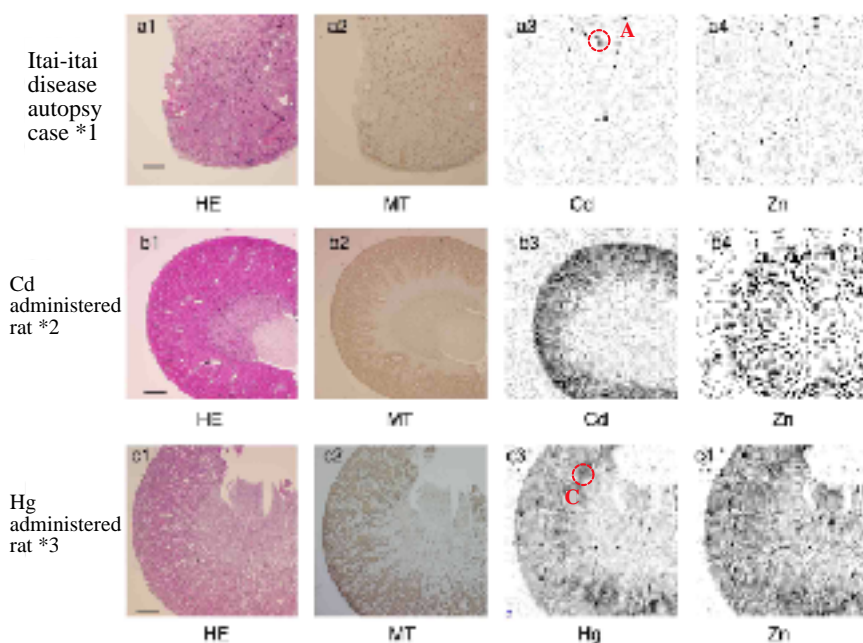


## Imaging of Heavy Metal Distribution in Thin Tissue Sections of Itai-itai Disease Kidney and Rat Kidney by X-Ray Fluorescence Analysis

Itai-itai disease is an endemic disease of Toyama prefecture, Japan, and once was a big social problem. It has been claimed that Cd-polluted Jinzu River water caused this disease based on epidemiological studies of patient

distribution. However, the pathological contribution of Cd to itai-itai disease is still controversial. The kidney is one of the major organs damaged in itai-itai disease, and it is of great interest to investigate Cd distribution in itai-itai diseased kidneys since it is known that their Cd concentration is lower than that of non-itai-itai-disease kidneys.

On the other hand, the relationship between Hg intoxication and Minamata disease is well established. The proximal urinary tubule of the kidney is one of the target tissues for Hg toxicity, and the precise location in the proximal urinary tubules (segments [1]) sensible for Hg toxicity has



**Fig. 1.** Formaline fixed paraffin embedded kidney tissue sections. Section thickness is  $3\ \mu\text{m}$  for HE and MT,  $6\ \mu\text{m}$  for microbeam X-ray fluorescence (XRF) analysis (Cd, Zn, and Hg). HE=hematoxylin-eosin staining, MT=immunohistochemistry with anti-metallothionein antibody. DAB was used as chromogen (the brown color is the signal). Bar = 1 mm.

\*1 Cd concentration is 16 ppm in the kidney cortex and 13 ppm in the medulla by AAS analysis. XRF: 32 keV X-rays were utilized for excitation. Beam size was approximately  $100\ \mu\text{m}$ . The counting time was 2 sec/spot. Spatial resolution is  $125\ \mu\text{m}/\text{step}$ .

\*2 Young male Wistar rat was used. Cd was administered by intraperitoneal injection of 2 mg Cd/ml  $\text{CdCl}_2$  solution in distilled water, 2 mg Cd/kg body weight. After eight weeks of breeding with standard laboratory food and water, the animal was sacrificed. Cd concentration is 43 ppm in the cortex, 3 ppm in the medulla by AAS analysis. XRF condition was same as shown in \*1 above.

\*3 Young male Wistar rat was used. Hg was administered by intraperitoneal injection of 1 mg/ml  $\text{HgCl}_2$  solution in distilled water. After the first injection of 1 mg  $\text{HgCl}_2$ /kg body weight, the animal was bred for two weeks, injected with 2 mg  $\text{HgCl}_2$ /kg body weight for the second time, bred for one week, and sacrificed. The Hg concentration is 32 ppm in the cortex, 1.2 ppm in the medulla by AAS analysis. XRF: 13 keV X-rays were utilized for excitation. The beam size was approximately  $100\ \mu\text{m}$ . The counting time was 2 sec/spot. Spatial resolution is  $125\ \mu\text{m}/\text{step}$ .

been analyzed using dissected nephrons. However, the direct analysis of Hg distribution in Formalin-fixed-paraffin-embedded tissue sections routinely used in histopathological study has not been reported.

So far, it is not possible to visualize Cd distribution in kidney sections by either EPMA or SIMS. Microprobe X-ray fluorescence analysis at beamline BL39XU [2] made this possible for the first time [3] (Fig. 1 a3). As a positive control, Cd-administered rat kidney was also analyzed (Fig. 1 b3). Pictures of hematoxylin-eosin (HE) stained section and immunohistochemistry with anti-metlothionein (MT) antibody are also shown since Cd is known to exist bound to MT in the kidney (Fig. 1).

For Cd analysis, 32 keV X-rays were utilized for K-excitation. In Cd-administered rat kidney, Cd is distributed widely in the cortex (Fig. 1 b3). The distribution pattern of Cd matches well with that of MT, compatible with the biochemical findings suggesting that Cd exists bound to MT in the kidney. The distribution of MT is limited to the epithelium of the proximal urinary tubules, suggesting that segment S1+2 is the main portion of the Cd storage. In itai-itai diseased kidney, the Cd signal is limited to several spots in the medulla

while the Cd signal in the cortex is background level (Fig. 1 a3). These spots seem to be corresponding to collecting urinary ducts, which show a strong signal in anti-MT immunohistochemistry (Fig. 1 a2). However, the precise identification of these spots should be carried out in the future since the outline of the kidney structure is not visible and the distribution of MT is wider than that of the Cd spots. The distribution pattern of Zn partially corresponds to that of Cd, suggesting that some part of Zn also exists bound to MT. The XRF spectra measured in spots A and B (blank) are shown in Fig. 2.

As a comparison, distribution of Hg in Hg-administered rat kidney was also analyzed (Fig. 1 c3). Although it is known that the cause of Minamata disease is organic Hg including methyl-Hg,  $\text{HgCl}_2$  was administered to the rat as a pilot study. In the case of Hg analysis, 13 keV X-rays were utilized for L-excitation. Images of Hg and Zn in paraffin embedded tissue section are shown in Fig. 1 c3 and c4. In this experiment, a strong Hg deposition was observed in the area between the cortex and the outer medulla, suggesting that segment S3 of the proximal urinary tubule is the main portion of Hg deposition. The distribution pattern of Hg does not correspond to that of MT

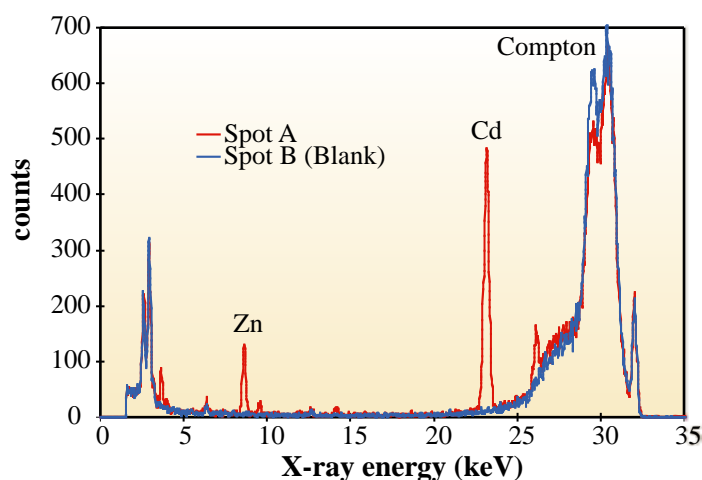
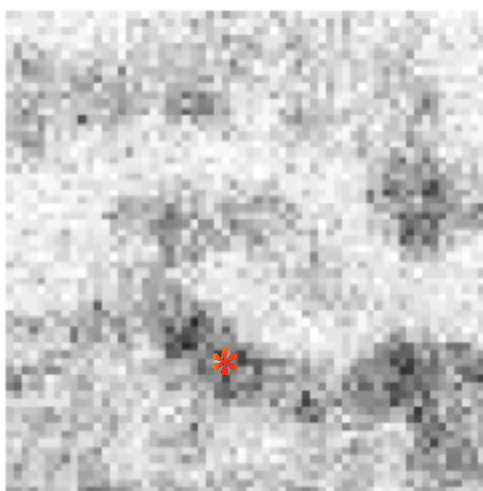


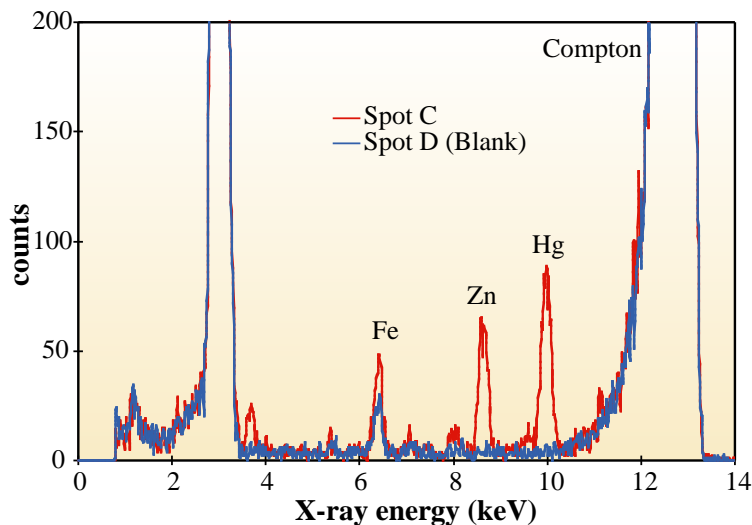
Fig. 2. XRF spectra measured in spots A and B (blank) in Fig. 1 a3.

very well, which suggests the existence of other binding proteins for Hg. The XRF spectra measured in spots C and D (blank) are shown in Fig. 3. With this X-ray energy an X-ray microbeam produced with the newly installed Kirkpatrick-Baez mirror system [4,5] was also available. The beam size was  $4 \mu\text{m} \times 4 \mu\text{m}$  in this measurement, and Fig. 4 shows the resultant image of a small area around spot C in Fig. 1 c3.

Microbeam X-ray fluorescence analysis is found to be a useful method to analyze heavy metal distribution in thin tissue sections. Distributions of Cd and Hg in paraffin embedded human and rat kidneys were demonstrated for the first time. This method will be an important tool to analyze heavy metal toxicosis, including itai-itai disease and Minamata disease.



*Fig. 4. Hg imaging of a small area around spot C in Fig. 1 c3. XRF: The energy of X-ray for excitation was 13 keV. The beam size was approximately  $4 \mu\text{m} \times 4 \mu\text{m}$ . The counting time was 2 sec/spot. Spatial resolution is  $5 \mu\text{m}/\text{step}$ . The signal seems to be located in the epithelium of the urinary tubules since the width of band-like Hg positive area (\*) is approximately the same as the diameter of the urinary tubules.*



*Fig. 3. XRF spectra measured in spots C and D (blank) in Fig. 1 c3.*

Kiyoshi Takagawa<sup>a</sup> and Shinjiro Hayakawa<sup>b</sup>

(a) Toyama Medical and Pharmaceutical University

(b) Hiroshima University

E-mail: takagawa@ms.toyama-mpu.ac.jp

## References

- [1] W. Kriz and L. Bankir, *Kidney Int.* **33** (1988) 1.
- [2] S. Hayakawa *et al.*, *J. Synchrotron Rad.* **5** (1998) 1114.
- [3] J. Kawai *et al.*, *J. Trace Microprobe Tech.* **19-4** (2001) 541.
- [4] S. Hayakawa, N. Ikuta, M. Suzuki M. Wakatsuki and T. Hirokawa, *J. Synchrotron Rad.* **8** (2001) 328.
- [5] S. Hayakawa, S. Tohno, K. Takagawa, A. Hamamoto, Y. Nishida, M. Suzuki, Y. Sato and T. Hirokawa, *Anal. Sci.* - in press.