Lithium Inhibition of Bone Mineralization and Osteoid Formation

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A B S T R A C T Lithium chloride administration to growing rats, which resulted in circulating lithium levels of 1.4 meq/liter, was attended by significant suppression of bone mineralization and organic matrix synthesis as assessed by tetracycline labeling and histological quantitation of osteoid, respectively. These effects of lithium were not associated with changes in animal behavior, nor were there any significant differences in blood levels of calcium, phosphorus, alkaline phosphatase, creatinine, pH, or parathyroid hormone. The data suggest that lithium inhibition of bone mineralization is secondary to suppression of osteoid formation.

INTRODUCTION

Although lithium salts have been proven effective therapeutic agents for manic-depressive disorders (1) adverse effects on bone and mineral metabolism have been considered potentially hazardous side effects. Lithium treatment in man has been reported to induce hypercalcemia with elevated levels of parathyroid hormone and magnesium (2), hypocalciuria without alterations in response to parathyroid hormone (3–5), and renal tubular acidosis (6). In the rat, lithium has been noted to have no effect on serum calcium (7, 8) or to result in hypercalcemia (9) and hypercalciuria (9–12). The response of serum magnesium of animals treated with lithium has also been controversial with both normal

(7, 8) and elevated (9) values recorded. Moreover, both enhanced (13) and inhibited (14) phosphaturic responses to parathyroid hormone have been attributed to this ion.

Previous studies demonstrated no changes in either bone composition or collagen metabolism in the rat after 6 wk of lithium treatment (6, 7). In contrast to these observations, data herein reported indicate that lithium does in fact inhibit in vivo bone mineralization and osteoid formation and support the hypothesis that the systemic effects of lithium on mineral metabolism are partially mediated by its inhibitory effect on bone mineralization.

METHODS

Eight 5-wk-old female Holtzman rats were treated for 4 wk with daily intraperitoneal injections of lithium chloride dissolved in sterile distilled water. The daily dose of lithium was 4 meq/kg body weight, an amount which resulted in circulating lithium levels of 1.4 meq/liter 90 min after injection. Eight control rats were injected with an equal volume of sterile distilled water. Rats were kept in metabolic cages and given distilled water ad libitum. Each control animal was pair fed with an individual lithium-treated animal.

On the 11th and 25th day of lithium treatment all rats were injected with 2.7 mg tetracycline intraperitoneally. On the 28th day, the seventh caudal vertebra of each rat was removed and embedded in methyl methacrylate with the distal surface placed en face against the bottom of the embedding vial. To assure transverse sectioning, the block was trimmed so that all surfaces were parallel or perpendicular to the distal surface of the bone, and mounted on an Isomet 11-1180 low speed saw (Buehler Ltd., Evanston, Ill.) with the distal surface parallel to the blade. Two
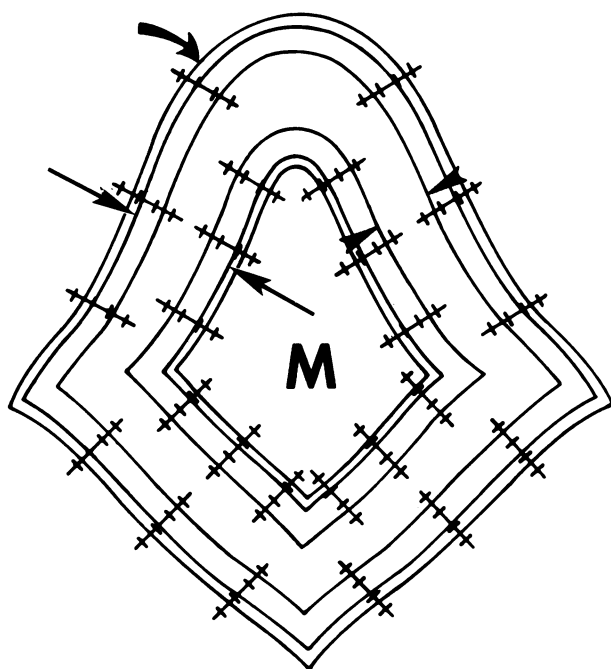
FIGURE 1 . Schematic representation of a cross-section of the seventh caudal vertebra. M = medullary cavity; curved arrow = periosteum. The distances between the fluorescent labels representing tetracycline administered on day 11 (arrowhead) and day 25 (straight arrow) were measured as illustrated at three equidistant points on each surface with a linear micrometer.

sequential sections, each approximately 150 μm thick, were taken from the midpoint of the vertebra and identified as the nadir of two converging planes. These sections were then hand ground to a thickness of 80–95 μm. The cortical bone and medullary cavity areas were measured under dark field microscopy using a Merz-Shenk integrating eyepiece (15). These parameters were determined by counting the number of "hits" on bone or medullary cavity.

The remaining histometric parameters were based upon tetracycline labeling and measured by fluorescent microscopy. Fig. 1 is a schematic representation of the cross section of the seventh caudal vertebra as viewed in a fluorescent microscope. The distance between the midpoints of the two fluorescent labels was measured at three equidistant points of each of the four sides of the periosteal and endosteal surfaces, respectively. Thus, the periosteal mineralization rate (PMR)[1] and endosteal mineralization rate (EMR) were quantitated by measuring the distances between the midpoints of the two fluorescent labels at 12 points on the periosteal and endosteal surface of two sequential sections from each control and experimental animal. The mean distance of the 24 points on the periosteal and endosteal surface for each animal was then divided by 14, the number of days between the administration of the two doses of the antibiotic, and expressed as the PMR and EMR in micrometers per day for that animal.

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[1] *Abbreviations used in this paper*: ELEBM, endosteal linear extent of bone mineralization; EMR, endosteal mineralization rate; PLEBM, periosteal linear extent of bone mineralization; PMR, periosteal mineralization rate.

The extent of periosteal and endosteal surface exhibiting a double label, and therefore undergoing mineralization, was quantitated and expressed as the linear extent of bone mineralization. The periosteal linear extent of bone mineralization (PLEBM) and endosteal linear extent of bone mineralization (ELEBM) were quantitated by counting the fraction of parallel lines of the integrating eyepiece which intercepted the double fluorescent labels on the periosteal and endosteal surfaces of each section and multiplying this fraction by Nr/V, where N represents the total number of intercepts, r is the distance between two parallel lines on the ocular reticule times π, and V is the cross sectional area of the histological section.

The amount of osteoid present was quantitated from longitudinal tibial sections of four lithium-treated and their respective pair-fed control rats. Each section was cut on a Jung-Sledge microtome to a thickness of 5 μm and stained by a modification of the Masson technique. The percentage of trabecular bone composed of or covered by osteoid was determined with a Merz-Shenk eyepiece as above. The amount of osteoid present was expressed as the ratio of volume occupied by osteoid to that occupied by osteoid plus mineralized bone (% matrix) and as the ratio of bone surface covered by osteoid to total bone surface (% surface).

The rats were phlebotomized at the time of sacrifice and calcium (16), phosphorus (17), alkaline phosphatase (18), creatinine, pH, and lithium levels (19) determined. Parathyroid hormone levels were determined with a carboxyterminal assay as previously described (20). Probabilities of difference were calculated with the paired t test.

RESULTS

Lithium treatment did not alter the gross behavior of the animals. The weights of the control and lithium-treated rats were similar both before (105±5 vs. 108±4 g) and at the conclusion (200±3 vs. 205±6 g) of lithium treatment. As shown in Table I, lithium treatment did not alter the blood values of calcium, phosphorus, alkaline phosphatase, parathyroid hormone, creatinine, or pH.

Examination of the seventh caudal vertebra revealed no significant difference in the cortical bone area between control and lithium-treated animals, nor was

TABLE I

Chemical Values in Control and Lithium-Treated Animals

	Ca	Pi	Alkaline phosphatase	Parathyroid hormone	Creatinine	pH
	mg/dl	mg/dl	units	μeq/ml	mg/dl	
Control	10.3 ±0.2	7.3 ±0.3	127 ±15	27 ±4	0.43 ±0.03	7.44 ±0.03
Lithium-treated	10.4 ±0.2	7.0 ±0.5	136 ±8	25 ±2	0.43 ±0.02	7.43 ±0.04
	NS	NS	NS	NS	NS	NS

Values represent the mean±SEM of six–eight animals in each group, and are the same animals whose bones were used to quantitate mineralization rates and osteoid formation.

TABLE II
Kinetic Parameters in Control and Lithium-Treated Animals

	Histometric data					
	Cortical bone area	Medullary cavity area	PLEBM	ELEBM	PMR	EMR
	mm^2	mm^2	mm/mm^2	mm/mm^2	$\mu m/day$	$\mu m/day$
Control	2.31±0.06	1.68±0.13	1.49±0.06	1.02±0.12	3.57±0.43	1.49±0.22
Lithium-treated	2.30±0.13	1.50±0.09	1.50±0.34	0.82±0.25	1.98±0.60	0.98±0.34
Probability of difference	NS	NS	NS	NS	0.05	NS

Values represent the mean±SEM of eight animals in each group. Experimental conditions and techniques appear in the text.

the mean medullary cavity area of the lithium-treated animals significantly different from that of the control group (Table II). Likewise, the periosteal and endosteal linear extent of bone mineralization were similar in the control and lithium-treated animals indicating that the extent of bone surface undergoing mineralization was not affected by lithium. However, as shown in Table II, lithium treatment resulted in a significant decrease in the PMR. Representative ultraviolet photomicrographs of the periosteal surface of tetracycline double-labeled control and lithium bones are illustrated in Fig. 2A and B, respectively. The mean value of the EMR was also lower in the lithium-treated than in the control animals, although the changes were not statistically significant (Table II).

Since tetracycline is only deposited in bone that is undergoing mineralization, the decrease in PMR in the lithium-treated animals could be attributed either to inhibition of the mineralization of osteoid or inhibition of collagen formation with a resultant decrease in the mineralization rate because of diminished osteoid formation. As shown in Table III, the volume of osteoid present in the tibias of the lithium-treated rats was significantly less than that in the control animals when expressed either as the ratio of osteoid to mineralized bone plus osteoid (% matrix) or as the ratio of bone surface covered by osteoid to total bone surface (% surface). This reduction in osteoid is graphically demonstrated in Fig. 3A and B, representative photomicrographs of tibial sections of control and lithium-treated bones, respectively.

DISCUSSION

This study demonstrates that blood lithium levels approximating those achieved in psychotic patients treated with this agent (21) resulted in decreased bone mineralization rates and diminished osteoid formation in the rat. Our control value for the periosteal mineralization rate, 3.57±0.43 $\mu m/day$, correlates with the value of 3.2 $\mu m/day$ reported by previous investi-

gators as the periosteal formation rate in the femur of rats of similar weight (22). Subsequent studies by others with the rat tibia have reported periosteal and endosteal formation rates of 6.0 $\mu m/day$ and 2.9 $\mu m/day$, respectively (23). The discrepancies in the reported bone mineralization rates in rats (22–25) are most likely a result of differences in the experimental conditions, the weights and ages of the animals, and the bones studied. Both the rat tibia and femur are complicated by structural asymmetry and cortical drift (25–26). Hence, the rates of growth of these bones vary considerably from one area to another within the section being measured (25). This applies to all hollow bones in the rat skeleton with the exception of the vertebrae which are uncomplicated by cortical drift (25).

We were unable to demonstrate a statistically significant inhibitory effect of lithium on endosteal mineralization rate (Table II). This is most likely because of the fact that endosteal bone formation as indicated by a well-defined, regular tetracycline-labeled endosteal margin commences in the caudal vertebrae in the 130–150-g animal (25). Our animals achieved this weight after approximately 7 days of lithium treatment. Hence, while mineralization was occurring at the periosteal surface during the entire 4 wk of lithium

TABLE III
Histomorphometric Analysis of Rat Tibias

	Percent of matrix occupied by osteoid	Percent of surface covered by osteoid
	%	
Control	3.17±0.55	9.56±1.73
Lithium-treated	0.06 ± 0.06	0.57±0.27
	$P < 0.025$	$P < 0.01$

Values represent the mean±SEM of four control and four lithium-treated rats and are the same rats whose kinetic parameters appear in Table II.
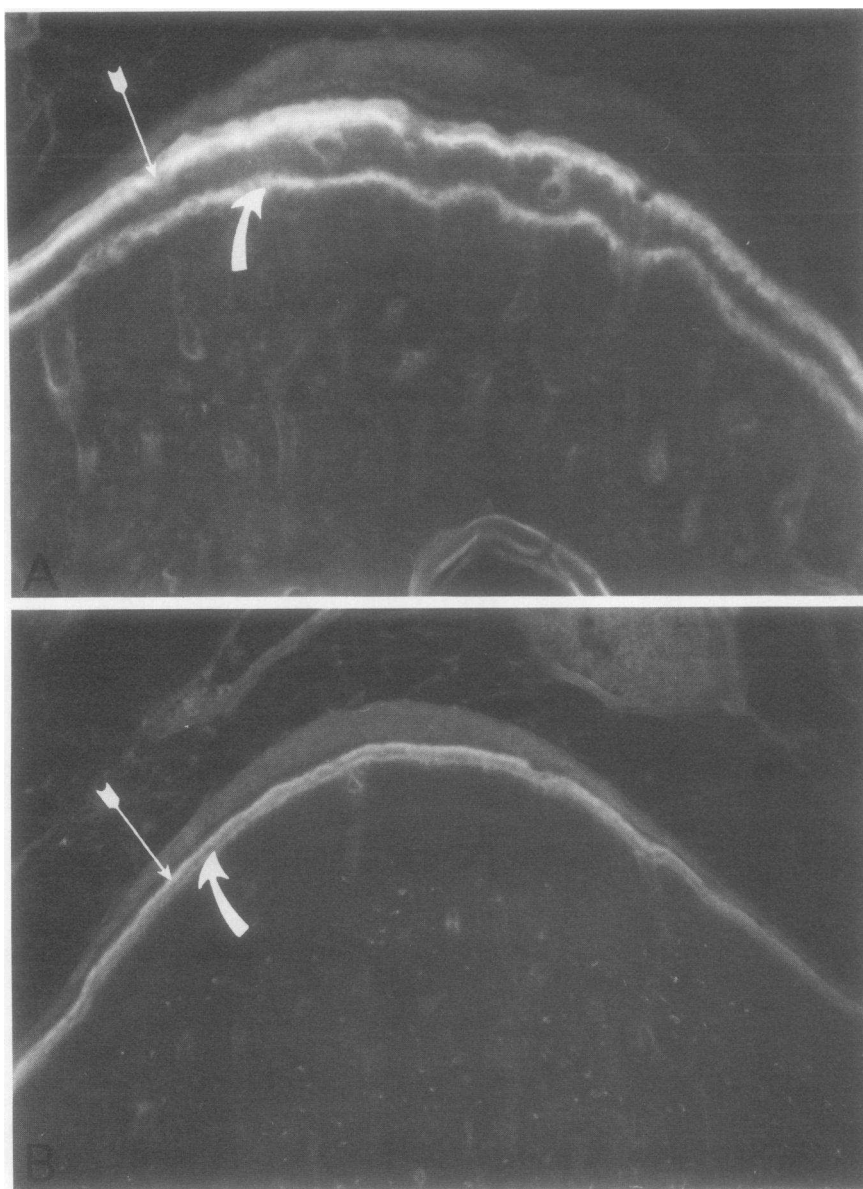
FIGURE 2 Periosteal double fluorescent tetracycline labels in (A) control and (B) lithium-treated rats. The label administered 3 days before sacrifice (straight arrow) and that administered 17 days before sacrifice (curved arrow) are much closer in the lithium-treated than control animal, representing a decreased rate of periosteal bone formation (undecalcified, unstained fluorescent micrographs, ×100).

treatment, endosteal mineralization was only proceeding during the last 3 wk of therapy.

Previous studies by others have employed medullary cavity area as an index of increased (23) or decreased (27) bone resorption. Despite suppression of bone formation associated with lithium treatment, we did not observe significant changes in medullary cavity or cortical bone area. It should be appreciated, however, that these are relatively short-term studies. As such, net change in bone mass as measured by medullary cavity and cortical bone area may lag behind alterations in the rate of mineralization. Furthermore, if in more prolonged experiments, the trend towards a decrement in medullary cavity area with lithium treatment proves to be significant, it may reflect suppression of bone resorption as well as formation, as obtains in the remodelling human skeleton during states of glucocorticoid excess (28).
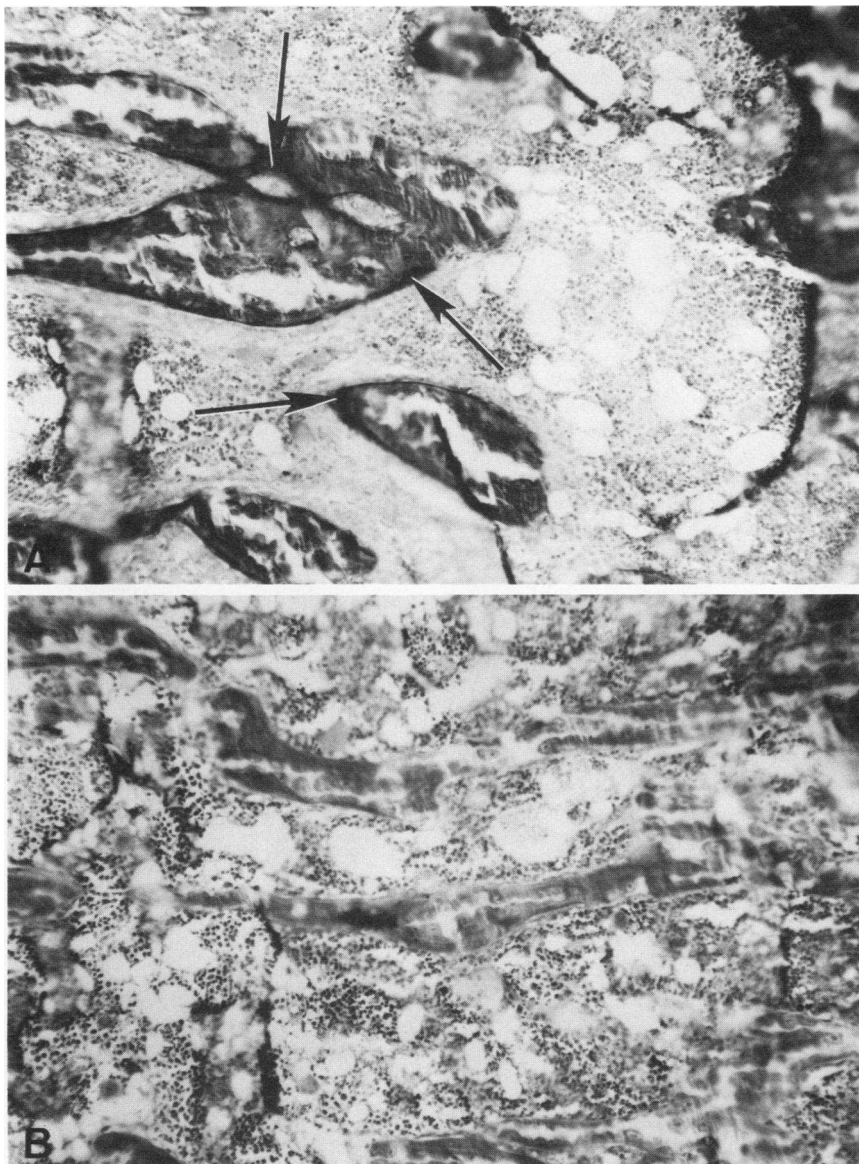
FIGURE 3 Longitudinal sections of tibias of (A) control and (B) lithium-treated rats. Osteoid (arrows) which is prominent in metaphyses of control rats is virtually absent in lithium-treated animals (undecalcified, modified Masson, ×100).

The discrepancies between our in vivo data and those in vitro results previously reported by others (7, 8) are most likely a function of experimental conditions and the parameters measured, because in previous studies either the rate of collagen resorption in vitro and/or bone wet weight was quantitated. Our data support the hypothesis that the previously reported systemic effects of lithium on mineral metabolism in man and experimental animal models (2–14) may be a function of the varying skeletal response to the lithium ion.

ACKNOWLEDGMENTS

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