We have studied the deposition of calcium salts in the autopsied intestines which have not been described previously as far as we were able to determine. In order to clarify the incidence, predisposing conditions, mineral compositions of the deposited materials and clinical significance of this phenomenon, we examined 76 cases of consecutive autopsied small intestines by von Kossa’s staining. Deposited calcium salts were further examined by electron microscopically, energy-dispersive X-ray spectroscope and electron diffraction analysis. Deposition of calcium salts was observed in the small intestines of 13 cases. Among them, 10 cases were accompanied by hypercalcemia. Deposition of calcium salts was mainly observed in smooth muscle cells of the proper muscle layers and ganglion cells of the Auerbach’s myenteric plexus. Intestinal calcinosis was frequently accompanied by deposition of calcium salts in the proper muscle layers of esophagus and large intestine, renal tubules and cardiac myocardial cells. Electron microscopically, the calcium salts were identified as needle-shaped crystals and located on the surface of the extracellular-collagen bundles, in the cytoplasm, mitochondria and nucleus of the smooth muscles cells. Energy-dispersive X-ray spectroscopy and electron diffraction analysis suggested the deposited calcium salts as hydroxyapatite. Two patients among the six cases with moderate to severe calcium deposition showed clinical manifestation of paralytic ileus. In conclusion, intestinal calcinosis was frequently observed mostly associated with hypercalcemia. Calcium salts of hydroxyapatite were deposited to the smooth muscle cells and the Auerbach’s myenteric plexus of the muscular layer. Deposition of calcium salts might occasionally causes the paralytic ileus but clinical significance of this lesion requires further examination.

Key words: calcification, intestine, hydroxyapatite

Introduction

Calcification is the deposition of calcium salts in tissues other than bones and teeth. Pathological calcification can be divided into two types, dystrophic and metastatic. Dystrophic calcification occurs in injured tissues in the absence of a systemic mineral imbalance. Metastatic calcification occurs in normal tissues when predisposing conditions exist. Hypercalcemia due to hyperparathyroidism, hypervitaminosis D, tumors secreting PTH-related protein and osteolytic bone metastasis are frequent causes of metastatic calcification.

The most frequent sites of metastatic calcification are the kidneys, lungs and heart. In the gastrointestinal tract, metastatic calcification is sometimes observed in
the fundic glands of the stomach. The preferential metastatic calcification of the stomach and lungs may be partly explained by the alkaline environment due to secretion of free hydrogen ions (stomach) and decreased PaCO₂, which promotes precipitation of calcium-phosphate salts (lung).

We have experienced an autopsy case of a patient who suffered from atypical mycobacterial pneumonia and long-term paralytic ileus of unknown etiology lasting to his death. Autopsy revealed laminar deposition of calcium salts mainly in the proper muscular layer of both the small and large intestines, which have not been described in the literature previously as far as we were able to determine.

In order to clarify the incidence, predisposing conditions, mineral compositions of the deposited materials and clinical significance of this phenomenon, we have studied the calcium deposition in 76 cases of consecutive autopsied small intestines by histological and electron microscopic techniques. We have determined that this phenomenon is rather frequently observed in the small intestine, mostly associated with hypercalcemia, and that the deposits are composed of hydroxyapatite.

**Materials and Methods**

**Autopsy samples and histological examination**

76 cases of consecutive autopsied small intestines were examined at Tokyo Medical and Dental University from 2001 to 2002. The resected organs were fixed with phosphate-buffered 20% formaldehyde. One to four pieces of the samples were excised from the small intestine, esophagus, stomach, duodenum, colon, heart, kidney and lung and embedded in paraffin. Sections of 4 μm thickness were obtained and were stained with hematoxylin-eosin and von-Kossa’s stainings.

**Energy-dispersive X-ray spectroscopy**

In order to study the composition of the calcium salts, sections of 10 μm thickness were spatter-coated with osmium, observed by a scanning electron microscope (S-4500; Hitachi) and then analyzed by an energy-dispersive X-ray spectroscope (EMAX-7000; Horiba, Kyoto, Japan).

**Transmission electron microscopic study and electron diffraction study**

For the transmission electron microscopic observations, formaldehyde-fixed tissues were again fixed with 2.5% glutaraldehyde in 0.01 M phosphate-buffered saline, post-fixed with 2% osmium tetroxide, dehydrated in ethanol and embedded in epoxy resin. Ultrathin nondecalcified sections were stained with uranyl acetate and lead citrate and then examined by transmission electron microscopy (Hitachi H-7100).

Some ultrathin sections were used for electron diffraction, and atomic structure-related D-spacings of diffraction patterns were compared to those of standard samples of hydroxyapatite derived from sialolithiasis.

All the examinations were carried out in accordance with the ethical standards of Tokyo Medical and Dental University.

**Results**

**Incidence and clinical findings of intestinal calcinosis (table 1,2)**

Intestinal calcinosis was observed in the small intestines of 13 cases (17.1%) among the 76 small intestines of consecutive autopsy cases. There was no significant difference in the gender (Fisher’s exact probability test), the age or the duration of the primary disease (Student’s t-test) between the patients with and without intestinal calcinosis. In laboratory examinations before death, 10 cases were accompanied by hypercalcemia. The incidence of hypercalcemia in the

<table>
<thead>
<tr>
<th>Intestinal Calcinosis</th>
<th>Gender (Cases)</th>
<th>Age (years)</th>
<th>Duration of disease(month)</th>
<th>Concentrations of serum calcium</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)</td>
<td>M/F=11/2</td>
<td>71 ± 8</td>
<td>46 ± 58</td>
<td>≥ 10.7 mg/dl 10.7 mg/dl &lt; Ave. ± S.D. (mg/dl)</td>
</tr>
<tr>
<td>(-)</td>
<td>M/F=47/16</td>
<td>68 ± 12</td>
<td>44 ± 84</td>
<td>11.5 ± 1.50</td>
</tr>
</tbody>
</table>

M: Male  
F: Female  
Ave: Average  
S.D: Standard Deviation
patients with intestinal calcinosis was significantly higher than that in the patients without intestinal calcinosis (Fisher’s exact probability test, P value = 0.025). However, the concentrations of serum calcium were not significantly different between the patients with and without intestinal calcinosis (Student’s t-test). The brief clinical histories and autopsy findings of the patients with intestinal calcinosis are summarized in table 2. Only 4 patients were nourished by intravenous hyperalimentation. The etiologies of hypercalcemia were metastatic bone tumors (4 cases), multiple myeloma (1 case), granulomatous infections (2 cases) and unknown origins (3 cases). Serum calcium levels were normal in other 3 cases. One of 3 cases suffered from plasma cell myeloma with broad involvement of bone marrow. The other two cases did not show any bone lesion at the autopsy other than osteoporosis. Intestinal calcinosis was not observed in other 27 cases with hypercalcemia.

Sites and patterns of calcium salt deposition

Deposits of calcium salts were mainly observed in the smooth muscle cells of the proper muscle layers. Deposited calcium salts were slightly basophilic in HE staining (figure 1A) and clarified by von Kossa’s staining (figure 1B). In the transversal section of the ileal wall (case 1), the deposition of calcium salts was lesser in the mesenteric portion. Deposits of calcium salts were also observed in the ganglion cells of the Auerbach’s myenteric plexus (figure 1C,D). In 11 of the 13 cases, calcium salts were more heavily deposited in the smooth muscle cells than in ganglion cells (table 3). In the submucosal layers of 2 cases, deposits of calcium salts was observed in the median layers of the small arteries and arterioles (figure 1E,F). Distinct deposits of calcium salts to the muscularis mucosae was not observed in any case.

In the case of mild deposition, calcium salts were scattered in the smooth muscle layers. In the severely affected areas, laminar deposition of calcium salts involved both smooth muscle layers and nervous plexus. When the degrees of calcium deposition were classified as mild (scattered deposits of calcium salts, (figure 2A,B), intermediate (composed of both scattered and laminar deposits, (figure 2C,D) and severe (the laminar deposits occupy more than 75% of the length, (figure 2E,F), 7 cases (54 %), 3 cases (23 %) and 3 cases (23 %), respectively corresponded to each of the three types. The degree of hypercalcemia (table 2) and severity of calcium deposits (table 3) did not correlate with each other (Kruskal-Wallis test, P value = 0.366). When the remaining sample of small intestine with severe calcium deposition (case 1) was again fixed with non-buffered 10% formaldehyde for 5 days and then microscopically examined, deposition of calcium salts was not observed on the HE specimen and was barely identified even by von Kossa’s staining (data, not shown), suggesting the acid-sensitive nature of the deposited calcium salts.

The degrees of calcium deposition in the jejunal and ileal portions of the small intestine were compared in case 1 and equivalent deposits were observed in the smooth muscle cells than in ganglion cells (table 3). In the submucosal layers of 2 cases, deposits of calcium salts was observed in the median layers of the small arteries and arterioles (figure 1E,F). Distinct deposits of calcium salts to the muscularis mucosae was not observed in any case.

In the case of mild deposition, calcium salts were scattered in the smooth muscle layers. In the severely affected areas, laminar deposition of calcium salts involved both smooth muscle layers and nervous plexus. When the degrees of calcium deposition were classified as mild (scattered deposits of calcium salts, (figure 2A,B), intermediate (composed of both scattered and laminar deposits, (figure 2C,D) and severe (the laminar deposits occupy more than 75% of the length, (figure 2E,F), 7 cases (54 %), 3 cases (23 %) and 3 cases (23 %), respectively corresponded to each of the three types. The degree of hypercalcemia (table 2) and severity of calcium deposits (table 3) did not correlate with each other (Kruskal-Wallis test, P value = 0.366). When the remaining sample of small intestine with severe calcium deposition (case 1) was again fixed with non-buffered 10% formaldehyde for 5 days and then microscopically examined, deposition of calcium salts was not observed on the HE specimen and was barely identified even by von Kossa’s staining (data, not shown), suggesting the acid-sensitive nature of the deposited calcium salts.

The degrees of calcium deposition in the jejunal and ileal portions of the small intestine were compared in case 1 and equivalent deposits were observed in

### Table 2. Clinical summary of the cases with intestinal calcinosis

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age</th>
<th>Autopsy diagnosis</th>
<th>IVH</th>
<th>Ca(mg/dl)</th>
<th>P(mg/dl)</th>
<th>PTH(pg/dl)</th>
<th>Bone finding</th>
<th>Aliment. symp.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>53</td>
<td>Pulmonary atypical mycobacteriosis</td>
<td>(+)</td>
<td>14.6</td>
<td>7.9</td>
<td>1.1</td>
<td>osteoporosis</td>
<td>ileus</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>70</td>
<td>Sigmoid colon cancer</td>
<td>(+)</td>
<td>11.0</td>
<td>3.6</td>
<td>N.E.</td>
<td>metastasis with necrosis (-)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>77</td>
<td>Multiple myeloma</td>
<td>(-)</td>
<td>10.0</td>
<td>2.6</td>
<td>N.E.</td>
<td>plasma cell myeloma (-)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>81</td>
<td>Miliary tuberculosis</td>
<td>(-)</td>
<td>11.1</td>
<td>3.9</td>
<td>95</td>
<td>granuloma, caseous necrosis (-)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>65</td>
<td>Parotid gland cancer</td>
<td>(-)</td>
<td>19.9</td>
<td>3.6</td>
<td>N.E.</td>
<td>metastasis (-)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>79</td>
<td>Old myocardial infarction</td>
<td>(-)</td>
<td>9.7</td>
<td>2.9</td>
<td>N.E.</td>
<td>no finding</td>
<td>ileus</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>76</td>
<td>Old bronchopneumonia</td>
<td>(+)</td>
<td>13.8</td>
<td>2.0</td>
<td>11</td>
<td>osteoporosis</td>
<td>(-)</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>63</td>
<td>Glioblastoma multiforme</td>
<td>(-)</td>
<td>13.4</td>
<td>5.6</td>
<td>N.E.</td>
<td>no finding</td>
<td>(-)</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>76</td>
<td>Hepatocellular carcinoma</td>
<td>(-)</td>
<td>11.6</td>
<td>2.8</td>
<td>N.E.</td>
<td>metastasis (-)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>72</td>
<td>Chronic interstitial pneumonia</td>
<td>(+)</td>
<td>11.2</td>
<td>5.5</td>
<td>N.E.</td>
<td>osteoporosis</td>
<td>bleeding colitis</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>66</td>
<td>Hepatocellular carcinoma</td>
<td>(-)</td>
<td>11.0</td>
<td>5.1</td>
<td>N.E.</td>
<td>exudative osteomyelitis</td>
<td>(-)</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>74</td>
<td>Lung small cell carcinoma</td>
<td>(-)</td>
<td>11.0</td>
<td>3.2</td>
<td>N.E.</td>
<td>metastasis</td>
<td>(-)</td>
</tr>
<tr>
<td>13</td>
<td>M</td>
<td>63</td>
<td>Malignant lymphoma</td>
<td>(-)</td>
<td>10.3</td>
<td>3.6</td>
<td>N.E.</td>
<td>no finding</td>
<td>(-)</td>
</tr>
</tbody>
</table>

IVH: intravenous hyperalimentation
Ca: concentration of serum calcium
P: concentration of serum phosphorus
PTH: concentration of serum parathyroid hormone
Aliment. symp.: Alimentary symptoms before death
N.E.: not examined
*(−): No symptoms
Figure 1. Sites of calcium deposition in the ileum
A.C.E: HE staining. B.D.F: von Kossa’s staining. A.B: Calcium deposits in the smooth muscle cells of the external longitudinal fascicles of the proper muscle layer. Bars show 500 μm. Deposited calcium was slightly basophilic in HE staining (A) and was clarified by von Kossa’s staining where slight deposition of calcium was found also in the Auerbach’s myenteric plexus (B). C.D: Calcium deposits in the ganglion cells of Auerbach’s plexus (arrow) and bundles of smooth muscle cells (arrowheads). E.F: Calcium deposits in the media of the submucosal arterioles (arrowheads).
Figure 2. Grading of calcium deposition in the ileum

A.B: Slight degree. Calcium salts were deposited scatteringly in the smooth muscle cells (arrowheads). Small amounts of calcium salts were also deposited in the Auerbach’s myenteric plexus (arrow), visualized by von Kossa’s staining. Bars show 500\,\mu m. C.D: Moderate degree. In addition to the scattered deposition of calcium salts (arrowheads) in the smooth muscle cells, short lamina of calcium salts (arrow) were observed in the external muscle layer. Bars show 2 mm. E.F: Severe degree. Laminar deposition of calcium salts involved both the external longitudinal muscular fascicle (arrowheads) and the Auerbach’s myenteric plexus (arrow). Muscularis mucosae was free from calcium deposits. Bars show 5 mm.
them. Intestinal calcinosis was accompanied by calcium deposition in the proper muscular layer of the esophagus, stomach, duodenum and large intestine (table 3). In the cases without intestinal calcinosis, calcium deposits could not be observed in any other portion of the alimentary tract. In addition, the degree of calcium deposition was most prominent in the ileal portion of the small intestine in each case of intestinal calcinosis (table 3).

Intestinal calcinosis was also frequently accompanied by calcinosis of extra-alimentary organs. Among them, calcium deposition of renal tubules was most common and were observed in 9 of the 13 intestinal calcinosis cases. In addition to the kidneys, 4 of the 13 cases showed calcium deposits of the myocardial cells in the hearts (table 4). Deposition of calcium salts in the heart (P value = 0.0024) and kidney (P value = 0.048) was significantly correlated with intestinal calcinosis (Fisher’s exact probability test).

**Transmission electron microscopic examination**

By the electron microscopic examination of 6 cases, calcium salts were identified as needle-shaped crystals, 10 nm in diameter and 100 nm in length which is a typical feature of hydroxyapatite crystals. In the extracellular areas, crystals of calcium salts existed on the surface of extracellular collagen fibers (figure 3A). In addition to the intracytoplasmic deposition (figure 3B), calcium salts were also found in the mitochondria (figure 3C) as well as in the nucleus of the smooth muscle cells (figure 3B).

**Energy-Dispersive X-ray spectrometry examination and electron diffraction analysis of the deposited calcium salts**

Distinct peaks of calcium and phosphorus were

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**Table 3.** Distribution, degree and mineral composition of calcium deposition in the alimentary tract

<table>
<thead>
<tr>
<th>Case</th>
<th>Deposition sites</th>
<th>Ileum</th>
<th>Esophagus</th>
<th>Stomach</th>
<th>Duodenum</th>
<th>Colon</th>
<th>Ca/P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ELF&gt;Ggl</td>
<td>+++</td>
<td>(+)</td>
<td>(−)</td>
<td>(+)</td>
<td>(+)</td>
<td>1.51</td>
</tr>
<tr>
<td>2</td>
<td>ELF&gt;A,a&gt;Ggl</td>
<td>+++</td>
<td>(+)</td>
<td>N.E.</td>
<td>N.E.</td>
<td>N.E.</td>
<td>1.69</td>
</tr>
<tr>
<td>3</td>
<td>ELF&gt;Ggl</td>
<td>+++</td>
<td>(−)</td>
<td>(−)</td>
<td>(+)</td>
<td>(+)</td>
<td>1.47</td>
</tr>
<tr>
<td>4</td>
<td>Ggl</td>
<td>(++)</td>
<td>N.E.</td>
<td>N.E.</td>
<td>N.E.</td>
<td>N.E.</td>
<td>1.31</td>
</tr>
<tr>
<td>5</td>
<td>ELF&gt;A,a&gt;Ggl</td>
<td>(++)</td>
<td>(−)</td>
<td>(+)</td>
<td>N.E.</td>
<td>(−)</td>
<td>1.62</td>
</tr>
<tr>
<td>6</td>
<td>ELF&gt;Ggl</td>
<td>(+)</td>
<td>(++)</td>
<td>N.E.</td>
<td>(++)</td>
<td>N.E.</td>
<td>1.52</td>
</tr>
<tr>
<td>7</td>
<td>ICF&gt;ELF</td>
<td>(+)</td>
<td>N.E.</td>
<td>(+)</td>
<td>N.E.</td>
<td>N.E.</td>
<td>1.51</td>
</tr>
<tr>
<td>8</td>
<td>ICF</td>
<td>(+)</td>
<td>(−)</td>
<td>(−)</td>
<td>N.E.</td>
<td>(−)</td>
<td>1.28</td>
</tr>
<tr>
<td>9</td>
<td>ELF&gt;Ggl</td>
<td>(+)</td>
<td>(−)</td>
<td>(−)</td>
<td>(−)</td>
<td>(−)</td>
<td>1.68</td>
</tr>
<tr>
<td>10</td>
<td>ICF&gt;Ggl</td>
<td>(+)</td>
<td>(−)</td>
<td>(−)</td>
<td>(−)</td>
<td>(−)</td>
<td>1.37</td>
</tr>
<tr>
<td>11</td>
<td>Ggl</td>
<td>(+)</td>
<td>(−)</td>
<td>(−)</td>
<td>(−)</td>
<td>(−)</td>
<td>1.48</td>
</tr>
<tr>
<td>12</td>
<td>ICF</td>
<td>(+)</td>
<td>N.E.</td>
<td>(−)</td>
<td>N.E.</td>
<td>(−)</td>
<td>1.2</td>
</tr>
<tr>
<td>13</td>
<td>ICF&gt;ELF</td>
<td>(+)</td>
<td>(+)</td>
<td>(−)</td>
<td>(+)</td>
<td>(++)</td>
<td>1.12</td>
</tr>
</tbody>
</table>

*: Sites of calcium deposition in the small intestine (ileum)
ICF: internal circular fascicle of proper muscle layer
ELF: external longitudinal fascicle of proper muscle layer
A.a: arteries and arterioles
Ggl: ganglion
(−): not detected; (+): slight; (++): moderate; (+++): severe degree
Ca/P: calcium and phosphorus mass ratios by the Energy-Dispersive X-ray Spectrometry examination
N.E.: not examined

**Table 4.** Correlation of calcium deposition between ileum and other organs

<table>
<thead>
<tr>
<th>Small intestine (Ileum)</th>
<th>Esophagus</th>
<th>Stomach</th>
<th>Duodenum</th>
<th>Colon</th>
<th>Heart</th>
<th>Kidney</th>
<th>Lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>(−)</td>
<td>(−)</td>
<td>(−)</td>
<td>(−)</td>
<td>(−)</td>
<td>(−)</td>
<td>(−)</td>
<td>(−)</td>
</tr>
</tbody>
</table>

(+): calcium deposition detected
(−): no calcium deposition
**Figure 3.** Electron microscopic findings of deposited calcium salts

A. Fibrils of calcium salts, 100 nm in length and 10 nm in diameter, were deposited at the surface of intercellular collagen fibers (arrows). Bar shows 200 nm.

B,C. Fibrils of calcium salts were also observed in the cytoplasm (B, white arrow), nucleus (B, arrowheads) and mitochondria of the smooth muscle cells (C, arrow). Black arrows indicate nuclear membrane (B). Bar shows 100 nm (B) and 50 nm (C) respectively.

**Figure 4.** Characterization of deposited calcium salts

A: Energy-dispersive analysis shows that deposited salts consist of calcium (Ca) and phosphorus (P). Peaks of magnesium could not be detected. B: Electron diffraction analysis reveals that calcium salts deposited in the ileum (case 1, left) show a pattern identical to that of the standard sample of hydroxyapatite (right).
observed in all the 13 cases (figure 4A). Other elements, such as magnesium, could not be detected in significant amounts. The mass rations of calcium and phosphorus ranged from 1.12 to 1.69. Electron diffraction analysis of three cases (case 1,3,5) revealed that all the examined calcium salts showed a pattern identical to the standard sample of hydroxyapatite (figure 4B).

Clinical significance of calcium salt deposition in the intestine

When clinical histories of the 13 patients with intestinal calcinosis were reviewed, 2 patients (cases 1 and 6) showed obvious signs of paralytic ileus with dilatation of the small intestine, and one patient showed intestinal bleeding probably due to bacterial colitis (case 10). Other patients did not show evident alimentary symptoms. In one patient (case 1), ileus lasted for 10 months until the death of the patient. In the other patient (case 6), the signs of ileus was evident only for a few days before death. In both of the cases, autopsy did not reveal any findings in the intestine other than calcinosis of severe and moderate degree, respectively.

Discussion

Deposition of calcium salts in the proper muscle layers and Auerbach’s myenteric plexus has not been described previously in the literature, as far as we were able to determine. In a report concerning rectal biopsies of patients with spinocerebellar degeneration, calcium deposits of Meissner’s ganglion cells and smooth muscle cells, probably derived from muscularis mucosae, were described. The pattern of calcium deposition to the smooth muscle cells and ganglion cells described in that report is similar to our observation, however, the localization in the intestinal wall and predisposing conditions are different. We suggest that this pathological change has not been demonstrated previously in spite of the frequent incidence of 17.1% in the autopsied small intestines because acid-sensitive deposits of calcium salts might be dissolved in non-buffered acidic formalin fixative, as we have shown in this study. Acidic formalin is generally used as a fixative in autopsy cases. In addition, calcinosis was so faint in most of the cases that it might be easily overlooked if only the routine hematoxylin-eosin staining is done.

In metastatic calcification, most of the calcium salts were hydroxyapatite. In the cases of pulmonary and arterial calcification, deposits were composed of whitlockite, which contained magnesium and were round particles as examined by electron microscopy. In our study, X-ray spectrometry revealed that calcium salts contained phosphorus but did not contain other mineral element, such as magnesium, in any of the cases. In most of the cases, the measured Ca/P ratios were close to that of hydroxyapatite, 1.5, which suggested that the deposits were hydroxyapatite. In the cases with severe deposition, the needle-like shape of the deposited crystals, as examined by electron microscopy, and the patterns of electron diffraction analysis proved that the observed deposits indeed were hydroxyapatite. The disappearance of the deposits in acidic formalin can be explained by the acid-sensitive nature of hydroxyapatite.

Similar to metastatic calcification of other sites, hypercalcemia was a distinct condition predisposing to intestinal calcinosis. Hyperphosphatemia due to chronic renal failure is another frequent condition predisposing to metastatic calcification, however, our study included only one patient on chronic hemodialysis, and in this patient we could not find intestinal calcinosis. Primary and metastatic bone tumors are frequently sources of hypercalcemia. Participation of granulomatous inflammation has also been observed. This is explained by secretion of 1.25-vitamin D by epithelioid cells and increased absorption of calcium ions from the intestine. However, intestinal calcinosis was observed in only 27% of the cases with hypercalcemia, and the degree of hypercalcemia and severity of calcium deposition did not seem to correlate with each other, as is the case in pulmonary calcinosis. Moreover, hypercalcemia was not observed in 3 cases of intestinal calcinosis. Thus, in addition to the serum levels of calcium and phosphorus, other factors in the local environment, such as activity of alkaline phosphatase or alkaline pH may also play important roles in the process of calcium deposition in the intestine.

We have named this phenomenon “intestinal calcinosis” because we first noticed this pathological change in the small intestine, but this change could also be found in other portions of the alimentary tract, such as the esophagus, to a lesser degree. Thus, the term of “alimentary calcinosis” might be more precise. As observed in the cases of intestinal calcinosis, metastatic calcification usually involves multiple organs, and the preferential organs are lungs, stomach, kidneys and heart. The alkaline environment of the lungs, kidneys and stomach is caused by excretion of
CO₂ or hydrogen ions, which can partly explain the susceptibility to calcium deposition in these organs. However, it is difficult to apply this explanation to the calcification of the heart and intestines.

In the small intestine, the smooth muscle layers, nerve plexus, submucosal small arteries and arterioles were the sites of calcium deposition. Although metastatic calcification is not common in the smooth muscle cells, calcification of the smooth muscle cells of subcutaneous arteries are occasionally observed in patients with chronic renal failure, referred to as calciphylaxis. Thus, smooth muscles cells are the potential sites of metastatic calcification. This consideration is supported by the in vitro experiment of calcification induced by the cultured aortic smooth muscle cells. On the other hand, calcium deposition in the autonomic nerves and ganglion cells has been described only in a few patients of spinocerebellar degeneration. These cases will not be discussed here because their predisposing conditions are different from those of intestinal calcinosis. However, the deposition pattern involving both submucosal ganglion cells and smooth muscle cells in the two patients of spinocerebellar degeneration resembles that of intestinal calcinosis. Involvement of the different cellular lineages suggests the existence of a common process of calcification shared by smooth muscle cells and ganglion cells. This process might be initiated by the influx of calcium ions into the cytoplasm during depolarization.

The previous ultrastructural studies of renal, cardiac and pulmonary calcification demonstrated that the calcium salts deposited in metastatic calcification preferentially deposited in the extracellular matrix, such as basement membrane, elastic fibers and collagen fibers. In addition, mitochondria in the renal tubular epithelium, myocardial cells, smooth muscle cells and ganglion cells are the intracytoplasmic organelles where calcium salts are preferentially deposited. The ultrastructural sites of calcium deposition at the surface of the extracellular collagen fibers and in the mitochondria of the smooth muscle cells suggest that intestinal calcinosis shares a common process of calcification with pulmonary, renal and myocardial calcification. In contrast, calcium deposition to the nuclei in smooth muscle cells are unique findings in intestinal calcinosis. Nuclei of muscle cells are considered to be the preferential organelle, next to mitochondria and sarcoplasmic reticulum, for accumulation of calcium ions entering the cells from the extracellular space. Thus, intranuclear calcification might occur through the characteristic influx of calcium ions in the smooth muscle cells.

Metastatic calcification of the heart is clinically symptomatic when involving the conducting pathways. Most patients with pulmonary calcification are asymptomatic, but patients with severe deposition show restrictive and diffusion ventilatory defects. The distribution of calcium deposition to the proper muscle layer and the Auerbach’s myenteric plexus is similar to that of intestinal amyloidosis, which often manifest the symptoms of motility disorders. Accordingly, intestinal calcinosis is likely to affect motile function rather than absorption. In our study, two patients among the six cases with moderate to severe calcium deposition showed clinical manifestations of paralytic ileus. At the present time, the clinical significance of intestinal calcinosis is not conclusive because the number of intestinal calcinosis cases is too limited, and there is no previous report clearly describing the relationship between hypercalcemia and paralytic ileus so far as we could examined. However, a few previous reports described the alimentary complication of ileus in the patient of peripheral T-cell lymphoma including adult T-cell lymphoma (ATL). ATL often accompanies hypercalcemia and rarely shows calcium deposits in the subserosal layer of the small intestine. Thus, intestinal calcinosis might be one of the cause of ileus in the patients of peripheral T-cell lymphoma, in addition to infiltration of tumor cells, strongyloidiasis and acquired aganglionosis.

In conclusion, we have demonstrated the autopsy cases of intestinal calcinosis. Most of the cases were associated with hypercalcemia due to primary or metastatic bone tumors or granulomatous disease. Calcium salts of hydroxyapatite were deposited to the smooth muscle cells and the Auerbach’s myenteric plexus of the muscular layer. Deposition of calcium salts might occasionally cause paralytic ileus but assessing the clinical significance of this lesion requires further examination.

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