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# Histomorphometric Study of Ribs with Looser Zones in Itai-Itai Disease

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**Abstract.** Twelve Looser zones and 17 healing bands of the ribs obtained from autopsy cases of Itai-itai disease were analyzed by bone histomorphometry. Furthermore, proper cancellous tissue of the ribs from 24 autopsy cases of Itai-itai disease with Looser zones or with the healing bands, 27 autopsy cases of Itai-itai disease without such lesions, and 29 control cases were studied by the same method to pursue the histogenesis of Looser zones. In translucent zones of Looser zones, 94% of the cancellous bone was occupied by thick woven bone in which 72% was woven osteoid and 22% was woven mineralized bone. In adjacent scleroses, 71% of the cancellous bone was occupied by woven bone in which 37% was woven mineralized bone, and 34% was woven osteoid; 53% of the cancellous bone consisted of mineralized bone. As compared with those in translucent zones, woven osteoid was decreased, and mineralized bone was increased significantly in the cancellous bone of adjacent scleroses. A significant increase of lamellar mineralized bone and a decrease of woven bone in healing bands were observed as compared with those in Looser zones. These findings suggest that the healing starts from the edge of the Looser zone, and slowly proceeds toward the center. In the cancellous bone of the ribs, the volume, thickness, and surface of osteoid and woven bone were significantly increased in patients with Itai-itai disease, with Looser zones as compared with those without Looser zones. It was concluded that Looser zones seem to occur in severe osteomalacic bones that contain abundant woven bone in the patients of Itai-itai disease.

**Key words:** Looser zone — Itai-itai disease — Histomorphometry — Woven bone — Osteomalacia.

Itai-itai disease is considered to be caused by parenteral chronic cadmium intoxication, and most patients are female and suffer from severe osteomalacia associated with osteoporosis [1–5]. In 76 autopsy cases of Itai-itai disease (1979–1992), Looser zones were observed in 46 (61%). A Looser zone has the following radiographic characteristics: a broad band of lucency that is perpendicular to the cortical surface with parallel margins, minimal or absent callus, and minimal marginal sclerosis. In 1920, Looser first gave the classical description of this feature [6]. These findings have since been reported by numerous investigators [7–11] as the radiologically important hallmark of osteomalacia. On the other hand, histological analyses of these lesions are few. Only a few descriptions have shown that they consist of unmineralized fracture callus or new uncalcified bone [6, 8, 12]. To clearly characterize the histomorphologic findings of Looser zone, Looser zones and their healing bands observed in the ribs of the cases with Itai-itai disease were examined by bone histomorphometry in the present study. The cancellous bones of the ribs were also examined with the same method to show the relationship between Looser zones and their background lesions, if any. We observed various amounts of woven bone composed of irregularly orientated collagen fibers in most specimens of Itai-itai disease. Therefore, several parameters that can evaluate the quality of woven osteoid and woven mineralized bone were added.

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## Materials and Methods

Autopsy cases of Itai-itai disease were examined in Study 1 and Study 2. The clinical diagnosis was based on the opinion of the Ministry of Health and Welfare with regard to Itai-itai disease in Toyama Prefecture [13]. The diagnosis of Itai-itai disease is made when patients fulfill the following four criteria [14]: (1) to live in an area polluted with cadmium; (2) have symptoms that appear in adults (especially after menopause) and are not congenital; (3) exhibit renal tubulopathy, and (4) have osteomalacia accompanied by osteoporosis (according to biopsy or radiogram).

### *Study 1: Bone Histomorphometric Analysis of Looser Zones and Healing Bands in the Ribs*

Twelve Looser zones of the ribs (Fig. 1a,b) from six autopsy cases of Itai-itai disease, aged 76–91 years, and 17 healing bands in the ribs (Fig. 2a,b) from 12 autopsy cases of Itai-itai disease, aged 62–97 years, were examined. Looser zones and their healing bands were identified by radiographs. These lesions were cut at the midline parallel to the long axis of the bones. The specimens were fixed in 15% buffered formalin and postfixed in 0.5% cyanuric chloride solution containing 1% N-methylmorpholin for 1 day. They were then decalcified with 10% EDTA-4Na in distilled water, embedded in paraffin, sectioned at 4 μm thick, and stained with hematoxylin and eosin according to Yoshiki's method [15–17]. In these preparations the osteoid tissue is deeply stained with eosin, and the previous reports [18–20] have verified the correlation to nondecalcified, plastic-embedded sections.

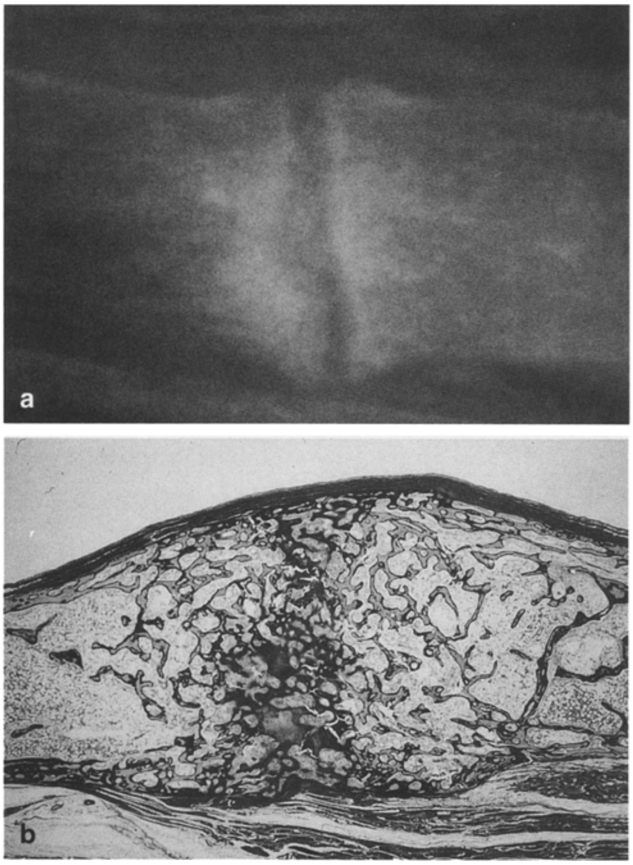


**Fig. 1.** (**a**) Roentgenographic view of a Looser zone in the rib. (**b**) Rupe view of the histologic section from the same portion as in Figure 1a (hematoxylin and eosin stain with Yoshiki's method ×2).

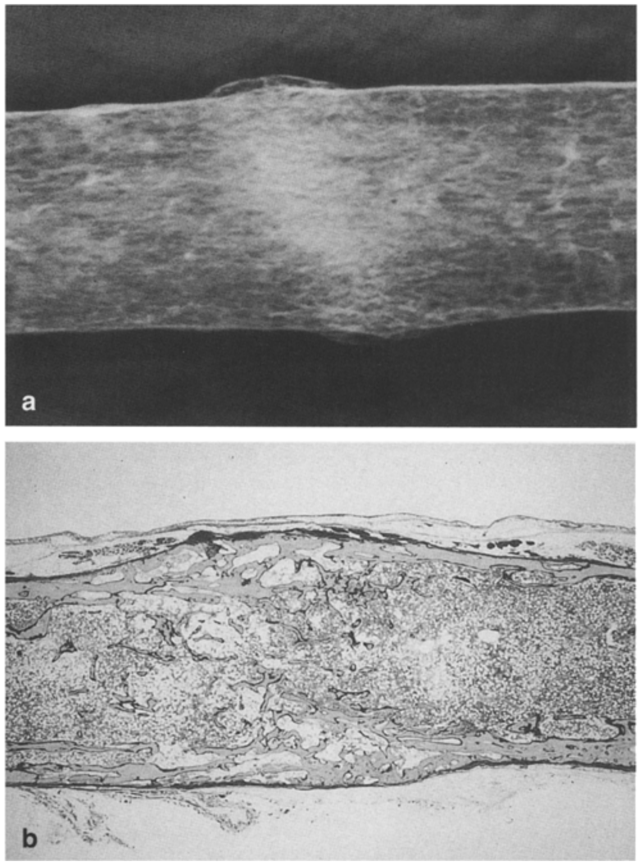


**Fig. 2.** (**a**) Roentgenographic view of a healing band in the rib. (**b**) Rupe view of the histologic section from the same portion as in Figure 2a (hematoxylin and eosin stain with Yoshiki's method ×2).

The translucent zone and the adjacent scleroses of a Looser zone in radiographs were identified histologically on the specimens. Translucent zones were 1–2.5 mm (mean 1.5) in width and adjacent scleroses were 2–4 mm (mean 2.7) in width. In a healing band, we expressed a width of 1.5 mm in the center as the central area and a width of 2.0 mm in the edge as the marginal area. Cancellous bone in each area was quantitatively analyzed by a semiautomatic method [21] and 15–20 optical fields were analyzed at a magnification of ×90. We used static parameters described by Parfitt et al. [22] as follows: (1) bone volume, the fraction of tissue volume occupied by cancellous bone (BV/TV, %); (2) mineralized volume, the fraction of tissue volume occupied by mineralized bone (Md.V/TV, %); (3) osteoid volume, the fraction of bone volume occupied by osteoid (OV/BV, %); (4) osteoid thickness, the mean distance between mineralization fronts and osteoid surfaces (O.Th, μm); (5) osteoid surface, the fraction of trabecular surface covered by osteoid (OS/BS, %); (6) osteoblast surface, the fraction of trabecular surface covered by osteoblasts (Ob.S/BS, %); (7) eroded surface, the fraction of trabecular surface covered by eroded surface (ES/BS, %); (8) osteoclast surface, the fraction of trabecular surface covered by osteoclasts (Oc.S/BS, %); (9) cartilage volume, the fraction of tissue volume occupied by cartilage (Cg.V/TV, %); (10) fibrous tissue volume, the fraction of tissue volume occupied by fibrous tissue (Fb.V/TV, %). Then four parameters that can evaluate the quality of both osteoid and mineralized bone components were added as follows: (11) woven osteoid volume, the fraction of bone volume occupied by woven osteoid (Wo.OV/BV, %); (12) lamellar osteoid volume, the fraction of bone volume occupied by lamellar osteoid (Lm.OV/BV, %); (13) woven mineralized volume, the fraction of bone volume occupied by woven mineralized bone (Wo.Md.V/BV, %); and (14) lamellar mineralized volume, the fraction of bone volume occupied by lamellar mineralized bone (Lm.Md.V/BV, %). These woven osteoid and woven mineralized bones were observed by polarizing light.

The data are indicated as mean ± standard error of the mean (SEM). The significance of differences among groups was evaluated by Student's *t*-test.

### *Study 2: Bone Histomorphometry on the Proper Cancellous Tissue in the Ribs*

Twenty-four autopsy cases of Itai-itai disease with Looser zones or healing bands (Group 1), aged 62–97 years (mean ± SD, 80.2 ± 7.6), and 27 autopsy cases of Itai-itai disease without such lesions (Group 2), aged 69–88 years (78.3 ± 5.3), were examined. All subjects were female. For the control, 29 autopsy cases of female, aged 61–91 years (77.9 ± 8.6) were selected. None had a history of immobilization, or any organic, endocrine/metabolic disease. In Group 1, materials were taken from the sites 2–3 cm from Looser zones (or the healing bands) in the ribs. In Group 2 and the control subjects, materials were taken from the medial region of the right eighth rib. Then, the specimens were prepared with the same method as in Study 1. The entire cancellous tissue area was quantitatively analyzed by the same method as in Study 1 and at least 60 optical fields were analyzed. We used the same parameters as in Study 1 except for cartilage volume and fibrous tissue volume. All data were given as mean ± SEM. The significance of differences among groups was evaluated by one-way analysis of variance (ANOVA).

**Table 1.** Histomorphometric values of Looser zone and healing band[a]

| | Looser zone (n = 12) | | Healing band (n = 17) | |
|---|---|---|---|---|
| Parameters | Translucent zone | Adjacent scleroses | Central area | Marginal area |
| BV/TV (%) | 59.99 ± 1.52 | 53.71 ± 3.10 | 28.31 ± 2.57[c,d] | 18.13 ± 2.06[e] |
| Md.V/TV (%) | 14.91 ± 1.42[b] | 28.73 ± 2.49 | 22.24 ± 1.66[c,d] | 13.92 ± 1.61[e] |
| OV/BV (%) | 75.47 ± 2.26[b] | 46.81 ± 3.19 | 18.94 ± 3.63[d] | 21.33 ± 3.65[e] |
| O.Th (μm) | ND | 23.77 ± 1.53 | 13.76 ± 1.52 | 13.68 ± 1.51[e] |
| OS/BS (%) | 87.66 ± 3.41 | 86.09 ± 3.93 | 60.56 ± 6.54[d] | 65.81 ± 6.55[e] |
| Ob.S/BS (%) | 2.77 ± 0.95 | 2.37 ± 0.60 | 0.58 ± 0.17[d] | 0.59 ± 0.24[e] |
| ES/BS (%) | 8.24 ± 1.64 | 9.09 ± 1.50 | 14.50 ± 1.90[d] | 11.80 ± 2.25 |
| Oc.S/BS (%) | 0.52 ± 0.11 | 0.39 ± 0.09 | 0.22 ± 0.05[d] | 0.11 ± 0.04[e] |
| Cg.V/TV (%) | 2.39 ± 0.63[b] | 0.10 ± 0.08 | 0 ± 0[d] | 0 ± 0 |
| Fb.V/TV (%) | 2.34 ± 0.58[b] | 0.18 ± 0.08 | 0 ± 0[d] | 0 ± 0[e] |

[a] Data are mean ± SEM
[b] Significantly different from adjacent scleroses, $P < 0.01$
[c] Significantly different from marginal area, $P < 0.01$
[d] Significantly different from translucent zone, $P < 0.05$
[e] Significantly different from adjacent scleroses, $P < 0.05$
ND, not determined

**Table 2.** Histomorphometric values of Looser zone and healing band by additional parameters[a]

| | Looser zone (n = 12) | | Healing band (n = 17) | |
|---|---|---|---|---|
| Parameters | Translucent zone | Adjacent scleroses | Central area | Marginal area |
| Wo.OV/BV (%) | 71.80 ± 2.30[b] | 33.58 ± 2.67 | 1.96 ± 0.47[c] | 2.08 ± 0.59[d] |
| Lm.OV/BV (%) | 3.67 ± 0.76[b] | 13.23 ± 1.95 | 16.98 ± 3.20[c] | 19.25 ± 3.20 |
| Wo.Md.V/BV (%) | 21.91 ± 2.03[b] | 36.50 ± 2.65 | 10.85 ± 2.38[c] | 9.59 ± 2.30[d] |
| Lm.Md.V/BV (%) | 2.62 ± 0.43[b] | 16.68 ± 1.86 | 70.21 ± 3.94[c] | 69.09 ± 3.79[d] |

[a] Data are mean ± SEM
[b] Significantly different from adjacent scleroses, $P < 0.001$
[c] Significantly different from translucent zone, $P < 0.01$
[d] Significantly different from adjacent scleroses, $P < 0.001$

## Results

### *Study 1: Looser Zones and Healing Bands*

Tables 1 and 2 show the histomorphometric data on Looser zones and healing bands in cases of Itai-itai disease.

*Looser Zones.* In translucent zones, 75% of the cancellous bone consisted of thick osteoid (Fig. 3a), and 94% was occupied by woven bone in which 72% was woven osteoid and 22% woven mineralized bone (Fig. 3b). There was very little (6%) lamellar bone. In adjacent scleroses, 47% of the cancellous bone consisted of osteoid and 53% mineralized bone (Fig. 4a). Then, 71% was occupied by woven bone in which 37% was woven mineralized bone and 34% was woven osteoid (Fig. 4b). Translucent zones and the adjacent scleroses were compared. Bone volume was not significantly different between the former and the latter. Mineralized volume was significantly increased in adjacent scleroses as compared with translucent zones. On the other hand, osteoid volume was significantly decreased in adjacent scleroses as compared with translucent zones. When osteoid, osteoblast, eroded, and osteoclast surfaces between translucent zones and the adjacent scleroses were compared, no significant differences were noted. Cartilage volume and fibrous tissue volume were significantly increased in translucent zones compared with adjacent scleroses. There was a significant increase in woven osteoid volume in translucent zones compared with adjacent scleroses, whereas lamellar osteoid volume, woven mineralized volume, and lamellar mineralized volume were significantly decreased in translucent zones compared with adjacent scleroses.

*Healing Bands.* Bone volume and mineralized volume in the central area were significantly increased compared with those in the marginal area. The other parameters did not significantly differ between central and marginal area. In the healing bands, approximately 20% of cancellous bone consisted of osteoid and 80% consisted of mineralized bone (Fig. 5a); 87% was occupied by lamellar bone in which 70% of cancellous bone was lamellar mineralized bone (Fig. 5b).

*Looser Zones Compared with Healing Bands.* Bone volume, osteoid volume, osteoid thickness, osteoid surface, osteoblast surface, and osteoclast surface were significantly decreased in the healing bands as compared with Looser zones. In the centers, mineralized volume of the healing bands was significantly increased compared with that of

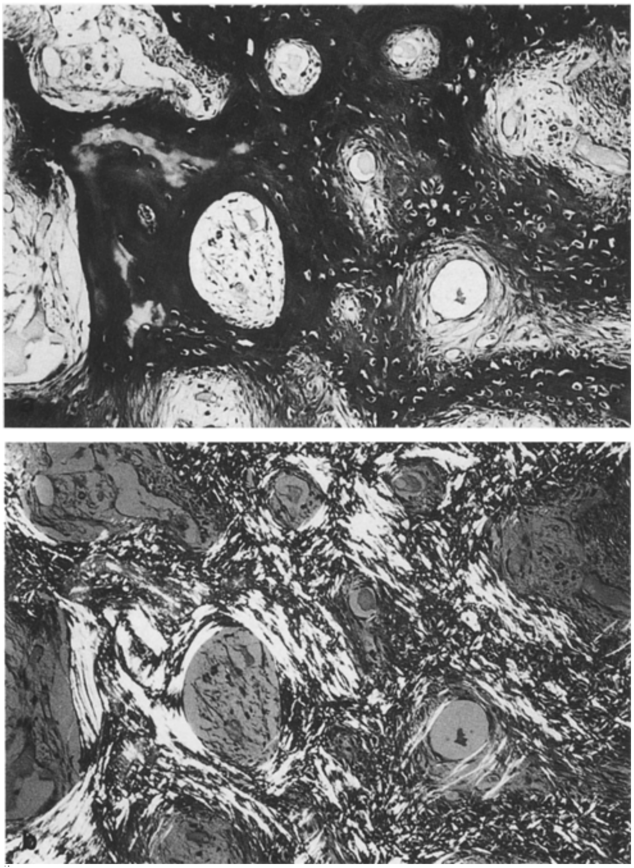

**Fig. 3.** **(a)** Light microscopic view of a translucent zone consisting of a large amount of osteoid (hematoxylin and eosin stain with Yoshiki's method ×40). **(b)** Polarizing light microscopic view of the same portion as in Figure 3a, mostly composed of woven bone.

Looser zones, whereas in the edge, it was significantly decreased. There was a significant decrease in woven osteoid and woven mineralized bone of the healing bands compared with those of Looser zones. Lamellar osteoid volume (only in the centers) and lamellar mineralized volume of the healing bands were significantly increased compared with those of Looser zones.

*Study 2: Proper Cancellous Tissue*

Tables 3 and 4 show the histomorphometric data from the cases of Itai-itai disease with Looser zones (Group 1), the cases without these lesions (Group 2), and the control cases. Table 3 shows that the values for the three groups, except for mineralized volume and osteoclast surface, are significantly different. Bone volume, osteoid volume, osteoid thickness, and osteoid surface were significantly higher in Group 1 than in Group 2. Eroded surface was significantly lower in Group 1 than in Group 2. Mineralized volume, osteoblast surface, and osteoclast surface did not significantly differ between Groups 1 and 2. Table 4 shows that Wo.OV/BV, Lm.OV/BV, Wo.Md.V/BV, and Lm.Md.V/BV are significantly different: Wo.OV/BV, Lm.OV/BV, and Wo.Md.V/BV were significantly higher in Group 1 than in Group 2. Lm.Md.V/BV was significantly lower in Group 1 than in Group 2. These results suggest that in the patients

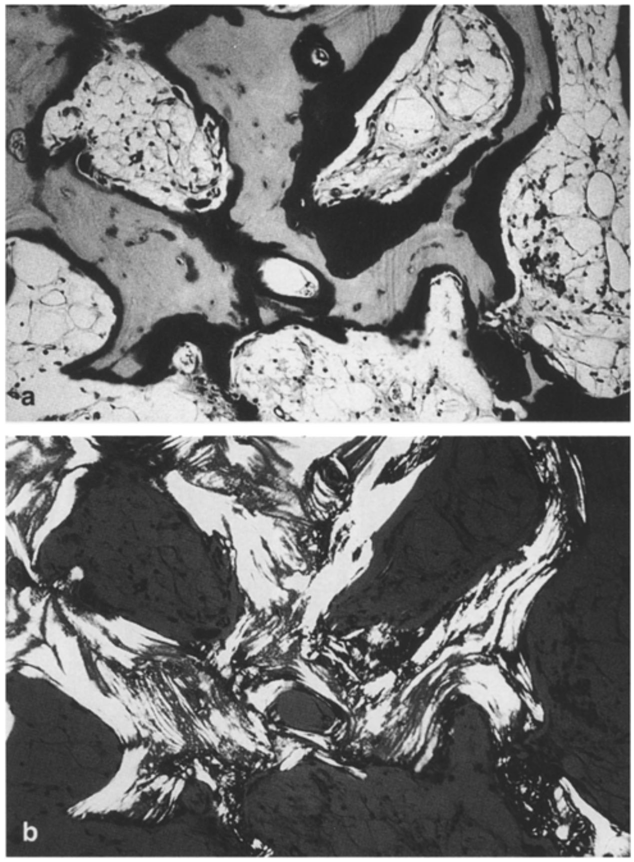

**Fig. 4.** **(a)** Light microscopic view of the adjacent sclerosing portion, composed of osteoid and mineralized bone, the latter being markedly increased (hematoxylin and eosin stain with Yoshiki's method ×40). **(b)** Polarizing light microscopic view of the same portion as in Figure 4a, showing marked decrease of the woven osteoid and marked increase of the lamellar osteoid and mineralized bone.

with Itai-itai disease, Looser zones occur in severe osteomalatic bone in which lamellar bone is decreased and woven bone is increased (Fig. 6a,b).

## Discussion

In this study, the quantitative and qualitative histologic findings of Looser zones and their healing stages (healing bands) were characterized by bone histomorphometric analysis, adding the parameters that can distinguish between woven structure and lamellar structure of the bone. As to Looser zones, in the translucent zone, abundant woven osteoid and as much as 72% and 22% of woven mineralized bone were present; there was little (6%) lamellar bone. In the adjacent scleroses, woven osteoid was 34% and mineralized bone was 53%, including 37% of woven mineralized bone. Compared with the translucent zone, woven osteoid was decreased, and mineralized bone and lamellar osteoid were increased significantly in the adjacent scleroses. In the healing bands, there was no significant difference quantitatively and qualitatively between the center and the edge of the lesion, except that bone volume was more abundant in the center. Compared with Looser zones, the cancellous bones were scantier, and instead of decrease of osteoid vol-

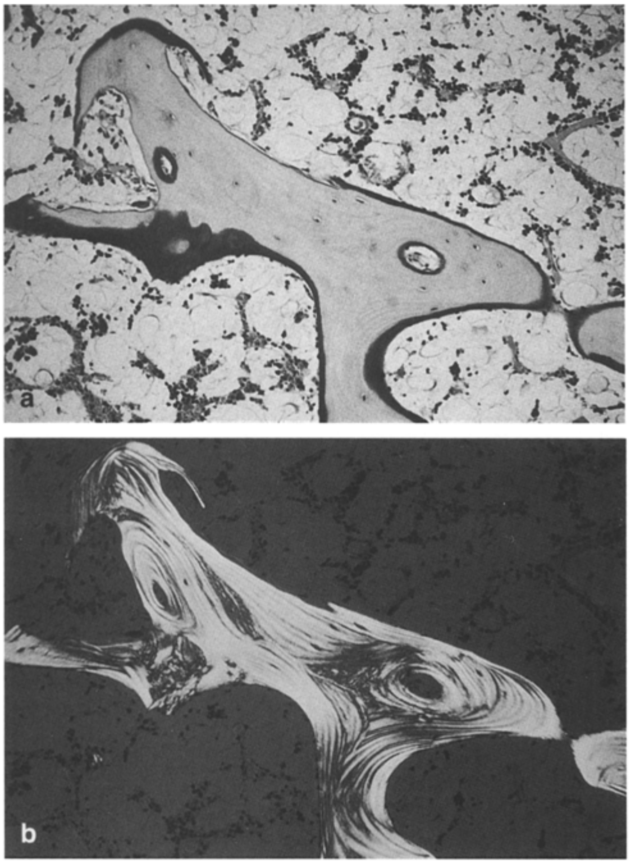

**Fig. 5.** **(a)** Light microscopic view of the healing band, mostly composed of mineralized bone (hematoxylin and eosin stain with Yoshiki's method ×40). **(b)** Polarizing light microscopic view of the same portion as in Figure 5a, mostly composed of lamellar bone, showing marked decrease of the woven bone.

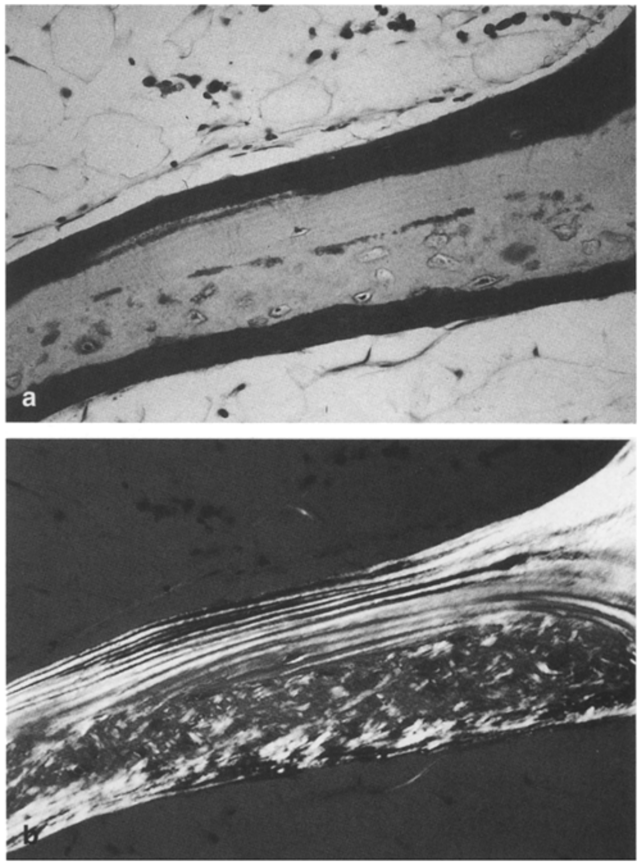

**Fig. 6.** **(a)** Light microscopic view of the cancellous bone in a case of Itai-itai disease with Looser zone, showing decrease of mineralized bone and marked increase of osteoid (hematoxylin and eosin stain with Yoshiki's method ×80). **(b)** Polarizing light microscopic view of the same portion as in Figure 6a, showing marked increase of the woven bone.

**Table 3.** Histomorphometric values of the proper cancellous tissue[a]

| Parameters | Group 1 (n = 24) | Group 2 (n = 27) | Control (n = 29) |
|---|---|---|---|
| BV/TV (%) | 9.06 ± 1.04[b,c] | 5.71 ± 0.55 | 6.19 ± 0.57 |
| Md.V/TV (%) | 5.32 ± 0.63 | 4.48 ± 0.39[d] | 6.11 ± 0.57 |
| OV/BV (%) | 38.51 ± 3.69[b,c] | 19.50 ± 2.18[d] | 1.41 ± 0.18 |
| O.Th (μm) | 22.37 ± 1.50[b,c] | 14.65 ± 0.98[d] | 7.00 ± 0.32 |
| OS/BS (%) | 84.79 ± 3.39[b,c] | 73.83 ± 3.93[d] | 12.19 ± 1.22 |
| Ob.S/BS (%) | 1.52 ± 0.21[c] | 1.15 ± 0.23[d] | 0.57 ± 0.14 |
| ES/BS (%) | 2.54 ± 0.39[b,c] | 5.10 ± 0.86 | 6.02 ± 0.88 |
| Oc.S/BS (%) | 0.09 ± 0.03 | 0.10 ± 0.04 | 0.04 ± 0.02 |

[a] One way ANOVA. Data are mean ± SEM
[b] Significantly different from Group 2 at the 0.05 level
[c] Significantly different from Control at the 0.01 level
[d] Significantly different from Control at the 0.05 level

**Table 4.** Histomorphometric values of the proper cancellous tissue by additional parameters[a]

| Parameters | Group 1 (n = 24) | Group 2 (n = 27) | Control (n = 29) |
|---|---|---|---|
| Wo.OV/BV (%) | 8.58 ± 1.46[bc] | 1.78 ± 0.29 | 0.01 ± 0.01 |
| Lm.OV/BV (%) | 29.93 ± 2.73[bc] | 17.72 ± 2.03[d] | 1.41 ± 0.18 |
| Wo.Md.V/BV (%) | 6.44 ± 0.67[bc] | 3.67 ± 0.54[d] | 0.09 ± 0.04 |
| Lm.Md.V/BV (%) | 55.05 ± 3.80[bc] | 76.64 ± 2.31[d] | 98.48 ± 0.18 |

[a] One way ANOVA. Data are mean ± SEM
[b] Significantly different from Group 2 at the 0.001 level
[c] Significantly different from Control at the 0.001 level
[d] Significantly different from Control at the 0.001 level

ume, mineralized bone volume was increased significantly in the healing bands. In addition, we observed significant increase of lamellar bone and decrease of woven bone in the healing bands compared with those in Looser zones. In the healing bands, 70% of the cancellous bone was occupied by lamellar mineralized bone.

Looser [6] believed that these incomplete linear translucencies in the bone were not due to a sudden break in continuity but to a local remodeling of the bone, and for this reason he used the word "Umbauzonen" (areas of reconstruction) to describe them. As already mentioned, Looser zone begins as an insufficiency type of minor stress fracture in bones subjected to great compression stress [7–10, 23, 24]. The mechanical function of the mineral matrix is to provide resistance to compression. The deficiency of the mineral matrix in osteomalacia may explain the predilection sites of Looser zone. The concept that the lesions begin as minor fractures in bones that are pathologically weakened is acceptable. However, several reports [25–28] have documented patients with symmetric, nonhealing Looser zones in the absence of histologic evidence of osteomalacia, indicating that insufficiency fractures, including Looser zones, do not always occur in osteomalacia.

In the present study, we observed abundant amounts of woven bone with a variable content of fibrous tissue and cartilage in the translucent zone. This evidence suggests that Looser zones begin as minor fractures. We observed that the amount of calcified and lamellar bone were markedly increased in the adjacent sclerosis compared with the translucent zone. These findings suggest that the healing starts from the edge of the Looser zone, and proceeds slowly toward the middle. In usual fractures the callus, the first-formed woven bone, is rapidly covered and eventually replaced by lamellar bone. The healing process in the Looser zone differs markedly from that in this usual fracture.

In patients with Itai-itai disease, the volume, thickness, and surface of osteoid were significantly increased in the bones with Looser zones as compared with those without Looser zones. Previous reports [29, 30] have shown that the volume limit above which was frequent in Looser zones was approximately 20%. Our results coincided well with those reports. We observed various amounts of woven bone composed of irregularly orientated collagen fibers in most patients with Itai-itai disease. Compared with bones without Looser zones, the amount of woven bone (15% of the cancellous bone) was significantly abundant, and the amount of lamellar bone was significantly little in the bones with these lesions. A previous report [31] has shown that the mineral matrix of bone does not affect its tensile strength, which depends largely on the quality and orientation of the collagen fibers. In the cases of Itai-itai disease, Looser zones have occurred most frequently in the ribs which are subjected to both compression stress and tensile strength by respiratory motion. These results have shown that Looser zones occurred in severe osteomalacic bones that were weak against both compression and tensile strength in the patients of Itai-itai disease.

Itai-itai disease is observed among the inhabitants of cadmium-polluted areas, and the damage to renal tubules has been demonstrated by urinary and serum chemistries [2, 32, 33] as well as histologic findings of the renal tubules (tubulopathy) [34]. An injury to the renal tubules is introduced also in the experimental studies of cadmium intoxication [2, 35, 36]. These studies support the idea that the tubulopathy in Itai-itai disease is attributed to cadmium exposure. Osteopathy in Itai-itai disease, therefore, seems to be due to renal tubular dysfunction and eventually to chronic renal failure. These changes are generally called "renal osteodystrophy," or Fanconi's syndrome. It is said that the histologic osseous changes take the form of osteitis fibrosa, osteomalacia, osteoporosis, etc. Pathogenetically, the advancement of the study on the metabolism of minerals and vitamin D has made it clear that these changes are attributed to the disorder of mineral homeostasis, including the defect of synthesis of $1,25(OH)_2D_3$, secondary hyperparathyroidism, hypocalcemia, and hyperphosphatemia. In Itai-itai disease we have observed various amount of woven bone without the findings of secondary hyperparathyroidism, including histologic osteitis fibrosa, increased resorptive activity, as well as increased serum parathyroid hormone level. On the other hand, some reports have shown that cadmium reduces an osteoblastic activity *in vitro* [37, 38] and inhibits a clonal osteogenic cell *in vitro* [39]. These observations and our results suggest that, due to a direct cadmium interference with osteoblastic function, osteoid maturation and mineralization have been impaired in Itai-itai disease.

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