Tohoku J. exp. Med., 1987, **152**, 151-172

Epidemiology of Renal Tubular Dysfunction in the Inhabitants of a Cadmium-Polluted Area in the Jinzu River Basin in Toyama Prefecture

KEIKO AOSHIMA

Department of Public Health, Faculty of Medicine, Toyama Medical and Pharmaceutical University, Toyama 930-01

AOSHIMA, K. *Epidemiology of Renal Tubular Dysfunction in the Inhabitants of a Cadmium-Polluted Area in the Jinzu River Basin in Toyama Prefecture.* Tohoku J. exp. Med., 1987, **152** (2), 151-172 —— Urinary β_2-microglobulin (β_2-m), α_1-microglobulin (α_1-m), amino-nitrogen, glucose, calcium, phosphorus, cadmium concentrations, and pH values were analyzed in urine samples from 187 females aged 55-66 years in the Jinzu River basin, which is known to be a cadmium-polluted area, and from 32 controls living in two adjacent reference areas in 1983-1984. Mean urinary β_2-m, α_1-m, amino-nitrogen, glucose, cadmium concentrations and pH values in the inhabitants of the Jinzu River basin were significantly higher than those in the adjacent reference areas. Sixty-four inhabitants in a cadmium-polluted area were found to have renal tubular dysfunction with urinary β_2-m level exceeding 1 mg/g creatinine and urinary glucose level exceeding 100 mg/g creatinine. The severity of renal tubular dysfunction in several inhabitants were comparable to those of the patients with Itai-itai disease. Mean cadmium concentrations in rice (mean : 0.32-0.57 ppm) which has been daily consumed by the inhabitants of the Jinzu River basin were significantly higher than those in the reference areas (mean : 0.12-0.13 ppm). The close relationship between cadmium exposure and the degree of renal tubular dysfunction was well demonstrated by principal component analysis. ———— epidemiology ; renal tubular dysfunction ; β_2-microglobulin ; cadmium ; Itai-itai disease

The cause or pathophysiology of the Itai-itai disease, endemic osteomalacia in the Jinzu River basin, Toyama Prefecture, Japan, has been a controversial issue for many years. Arguments have been repeated as to whether or not Itai-itai disease is caused by cadmium-induced renal tubular dysfunction, or is simply due to vitamin D deficiency or malnutrition (Friberg et al. 1974 ; Kajikawa 1978 ; Takeuchi 1978 ; Nogawa 1981).

Received November 21, 1986 ; accepted for publication April 23, 1987.

Address for reprints : Keiko Aoshima, Department of Public Health, Faculty of Medicine, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-01, Japan.

The first systematic epidemiological studies on the Itai-itai disease were conducted in 1962 to determine the number of patients, and to clarify the cause of this disease (Kato and Kawano 1968 ; Friberg et al. 1974). Furthermore, the Toyama Prefectural Health Authorities performed a health survey on 15,000 inhabitants of the Jinzu River basin in 1967-1968 to circumscribe accurately the endemic areas and also to learn the incidence of Itai-itai disease. The results of examinations of about 6,000 inhabitants showed that the inhabitants of the endemic areas of Itai-itai disease had a significantly higher incidence of proteinuria with glycosuria than those in the non-endemic areas (Fukushima et al. 1974, 1975 ; Friberg et al. 1974 ; Nogawa 1981).

Kjellström et al. (1977) later showed that the women aged 51-60 years living in the Jinzu River basin had a high concentration of urinary β_2-microglobulin (β_2-m) having a high positive correlation between cadmium exposure. Kobayashi (1982a, b) further indicated that all inhabitants of nine hamlets in the west-side of the Jinzu River basin had higher cadmium concentrations in urine than that of the references, and the positive rates of urinary β_2-m detected by single radial immunodiffusion method were 30-100% of inhabitants over 40 years old in the Jinzu River basin. However, few detailed epidemiological studies have been performed concerning renal tubular dysfunction of the inhabitants in the total polluted areas of the Jinzu River basin with special reference to the levels of cadmium exposure indicated by urinary cadmium and cadmium concentration of rice consumed by the inhabitants.

The purpose of this study is firstly to clarify the distribution and frequency of renal tubular dysfunction showing prominent low-molecular weight proteinuria as well as glucosuria, among all female inhabitants in an age group 55 to 66 years old of 24 hamlets divided into 11 districts in the Jinzu River basin, as well as two reference areas in the adjacent Ida and Kumano River basins. Secondly, critical analyses were conducted on biological variables, including the amounts of cadmium in daily consumed rice, residence time, and levels of urinary cadmium, β_2-m, α_1-microglobulin (α_1-m), glucose and total protein, to confirm dose-response to cadmium-induced renal tubular dysfunction more accurately.

Materials and Methods

The research areas

The Jinzu River is 126 km in length and is the longest river in Toyama Prefecture (Fig. 1). It flows northwards and, at the Jinzu second dam (Fig. 2), begins to flow down gentler slopes to a fan-shaped deposit, then flows into the Japan Sea. The Jinzu River water has been supplied to the paddy fields in the west-side areas in the Jinzu River basin bounded by the Ushigakubi canal and the Ida River as well as the east-side areas bounded by the Kumano River for rice cultivation through several irrigation canals (such as Ohsawano, Ohkubo, Shinbo, Ushigakubi and Jinzu-gokuchi). About 30 kilometers upstream from this area, a lead and zinc mine and smeltery are located. The paddy fields in this area had been irrigated with cadmium-containing water discharged from the mine for many years, and the

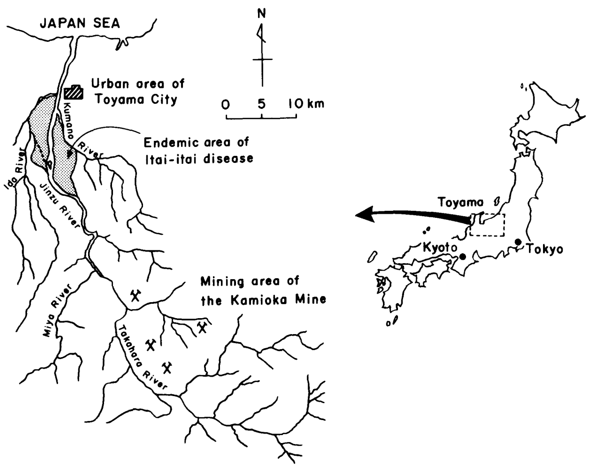

Fig. 1. Map showing the Jinzu River basin (after Morishita 1981).

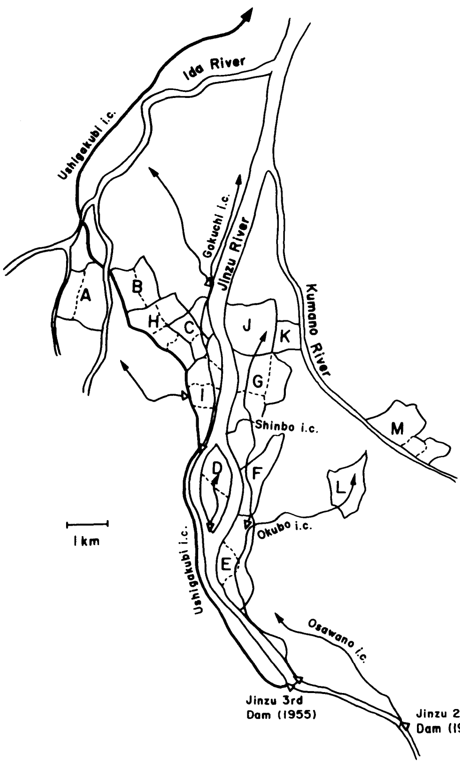

Fig. 2. Map showing the location of sampling sites. The letters A and M specify the reference areas which are irrigated by the Ida and Kumano River water, respectively ; B-L indicate the target areas irrigated by the Jinzu River water through several irrigation canals (i.c.).

soil and rice-grains were heavily polluted by cadmium (Morishita 1981 ; Yanagisawa et al. 1984).

Fig. 2 shows a map of the research area. Eleven districts, including 24 hamlets indicated by the letters B-L, were selected from the area located in the Jinzu River basin. The two reference areas including 5 hamlets, indicated by A and M in the map and located in the Ida and Kumano River basin, respectively, were selected in this study for comparison to the Jinzu River basin.

Subjects

This study was conducted for two years in 1983 and 1984. The first study was conducted in January, 1983, in the areas of A-G districts and the second study was conducted in June, 1984, in the areas of H-M districts. All female inhabitants of the target areas whose year of birth was between January 1, 1918, and December 31, 1927, were selected from the roster of resident registration and asked to participate in this study. At the time of study they were 55-66 years old. Twelve female patients with Itai-iai disease aged 60-80 years were also examined to compare their urinary components with those of the inhabitants. Six patients have lived in the target areas (B-L) and the others in the cadmium-polluted areas other than B-L districts in the Jinzu River basin. Residence history and occupational history were obtained from each participant through questionnaires.

Procedure of sample collection and urine analysis

The first-voided morning urine specimen was collected in a polypropylene container, which was previously washed with hydrochloric acid. Immediately after collection, the container was shaken gently and the urinary pH was measured with a digital pH meter using a glass electrode, and the specific gravity with a hydrometer (urinometer). The urine sample for the quantification of β_2-m was kept up to pH 6.0 by adding an ammonia solution. The urine sample for the determination of cadmium was acidified (1% v/v) with hydrochloric acid. The samples were stored at $-20^{\circ}C$ until the analysis was performed.

β_2-m was determined by a radio-immunoassay method (β_2-m Radioimmunoassay Kit, Eiken Chemical Co., Ltd., Tokyo). The α_1-m analysis was conducted by an enzyme-immunoassay of sandwich methods (α_1-m EIA Kit, Fuji Rebio, Tokyo). Glucose was determined by an enzymatic method (glucose oxidase, peroxidase) (Glucose C-Test Wako, Wako Pure Chemical Ind., Ltd., Osaka). Total protein was measured by the trichloracetic acid test (one volume of urine and five volumes of 3% trichloracetic acid). Amino-nitrogen was measured by the trinitro benzene sulfonic acid (TNBS) method (Fukushima and Kobayashi 1975), calcium by the OCPC method (Connerty and Briggs 1966), phosphorus by Fiske-Subbarow' method (Fiske and Subbarow 1925), and creatinine by Folin-Wu method (Bonsnes and Taussky 1945).

Cadmium analysis was performed using Chelex 100 resin (Kingston et al. 1978) and graphite furnace atomic absorption spectrometry (Nippon Jarrell-Ash AA-855 Instrument, Kyoto). A duplicate sample of 0.5 ml of urine was desiccated. The drying was followed by a wet digestion with concentrated nitric acid. Then 1 ml of 2 M acetic acid solution was added and mixed, after which 1 ml of 2 M ammonium solution was added and mixed. A two ml sample applied to a Chelex 100 resin column and eluted with 2 ml of 1 M ammonium acetate buffer (pH 5.2) to selectively elute Na, K, Ca and Mg. The amount of 0.5 ml water was added to remove residual ammonium acetate. Then cadmium was eluted using 2 ml of 0.5 N HNO_3 and collected in clean polyethylene bottles (Kato et al. 1984).

Sampling and analysis of rice

Polished rice grains were collected from the inhabitants of polluted and reference areas for analysis of cadmium. Cadmium in rice was analyzed by graphite furnace atomic absorption spectrometry, after the low-temperature ashing followed by acid extraction.

Statistical analysis

Urinary β_2-m, α_1-m, total protein, glucose, cadmium, amino-nitrogen, calcium and phosphorus sample data were all adjusted for creatinine concentration and expressed in term of g creatinine. After the concentration adjustment, logarithm-transformed data were used in all analyses, since the frequency distributions of transformed results were nearly normal. The results were expressed as the geometric mean and geometric standard deviation (GM (GSD)) or the arithmetic mean and arithmetic standard deviation (am$\pm$a S.D.). Means were compared by one-way analysis of variance and significance was estimated using the F of Snedecor with the appropriate degree of freedom. If the F value was significant, the means of cadmium-polluted areas were compared to the means of two reference areas using the least significant difference (LSD) methods (Snedecor and Cochran 1972). The x^2 test was used for testing the statistical significance of prevalence rate of renal tubular dysfunction between reference areas and cadmium-polluted areas. Multivariate analysis (Principal Component Analysis, PCA) was conducted to examine more inclusively the 1983 data taken from seven districts (A-G). This was done to establish the interrelationships among various environmental as well as biological variables.

Results

The characteristics of the subjects

The distributions and personal histories of participants in eleven districts in the Jinzu River basin (B-L) and adjacent reference areas (A and M) are presented in Table 1. The total number of women born in 1918 to 1927 was 247 in cadmium-polluted areas in the Jinzu River basin and 46 in the reference areas.

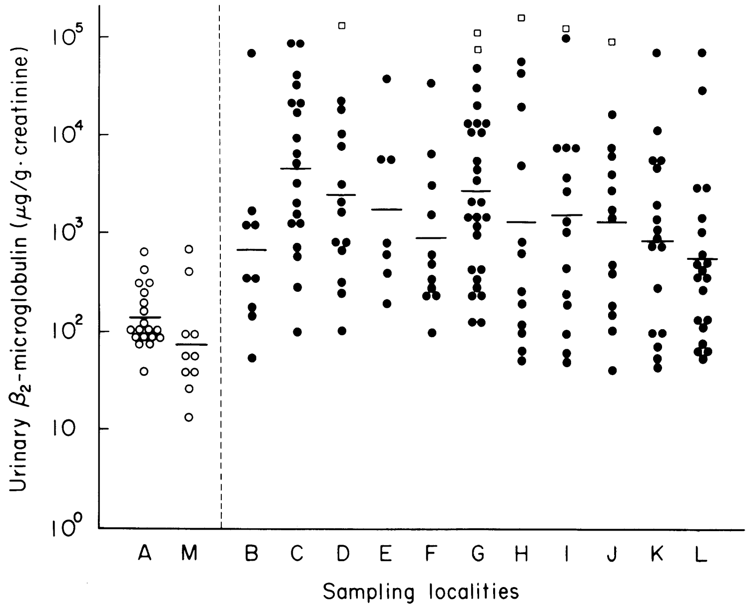

Fig. 3. The individual urinary β_2-microglobulin concentration in eleven districts in the Jinzu River basin (B-L) (●) and neighbouring reference areas (A, M) (○). The six patients with Itai-itai disease included among the subjects studied are indicated by open rectangles.

Two hundred and nineteen (74.7%) of the 293 women participated in this study. Most of the participants were engaged in agriculture, but some were teachers or commercial workers. Sixteen of the participants in the B, C, G and H districts have lived for less than 30 years in cadmium-polluted areas. Three of the residents in the A district in the reference areas have lived for about 20 years in the Jinzu River basin. Six subjects of the 187 participants among 247 target inhabitants of the Jinzu River basin were previously known as patients with Itai-itai disease.

Urinary levels of β_2-microglobulin, α_1-microglobulin, amino nitrogen, glucose, cadmium, calcium and phosphorus

The calculated geometric means and geometric standard deviations of urinary β_2-m, α_1-m, amino-nitrogen, glucose, cadmium, calcium and phosphorus for each sampling population are compiled in Table 2. These are the results for those who have lived in this region for more than 30 years. The patients with diabetes mellitus (two in B, one in F and I) were excluded from the statistical analyses (cf.

TABLE 1. *Distribution and personal histories of participants in eleven districts in the Jinzu River basin (B-L) and adjacent reference areas (A, M)*

Site	Number of target persons	Number of participants (%)	Number of participants residing for over 30 years in the Jinzu River basin (%)	Number of participants engaged in agriculture (%)
Reference area				
A	30	22 (73.3)	0 (0)	16 (72.7)
M	16	10 (62.5)	0 (0)	10 (100)
Total	46	32 (69.6)	0 (0)	26 (81.3)
Cadmium-polluted area				
B	29	17 (58.6)	11 (64.7)	14 (82.4)
C	26	23 (88.5)	19 (82.6)	19 (82.6)
D	21	14 (66.7)	14 (100)	14 (100)
E	9	7 (77.7)	7 (100)	7 (100)
F	22	12 (54.5)	12 (100)	9 (75.0)
G	44	30 (68.2)	28 (93.3)	27 (90.0)
H	18	17 (94.4)	13 (76.5)	12 (70.6)
I	20	16 (80.0)	16 (100)	13 (81.3)
J	20	14 (70.0)	14 (100)	12 (85.7)
K	18	17 (94.4)	17 (100)	17 (100)
L	20	20 (100)	20 (100)	20 (100)
Total	247	187 (75.7)	171 (91.4)	164 (87.7)

The target persons in this study are all female inhabitants of the target areas (A-M) whose year of birth was between January 1, 1918 and December 31, 1927.

TABLE 2-1. *Mean values and the results of the analysis of variance for various urinary components, such as β_2-microglobulin, α_1-microglobulin, amino-nitrogen, glucose, cadmium, calcium, phosphorus and creatinine in inhabitants of thirteen study areas (A-M) and patients with Itai-itai disease*

Sampling sites	Number of subjects	β_2-Microglobulin (μg/g creat.)	α_1-Microglobulin (μg/g creat.)	Amino-nitrogen (mg/g creat.)	Glucose (mg/g creat.)	Cadmium (μg/g creat.)
Reference area						
A	19	141.3 (1.9)*	2588.2 (2.0)*	—‡	46.8 (1.5)*	3.8 (2.2)*
M	10	74.1 (3.3)	—	141.3 (1.4)*	38.0 (1.4)	4.4 (2.8)
Total	29					
Cadmium-polluted area						
B	9	660.7 (7.6)[ac]	4819.4 (4.1)	—	64.6 (3.5)	9.3 (1.8)[bd]
C	19	4570.9 (7.1)[bd]	18967.0 (3.5)[b]	—	154.9 (5.5)[bd]	19.5 (1.6)[bd]
D	14	2431.6 (7.3)[bd]	11953.6 (3.7)[b]	—	172.1 (3.0)[bd]	21.4 (1.6)[bd]
E	7	1771.3 (6.4)[bd]	10459.2 (4.3)[a]	—	116.8 (3.3)	13.0 (1.7)[bd]
F	11	912.0 (5.5)[ad]	6053.4 (2.7)	—	182.0 (3.4)[bd]	11.5 (1.7)[bd]
G	28	2884.0 (7.2)[bd]	14190.5 (4.3)[b]	—	173.8 (5.0)[bd]	11.0 (1.6)[bd]
H	13	1349.0 (17.4)[bd]	—	263.0 (1.8)[d]	114.8 (6.2)	10.2 (2.3)[bd]
I	15	1548.8 (11.7)[bd]	—	257.0 (1.7)[d]	139.4 (4.7)[ac]	23.4 (1.7)[bd]
J	14	1409.3 (8.7)[bd]	—	257.0 (1.7)[d]	129.1 (5.0)	15.9 (1.7)[bd]
K	17	916.2 (7.9)[bd]	—	229.1 (1.6)[c]	64.6 (5.4)	11.7 (1.5)[bd]
L	20	583.4 (6.9)[bd]	—	208.9 (1.4)[c]	53.1 (3.5)	11.7 (1.7)[bd]
Total	167					
Itai-itai disease cases						
	12	109446.0 (1.5)[bd]	—	—	5967.6 (2.6)[bd]	18.1 (2.1)[bd]
Significance		$p<0.01$	$p<0.01$	$p<0.05$	$p<0.01$	$p<0.01$

* Values are represented as GM (GSD). † Values are represented as am ± as.D. ‡ No measurement taken.
Significant differences between the reference area at district A and each district in the cadmium-polluted areas; $^{a}p<0.05$, $^{b}p<0.01$.
Significant differences between the reference area at district M and each district in the cadmium-polluted areas; $^{c}p<0.05$, $^{d}p<0.01$.

TABLE 2-2.

Sampling sites	Number of subjects	Calcium (mg/g creat.)	Phosphorus (mg/g creat.)	pH	Specific gravity	Creatinine (g/liter)
Reference area						
A	19	190.5 (1.9)*	812.8 (1.9)*	6.02±0.53†	1.016±0.005†	0.68±0.27†
M	10	173.8 (2.0)	676.1 (1.5)	6.01±0.55	1.016±0.007	0.80±0.44
Total	29					
Cadmium-polluted area						
B	9	316.2 (1.4)[c]	724.4 (1.3)	6.57±0.55[ac]	1.015±0.005	0.66±0.31
C	19	218.8 (3.2)	691.8 (1.4)	6.56±0.51[bd]	1.013±0.003	0.65±0.42
D	14	272.2 (2.1)	1135.0 (1.3)[ad]	6.72±0.42[bd]	1.012±0.004[a]	0.34±0.14[bd]
E	7	330.1 (1.7)[c]	888.4 (1.4)	6.66±0.57[bd]	1.014±0.003	0.46±0.12[c]
F	11	371.5 (1.7)[bd]	1174.9 (1.6)[ad]	6.41±0.56	1.015±0.005	0.48±0.17[c]
G	28	251.2 (1.9)	794.3 (1.3)	6.30±0.47	1.013±0.005[a]	0.58±0.37
H	13	302.0 (1.6)[c]	645.7 (1.3)	6.37±0.59	1.011±0.005[bc]	0.44±0.21[ad]
I	15	275.4 (2.0)	741.3 (1.4)	6.37±0.60	1.013±0.005[a]	0.49±0.32[c]
J	14	322.8 (1.5)[ac]	576.8 (1.5)	6.42±0.72[a]	1.012±0.005[a]	0.52±0.27[c]
K	17	311.9 (1.4)[ac]	895.4 (1.4)	6.40±0.40[a]	1.014±0.004	0.53±0.23[c]
L	20	275.4 (1.7)	693.4 (1.6)	6.29±0.44	1.014±0.006	0.64±0.26
Total	167					
Itai-itai disease cases						
	12	145.6 (1.7)	614.2 (1.4)	6.61±0.53[bd]	1.009±0.003[bd]	0.41±0.20[ad]
Significance		$p<0.05$	$p<0.01$	$p<0.01$	$p<0.05$	$p<0.01$

Abbreviations are the same as those used in Table 2-1.

Tables 1 and 2).

β_2-Microglobulin in urine. The distribution of individual urinary β_2-m in thirteen districts is presented in Fig. 3. The β_2-m levels for reference groups in the A and M districts ranged from 43.7 to 590.0 μg/g creatinine (unadjusted; 0.05 to 0.35 mg/liter), and from 13.0 to 694.0 μg/g creatinine (unadjusted; 0.01 to 0.38 mg/liter), respectively. The geometric mean of β_2-m for the inhabitants of the A district was slightly higher than that for the inhabitants of the M district, although the difference was not significant ($p>0.05$).

There was a large variation in urinary β_2-m excretion of the inhabitants of the cadmium-polluted Jinzu River basin. The geometric means in the Jinzu River basin (B-L) had a significantly higher β_2-m excretion than the references (A and M) (Table 2). Four patients with Itai-itai disease and one inhabitant had extremely high β_2-m concentrations exceeding 10^5 μg/g creatinine, ranging from 111,901 to 167,640 μg/g creatinine (unadjusted; 15.9 to 35.1 mg/liter).

Twelve patients with Itai-itai disease exhibited exceedingly high urinary β_2-m excretion, ranging from 51,900 to 200,900 μg/g creatinine (unadjusted; 17.9 to 64.6 mg/liter), with a mean value of 109,446.0 μg/g creatinine. It should also be noted, however, that 29 persons except 6 patients with Itai-itai disease in the B-L districts showed an excretion level exceeding 10,000 μg/g creatinine. These values are comparable to those of patients with Itai-itai disease (cf. Fig. 3).

α_1-Microglobulin in urine. The α_1-m, which is a low-molecular weight glycoprotein (MW 33,000), was measured in seven districts (A-G) in 1983. The means in the four districts (C-E and G) except B and F were significantly higher than that in the reference (A) (Table 2). The correlation coefficient between α_1-m and β_2-m was 0.932 ($n=196$, $p<0.01$).

Amino-nitrogen in urine. The amino-nitrogen was measured in six districts (H-M) in 1984. The amino-nitrogen excretion of the inhabitants of the Jinzu River basin (H-L) was significantly higher than that of the reference (M) (Table 2).

Glucose in urine. The geometric means of glucose excretion in the two reference groups (A and M) were 46.8 and 38.0 mg/g creatinine, respectively (unadjusted; 3.3 mg/100 ml in both A and M, and ranged from 0.5 to 5.7 mg/100 ml in A and from 1.2 to 7.6 mg/100 ml in M, respectively). Glucose excretions of the cadmium-polluted groups (B-L) were much higher than the references. Notably those from C, D, F, G and I districts were significantly higher with the means ranging from 134.9 to 182 mg/g creatinine (Table 2).

The concentration of glucose of the patients with Itai-itai disease was significantly higher (mean: 5,967.6 mg/g creatinine; range: 1,218-20,858 mg/g creatinine) than those of the references and the inhabitants of cadmium-polluted areas. The geometric mean of unadjusted data of patients was 275.5 mg/100 ml.

Calcium and phosphorus in urine. The calcium excretion of the inhabitants of the Jinzu River basin was high. As shown in Table 2, significant differences

were detected in B, E, F, J and K areas compared to the references. The patients with Itai-itai disease, however, showed the lowest calcium excretion among all groups examined.

The phosphorus excretion of the inhabitants of only the D and F districts exhibited significantly higher than the references. However, in other areas including the two references (A and M), no significant difference was detected (Table 2). The levels of phosphorus excretion in the patients with Itai-itai disease were not necessarily high, with an average value of 614.2 mg/g creatinine.

Other urinary components. The means of urinary pH of the inhabitants of cadmium-polluted areas and the patients with Itai-itai disease were higher than those of the references (Table 2).

The mean values of specific gravity of the inhabitants (B-L) were lower than those of the references ; particulaly significant differences were detected between those of the references and H district (Table 2). The mean specific gravity of the patients with Itai-itai disease was the lowest among all groups examined.

The mean creatinine concentrations of the inhabitants of the Jinzu River basin (B-L) were lower than those of the references (A and M) (Table 2). A significant difference was found between those of the references and of the resi-

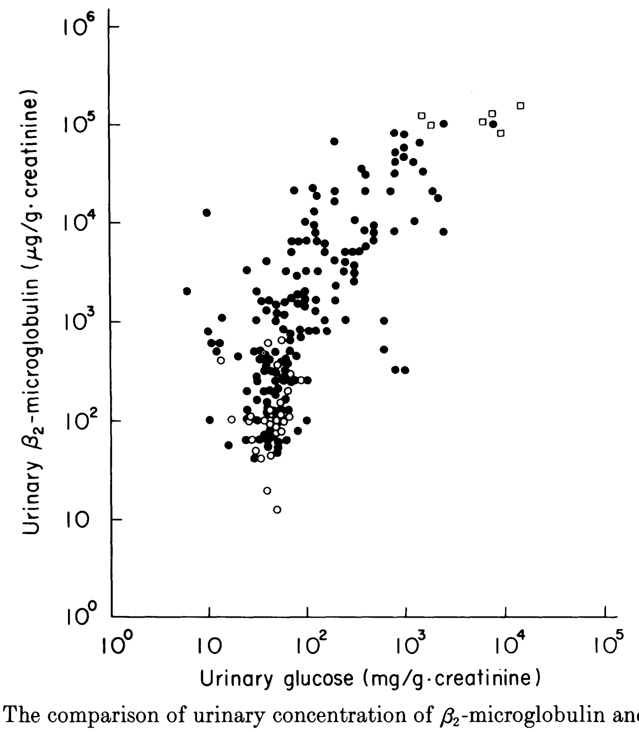

Fig. 4. The comparison of urinary concentration of β_2-microglobulin and glucose. ●, inhabitants of cadmium-polluted areas ($n=161$) ; ○, inhabitants in adjacent reference areas ($n=29$) ; □, patients with Itai-itai disease ($n=6$). correlation coefficient (r)=0.754 ($p<0.01$).

dents in D and H districts and patients. Since the correlation coefficient between creatinine and specific gravity was 0.730 ($n=196$, $p<0.01$), the decreased creatinine in the inhabitants seems to reflect the increased volume of urine.

Comparison of different urinary components among controls, cadmium-exposed groups, and patients with Itai-itai disease and prevalence of renal tubular dysfunction in thirteen districts

As shown in Table 2 and Fig. 3, the inhabitants of the cadmium-polluted areas exhibited exceedingly high excretions of β_2-m, α_1-m, glucose and amino-nitrogen. Fig. 4 shows the comparison of individual urinary concentrations of β_2-m and glucose. In this analysis, six patients with Itai-itai disease had the most elevated excretions of β_2-m and glucose among all the subjects examined. Several persons exhibited extraordinary high β_2-m and glucose excretions comparable to those of patients with Itai-itai disease.

Table 3 represents the number and proportions of the subjects who had renal

TABLE 3. *Prevalence of renal tubular dysfunction determined by urinary β_2-microglobulin exceeding 1 mg/g creatinine and urinary glucose exceeding 100 mg/g creatinine in eleven districts in the Jinzu River basin (B-L) and adjacent reference areas (A, M)*

Site	Number of subjects examined	Number of cases with renal tubular dysfunctions (%)
Reference area		
A	19	0 (0)
M	10	0 (0)
Total	29	0 (0)
Cadmium-polluted area		
B	9	1 (11.1)
C	19	8 (42.1)**
D	14	7 (50.0)**
E	7	3 (42.9)**
F	11	4 (36.4)**
G	28	15 (53.6)**
H	13	4 (30.8)**
I	15	7 (46.7)**
J	14	7 (50.0)**
K	17	5 (29.4)**
L	20	3 (15.0)*
Total	167	64 (38.3)**

Chi-square test; $^*p<0.05$, $^{**}p<0.01$, compared to the reference areas.

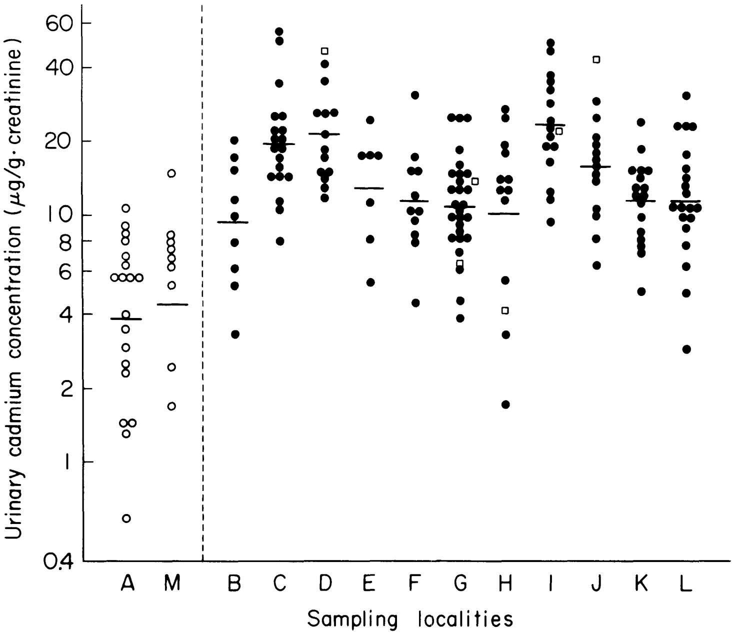

Fig. 5. The individual urinary cadmium concentration in eleven districts in the Jinzu River basin (B-L) and adjacent reference areas (A, M). For explanations, see Fig. 3.

tubular dysfunction as determined by urinary β_2-m level exceeding 1,000 μg/g creatinine and urinary glucose level exceeding 100 mg/g creatinine. No subjects with renal tubular dysfunction were detected in the reference areas. On the other hand, 64 subjects (38.3%) from the cadmium-polluted Jinzu River basin were detected to have renal tubular dysfunction. The high prevalence rates were detected in C, D, E, F, G, I and J districts adjacent to the Jinzu River ($p<0.01$).

Cadmium in urine

The distribution of urinary cadmium concentrations and its mean values in 13 districts is demonstrated in Fig. 5 and Table 2. There was a large variation in urinary cadmium even in the two reference areas. The means of cadmium concentration in two reference areas A and M were 3.8 and 4.4 μg/g creatinine, respectively. In spite of considerable overlaps, however, the mean values of the inhabitants of the Jinzu River basin (B-L) were significantly higher ($p<0.01$, Table 2) than those of the references. The patients with Itai-itai disease also had high urinary excretion of cadmium ranging from 6.6 to 110.6 μg/g creatinine. This result revealed that the inhabitants of the Jinzu River basin have been exposed to cadmium and exhibited the increased cadmium body-burden.

TABLE 4. *Cadmium concentration in polished rice harvested in eleven districts in the Jinzu River basin (B-L) and adjacent reference areas (A, M)*

Site	Number of household rice (%)	Mean ± S.E. (ppm)	Range (ppm)
Reference area			
A	15 (68.2)	0.13±0.04	0.06-0.20
M	10 (100)	0.12±0.03	0.07-0.17
Total	25 (84.1)		
Cadmium-polluted area			
B	14 (82.4)	0.32 ± 0.17^{ac}	0.10-0.81
C	10 (43.5)	0.40 ± 0.22^{bd}	0.14-0.73
D	14 (100)	0.40 ± 0.24^{bd}	0.02-1.02
E	7 (100)	0.38 ± 0.17^{ac}	0.09-0.62
F	9 (75.0)	0.24±0.13	0.06-0.52
G	28 (93.3)	0.39 ± 0.30^{bd}	0.03-1.22
H	8 (47.1)	0.33 ± 0.11^{ac}	0.18-0.56
I	11 (68.7)	0.61 ± 0.31^{bd}	0.03-0.96
J	11 (78.6)	0.36 ± 0.15^{bc}	0.10-0.67
K	15 (88.2)	0.36 ± 0.19^{bd}	0.13-0.92
L	20 (100)	0.37 ± 0.33^{bd}	0.08-1.48
Total	147 (79.7)		
Commercial rice			
	32 (15.7)	0.13±0.06	0.03-0.28
Significance		$p<0.01$	

Significant differences between the reference area at district A and each district in the cadmium-polluted area; $^{a}p<0.05$, $^{b}p<0.01$.

Significant differences between the reference area at district M and each distict in the cadmium-polluted area; $^{c}p<0.05$, $^{d}p<0.01$.

Cadmium in rice

The cadmium concentration in rice was also measured to evaluate the present exposure level to the subjects. Two hundred and four inhabitants (94.4%) supplied polished rice as a sample of their daily food for analysis. 84.8% of the rice samples (172 samples out of 204) were produced by themselves, and the rest (32 samples) were commercial rice. Table 4 shows the average cadmium contents in polished rice grains harvested in 13 districts. The cadmium content of polished rice in the reference areas A and M ranged from 0.06 to 0.20 ppm or from 0.07 to 0.17 ppm with an average content of 0.13 ppm and 0.12 ppm, respectively. The cadmium content of 32 commercially available rice samples ranged from 0.03

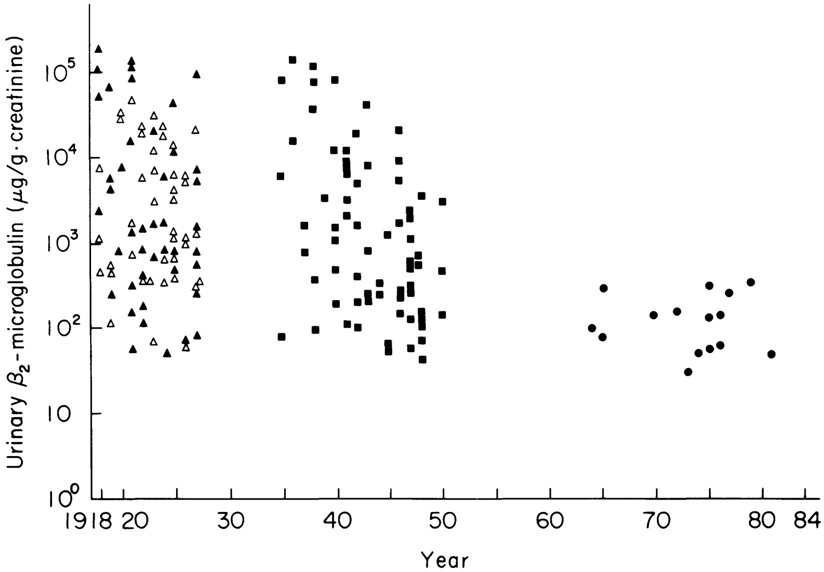

Fig. 6. The relationship between the urinary β_2-microglobulin concentration and the time of residence in the Jinzu River basin. People who have lived continuously in the present address since they were born (▲), people who have moved to the present address from other areas within the Jinzu River basin (△), people who moved into the Jinzu River basin from non-cadmium polluted areas during the years 1918–1927 (■) and people who have moved in the Jinzu River basin after 1964 (filled circles).

to 0.28 ppm with an average of 0.12 ppm, which did not differ significantly ($p>0.05$) from the levels of rice obtained from the reference areas. The means of cadmium content of home-grown rice in eleven districts in the Jinzu River basin (B-L) except F ranged from 0.32 to 0.57 ppm, which were significantly higher ($p<0.05$ or $p<0.01$) than those of the references. These results indicate that the inhabitants of the Jinzu River basin have had rice that contains a high concentration of cadmium for their daily food.

Relationship between renal tubular dysfunction and the time of residence in the Jinzu River basin

Fig. 6 shows the relationship between the urinary β_2-m concentrations and the time of residence in the Jinzu River basin. The subjects were divided into three groups based on the time of residence in the Jinzu River basin: group A, inhabitants who were born in the Jinzu River basin between 1918 and 1927; group B, those who moved into the Jinzu River basin from non-cadmium polluted areas during the time between 1933 and 1950 mainly due to marriage; and group C, those who have recently moved into the Jinzu River basin after 1964. Group A consisted of two subgroups of inhabitants: those who have lived continuously in the present address (A1), and others who have moved to the present address

TABLE 5. *Means of residence time (in years), urinary β_2-microglobulin, glucose, calcium, phosphorus, creatinine, cadmium and cadmium content in rice in different residence categories**

Group	A1	A2	B	C	ANOVA§
Number of subjects	44	45	69	15	
Age (year)	60.7 ±3.0†	60.6 ±2.7	60.9 ±3.0	61.0 ±2.8	NS
Residence time (year)	60.1 ±3.5†	58.7 ±5.2	40.6 ±4.7	10.4 ±4.9	$p<0.01$
β_2-Microglobulin (μg/g creatinine)	2287.7‡ (10.9)	1819.7 (6.1)	1258.9 (8.5)	109.6 (2.1)	$p<0.01$
Glucose (mg/g creatinine)	204.1‡ (5.4)	102.3 (3.3)	95.4 (4.4)	40.7 (1.7)	$p<0.01$
Calcium (mg/g creatinine)	295.1‡ (1.8)	288.4 (1.6)	275.4 (2.1)	275.4 (1.4)	NS
Phosphorus (mg/g creatinine)	741.3‡ (1.5)	741.3 (1.3)	851.1 (1.5)	707.9 (1.3)	NS
Cadmium (μg/g creatinine)	13.1‡ (1.9)	13.4 (1.8)	14.4 (1.7)	4.7 (2.1)	$p<0.01$
Specific gravity	1.012±0.004†	1.013±0.005	1.014±0.005	1.016±0.007	$p<0.05$
pH	6.44 ±0.47†	6.53 ±0.54	6.41 ±0.50	6.01 ±0.54	$p<0.01$
Creatinine (g/liter)	0.47 ±0.23†	0.56 ±0.29	0.55 ±0.31	0.70 ±0.38	NS
Cadmium in rice (ppm)	0.33 ±0.25†	0.34 ±0.23	0.35 ±0.29	0.21 ±0.19	NS

* A1, people who have lived continuously in the present address since they were born; A2, people who have moved to the present address from other areas within the Jinzu River basin; B, people who have moved into the Jinzu River basin from non-cadmium polluted areas during the time between 1918-1927; C, people who moved in the Jinzu River basin after 1964. † Values are represented as am± as.D. ‡ Values are represented as GS (GSD). § Analysis of variance (ANOVA), NS=not significant at the $p=0.05$ level.

from other areas within the Jinzu River basin.

Table 5 shows the mean age, years of residence in the Jinzu River basin and various urinary components in the four groups as defined above. Means of β_2-m, glucose and calcium were the highest in group A1. Fig. 6 also shows that even inhabitants who have lived in the Jinzu River basin since 1946 (group B) have high excretion levels of β_2-m over 10,000 μg/g creatinine. However, those who moved into this study area recently (group C) showed no increased excretion of β_2-m. These results indicate that the longer the inhabitants lived in the Jinzu River basin, the more β_2-m and glucose excretions increased. Therefore it appears that the degree of renal tubular dysfunction is related to the resident time and years in the Jinzu River basin.

Multivariate analysis on the various components related to renal tubular function

The principal component analysis was conducted with various environmental as well as biological variables, in which 97 inhabitants in the A-G districts who continuously consumed only their own home-grown rice were selected for this analysis.

The variables analyzed are residence time (in years) in the Jinzu River basin (X_1), specific gravity of urine (X_2), urinary pH (X_3), urinary creatinine (X_4), urinary glucose (X_5), urinary β_2-m (X_6), urinary total proteins (X_7), urinary α_1-m (X_8), urinary cadmium (X_9) and cadmium content in rice (X_{10}). The percentages of the total contribution were 43.37% in the first component (Z_1), 15.58% in the second component (Z_2), and 10.86% in the third component (Z_3), respectively. The first three components account for 69.80% of the total contribution (Table 6). Considering the factor loading of ten variables, the first principal component (Z_1) appears to indicate the levels of renal tubular dysfunction. Thus, the higher Z_1

TABLE 6. *Factor loadings, eigen values and their contribution percentages to the total variances obtained from principal component analysis of 10 variables*

Variables	Z_1	Z_2	Z_3	Z_4
Residence time (X_1)	0.597	0.227	0.569	0.210
Specific gravity (X_2)	−0.492	0.747	0.192	−0.085
pH (X_3)	0.525	−0.283	0.165	0.683
Creatinine (X_4)	−0.582	0.620	0.231	0.173
Glucose (X_5)	0.682	0.273	−0.201	−0.283
β_2-Microglobulin (X_6)	0.907	0.251	−0.096	0.112
Total protein (X_7)	0.821	0.180	−0.307	−0.102
α_1-Microglobulin (X_8)	0.885	0.265	−0.120	−0.006
Cadmium (X_9)	0.577	0.091	0.475	−0.270
Cadmium content in rice (X_{10})	0.219	−0.484	0.515	−0.403
Eigen value	4.337	1.558	1.085	0.887
Contribution (%)	43.367	15.576	10.855	8.866

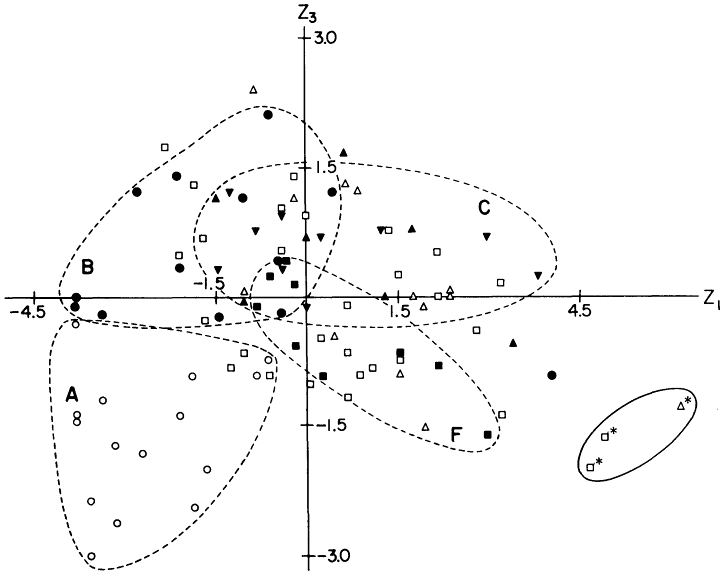

Fig. 7. Scatter diagram illustrating 97 inhabitants in the A-G districts distributed in the Z_1-Z_3 axis computed by Principal Component Analysis. ○, inhabitants in A district; ●, in B district; ▼ in C district; △, in D district; ▲, in E district; ■, in F district; □, in G district. Aster patients with Itai-itai disease.

values indicate the higher levels of renal tubular dysfunction, whereas higher levels in the third principal component (Z_3) reveal higher levels of cadmium intake from rice and higher urinary cadmium excretions without prominent renal tubular dysfunction in spite of a long period of residence in cadmium-polluted areas.

Therefore, as illustrated on the Z_1-Z_3 plane of the scatter diagram (Fig. 7), the distribution of the reference area (district A) is strictly confined to the third quadrant, while patients with Itai-itai disease are located in the fourth quadrant. The inhabitants of cadmium-polluted areas with high cadmium exposure at present but without prominent renal tubular dysfunction are found mainly in the second quadrant (e.g., district B) and in response to the increase in the levels of renal tubular dysfunction they are scattered over the plane from the first quadrant (e.g., district C). The inhabitants of cadmium-polluted areas with relatively low cadmium exposure at present but having renal tubular dysfunction are found mainly in the fourth quadrant (e.g., district F).

This result indicates that the PCA is a useful means to estimate the severity of renal tubular dysfunction and the exposure level of cadmium among the exposed population.

Discussion

Health surveys for all the residents over 45 years old in the Jinzu River basin have been performed by the Toyama Prefectural Health Authorities since 1967, but the results were not published except for those reported by Fukushima et al. (1974, 1975). The results of urinary examinations of inhabitants in the Jinzu River basin were reported previously by various authors (Hagino 1972 ; Kanai et al. 1976 ; Kjellström et al. 1977 ; Shiroishi et al. 1977 ; Kobayashi 1982a, b). However, a survey of low-molecular weight proteinuria for all the inhabitants of the entire Jinzu River basin and adjacent Kumano and Ida River basins has not been conducted. Therefore, in this study, an emphasis was made to clarify the distribution and frequency of renal tubular dysfunction among the female inhabitants in the age group 55 to 66 years old of the Jinzu River basin and its neighbours.

The β_2-m, α_1-m, amino-acid and glucose are filtered through the glomeruli and 99.9% of them are reabsorbed by the proximal tubules continuously. Consequently, increased urinary excretion of these substances indicates the disturbance of the proximal tubular function. Since the concentrations of urinary β_2-m and glucose of the inhabitants of reference areas did not exceeded 1,000 μg/g creatinine and 100 mg/g creatinine, respectively (Fig. 4), the renal tubular dysfunction was defined by the β_2-m and glucose exceeding these values (cf. Table 3). Shitomi et al. (1981) have also reported that the urinary β_2-m adjusted to a per gram creatinine showed a close relationship to the results of the proximal renal tubular function test and over 1,000 μg/g creatinine of urinary β_2-m indicated proximal renal tubular dysfunction. The results of the present epidemiological survey revealed that urinary β_2-m, α_1-m, amino-nitrogen, glucose and cadmium concentrations of the inhabitants of the Jinzu River basin were significantly higher than those of the inhabitants of the reference areas of the Ida and Kumano River basins (Table 2). On the other hand, none of the inhabitants of the reference areas which are adjacent to the areas irrigated by the Jinzu River water showed high β_2-microglobulinuria. This fact clearly indicates that the high prevalence of β_2-microglobulinuria is strictly confined to the Jinzu River basin. As the reference areas were selected from the neighbouring areas of the Jinzu River basin (Fig. 2), it is apparent that the occurrence of renal tubular dysfunction was restricted in the areas irrigated by the Jinzu River water. This fact likewise agrees well with the evidence that the Itai-itai disease has been found only in a clearly defined area. It is an area where the Jinzu River water has been used for irrigation of rice fields (Kato and Kawano 1968 ; Friberg et al. 1974).

Moreover, it is noteworthy that in the present study, different prevalence rates of renal tubular dysfunction were detected in the different areas within the Jinzu River basin. As shown in Table 3, the prevalence of renal tubular dysfunction in C, D, E, G, I and J districts adjacent to the Jinzu River showed remarkably high

prevalence rates of over 40%. On the other hand, prevalence rates in B and L districts apart from the Jinzu River showed relatively lower levels. This diffecrence in the prevalence rate of renal tubular dysfunction in each district except for D district was well in accordance with the prevalence of Itai-itai disease reported in 1968 (Kato and Kawano 1968; Friberg et al. 1974).

The subjects for the present study overlap to a considerable extent with those reported by Fukushima et al. (1974, 1975), who used as age group persons between 40 and 49 years when their survey was performed in 1967. In their study, none of the female inhabitants of the 40-49 age group in the endemic areas of Itai-itai disease showed the concurrent clinical proteinuria and glycosuria. It is, therefore, noteworthy that an exceedingly high prevalence of β_2-microglobulinuria and glucosuria was detected in the 55-66 age group in this study (Table 3). This fact indicates that during the past 16 years, renal tubular dysfunction has become apparent in this same target group as was studied by Fukushima et al. (1974, 1975) in 1967.

The cadmium concentration in rice was also measured in this study to evaluate the present exposure level to the inhabitants in the cadmium-polluted areas. The mean values in rice from the reference areas of the Ida and Kumano River basin (0.12-0.13 ppm) are in agreement with the mean value of 0.10 ppm from other parts of Toyama Prefecture (Kjellström et al. 1977). The mean cadmium concentration in rice in eleven districts in the Jinzu River bain (0.24-0.57 ppm) was about 2-5 times higher than in the reference groups (Table 4). The production of rice in the paddy fields of about 500 ha which were heavily polluted with cadmium was prohibited by law. However, in about 1,000 ha paddy fields which was polluted with cadmium, rice has been produced for private consumption. Therefore, the inhabitants of the Jinzu River basin are still exposed to a high level of cadmium intake through daily consumed food even today.

Considering all the evidence gathered in the present study, it can be concluded that a high prevalence of renal tubular dysfunction detected in the inhabitants of the Jinzu River basin was obviously caused by the environmental cadmium pollution.

The second objective of this study was to confirm the dose-response relationship of cadmium-induced renal tubular dysfunction. Kjellström et al. (1977) tried to clarify the dose-response relationship by comparing individual cadmium dose estimates with urinary β_2-m concentrations. They pointed out the difficulty of estimating the dose levels accurately, primarily due to the very long biological half-time of cadmium and also the requirements of a long-term exposure for the renal cortex concentrations to reach critical level.

In this study, a tendency towards higher β_2-m excretion with longer residence time was apparent (Fig. 6, Table 5). The correlation coefficients between cadmium contents in individual home-grown rice and β_2-m concentrations in urine ($r=0.354$, $n=147$), and between cadmium and β_2-m concentrations in urine ($r=$

0.463, $n = 196$) were statistically significant ($p < 0.01$). Furthermore, the present study clearly reveals that a considerable number of the inhabitants of the Jinzu River basin have conspicuous renal tubular dysfunction at present and they are still exposed to a high level of cadmium via food. This dose-response relationship was grasped with considerable accuracy by the results of multivariate analysis conducted in the present study (Fig. 7). That is, there occur some localized variations in the extent and prevalence rate of renal tubular dysfunction even within the Jinzu River basin (Table 3), but such local differences in the prevalence rate obviously reflect the extent of cadmium pollution and length of the exposure-time within this area.

It should also be noted here again that the exceedingly high excretion of low-molecular weight proteins and glucose found in the inhabitants of cadmium-polluted areas were almost comparable to those of the patients with Itai-itai disease (cf. Figs. 3 and 4). The results obtained in this study evidently exhibit that there occurs a large latent female population with renal tubular osteomalacia in the inhabitants of the Jinzu River basin. This is one of the most significant findings made in the present study. Furthr detailed clinical examinations of the inhabitants with renal tubular damage are needed in order to clarify the pathogenesis of renal tubular osteomalacia (Itai-itai disease) more accurately.

Acknowledgments

I would like to thank to Professor Minoru Kasuya and all members of the Department of Public Health, Faculty of Medicine, Toyama Medical and Pharmaceutical University, Professor Shoichi Kawano and Dr. Shuichi Nakayama of the Department of Botany, Faculty of Science, Kyoto University, Dr. Noboru Hagino of the Hagino Hospital in Fuchu-machi, Toyama and the students of 1983 and 1984 classes of the medical course for their advice and help throughout this study. This study was partly supported by Grant for Scientific Research No. 59030030 from the Ministry of Education, Science and Culture, Japan.

References

1) Bonsnes, R.W. & Taussky, H.H. (1945) On the colorimetric determination of creatinine by the Jaffe reaction. *J. biol. Chem.*, **158**, 581-591.
2) Connerty, H.V. & Briggs, A.R. (1966) Determination of serum calcium by means of orthocresolphthalein complexone. *Amer. J. clin. Path.*, **45**, 290-296.
3) Fiske, C.H. & Subbarow, Y. (1925) The colorimetric determination of phosphorus. *J. biol. Chem.*, **66**, 375-400.
4) Friberg, L., Piscator, M., Nordbelg, G.F. & Kjellström, T. (1974) *Cadmium in the Environment*, 2nd ed., Chemical Rubber Press, Cleveland, Ohio.
5) Fukushima, M. & Kobayashi, E. (1975) Studies on some urinary screening tests for renal dysfunction induced by cadmium exposure. 4. Determination of free amino acid in urine by 2, 4, 6-trinitrobenzene-1-sulfonic acid method. *J. Juzen med. Soc.*, **84**, 166-171. (abstracts in English)
6) Fukushima, M., Ishizaki, A., Nogawa, K., Sakamoto, M. & Kobayashi, E. (1974) Epidemiological studies on renal failure of inhabitants in "Itai-itai" disease endemic district. 1: Some urinary findings of inhabitants living in and around the endemic

district of the Jinzu River basin. *Jap. J. Hyg.*, **21**, 65-73. (in Japanese with English summary)

7) Fukushima, M., Ishizaki, A., Sakamoto, M., Nogawa, K. & Kobayashi, E. (1975) Epidemiological studies on renal failure of inhabitants in "Itai-itai" disease endemic district. 2: Observations on urinary abnormalities and cadmium excretion of the inhabitants selected for the close examination of "Itai-itai" disease. *Jap. J. Hyg.*, **22**, 217-224. (in Japanese with English summary)

8) Hagino, N. (1972) Disk electrophoresis of urine of the families inhabited in Itai-itai disease districts. *Med. Biol.*, **84**, 175-179. (Japanese)

9) Kajikawa, K. (1978) Pathogenesis of Itai-itai disease based on post-mortem studies. In: *Cadmium Studies in Japan : A Review*, edited by K. Tsuchiya, Elsevier, Amsterdam, pp. 286-295.

10) Kanai, M., Momose, T., Aizawa, T., Okudaira, S., Honma, T. & Hagino, N. (1976) Pathophysiological studies on β_2-microglobulin: Increased urinary excretion of β_2-microglobulin in patients with "Itai-itai" disease and their family members. *Saishin Igaku*, **31**, 1793-1799. (Japanese)

11) Kato, T. & Kawano, S. (1968) Review of past and present of "Itai-itai" disease. On the process of reseach development. *Curr. Med.*, **16**, 29-48. (Japanese)

12) Kato, T., Aoshima, K., Kubota, Y., Tanaka, M., Teranishi, H. & Kasuya, M. (1984) Simple pretreatment for the determination of heavy metals in blood and urine by flameless atomic absorption spectro-photometry. *Jap. J. Hyg.*, **39**, 349. (Japanese)

13) Kingston, H.M., Barnes, I.L., Brady, T.J. & Rains, T.C. (1978) Separation of eight transition elements from alkali and alkaline earth elements in estuarine and seawater with chelating resin and their determination by graphite furnace atomic absorption spectrometry. *Anal. Chem.*, **50**, 2064-2070.

14) Kjellström, T., Shiroishi, K. & Evrin, P.-E. (1977) Urinary β_2-microglobulin excretion among people exposed to cadmium in the general environment. An epidemiological study in cooperation between Japan and Sweden. *Environ. Res.*, **13**, 318-344.

15) Kobayashi, E. (1982a) An epidemiological study on the health effects of environmental cadmium. 1: Some urinary findings by sex and age. *Jap. J. Pub. Hlth*, **29**, 123-133. (in Japanese with English summary)

16) Kobayashi, E. (1982b) An epidemiological study on the health effects of environmental cadmium. 2: Some urinary findings by residence time in Cd-polluted area. *Jap. J. Pub. Hlth*, **29**, 201-207. (in Japanese with English summary)

17) Morishita, T. (1981) The Jinzu River Basin: Contamination of soil and paddy rice with cadmium discharged from Kamioka mine. In: *Heavy Metal Pollution in Soil of Japan*, edited by K. Kitagishi & I. Yamane, Japan Scientific Societies Press, Tokyo, pp. 107-124.

18) Nogawa, K. (1981) Itai-itai disease and follow-up studies. In: *Cadmium in the Environment*, *Part II*, edited by J.O. Nriagu, John Wiley & Sons, New York, pp. 643-689.

19) Snedecor, G.W. & Cochran, W.G. (1972) *Statistical Methods*. 6th ed., Iowa State University Press, Ames.

20) Shiroishi, K., Kjellström, T., Kubota, K., Evrin, P.-E., Anayama, M., Versterverg, O., Shimada, T., Piscator, M., Iwata, T. & Nishino, H. (1977) Urine analysis for detection of cadmium induced renal changes, with special reference to β_2-microglobulin. *Environ. Res.*, **13**, 407-424.

21) Shitomi, K., Saito, H., Nakano, A., Unakami, H., Takada, K., Sato, T., Furuyama, T., Yoshinaga, K., Arikawa, T. & Nagai, K. (1981) Urinary β_2-microglobulin for residents in an environmentally cadmium-polluted area. Studies of general and sexual differences, and a comparison with the results of proximal renal tubular function tests. *Jap. J. Nephrol.*, **23**, 45-62. (in Japanese with English summary)

22) Takeuchi, J. (1978) Cadmium vs. nutrition in Itai-itai disease. In: *Cadmium Studies in Japan : A Review*, edited by K. Tsuchiya, Elsevier, Amsterdam, pp. 283-286.

23) Yanagisawa, M., Shinmura, Y., Yamada, N., Segawa, A. & Kida, K. (1984) Heavy metal pollution and methods of restoration of polluted soils in the Jinzu River basin. *Bull. Toyama Agric. Exp. Stn.*, **15**, 1-110. (in Japanese with English summary)