Iron Lactate-Induced Osteopenia in Male Sprague-Dawley Rats

SHUUICHI MATSUSHIMA,1,2 MARIKO HOSHIMOTO,1 MIKINORI TORII,1 KIYOKAZU OZAKI,2 AND ISAO NARAMA2

1 Pathology Section, Drug Safety Evaluation, Developmental Research Laboratories, Shionogi & Co., Ltd., Toyonaka, Osaka, Japan
2 Research Institute of Drug Safety, Setsunan University, 45-1 Nagao-toge-cho, Hirakata, Osaka, 573-0101, Japan

ABSTRACT

Osteopenia was induced in rats fed a diet containing 50,000 ppm (5%) iron lactate for 2 or 4 weeks. Blood chemistry, urinalysis, and bone histomorphometry of the proximal tibial metaphysis were performed. Urinary pyridinoline and deoxypyridinoline and the osteoclast number per bone surface were selected for the measurement of dynamic resorption. The osteoclast surface, eroded surface, and osteoblast surface increased at both ends of the exposure periods, and bone resorption and formation both increased. The bone volume, trabecular thickness, and trabecular number decreased, and the secondary spongiosa of proximal metaphysis showed a marked bone loss. However, no mineralization defect was observed. At the end of the 2-week exposure period, biomarkers of osteoclasts and osteoblasts had increased the most, and the osteoblast surface, osteoclast surface, and osteoclast number per bone surface increased with prolonged exposure. The pathological changes of the bone lesion in iron lactate-overloaded rats were similar to those in rats of the osteoporotic model, because they consisted of changes reflecting the increase of bone resorption and formation without an osteomalacic change. However, the decline of serum parathyroid hormone (PTH) levels was different from that of the osteoporosis model rat.

We concluded iron-induced bone lesions probably differ from those of low turnover bone diseases.

Keywords. Iron lactate; osteopenia; PTH; osteoclast; osteoblast.

INTRODUCTION

Osteoporosis is one of the most frequent bone diseases affecting postmenopausal women (estrogen-depleted) and aged men. It is associated with low bone mass, resulting from acceleration of bone resorption and inhibition of bone formation (3). Bone lesions similar to postmenopausal osteoporosis have been induced by ovariectomy, thyroid-parathyroidectomy, immobilization, partial fifth-sixths nephrectomy, excess administration of glucocorticoid, and low-calcium diet in rats (2, 16, 19, 21, 23, 27, 34, 35). Some metals such as cadmium and aluminum induce osteomalacia (14, 15, 28, 33), but not osteoporosis.

Narama et al (1999) have previously reported eosinophilic gastroenterocolitis induced in rats given feed containing iron lactate for 3 months (25, 26). In a 2-week preliminary dose finding experiment (data not shown), we also observed excessive bone loss of secondary spongiosa similar to osteoporotic lesion in rats; however, the bone toxicity of iron lactate was not fully evaluated to find whether it was caused by malnutrition due to gastroenterocolitis or direct toxicity of iron lactate. We thought that the bone resorption induced by iron lactate started at an early stage of the exposure, because the tibia showed severe bone loss at 3 months after exposure. To evaluate early events of bone toxicity, we used 2- or 4-week iron lactate exposure groups and pair-fed control groups.

In the present study, we reproduced experimental osteopenia in male rats by exposure to an overload of iron lactate and investigated the pathological mechanisms of early bone lesions.

MATERIALS AND METHODS

Animals and Test Materials: Forty male Sprague-Dawley rats were obtained at 4 weeks of age from Ishibe Breeding Laboratory, Clea Japan Inc, and were acclimatized for 12 days until the start of the study. The rats were divided into 4 groups of 10 each, receiving 0 or 50,000 ppm iron lactate (Musashino Chemical, Japan) in a commercial diet (CRF-1, Oriental Yeast, Japan) for 2 or 4 weeks. The admixed diet was insufflated with nitric gas and kept sealed in light-resistant containers at 4°C to prevent oxidation before feeding. Each control group was pair-fed the diet throughout the exposure period. Rats were individually housed in polycarbonate cages in a barrier-sustained animal room, air-conditioned at 21°C ~ 25°C with a relative humidity of 40% ~ 70% with 15 air changes/h under a 12-h light/12-h dark cycle. All rats were supplied tap water ad libitum throughout the experimental periods. Body weight was measured twice and food consumption was measured 3 times weekly throughout the experimental periods. The body weights ranged from 150.8 to 198.3 g at the beginning of dosing. All rats were administered 20 mg/kg tetracycline hydrochloride (Nacalai Tesque, Inc, Japan) subcutaneously to double-label the bone at 5 and 2 days before necropsy. The rats were cared for according to the principles outlined in the guide for the care and use of laboratory animals prepared by the Japanese Association for Laboratory Animal Science and our institution.

Hematology and Blood Chemistry: At the end of the study, blood samples were collected from the posterior vena cava of all rats under pentobarbital sodium anesthesia (Nembutal® injection, Dainippon Pharmaceutical Co., Ltd., Japan) and treated with EDTA (Anglot/ET2, Nihon Shoji Co., Ltd, Japan). Routine hematological parameters were determined using an automated blood analyzer (THMS H*1, Bayer, Germany); white blood cell count, red blood cell count, hemoglobin concentration, hematocrit value, mean

Address correspondence to: Shuuichi Matsushima, Pathology Section, Drug Safety Evaluation, Developmental Research Laboratories, Shionogi & Co., Ltd., 3-1-1 Futaba-cho, Toyonaka, Osaka 561-0825, Japan. Fax: 06-6332-6385. E-mail: shuuichi.matsushima@shionogi.co.jp
corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, differential counts of leukocytes, and platelet counts. Routine blood chemistry parameters were also examined with an automated analyzer (Hitachi 7170, Japan); glucose, total protein, albumin, total cholesterol, creatinine, urea nitrogen, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase, creatine kinase, amylase, triglyceride, total bilirubin, sodium, potassium, chloride, calcium, inorganic phosphorus, and iron. Osteocalcin and PTH were measured with a commercial radioimmunoassay kit (Biochemical Technologies Inc, USA and Nichols Institute Diagnostics, USA).

Urinalysis: The day before the end of each study period, all rats were placed in individual metabolic cages, and 4-h urine samples were collected from the rats deprived of food. Calcium, inorganic phosphorus, and iron in urine were examined with an automated analyzer (Environmental Biological Life Science Research Center Inc, Japan). Pyridinoline and deoxypyridinoline were assayed by high performance liquid chromatography (Mitsubishi Chemical Safety Institute, Ltd, Japan).

Bone Histomorphometry and Histopathology: All rats were euthanized by exsanguination under anesthesia with sodium pentobarbital (Nembutal®, Dainippon Pharmaceutical Co, Ltd, Japan) at the end of study and each rat underwent a gross necropsy. The tibia and thyroid and parathyroid glands were removed, and the tibia were fixed in 10% neutral buffered formalin at 4°C and the thyroid and parathyroid glands were also fixed in 10% neutral buffered formalin but at room temperature. The right tibia was dehydrated with graded alcohol solution, embedded in methyl methacrylate (Osteo-Bed, Polyscience, Inc, UK), and cut into 3 μm-thick undecalci/bullet5ed sections (Rotary Microtome HM 350, Microm, Germany) of the proximal tibia, and stained with Goldner’s Masson trichrome and Toluidine blue then with Berlin blue for ferric iron. The left tibia was transferred to 10% ethylenediamineteraetic acid (EDTA) at 4°C, de- calcified 7 days on average, and embedded in hydroxyethyl methacrylate (Technovit® 7100, Kulzer & Co, GmbH, Germany). The proximal tibia was cut into 3 μm-thick sections. Decalcified plastic sections were stained with tartrate-resistant acid phosphatase (TRAP) as a marker enzyme of osteoclasts and additionally stained with Toluidine blue and hematoxylin-eosin then examined histopathologically and histomorphometrically. The thyroid and parathyroid glands were routinely processed and embedded in paraffin. These paraffin sections were stained with hematoxylin-eosin and Berlin blue for ferric iron and examined microscopically.

Histomorphometry of proximal tibial metaphysis was performed using a computer-digitizing image system consisting of a light microscope with a camera (Fuji HC-2000, Fujifilm, Japan), a flat scanner (ScanMaker, Microtek, Japan) and software (Image-Pro® Plus, Media Cybernetics®, USA). An area of about 6 mm² of secondary spongiosa was measured in the central proximal tibial metaphysis beginning at 1 mm distal to the growth plate-metaphyseal junction. Histomorphometric parameters measured and calculated for the undecalci/bullet5ed sections included the following: bone volume (BV/TV), osteoid volume (OV/BV), osteoid surface (OS/BS), osteoblast surface (Ob.S/BS), eroded surface (ES/BS), osteoclast surface (Oc.S/BS) as static parameters; trabecular thickness (Tb.Th), trabecular number (Tb.N), trabecular separation (Tb.Sp) for the architecture; and double-labeled surface (dLS/BS), mineral apposition rate (MAR) as dynamic parameters (29, 30). Tb.Th, Tb.N, and Tb.Sp were used for Parfitt’s equation (31). The dynamic parameters were measured from photomicrographs of the double-labeled surface that were taken under fluorescent light. The mineral apposition rate was calculated by dividing the labeling width by the number of days between the double labels with tetracycline hydrochloride. Decalcified sections were used for the measurement of TRAP-positive cells and pathological examination. The TRAP-positive cell counts were expressed as the number of multinuclear TRAP-positive cells, and used as a static parameter, osteocalcnum per mm of bone surface (N.Oc/BS).

Statistical Analysis: All data are expressed as the mean ± SD on the tables and in the figure. Body weights were analyzed by two-factor ANOVA. Statistical significance was also analyzed by Student’s t-test for body weight, hematology, blood chemistry, urinalysis, and bone morphometric parameters, comparing the 2- and 4-week exposure groups with the respective pair-fed control group, and also between 2- and 4-week exposure groups. The probability level was set at 0.05 or 0.01 for the criteria of significance.

RESULTS

Body Weight (Figure 1): During the first 2 weeks, the body weight of the iron lactate exposure group was higher than the pair-fed control group. However, during the following 2 weeks, the iron lactate exposure group was consistently lower than the pair-fed control group.

Hematology and Blood Biochemistry (Table 1 and Table 2): In the 2-week exposure group, the white blood cell count and number of basophils were significantly higher, yet the red blood cells and platelet counts were significantly lower than in the 2-week, pair-fed control group. In the 4-week exposure group, hemoglobin concentration, mean corpuscular volume, mean corpuscular hemoglobin, and platelet counts were also significantly higher than in the 4-week, pair-fed control group. However, these hematological changes were marginal.

The blood biochemistry showed similar changes among the 2- or 4-week exposure groups. In the 2-week exposure group, osteocalcin (+53%), the activity of alkaline phosphatase (+67%) and inorganic phosphorous level (+45%) as markers of bone formation were significantly higher, yet PTH (−53%) was significantly lower than in the 2-week, pair-fed control group. In the 4-week exposure group, osteocalcin (+59%), alkaline phosphatase (+28%), and inorganic phosphorous (+36%) were significantly higher, whereas PTH (−55%) was significantly lower than in the 4-week, pair-fed control group. Between the 2- and 4-week exposure groups, alkaline phosphatase and inorganic phosphorus were 30% and 14% lower in the 4-week exposure group than in the 2-week exposure values, respectively.

Urinalysis (Table 3): The results of urinalysis were similar among the 2- or 4-week exposure groups. In the 2-week
FIGURE 1.—Body weight changes of pair-fed control and iron lactate-overloaded rats. Each point represents the mean±SD (N = 10). **Statistically significant against respective control group at p < 0.01.

exposure group, pyridinoline and deoxypyridinoline as markers of the collagen-breakdown product were 35% and 103% significantly higher than in the 2-week, pair-fed control group, respectively. Iron and calcium were also 59-fold and 27-fold significantly higher, respectively, and inorganic phosphorous was 99% significantly lower than in the 2-week, pair-fed control group. In the 4-week exposure group, pyridinoline and deoxypyridinoline were 39% and

| Table 1.—Hematological findings in dietary iron lactate-overloaded rats for 2 or 4 weeks. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Group | Dose (ppm) | No. of rats | WBC (×10³/µl) | RBC (mill) | HGB (g/dl) | HCT (%) | MCH (pg) | MCHC (g/dl) | PLT (×10³/µl) | NEUT (%) | LYMPH (%) | MONO (%) | EOS (%) | BAS (%) |
| 2-week Control | 0 | 10 | 3.7 ±0.7 | 7.1 ±0.3 | 14.6 ±1.8 | 37.5 ±1.2 | 58.5 ±1.2 | 20.7 ±0.5 | 35.4 ±0.8 | 1,598.5 ±336.0 | 18.8 ±7.5 | 79.5 ±1.3 | 2.6 ±1.3 | 0.7 ±0.6 |
| Iron lactate | 50,000 | 10 | 4.9** ±1.4 | 6.7** ±0.3 | 14.2 ±1.3 | 39.8 ±3.0 | 59.4 ±1.1 | 21.2 ±0.4 | 35.6 ±1.2 | 1,281.2** ±110.3 | 15.5 ±5.0 | 79.5 ±0.6 | 2.2 ±0.9 | 1.8 ±0.7 |

Values are expressed as mean± standard deviation.
* **Statistically significant against respective control group at p < 0.05 and p < 0.01.

TABLE 2.—Blood chemistry dietary iron lactate-overloaded rats for 2 or 4 weeks.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (ppm)</th>
<th>No. of rats</th>
<th>Osteocalcin (ng/ml)</th>
<th>PTH (pg/ml)</th>
<th>T. Pro (g/dl)</th>
<th>Alb (g/dl)</th>
<th>Crea (mg/dl)</th>
<th>UN (u/l)</th>
<th>AST (u/l)</th>
<th>ALT (u/l)</th>
<th>ALP (u/l)</th>
<th>LD (u/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-week</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>10</td>
<td>92.7 ± 3.6</td>
<td>±13.6</td>
<td>±4.1</td>
<td>±0.2</td>
<td>±1.3</td>
<td>3.2</td>
<td>±6.7</td>
<td>±5.3</td>
<td>±10.2</td>
<td>±2.4</td>
</tr>
<tr>
<td>Iron lactate</td>
<td>50,000</td>
<td>10</td>
<td>141.4 ± 15.3**</td>
<td>±21.7</td>
<td>±6.0</td>
<td>±0.2</td>
<td>±0.03</td>
<td>±3.9</td>
<td>±19.2</td>
<td>±20.1</td>
<td>±251.1</td>
<td>±34.7</td>
</tr>
<tr>
<td>4-week</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>10</td>
<td>81.8 ± 7.0</td>
<td>±15.0</td>
<td>±0.2</td>
<td>±3.1</td>
<td>±51.2</td>
<td>±40.5</td>
<td>±5.5</td>
<td>±88.3</td>
<td>±15.6</td>
<td>±76.1</td>
</tr>
<tr>
<td>Iron lactate</td>
<td>50,000</td>
<td>10</td>
<td>130.3 ± 15.7**</td>
<td>±32.8</td>
<td>±6.3</td>
<td>±0.3</td>
<td>±2.6</td>
<td>±74.1</td>
<td>±5.0</td>
<td>±45.2</td>
<td>±155.9</td>
<td>±203.8</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation.

**Statistically significant against respective control group at p < 0.05 and p < 0.01.

††Statistically significant against 2-week exposure group at p < 0.05 and p < 0.01.

PTH: parathyroid hormone; Glu: glucose; T. Pro: total protein; Alb: albumin; Crea: creatinine; UN: urea nitrogen; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; LD: lactate dehydrogenase; CK: creatine kinase; AMY: amylase; T. Bil: total bilirubin; T. Cho: total cholesterol; T. Glu: triglyceride; IP: inorganic phosphorus; Ca: calcium; Na: sodium; K: potassium; Cl: chloride; Fe: iron.

111% significantly higher than in the 4-week, pair-fed control group, respectively. Iron and calcium were also 87-fold and 56-fold significantly higher, and inorganic phosphorus was 99% significantly lower than in the 4-week, pair-fed control group, respectively.

Pyridinoline and deoxypyridinoline were 32% and 39% lower in the 4-week exposure group than in the 2-week exposure group, respectively. These results showed that the osteoclast decreased with prolonged exposure.

Histopathology: Narrowing and disappearance of trabecula in the secondary spongiosa occurred in both 2- and 4-week exposure groups and were often accompanied by thinning of the primary spongiosa (Figures 2, 3). Many osteoclasts accumulated at the eroded surface of the bone trabecula. Active osteoblasts characterized by cuboidal or columnar shapes were also present adjacent to the bone surface (Figure 4). No positive reaction was detected on the surface of the trabecula in the tibia by Berlin blue staining, and no significant changes were observed in the parathyroid gland.

Bone Histomorphometry (Table 4): The static parameters were markedly high in the 2-week exposure group such as the osteoclast surface (+107%), eroded surface (+55%), and osteoblast surface (+115%), and the osteoclast number per bone surface (+52%) and trabecular separation (+47%) were also significantly higher than in the 2-week, pair-fed control group. On the other hand, bone volume (−43%), trabecular thickness (−24%), and number of trabecula (−24%)

TABLE 3.—Urinalysis in dietary iron lactate-overloaded rats for 2 or 4 weeks.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (ppm)</th>
<th>No. of rats</th>
<th>PYR (pM/M-Cr)</th>
<th>D-PYR (pM/M-Cr)</th>
<th>Fe (μg/dl)</th>
<th>IP (mg/dl)</th>
<th>Ca (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-week</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>10</td>
<td>77.9 ± 4.6</td>
<td>±9.6</td>
<td>±6.4</td>
<td>±19.7</td>
<td>±155.9</td>
</tr>
<tr>
<td>Iron lactate</td>
<td>50,000</td>
<td>10</td>
<td>105.7 ± 10.7</td>
<td>±10.7</td>
<td>±139.3**</td>
<td>±392.2**</td>
<td>±82.4**</td>
</tr>
<tr>
<td>4-week</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>10</td>
<td>51.2 ± 7.9</td>
<td>±7.9</td>
<td>±7.0</td>
<td>±3.1</td>
<td>±78.2</td>
</tr>
<tr>
<td>Iron lactate</td>
<td>50,000</td>
<td>10</td>
<td>71.24**±†</td>
<td>±4.9</td>
<td>±45.5</td>
<td>±76.9</td>
<td>±11.4</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation.

**Statistically significant against respective control group at p < 0.01.

††Statistically significant against 2-week exposure group at p < 0.01.

PYR: pyridinoline; D-PYR: deoxypyridinoline; IP: inorganic phosphorus; Ca: calcium; Fe: iron.
were significantly lower than in the 2-week, pair-fed control group. Similar histomorphometry was observed in the 4-week exposure group. The static parameters, osteoclast surface (+60%), eroded surface (+90%), osteoblast surface (+157%), osteoclast number per bone surface (+122%), and trabecular separation (+82%), were significantly higher, whereas the bone volume (−51%), trabecular thickness (−24%), and trabecular number (−35%) were significantly lower than in the 4-week, pair-fed control group. In the 2- or 4-week exposure groups, the dynamic parameters of the double-labeled surface were significantly lower than in the pair-fed control groups, respectively, but the mineral apposition rate was not significantly different from that in the pair-fed control groups, respectively. Between 2- and 4-week exposure groups, bone volume (−17%) was significantly lower in the 4-week exposure group, yet trabecular separation (+33%) was significantly higher than in the 2-week exposure group.

With prolongation of the exposure period, the osteoclast surface and osteoclast number per bone surface increased, leading to decrease of bone trabecular.

DISCUSSION

The effect of iron overloading on bone resorption has not been subjected to biochemical or histomorphometrical study, although much effort has been expended in trying to clarify the mechanism of toxic or carcinogenic effects of iron on the liver, kidney, and other organs by detailed histopathology of iron toxicity (4, 8, 9, 12, 17, 18, 20, 32, 36, 37, 40). We observed bone loss of the secondary spongiosa in rats in a preliminary 3-month toxicity study of iron-lactate and conducted the present study to induce bone lesions and clarify the early changes using histomorphometry and biochemical markers of bone turnover.

Marked increase of urine pyridinoline and deoxypyridinoline, serum osteocalcin, and alkaline phosphatase consisted of increase of osteoclasts and osteoblasts. Bone volume and trabecular number decreased after 2 weeks and secondary spongiosa were found in the tibial region, showing marked bone loss. From the standpoint of increased bone metabolic markers, overload of iron lactate increased both bone resorption and formation. However, because of the increase of bone resorption that exceeded bone formation, the net bone volume gradually decreased. The decrease of the trabecular in the second spongiosa is similar to experimental osteoporosis (16, 19, 21–24, 27, 34, 35, 39).

Osteoclasts secrete tartrate-resistant acid phosphatase in which the binuclear iron center is important for regulating activity (11). Electrons from ferrous ions are transferred to molecular di-oxygen in a reversible process (13). Therefore, ferrous iron appears to directly stimulate the enzyme for resorption by osteoclasts and may have played a role in triggering bone lesions in the 2-week study. The definite role of ferrous iron for osteoclasts must be different from ferric
iron, and this may be why the lesion in the present study was different from those observed in other studies (6, 8, 31, 32).

Our results indicated the following steps for the formation of bone lesions in iron-overloaded rats: a) ferrous iron affects bone resorption and formation; however, the excessive bone resorption causes decrease of the net bone volume; b) ferrous iron mediates hydroxy radical formation or decreases the pH by oxidative stress (6), affects the bone matrix and increases osteoclasts, and causes excessive bone resorption; c) ferrous iron directly affects osteoclasts and increases them, and osteoblasts could be increased by the stimulation.

In experimental osteoporosis, PTH secretion, bone resorption and formation, serum osteocalcin and alkaline phosphatase, urinary pyridinoline, and deoxypyridinoline values increase simultaneously (21, 27, 38). However, in 2- or 4-week exposure groups in the present study, most of the other biochemical markers increase and PTH secretion decreases. In other words, iron lactate induces bone resorption but does not accompany PTH secretion.

Some low turnover bone diseases are caused by iron or aluminum, which induce osteomalacia in dialysis patients. These bone diseases display PTH hyposecretion, excessive osteoid of the bone surface, mineralization defect, and the disappearance of fluorescent labels (1, 5, 10, 32, 36, 37). In the present study, bone resorption progressed rapidly, but was not accompanied by any mineralization defect. Thus, iron lactate-induced bone lesions probably differ from these low turnover bone diseases.

PTH secretion is mainly regulated by the serum calcium level, and PTH increases the activation of osteoclastic resorption and mobilizes calcium from the skeletal reserves into the extracellular fluids. PTH also regulates the uptake of calcium or excretion of phosphorus, leading to calcium hyposecretion or phosphaturia in the kidney (7). The administration of iron lactate results in a decrease in PTH and an increase in serum phosphorus but does not affect the serum calcium. Narama et al (1999) described elevated serum BUN and creatinine levels in iron-overloaded rat, which suggested slight but consistent renal toxicity without morphological evidence of cytotoxicity (26). This adverse effect on the renal tubule epithelium may lead to calcuria. As the serum calcium level is normal in this study, iron lactate does not affect the ability to absorb calcium from the intestinal epithelium. According to these biochemical data, the decrease of PTH promotes the reabsorption of phosphorus, drains phosphorus from the urine, suppresses the reabsorption of calcium, and leads to calcuria.

No lesion was observed in the parathyroid gland and the cause of hyposecretion of PTH remains unclear, as does the reason that the bone formation was increased under low PTH. Further study with exogenous PTH-loaded animals should clarify the effect of iron lactate.

ACKNOWLEDGMENT

The authors wish to thank Mr. Muneaki Yugawa of Musashino Chemical Laboratory Co, Ltd, for providing the iron lactate test material.

REFERENCES