mineralized woven bone (osteoid) constituted the majority of the newly synthesized tissue, comprising 11.5±4.6% of the bone volume (Fig. 4). Deposition of tetracycline in wide bands at the woven osteoid bone interface and as broad isolated foci marked the abnormal mineralization within this tissue (Table III). Consistent with this, the cMAR was decreased while estimates of MLT revealed increased values (Table III). The presence of woven bone and a coincident increase in the number of osteoblasts within the tissue (Table II) indicate that an accelerated rate of matrix synthesis underlies the new bone formation. Indeed, the concomitant appearance of marrow fibrosis provides further evidence that aluminum stimulation of mesenchymal cell proliferation (and differentiation) is responsible for the observed changes. In any case, aluminum had no effect on lamellar osteoid since this tissue persisted in normal

Figure 1. Microscopic appearance of bone obtained from a beagle treated with low-dose aluminum for 16 wk. (*Top*) Villanueva-stained specimen showing protuberances of bone projecting beyond the normal trabecular contour. Blue-stained osteoid covers these "buds" of bone. (*Bottom*) Same section viewed under polarized light demonstrates that the excrescences of bone maintain a lamellar structure. In addition, the cement line (*arrow*) is unscalloped, suggesting uncoupled bone formation. × 200.



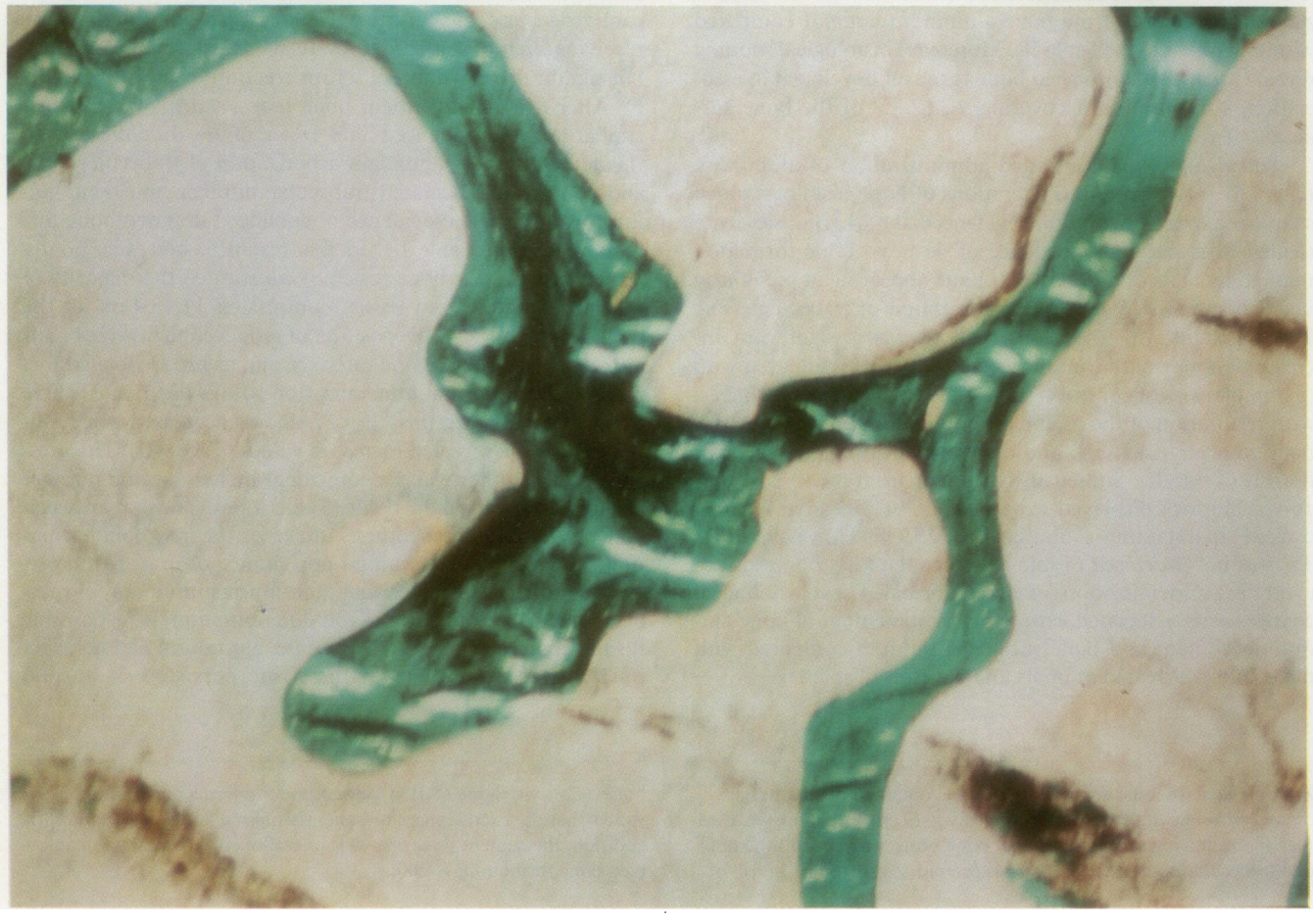


Table III. Dynamic Indices of Trabecular Bone in Control and Aluminum-treated Beagles

	Control	Low dose aluminum		High dose aluminum	
		8 wk	16 wk	8 wk	16 wk
Mineral apposition rate [MAR] ($\mu m/d$)	0.69±0.08[a]	0.82±0.12[a]	0.97±0.16[a]	0.92±0.08[a]	1.03±0.14[a]
Corrected mineral apposition rate [cMAR] ($\mu m/d$)	0.50±0.06[b]	0.42±0.06[b]	0.44±0.07[b]	0.17±0.04[a]	0.16±0.09[a]
Mineralization lag time [MLT] (d)	21.1±2.9[a]	26.6±3.8[ab]	30.8±4.2[b]	289.0±113.1[c]	204.4±65.4[c]
Mineralization front [MS/OS] (%)	71.9±2.92[c]	51.9±5.6[b]	46.0±4.2[b]	15.3±3.0[a]	14.6±6.3[a]
Mineralizing surface [MS/BS] (%)	8.9±1.1[b]	5.7±0.8[a]	6.7±0.7[ab]	5.3±1.5[a]	3.9±1.2[a]
Bone formation rate [BFR/TV] ($\%/yr$)	16.3±1.58[b]	9.2±1.56[a]	18.8±6.75[b]	10.7±2.53[a]	6.6±4.01[a]

Data were compared by one-way ANOVA and Tukey's multiple range test. Values that do not share a common superscript are significantly different at $P < 0.05$. * Values represent mean±SEM.

amounts (Table II). These complex alterations occurred in association with a concomitant reduction in active bone resorption and an increase of the bone aluminum content and aluminum-covered bone surface (Table II).

Continuation of high-dose aluminum treatment for up to 16 wk resulted in further de novo bone formation. In this regard, bone volume continued to increase as did the trabecular number (Table II). In contrast, while mineralization remained abnormal, the presence of diminished woven osteoid indicated that heterogeneous calcification of this tissue was more complete (Fig. 5, Table III). Thus, the proportion of mineralized bone that is woven significantly increased above that evidenced after 8 wk of therapy (1.5±0.7 vs. 7.6±1.7%).

Discussion

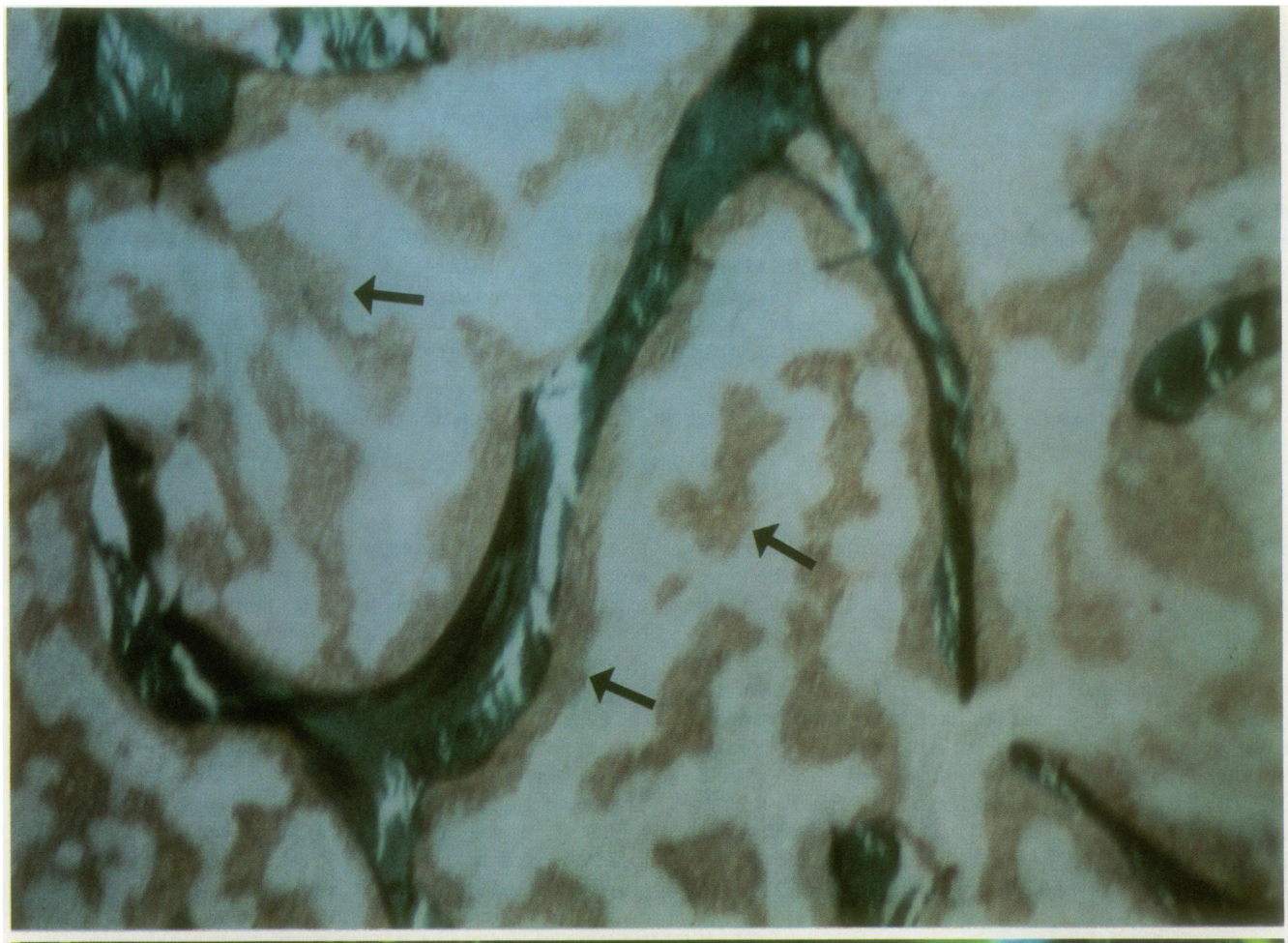
Pharmacological induction of a positive bone balance remains the goal of therapy in a variety of osteopenic disorders. However, in general, bone formation in the adult human skeleton serves a replacement function, occurring only at locations where bone resorption has been recently completed. Thus, drug development efforts have been limited and have resulted in selection of agents that alter the relationship between bone resorption and formation in order to effect a change in bone volume. In contrast, drugs that directly evoke the histogenesis of new bone (without a preceding resorptive stage) have not been identified.

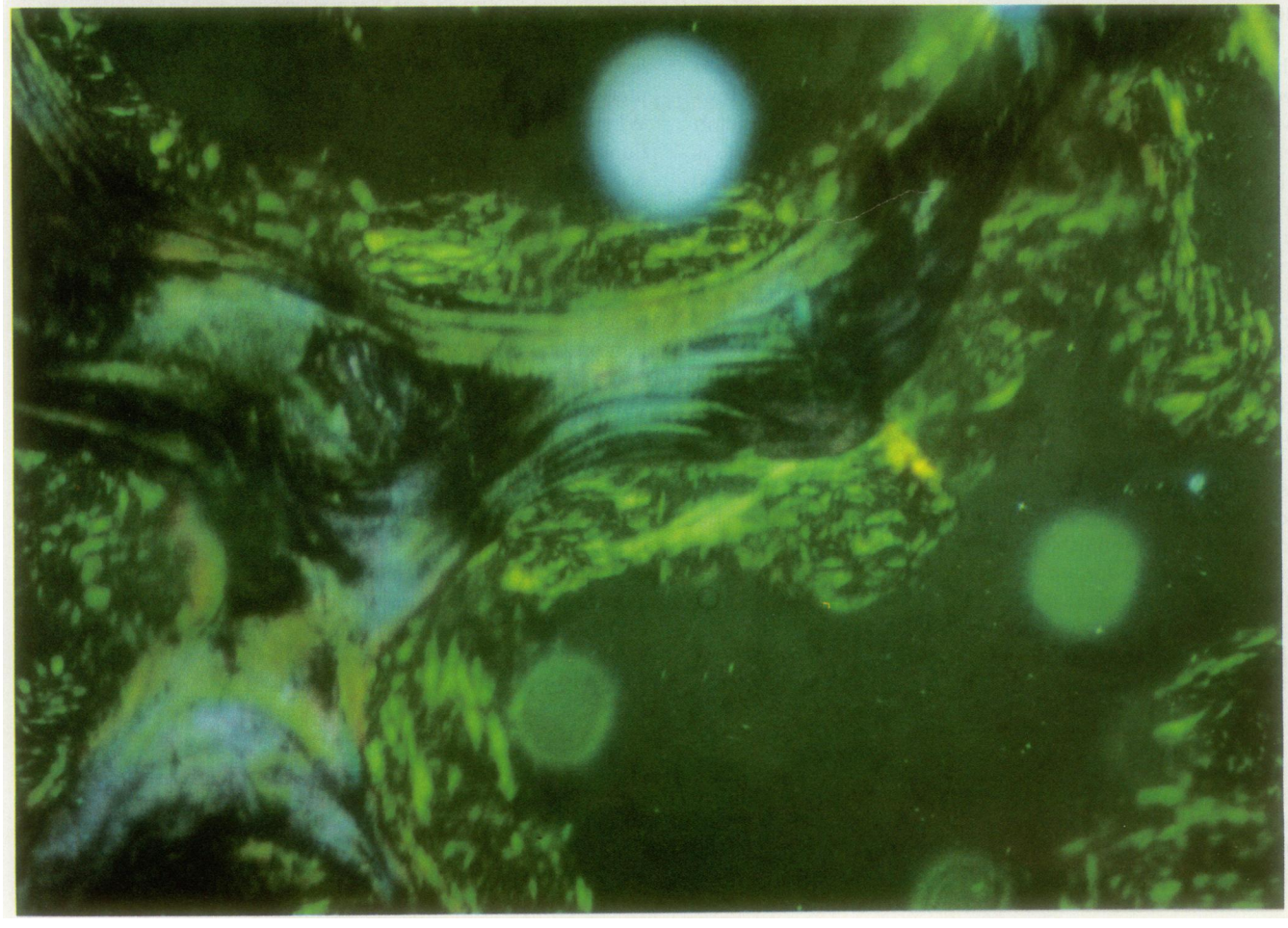
The data in this study illustrate, however, that aluminum administration stimulates uncoupled bone formation and induction of a positive bone balance in adult beagles that exhibit bone remodeling dynamics similar to those found in man. Several observations support this conclusion. First, bone biopsies that display an increased bone volume concurrently manifest normal or diminished resorptive activity. Second, long-term, low-dose aluminum treatment results in the appearance of localized excrescences of mineralized bone over unscalloped cement lines, consistent with de novo bone formation (23). Third, at higher doses aluminum stimulates deposition of woven bone over the majority of the trabecular envelope and, most notably, in the marrow space. And finally, an increased number of trabeculae, as well as enhanced bone volume, marks the new bone formation. This apparent stimulation of bone histogenesis contrasts with the effects of other pharmacological agents that solely alter function of existing bone remodeling units (24).

The mechanism(s) underlying the aluminum-mediated enhancement of bone formation remain unknown. However, the effects depend on variations of exposure time and/or dose. Treatment with low-dose aluminum for 8 wk paradoxically results in histological changes (Tables II and III) similar to those observed in disorders that exhibit low bone turnover (13). In contrast, longer term treatment or therapy with higher doses causes a graded stimulation of cellular activity and new bone formation (Tables II and III). These observations suggest that aluminum may exert both inhibitory and stimulatory effects on osteoblasts. Indeed, a similar aluminum-induced biphasic response of osteoblasts in vitro has been observed (25). Whether the divergent activities in vivo represent direct actions or are mediated by hormonal/metabolic influences cannot be determined from this study. While a decreased serum phosphorus concentration did occur in all groups, the magnitude of the changes was uniformly similar, making it unlikely that such a constant alteration evoked disparate histomorphometric findings. Thus, it appears likely that the stimulatory effects constitute a compensatory response to an initial inhibition of osteoblast function.

In any case, the positive effects of aluminum on osteoid synthesis are undoubtedly mediated by an increase in both the number and activity of osteoblasts. In this regard, the enhanced bone volume observed after 16 wk of low-dose treatment occurred concomitantly with restoration of the depleted osteoblast pool, while high-dose therapy resulted in an overt increase in the number of osteoblasts (Table II). Moreover, deposition of woven osteoid by the osteoblasts after treatment with higher doses of aluminum is itself indicative of enhanced cellular activity (23) and is very similar to the rapid bone proliferative response which occurs during fracture healing. In addition, the concurrence of peritrabecular fibrosis and in-

Figure 2. Microscopic appearance of a 5-μm-thick Goldner-stained histologic section of bone obtained from a beagle treated with low-dose aluminum for 16 wk (*top*) compared with that of a normal beagle (*bottom*). Mineralized trabecular bone from the dog receiving aluminum is marked by "arborization" within the marrow space, reflecting the continuum of this proliferative response. × 100.





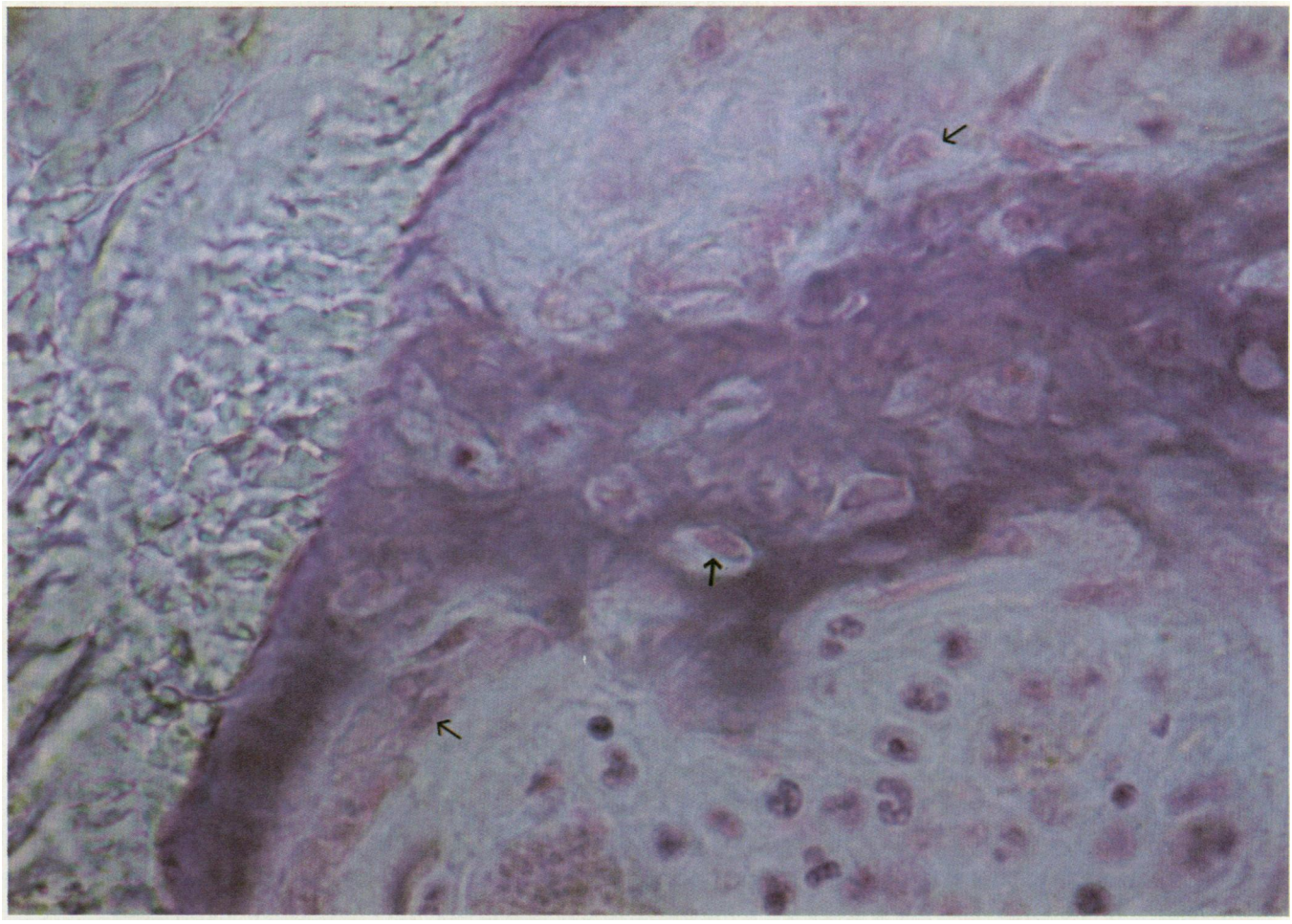


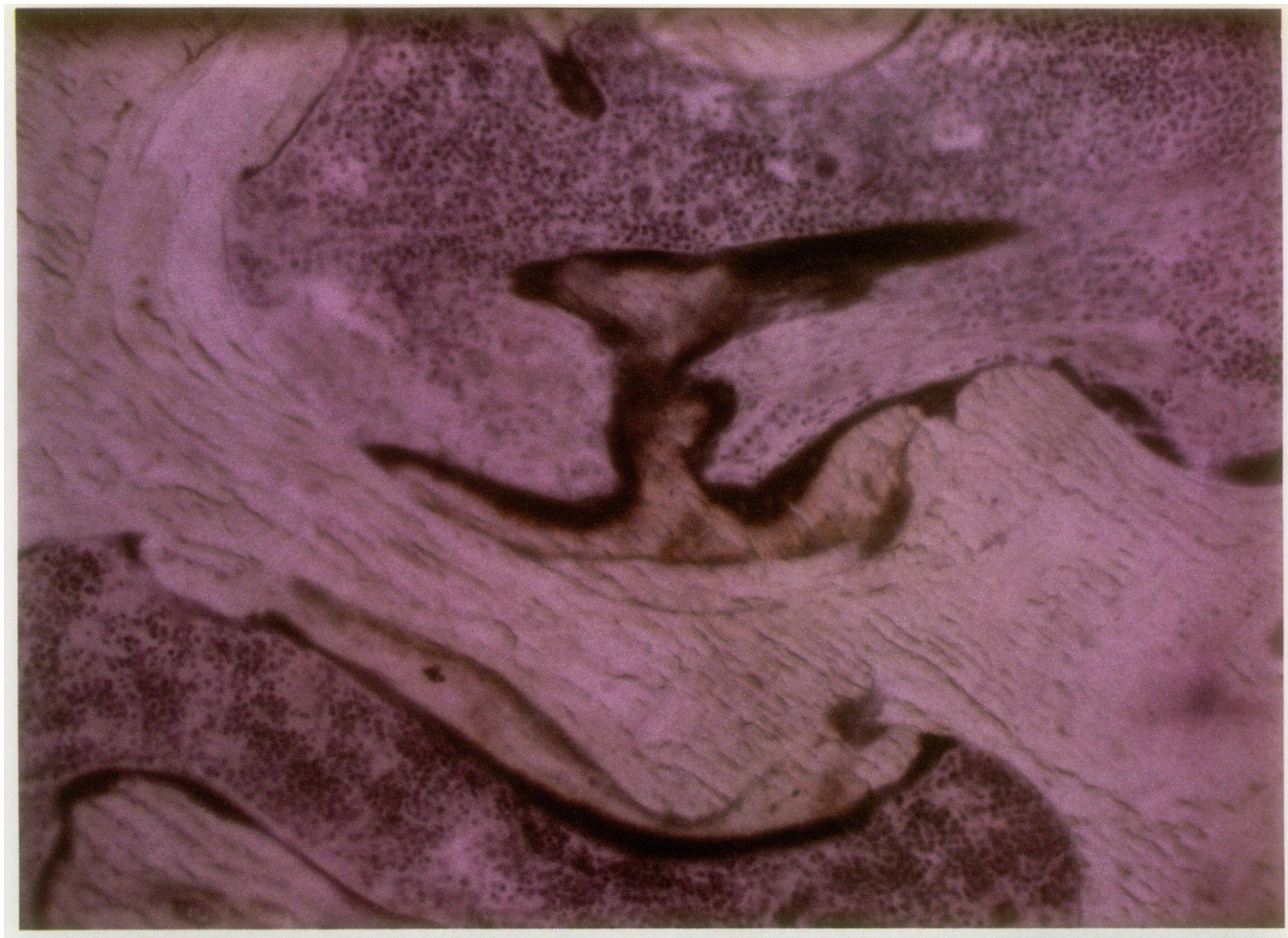
Figure 4. Villanueva-stained 5-μm-thick section of bone after 8 wk of high-dose aluminum treatment. Plump osteoblasts (*arrows*) are seen both upon the trabecular surface and within blue-stained woven osteoid. × 400.

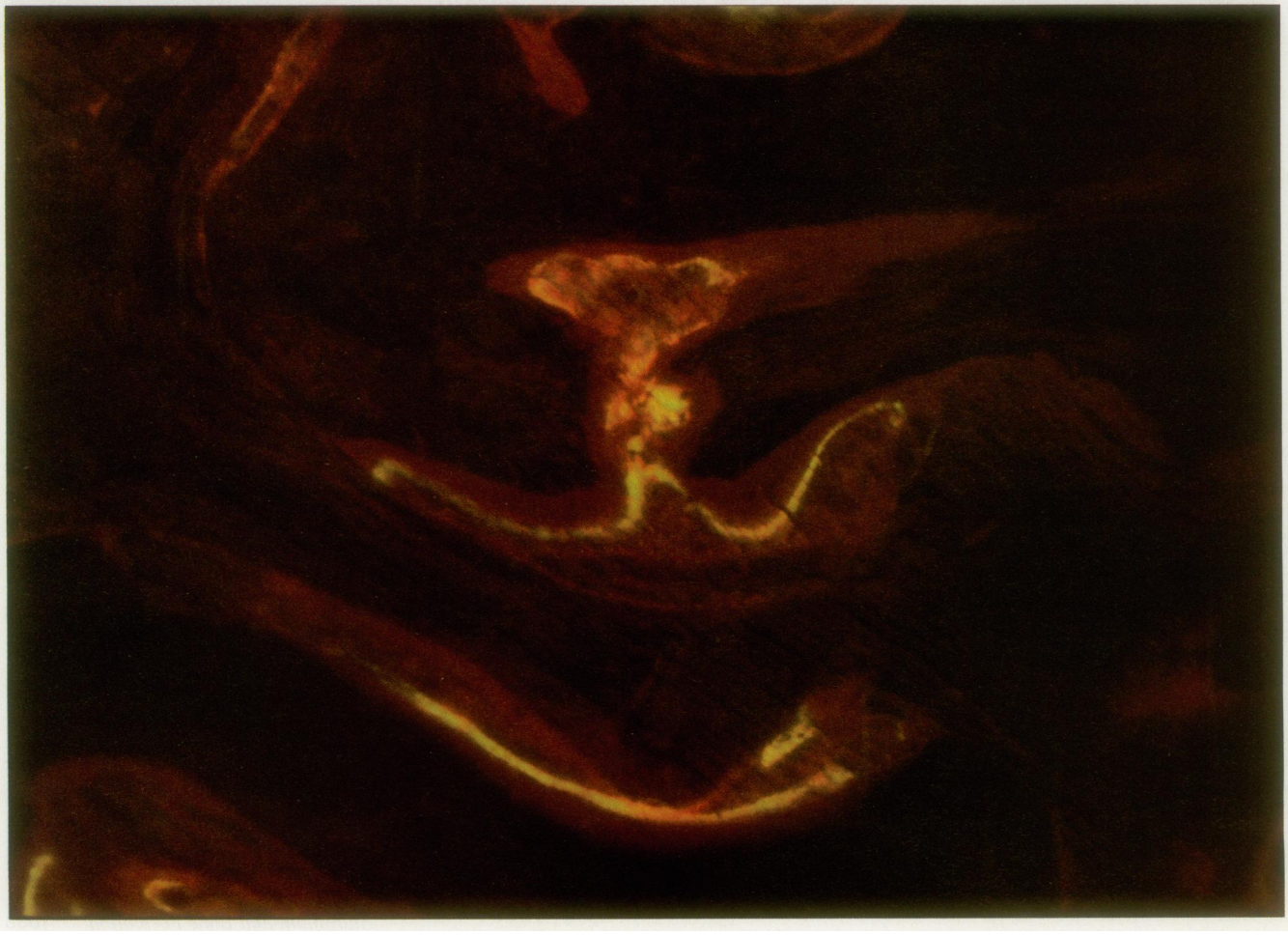
creased bone volume provides further evidence that an increase in the number and activity of osteoblasts mediates the positive effects of aluminum (Table II). In this regard, aluminum most likely stimulates proliferation (and differentiation) of a mesenchymal precursor which results in enhanced production and activity of both fibroblasts and osteoblasts. Several in vitro studies support this possibility. Data from these investigations indicate that aluminum can directly stimulate proliferation of both fibroblasts (26) and osteoblasts (25).

The apparent increase in osteoblast activity was incomplete. While enhanced cellular function resulted in synthesis and secretion of woven osteoid (Figs. 3 and 4), mineralization of the tissue (an apparent osteoblast-mediated event) was abnormal (Table III). Indeed, after 8 wk of high-dose treatment, little calcification was evident. Although the mineralization defect persisted with further therapy, at 16 wk heterogeneous calcification of the vast majority of the woven tissue became evident (Fig. 5). Whether the increased rate of bone synthesis contributed to the mineralization defect or the abnormal calcification resulted solely from an unrelated aluminum effect remains unknown. In any case, the defective mineralization and the enhanced rate of osteoid synthesis resulted in an increased mean osteoid seam width (Table II). Moreover, the existence of abnormal calcification prohibits estimating the rate of osteoid synthesis by calculation of the BFR/TV. Consequently, an increased BFR/TV does not mark the de novo bone formation that occurred after high dose aluminum treatment (Table III).

In addition, we did not observe an enhanced BFR/TV after 16 wk of low-dose therapy, in spite of apparent adequate mineralization (Table III). This aberration may be due to at least two factors. These are (*a*) completion of bone forming activity before the biopsy, or (*b*) formation of new bone at sites that have not been previously resorbed (uncoupling) combined with a reduction in the remodeling space due to the concomitant decrease of bone resorption. Indeed, the unscalloped ce-

Figure 3. Histologic findings in bone after 8 wk of high-dose aluminum therapy. (*Top*) Low power view of a 5-μm-thick Goldner-stained section reveals widespread deposition of brown staining osteoid over the trabecular envelope and within the marrow space (*arrows*). × 40. (*Bottom*) High power view of a 5-μm-thick Goldner-stained section under polarized light illustrates that the newly deposited osteoid has a woven structure. × 200.





ment lines and excrescences of bone are consistent with the latter interpretation. While we cannot discriminate between these possibilities, the evidence of new bone formation remains abundantly clear.

Both prostaglandin E_2 and fluoride induce histologic changes in bone similar in some respects to those observed in the aluminum-treated beagles (27, 28). High-dose fluoride treatment induces production of poorly mineralized woven bone in excessive amounts (27). However, new tissue deposits not only on endosteal surfaces, as we observed after aluminum therapy, but on periosteal surfaces as well. Moreover, the enhanced bone formation follows increased resorption (29). Consequently, the increase in bone mass occurs secondary to thickening of existing trabeculae and not to production of new units (30). A similar disparity likely exists between prostaglandin E_2 and aluminum effects. However, studies of the prostaglandin have been performed in rodent models that do not exhibit bone remodeling similar to that found in the beagle (28, 31). Thus, comparisons are difficult. Nevertheless, the exceptional effect of aluminum to stimulate bone formation without increasing resorption remains uniquely different from that of both prostaglandin E_2 and fluoride (both of which enhance resorption) (29, 32).

In contrast to our studies, considerable clinical and epidemiological evidence indicates that aluminum may be a pathogenetic factor underlying the development of various bone diseases which have as their hallmark abnormal mineralization and decreased bone resorption and formation (1–7, 13). However, attempts to produce animal model systems that exhibit such aluminum-mediated osseous abnormalities have provided conflicting data (8–12, 14). Experimental aluminum exposure in normal animals has resulted in no discernable bone pathology (14) or induction of diverse abnormalities ranging from defective mineralization (consistent with osteomalacia) (8, 9, 11) and decreased bone formation (33) to stimulation of resorption (12) and mineral apposition rate (10), changes indicative of increased bone turnover. However, these studies have been confounded by several factors. First, the doses of aluminum employed have often been large (exceeding 5 mg/kg per d), which may predispose to nonspecific toxicity (9). Indeed, exposure has been terminated abruptly in several studies due to a high attrition rate (9). Moreover, aluminum-treated animals have frequently exhibited significant weight loss due to poor intake of food and inadvertent nephrotoxicity (8, 9, 11). Second, aluminum treatment has generally been short, precluding evaluation of longer-term effects (8, 12). Third, use of large doses of aluminum has resulted in liver toxicity, causing a decrease of 25 hydroxyvitamin D levels which may complicate interpretation of bone histomorphometric changes (33, 34). And finally, the majority of studies have used rodents as the animal models (8–10, 12). Because the bone in these animals only undergoes modeling (31), their bone physiology is fundamentally different from that in adult dogs and humans. This difference may result in varying responses to aluminum. In any case, the histologic abnormalities which have been observed in these animal models are similar, but, in general, not entirely comparable, to the pathologic findings attributed to aluminum in man (6). In our study we administered aluminum intermittently and observed abnormal bone mineralization dynamics and inhibition of bone resorption, changes similar to those reported in some animal studies and found in man with aluminum-associated bone disease. More importantly, we coincidentally noted de novo synthesis of woven osteoid, which results in an increase in bone volume and in trabecular number and represents a unique aluminum-related effect that has not been previously reported. While we cannot determine with certainty the cause for these discrepancies, it appears likely that the aluminum-related osteodystrophies of man and aluminum-induced bone diseases previously reported in animal models occur secondary to the interaction of aluminum with a permuted metabolic milieu. This milieu may encompass uremia, among other abnormalities.

Regardless, the interrupted administration of aluminum to an animal model with dynamic bone remodeling and an uncompromised metabolic state, as described in this paper, produces de novo bone formation. Although many questions remain about the mechanisms of action, potential marrow toxicity (secondary to peritrabecular fibrosis), bone strength, remodeling of woven bone, and the ability to sustain the increased volume, aluminum appears to be a pharmacologic agent which can increase osteoblast number, stimulate osteoblastic collagen synthesis, and thereby induce bone histogenesis in spite of inhibiting mineralization. As such, aluminum may serve as a model to develop potential effective therapies for the osteopenic disorders.

Acknowledgments

We are indebted to Ms. Janice Almond and Ms. Jeane Raye for excellent technical assistance.

This study was supported in part by grants from the National Institutes of Health (AM-27032, DK-38015) and a research grant from the National Foundation (1-852). Dr. Quarles is a recipient of a Young Investigator Award (AR-37308) from the National Institute of Arthritis, Metabolism, and Digestive Diseases.

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Figure 5. Light and fluorescent photomicrographs of a bone biopsy from a beagle treated with high-dose aluminum for 16 wk. (*Top*) Villanueva-stained section showing an excrescence of bone on the trabecular surface. In contrast to the biopsies obtained after 8 wk of high-dose treatment (Figs. 3 and 4), a marked reduction in unmineralized woven osteoid is evident. (*Bottom*) Same section viewed under UV light showing the heterogenous deposition of tetracycline in woven tissue. This mineralization is responsible for the diminution in woven osteoid after prolonged high-dose treatment. × 200.

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