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Aluminum-Induced Osteogenesis in Osteopenic Rats with Normal Renal Function

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Abstract. Previous studies have shown a different effect of aluminum (Al) on bone metabolism in animals with chronic renal failure and conversely, positive osteogenic effects in animals with normal renal function. The aim of this study was to evaluate the effect of aluminum on bone metabolism in osteopenic rats. We studied male Wistar rats with severe osteopenia induced by adding NH4Cl (2%) to the drinking water over a 6-month period. The rats were divided into two groups and followed for 4 months. The Aluminum group (G1) received AlC13 intraperitoneally (10 mg/kg/5 days/week) (n = 8); the Control group (G2) did not receive any treatment after stopping the administration of NH4Cl (n = 5). In all animals we measured biochemical markers (serum Ca, P, Cr, Al, osteocalcin, hydroxyproline) as well as bone mineral density and bone histomorphometry (BV/TV, CTh, ObS/BS, OTh, and NOc/TV). Bone aluminum content, measured by atomic absorption spectrometry, was 101.6 ± 13 μg/g in the Al overloaded group and 1.31 ± 0.14 in controls. Bone mineral density, evaluated by dual X-ray absorptiometry (DXA) at the proximal extremity of the tibia was significantly higher in G1 (0.292 ± 0.01 g/cm^2 versus 0.267 ± 0.02 g/cm^2). No significant differences were found between the biochemical markers. In the histomorphometric parameters we observed significant differences in G1 compared with G2: an increase in BV/TV (18.59 ± 5.6 versus 7.69 ± 3.08%) and in CTh (0.52 ± 0.06 versus 0.36 ± 0.07 mm) with a moderate increment of the osteoid thickness (14.05 ± 4.72 versus 5.25 ± 0.9 μm) ($P < 0.05$). Changes in others parameters and the relationship between biochemical parameters of bone remodeling, Al, and histology were analyzed. These findings indicate that in rats with normal renal function, Al is able to induce bone formation even when osteopenia is present.

Key words: Aluminum — Osteogenesis — Osteopenic rats — Bone formation — Bone mass — Biochemical markers.

The toxic effects of aluminum (Al) on the bone have been confirmed clinically, epidemiologically, and experimentally upon observation of its capacity to induce two types of histological lesions: osteomalacia and adynamic bone disease [1]. It has been shown that Al can depress parathyroid activity and interfere with the mineralization process [2, 3]; these negative effects are obtained in the presence of chronic renal failure, a circumstance in which the calcium-PTH-vitamin D axis is altered. By contrast, in experimental animals with normal renal function, Al deposition in bone does not necessarily produce alterations in mineralization. Two contrasting effects have been described: a mineralization defect which produces osteomalacia, reversible by the administration of vitamin D [4], and a bone formation stimulus [5–7]. The latter is proportional to the dosage of Al administered and to the exposure time, showing the formation of new trabeculae, a decrease in bone reabsorption, an increase in osteoblast number, and partial mineralization defects [6].

At the cellular level, many different responses to Al exposure have been described: in the function of the type of cell—inhibition of osteoblasts and stimulation of preosteoblasts—and of the culture situation—inhibition in subconfluence or mythogenic stimulus and an increase in the synthesis of collagen in confluence [8].

This diversity of responses has been justified by the effect of the degree of maturity or differentiation—preosteoblasts and mature osteoblasts—, by interactions with systemic hormones and/or local bone growth factors, and/or by differences in Al speciation [9].

Until now, *in vivo* findings were obtained from normal bones. A possible clinical application makes it interesting to see the effect of Al on osteopenic bone. Therefore, the objective of this study was to evaluate the effect of aluminum administration on bone, in a model of osteopenia induced by chronic acid overload in rats with normal renal function.

Material and Methods

Study Protocol

Male Wistar rats (n = 20) 6 months old and weighing 350 g were used. The study was divided into two phases. In the first, osteopenia was induced. The animals were divided into two groups: Group I (n = 13) received a chronic acid overload with 2% ammonium chloride in deionized drinking water for 6 months as an osteopenia inducer; Group II (n = 7) animals were not manipulated. At the end of this phase, the effect of the chronic acid overload on bone was verified by bone densitometry *in vivo* and also by biochemical studies, to be detailed later.

In the second phase, only the osteopenic rats from GI were

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included, distributed into two groups of homogeneous weight: Aluminum Group (n = 8), in which ammonium chloride in drinking water was stopped and intraperitoneal aluminum chloride was started at 1 mg of aluminum element, 5 times per week for 4 months (total dose 80 mg of aluminum element); Control Group (n = 5); in which ammonium chloride in drinking water was stopped and no treatment was given for 4 months. At the end of the second phase the biochemical studies and bone densitometry *in vivo* were repeated and the animals were then sacrificed. Both tibias were extracted: the right for the determination of aluminum content, and the left for *in vitro* bone densitometry, histological study, and histomorphometry.

The rats were kept under the same standard conditions for both phases, in cages of three to four animals (except for the collection of urine samples which was done in metabolic cages), at a stable temperature of 20°C, with drinking water *ad libitum* and a maintenance diet with 0.6% calcium, 0.59% phosphorus, and 2020 UI/kg of vitamin D3. All procedures (densitometry *in vivo,* blood withdrawal from the jugular vein, and final euthanasia) were carried out under anesthesia, using either inhaled ether or, if stronger anesthesia was required, pentobarbital (Nembutal, 40 mg/kg of weight, intraperitoneal).

Biochemical Studies

In both phases, calcium, phosphorus, creatinine, total protein, and serum osteocalcin were determined. In addition, at the end of the second phase, serum biochemical studies were completed, determining PTH, pH, bicarbonate, ionic calcium, and aluminum. In 24-hour urine, calcium, phosphorus, creatinine, and hydroxyprolin were determined, and calcium/creatinine (Ca/Cr), hydroxyprolin/creatinine (HYP/Cr) indexes, tubular reabsorption of phosphates, and creatinine clearance were calculated. At the end of the second phase, determination of aluminum in 24-hour urine was also included.

Osteocalcin and PTH were determined by radioimmunoassay (RIA) (Antirat Osteocalcin, Biomedical Technologies and PTHmm, Incstar Corp, respectively); pH, bicarbonate and total serum and ionic calcium by automatic analyzer (Corning pH/Blood Gas analyzer and Ciba-Corning, respectively). Hydroxyprolin was determined by spectrophotometry (Hypronosticon, Organon Teknica GmbH); serum and 24-hour urinary Al determinations were done by graphite furnace atomic absorption spectrometry following the usual procedures [10]. Other parameters were determined by standard colorimetric techniques.

Bone Densitometric Studies

Bone density was determined by dual x-ray absorptiometry, (DXA) Hologic QDR 100 (Hologic Inc., Waltham, MA) using a collimator diameter of 0.9 mm on the X-ray output source and specific software for small animals (Ultrahigh resolution V 4.26). For *in vivo* densitometries, which included exploration of the lumbar and caudal spine, the animals were anesthetized with sodium pentobarbital and ether, immobilized in hyperextension using a frame support (Fig. 1). Densitometries on isolated tibias were carried out in a cubic recipient containing wheat flour in sufficient quantity (2.5 cm thickness) to equalize the attenuation coefficients of the soft tissues.

The lumbar densitometry included the first 5 lumbar vertebrae (taking the last rib as reference) and the caudal densitometry included 7 caudal vertebrae (taking the first of the smooth part of the tail as reference). Densitometry of the isolated tibias was done at one-eighth proximal level. The densitometric parameters evaluated included projection area (cm2), bone mineral content (BMC, mg), and bone mineral density (BMD, mg/cm2). The coefficient of variation of the *in vivo* technique in our unit is <1%; the accuracy, considering the correlation BMC/dry weight of the ash is $r = 0.97$, $P < 0.001$ in isolated vertebrae and $r = 0.99$, $P < 0.001$ in long bones, and the correlation coefficients between BMD *in vivo* with respect to BMD *in vitro* is $r = 0.94$, $P < 0.001$ [11, 12]. These figures are similar to those obtained by other authors [13].

Fig. 1. Bone density measurement at lumbar and caudal vertebras. The rats were anesthesized and immobilized in hyperextension with the frame support.

Studies on Tissues

Before sacrificing the animals, they were intraperitoneally marked with oxytetracycline (Terramycine 20 mg/kg), for 2 consecutive days; after 10 days of rest, the dose was repeated for another 2 days, and the animals were sacrificed 24 hours later. The right tibia was used for quantification of Al by atomic absorption spectrometry [10, 14], and the left tibia was used for densitometry (already described), histology and histomorphometry.

Histological study was done on the proximal metaphysis of the left tibia. The bone specimens were fixed with ethanol and soaked in methylmetacrilate [14]. The histological cuts, 6 and 10 μm thickness, were made with a microtome Polycut S Reicher-Jung. After the deplastification of the cuts, Von Kossa-Ponceau stains of Xilidin-Orange G, trichromic from Masson-Goldner, tricarboxylic aurin acid (aluminon), solochrome of azurine and Perls were used [14]. The histomorphometric analysis was done by a semiautomatic morphometer Videoplan (Zeiss), with a Polyvar Reichert computer-assisted microscope. The histomorphometric parameters evaluated were trabecular perimeter, bone volume (BV/TV), cortical bone thickness (CTh), osteoblast surface (Ob. S/BS), osteoid thickness (O Th), and osteoclast number ((N Oc/T Ar).

Statistical Analysis

Statistical analysis of the results was done with Systat and Sigma

Table 1. Biochemical parameters at the end of both phases[a]

	First phase		Second phase	
	Group I (n = 13)	Group II (n = 7)	Aluminum (n = 8)	Control (n = 5)
Serum				
Ca (mg/dl)	10.5 ± 0.70	10.6 ± 0.63	10.6 ± 0.56	10.5 ± 0.62
P (mg/dl)	6.70 ± 0.59[b]	7.77 ± 0.22	7.40 ± 1.53	8.34 ± 2.25
Cr (mg/dl)	0.68 ± 0.05	0.69 ± 0.04	0.70 ± 0.08	0.67 ± 0.07
Prot (g/l)	7.04 ± 0.33[b]	7.38 ± 0.22	7.33 ± 0.31	7.66 ± 0.45
Oc (ng/ml)	19.7 ± 3.05[d]	23.4 ± 5.8	21 ± 19.7	13.4 ± 2.5 Δ
pH			7.15 ± 0.05[b]	7.27 ± 0.05[b]
Bic. (mmol/l)			21.5 ± 4.6	22 ± 2.7
Ca^{2+} (mg/dl)			5.7 ± 0.5	4.8 ± 1.9
PTHmm (pmol/l)			257 ± 89	237 ± 95
Urine				
Ca (mg/24 h)	75.9 ± 28.8[c]	20.4 ± 10.4	37.7 ± 27.1	14.6 ± 5.6 Δ
Ca/Cr (mg/mg)	0.56 ± 0.27[c]	0.13 ± 0.07	0.28 ± 0.32	0.09 ± 0.03 Δ
HYP/Cr (μg/mg)	0.41 ± 0.20[c]	0.20 ± 0.03	0.42 ± 0.36	0.30 ± 0.09
ClCr (ml/min)	1.41 ± 0.19	1.42 ± 0.43	0.89 ± 0.68	1.43 ± 0.70
TRP (%)	93 ± 4	91 ± 3	91 ± 3	94 ± 2

[a] First phase: Group I rats with chronic water administration of ClNH4 (2%); Group II rats without any manipulation. Second phase: Aluminum group rats administered AlCl3 intraperitoneally; Control group rats without any treatment
[b] $P < 0.05$; [c]$P < 0.001$; [d]$P = 0.07$ intergroup differences in either of the phases; [e]Δ $P < 0.05$ differences in baseline values (paired T-test)
Ca = calcium, P = phosphorus, Cr = creatinine, Prot = total serum proteins, Oc = osteocalcin, Bic = bicarbonate, Ca^{2+} = ionic calcium, PTH = parathormone, HYP = hydroxyproline, ClCr = clearance of creatinine, TRP = tubular reabsorption of phosphate

(Horus Hardware) support, using the Student's *t* test for paired and unpaired data, and the Mann-Whitney as a nonparametric test. The relation between variables was studied by the Pearson linear correlation coefficient. The results are expressed as mean ± standard deviation; differences were considered significant at $P < 0.05$.

Results

First Phase

Administration of ammonium chloride in the GI animals stopped the ponderal growth; their weight at the end of 6 months was significantly lower than that of GII (365 ± 55 versus 535 ± 60 g; $P < 0.001$) and induced a significant decrease in total proteins and serum phosphorus together with a marked increase in 24-hour urinary calcium and Ca/Cr and HYP/Cr indexes; the decrease of osteocalcin was at the limits of statistical significance (Table 1). In GI, BMD values were significantly lower than those in GII, at both the lumbar spine (223 ± 22 versus 254 ± 17 mg/cm2, $P < 0.005$) and caudal spine (259 ± 19 versus 276 ± 19 mg/cm2, $P < 0.05$).

Second Phase

The increment of weight throughout the 4 months of study was similar in the rats of both groups ($P < 0.01$): The Aluminum group increased from 367 ± 34 to 430 ± 51 g, and the Control group increased from 373 ± 60 to 438 ± 62 g. There were no differences between groups in final weight.

Biochemical Parameters. There were no significant intergroup differences in the final biochemical parameters. However, the levels of osteocalcin, 24-hour urinary calcium, and Ca/Cr and HyP/Cr indexes tended to be higher in the Aluminum group (Table 1), showing a great dispersion. Evolutively, and compared with the final values of the first phase (Group I), the Control group showed a significant decrease (paired t test) in the values for osteocalcin, 24-hour urinary calcium, and Ca/Cr ($P < 0.05$). These differences were not observed in the evolution of the Aluminum group, in which a tendency to decrease 24-hour urinary calcium (from 75.9 ± 28.8 to 37.7 ± 27.1 mg/24 hour) was noted, although without reaching significant differences (Table 1). In spite of this, upon analyzing all the animals as a group in the second phase, the correlation coefficients among the various biochemical markers of bone metabolism were high and significant (Table 2).

Serum pH was lower in the Aluminum group without differences in bicarbonate, ionic calcium, or PTH (Table 1). The levels of serum, urinary, and tibia aluminum were, as expected, significantly higher in the Aluminum group (Table 3). In this group, the serum Al concentration correlated with its elimination in urine ($r = 0.85$; $P < 0.01$) and with the concentration of Al in the tibia ($r = 0.71$; $P < 0.05$). The correlation between Al excretion in urine, evaluated as Al/Cr coefficient, and tibia Al concentration was also significant ($r = 0.89$; $P < 0.01$).

Upon analysis of the animals in the Aluminum group, done separately, the correlations between biochemical parameters, including in this case serum and bone Al, improved with respect to the global analysis done with both groups. This was especially marked in the inverse relationship between serum and bone Al and markers of bone re-

Table 2. Relationship among the biochemical parameters and aluminum levels

Both groups	Osteocalcin	Ca/Cr	HYP/Cr	
Ca/Cr	0.93			
HYP/Cr	0.78	0.92		
Urine Ca	0.89	0.96	0.81	
Aluminum	**Osteocalcin**	**Ca/Cr**	**HYP/Cr**	**Urine Ca**
Ca/Cr	0.93			
HYP/Cr	0.80	0.94		
Urine Ca	0.92	0.96	0.84	
Serum Al	–0.96	–0.92	–0.85	–0.89
Bone Al	–0.62	–0.68	–0.73	–0.67

In both groups, significance of r = 0.80, $P < 0.01$; r > 0.80, $P < 0.001$. Below, only the Aluminium group, significance of r < 0.71 NS, r = 0.83, $P < 0.05$; r = 0.93, $P < 0.01$, and r > 0.93, $P < 0.001$.
Ca/Cr = ratio calcium/creatinine, HYP/Cr = ratio hydroxyproline/creatinine, U Ca = urine calcium (in all cases 24-hour urine)

absorption (Ca/Cr, HYP/Cr and urinary calcium), and also with a parameter of formation as osteocalcin (Table 2; Fig. 2).

Bone Mineral Density. Evolutive analysis of the behavior of bone mass from the first to the second phase showed that the two groups, Aluminum and Control, increased values significantly ($P < 0.05$) with respect to basal figures, both in the lumbar spine (from 226 ± 13 to 243 ± 17 mg/cm2 in Group 1 and from 219 ± 36 to 248 ± 30 mg/cm2 in Group 2) and caudal spine (from 253 ± 11 to 273 ± 15 mg/cm2 in Group 1 and from 252 ± 25 to 264 ± 28 mg/cm2 in Group 2). In the lumbar region, the highest value was obtained in the Control group, in the caudal region, the highest was in the Aluminum group which showed the highest values, without significant differences between the two groups. On the contrary, in the densitometry on isolated bone (upper tibia), BMD values were significantly higher in the group that received Al compared with the Control group (Fig. 3).

Bone Histology. The Aluminum group showed an increase of trabecular bone, predominantly in the center of the medullar cavity (Fig. 4a) whereas the cortical regions showed normal thickness and appearance. Using polarized light microscopy, an increase of lamellar osteoid was observed with some areas of up to five lamellas in thickness, placed sheetwise (Fig. 5). All rats showed tetracycline labels, the majority of them doubles; some animals also showed single and partially blurred labels. The aluminon and the azurine solochrome stainings showed Al deposits on all surfaces at the mineralization front. In addition, azurine solochrome demonstrated Al deposits on neoformed and mineralized trabeculae as well as in the intertrabecular connections (Fig. 6). Rats in the Control group showed focal losses of trabecular bone with normal cortical thickness (Fig. 4b), no significant alterations in cellularity, and a few tetracycline labels.

Bone Histomorphometry. In the Aluminum group, histomorphometry showed a significant increase in bone volume, cortical thickness, osteoid thickness, and osteoclast number (Table 4). Technical difficulties, mainly partial decoloration of the sample used, prevented the measurement of the dynamic indexes derived from tetracycline labeling.

Serum and tibia Al concentrations showed weak correlation coefficients. The only histomorphometric parameter that showed a significant correlation with Al concentration at the tibia was the BV/TV (r = 0.74, $P < 0.05$); serum aluminum and BV/TV were at the limit of significance (r = 0.58; $P = 0.07$). In the Aluminum group, BMD in the proximal tibia correlated with trabecular perimeter (r = 0.66, $P < 0.05$).

Discussion

The chronic administration of ammonium chloride in drinking water is one of the classic models of induced experimental osteopenia [15, 16]. In this study, mature 6-month-old rats were chosen because there is no interference in their growth process which is already finalized at that age [17], despite the ponderal gain increase. In the first phase, after 6 months of acid overload, rats in Group I did not increase significantly in weight, whereas the rats from Group II, not having received the acid overload, showed a marked increase in weight, as has been observed in previous studies [15, 18].

The densitometric and biochemical results observed in Group I at the end of the first phase showed a clear osteopenia with increase in bone reabsorption (Ca/Cr and HyP/Cr) and normal or slightly decreased signs of bone formation (osteocalcin at significance limit). Similar results have been observed previously with a marked decrease in bone and trabecular volume [16, 18], probably reflecting the participation of bone in the control of chronic metabolic acidosis [19].

In the second phase, both Aluminum and Control groups showed a significant weight gain, likely due to the disappearance of the negative effect of the acidosis on protein metabolism. Regarding bone remodeling markers, in the Control group, a decrease of all markers was observed. By contrast, the Aluminum group showed less changes and more dispersed values in bone remodeling markers, despite the fact that in this group we observed a greater bone formation in the bone histomorphometry.

In the Aluminum group, the Al concentration reached in serum and bone was very high, in the range observed in Al-induced osteomalacia in animals with chronic renal failure [20]. The same levels were referred to in previous studies carried out on dogs, in which Al was proven to have an osteogenic effect [5]. In spite of the magnitude of the Al overload, it did not induce significant changes in other bone metabolism parameters such as serum calcium, serum phosphorus, or parathyroid hormone (PTH).

Despite the different behavior observed in the evolution of bone remodeling markers in both groups, the final results, likely due to the dispersion of values in the Aluminum group, did not show significant differences between animals receiving Al and those not receiving it.

The important negative correlation observed between the serum Al levels (and even tissue Al) and the four biochemical markers of bone remodeling studied (Table 2), suggests that Al could have a negative effect on bone reabsorption and bone formation. This inverse relationship between Al and bone activity was highly significant with osteocalcin; there was a great dispersion of values and only the rats with very high serum Al (around 500 μg/liter) showed low serum osteocalcin levels (Fig. 2). However, this effect does not

Table 3. Aluminum measurement in serum, in 24-hour urine, and tibia at the end of the study in the two experimental groups

		Aluminum	Control	Significance
Serum Al	(μg/liter)	404.5 ± 108.4[a]	2.12 ± 1.59	$P < 0.001$
Urine Al	(μg/24 h)	124.5 ± 23[a]	0.23 ± 0.3	$P < 0.001$
Al/Cr	(μg/g 24 h)	97 ± 24[a]	0.1 ± 0.04	$P < 0.001$
Bone Al (Tibia)	(μg/g)	101.6 ± 13.4[a]	1.31 ± 0.14	$P < 0.001$

[a] $P < 0.001$

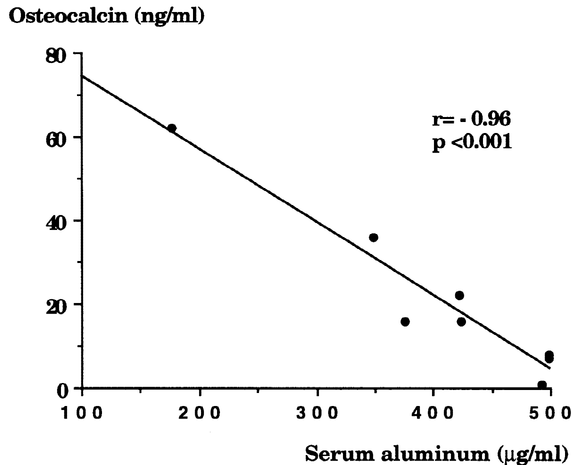

Fig. 2. Relationship between osteocalcin and serum aluminum in the Aluminum group.

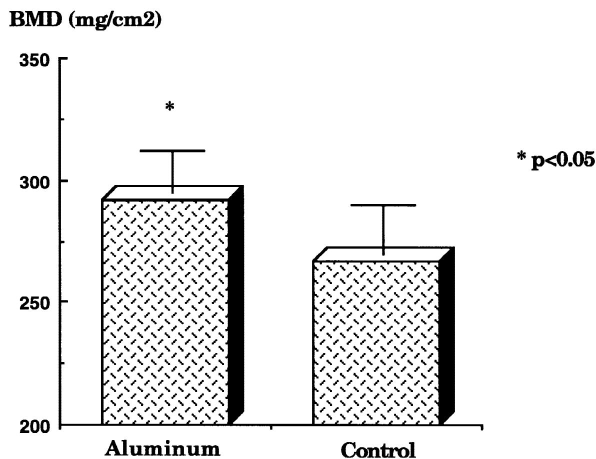

Fig. 3. Bone density (BMD) at the proximal end of the tibia. *$P < 0.05$ between groups.

imply that other expressions of osteoblastic function, such as synthesis of the protein matrix, are necessarily inhibited. In other experimental models it has been possible to inhibit the expression of osteocalcin without observing these changes in bone histology [21].

Independently of the above-mentioned intragroup relationship, the Al-treated rats, as a group, showed higher indexes of bone resorption in urine compared with controls, likely due to the significant decrease in pH induced by the Al chloride. In any case, apart from the effect of pH, previous studies have also pointed out the possibility that, in the presence of a chronic Al overload, biochemical markers of bone remodeling lose part of their current usefulness in the evaluation of bone remodeling [5, 8, 22]. In spite of these discrepancies, the correlations between the levels of serum and tissue Al and the biochemical markers of bone remodeling, and the correlations among the latter themselves, suggest that in both groups there was an adequate coupling between the bone reabsorption and bone formation processes.

The evolution of bone mass at the lumbar and caudal level *in vivo* was similar in both groups: at the end of phase I, the suspension of the acid overload was followed by an increase in BMD in both groups. The results obtained in the tibia, which for some authors is the most sensitive bone to show changes in BMD [23], demonstrated a significant increase in BMD in the Aluminum group, which is in keeping with the findings of the bone histomorphometry. The discrepancies observed in the densitometric results obtained in different bones may be explained by the different proportion of cortical and trabecular bone in the vertebrae of the rats. In the caudal area, the posterior arcs and the "star" distribution of cortical bone make the proportion of cortical bone greater compared with the upper vertebral areas. With the latter we demonstrated *de novo* bone formation in the Aluminum group, with a pattern similar to previous studies in dogs [5].

Table 4 shows that all parameters studied indicated a higher bone activity in rats treated with Al; in some of them the higher activity was particularly evident, such as in bone volume, with more than a twofold increase. The higher bone activity in the Al-treated group was localized mainly in the central areas of the medullar cavity (Fig. 4a). By contrast, in the control group, the few areas with some activity were localized close to the cortical and epyphiseal areas (Fig. 4b).

Among these parameters, the trabecular perimeter merits specific comments. Despite the difference in trabecular perimeter between Al-treated rats and Controls, it was the parameter together with the cortical thickness that showed the smallest difference. However, the trabecular perimeter correlated better than the other with BMD in the proximal tibia. The pattern of the Al deposition in bone in all the trabecular surfaces observed (Fig. 5) may partly explain this result; Al deposition may attenuate the X-ray transmission, as the hydroxyapatite as a result of that, the greater the surfaces covered by Al, the higher the BMD results.

As we have seen, overall, the Al chloride-treated rats showed an increased bone volume and *de novo* bone formation. Likewise, as has been reported in dogs [5, 6], there are also a few focal mineralization defects with increased osteoid thickness, no woven osteoid formation, and no medullar fibrosis. None of the changes observed resembles the osteomalacic findings observed with similar degrees of Al overload in the presence of chronic renal failure [20]. The

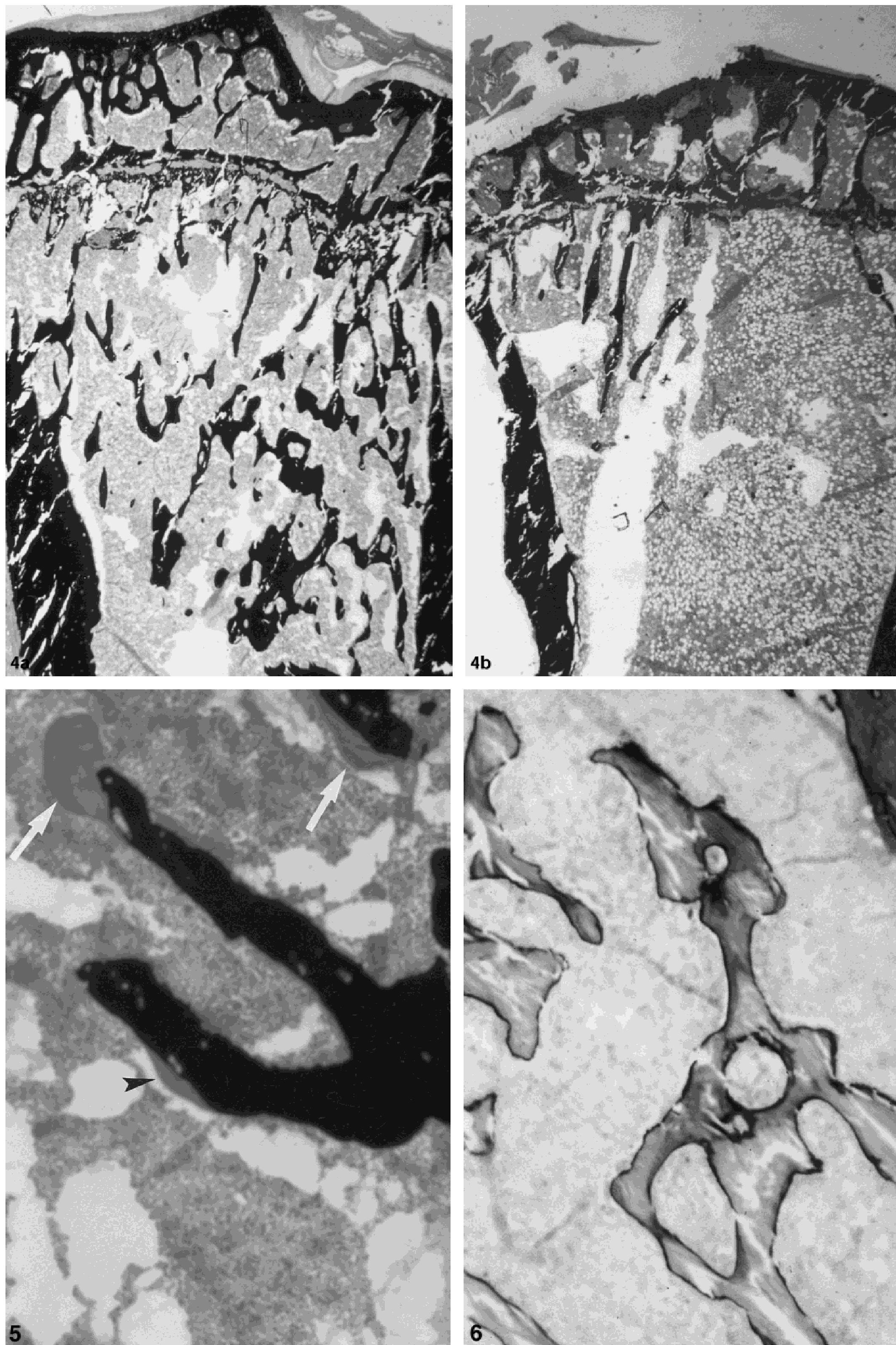

Fig. 4. (a) Aluminum group: proximal metaphysis of the left tibia with increased trabecular bone, predominantly in the center of the medullar cavity (Von Kossa ×25). **(b)** Control group: proximal metaphysis of the left tibia with a marked trabecular bone loss (Von Kossa ×25).

Fig. 5. Aluminum group: Lamellar osteoid with up to five lamellas in the Al-treated rats (Von Kossa ×100).

Fig. 6. Aluminum staining (Azurine solochrome ×100). Note the aluminum into the mineralized bone (intratrabecular) and also in all surfaces of the trabecula.

Table 4. Histomorfometric parameters at the proximal metaphysis of the tibia

	Aluminum	Control
Trabecular perimeter (mm)	44.0 ± 7.8[a]	30.5 ± 3.5
Bone volume (%)	18.59 ± 5.66[a]	7.69 ± 3.08
Cortical thickness (mm)	0.52 ± 0.06[a]	0.36 ± 0.07
Osteoblast surface (%)	3.79 ± 2.58[b]	2.04 ± 0.11
Osteoid thickness (μm)	14.05 ± 4.72[a]	5.25 ± 0.90
Osteoclast number (N Oc/mm2)	2.44 ± 0.52[a]	1.30 ± 0.01

[a] $P < 0.01$; [b] $P < 0.1$

disturbances in the metabolism of calcium, phosphorus, vitamin D, PTH, or the uremia itself [24], not present in our model, may account for the differences observed. In fact, using a combination of fluoride and Al, synergetic action increasing bone mass in ovariectomized rats has been observed [25]. On the other hand, using the same combination in rats with renal failure, they increased cellularity but the osteoid had severe mineralization defects instead of increasing the bone mass [26].

Several studies have attempted to find explanations for the variable effects of Al on bone metabolism [9]. The likely influence of the calciotropic hormones mentioned before and the local role of Al modifying the components of the surface membrane and the mineralization process [28–30] may have influenced our results. In addition, in our study, the higher osteoclastic and osteoblastic activity observed may have been partly explained by the degree of acidosis induced by the Al salt used, which may have counterbalanced some of the likely negative effects of Al on bone remodeling.

Other reports have suggested that Al may be implicated in the DNA synthesis of osteoblast by a G-protein, coupled-cation sensing mechanism [27], as trigger or part of the insuline-like growth factor regulatory system [28], as potential modifier of the final composition of the bone matrix [29], as regulator of the ionic composition of the surface membrane [30], and as modulator of the mineralization process [31, 32]. Its role on alkaline phosphatase production is still is a matter of controversy [29, 31–33]. More recent studies suggest that the final positive effect of Al on bone may not follow the classical pattern of coupling between bone reabsorption and bone formation [34].

No matter which are the mechanisms involved in this process, Al chloride has been able to induce *de novo* bone formation in rats with normal renal function and predominant trabecular osteopenia, having a histological pattern like that observed in osteoporosis. These important loses of trabecular bone, such as the corticosteroid-induced osteoporosis. Further studies comparing different aluminum salts and also the mechanical properties of the new bone formed are required.

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