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Effects of Dietary Cadmium on Rhesus Monkeys

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Ten male rhesus monkeys, each weighing 3.5 kg, were divided into four groups of 3, 3, 2, and 2, and were fed daily with 100 g pelleted food containing 300, 30, 3, and 0 ppm cadmium, respectively. Urine samples were collected every 2 weeks and blood samples every 4 weeks. One monkey each of the 300 and 30 ppm groups was autopsied for pathological examination and tissue cadmium determination at the week 24 of the experiment; the remaining 8 animals were killed after 55 weeks.

The lowest exposed group (3 ppm) did not show any specific biological response to cadmium over a period of 55 weeks. In the 30 ppm group, no significant changes were observed for up to 24 weeks, although cadmium concentration in the renal cortex and urine at 24 weeks were 300 μg/g wet weight and 18 μg/l., respectively. Plasma urea nitrogen and urine protein (quantitative determination) increased after 30 and 36 weeks. At 55 weeks of the experiment, qualitative tests were negative for low molecular weight proteinuria and glycosuria, and the results remained normal for renal and liver function tests and blood analysis, although cadmium concentrations in the renal cortex of two monkeys were 460 and 730 μg/g wet weight and those in the liver were 110 and 160 μg/g wet weight, respectively.

In the highest exposure group (300 ppm), urine cadmium increased to 250 μg/l. by 11 weeks, and urine retinol-binding protein, plasma GOT, GPT, and LDH increased after 12 weeks. Proteinuria (quantitative determination), glycosuria, aminoaciduria (panaminoaciduria), and erythrocytopenia were observed after 16 weeks, when urine cadmium was 500–900 μg/l. Hypohemoglobinopathy and proteinuria (qualitative determination) were observed after 20 and 24 weeks, while cadmium concentrations in the renal cortex and the liver were 760 and 430 μg/g wet weight at 24 weeks, respectively. Slightly depressed tubular reabsorption of phosphate, increased urine β_2-microglobulin, increased plasma urea nitrogen, and increased plasma α_2-globulin fraction (electrophoresis) were observed between 28 and 30 weeks of the experiment. Creatinine clearance and plasma cholinesterase decreased after 47 and 54 weeks, respectively. Cadmium concentrations in the renal cortex and the liver of two monkeys at 55 weeks were 350 and 580 μg/g wet weight and 410 and 630 μg/g wet weight, respectively. Pathological examinations revealed denaturation, destruction, and regeneration of the epithelial cells in renal proximal tubules, but no pathological changes in osseous tissues.

Critical cadmium concentration in the renal cortex was estimated to be 380 μg/g wet weight for low molecular weight proteinuria and 470 μg/g wet weight for proteinuria, glycosuria, and aminoaciduria. Critical concentration in the liver was also estimated to be 210 μg/g wet weight. The apparent biological half-time of cadmium in monkeys at autopsied stage was calculated to be 0.66, 6.4, 5.2, and 22.4 years for the 300, 30, 3, and 0 ppm groups, respectively.

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Introduction

Since the conclusion was drawn that itai-itai disease which occurred in the basin of the Jinzu River in Toyama Prefecture (*1*) was related to cadmium (*2*), much attention has been paid to health effects of

cadmium on inhabitants of cadmium-polluted areas in Japan. While health surveys have been performed in cadmium-polluted areas, animal experiments have been conducted to elucidate the mechanism and early detection of cadmium poisoning (*3*). However, most animal experiments have been carried out on mice and rats, and occasionally on rabbits. Therefore, experimental results are not always applicable directly to cadmium health effects on human. Besides, animals in those experiments have been exposed to cadmium at far higher dose levels than actual exposure levels in human. This is why it was very important to expose monkeys to cadmium at relatively low levels for a prolonged period. This experiment was primarily focused on the dose-response relationship of cadmium. As many clinical pathology determinations were performed as possible to find criteria for evaluating cadmium toxicity. Critical concentration and biological half time of cadmium in monkeys were also determined.

Materials and Methods

Ten male rhesus monkeys, *Macaca mulatta*, imported from India, were purchased after quarantine through the Shizuoka Prefecture Agricultural Corporation for Experimental Animals. They weighed 3.5 kg and were estimated to be 2.0–2.5 years old. After maintaining them for 10 weeks in steel cages with Oriental monkey pelleted food, they were divided into four groups at random after gradation by body weight; three monkeys of the first group were given 30 mg cadmium per day as cadmium chloride by oral route, three monkeys of the second group 3 mg cadmium/day, two monkeys of the third group 0.3 mg cadmium/day, and two monkeys of the last group 0 mg cadmium/day, respectively. Originally, in September 1976, cadmium was given to monkeys mixed into a 0.5 g biscuit; animals on high doses refused for several days to consume these biscuits. Subsequently, orange-flavored biscuits were given to these monkeys instead of ordinary cadmium biscuits, but these also were refused. Even starvation failed to cause the animals to eat the biscuits. Finally, the monkeys were given CLEA (Central Laboratory for Experimental Animals, Tokyo) monkey pelleted food containing 300, 30, 3, and 0 mg cadmium/kg with orange flavor; this food was readily accepted. The monkeys were given 100 g pelleted food containing cadmium at four levels and half an apple daily throughout the experimental period. The levels 30, 3, 0.3, and 0 mg cadmium/head/day group are termed the 300, 30, 3 and 0 ppm groups in the present paper. In addition 30 g ordinary pelleted food containing 0.13 mg cadmium/kg was provided per day starting in May of 1977.

At 2-week intervals, samples of urine and feces were collected for clinical pathology and cadmium assay. Immediately after collection, urine samples were divided into two parts: to one portion was added sodium azide to a final concentration of 0.1% prior to storage at 4°C. Urine cadmium was determined by direct Zeeman effect atomic absorption spectrophotometry (*4*). Urine samples were diluted with distilled water to a final concentration of approximately 20 ng/ml and acidified with nitric acid (final concentration 0.1*N*). The Hitachi Zeeman effect atomic absorption spectrophotometer, Type 170-70, equipped with Perkin-Elmer auto sampling system AS-1 was used for cadmium determination. Urine protein and amino acid were determined by the Tsuchiya-Biuret method (*5*) and the TNBS method (*6*). Acidity, protein (qualitative determination), and glucose were evaluated by tape. Plasma and urine creatinine were determined by the method of Folin-Wu (*7*), plasma and urine phosphate by that of Fiske-Subbarow (*8*), plasma calcium by atomic absorption spectrophotometry, plasma protein by refractometry, plasma GOT and GPT by the standard procedures of Japan Association of Digestive Systems (Reitman-Frankel unit) (*11*), plasma LDH by the method of Wroblewski (*12*), plasma leucine amino peptidase (LAP) by the method of Goldbarg and Rutenburg (*13*), plasma alkaline phosphatase (ALP) by the method of Kind and King (*14*). Isoenzymes of alkaline phosphatase and leucine aminopeptidase were also studied. Erythrocyte and leucocyte counts were determined by automatic blood cell counter (Coulter Counter) and hemoglobin by the cyanmethemoglobin method (*15*). Electrocardiograms and blood pressure were recorded under ketamine hydrochloride anesthesia after Hirayama's procedure (*16*) for A-B and ordinary limb leads. Blood pressure was also measured at 55 weeks by both indirect and direct methods. The latter measurement was performed with the use of a carotid artery catheter.

At the 24th week of the experiment, one monkey from each of the high exposure groups was killed for pathological examination and tissue cadmium determination; the remaining eight monkeys were killed after 55 weeks. For tissue cadmium determination, tissues were weighed at the level of 10^{-3} g accuracy and then kept at −20°C. At the time of cadmium determination, samples were thawed and wet-ashed with nitric acid and perchloric acid. Tissue cadmium was determined by a Varian-Techtoron atomic absorption spectrophotometer, Type AA-1100 equipped with background corrector BC-6 (D_2 lamp).

Results

Body Weight

Monkeys of the 0 ppm group gained weight at a rate of 21 g/week over a period of 55 weeks (Fig. 1). Some monkeys in the 3 and 30 ppm groups showed little gain in body weight. Monkeys in the 300 ppm group showed almost no gain in body weight over a period of 55 weeks.

Feces Weight

Monkeys of the 0, 3, and 30 ppm groups excreted 20-40 g/day feces in the early experimental period, but feces weight increased to 100 g/day at the end of the experiment. Feces weight of the 300 ppm group varied widely between individuals, but did not increase during the experiment.

Urine

Volume. Urine volume of the four groups showed no changes throughout the experiment.

pH. Urine pH was not changed by cadmium administration.

Urine Protein (Qualitative). The qualitative test for proteinuria was negative in the 0, 3, and 30 ppm groups throughout the experiment. One monkey of the 300 ppm group showed some initial proteinuria after 24 weeks, which became very marked after 50 weeks; another monkey showed proteinuria after 36 weeks.

Urine Protein (Quantitative). No increase was observed in urine protein of the 0 and 3 ppm groups over a period of 55 weeks (Fig. 2). The average urine protein concentration was 9.0 mg/dl. One monkey of the 30 ppm group showed a slight increase after the 36th week. Two of three monkeys

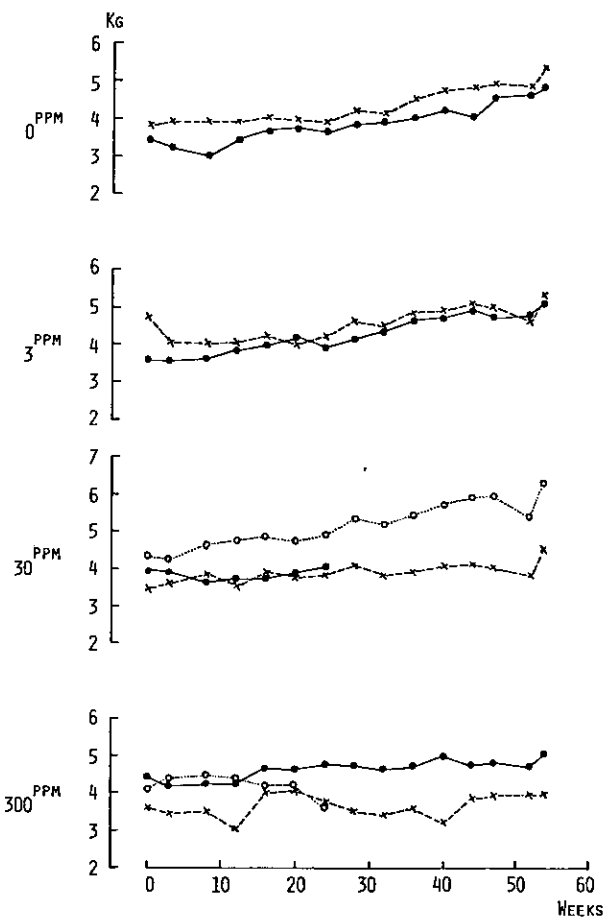

FIGURE 1. Body weights: 0 ppm group, (●) monkey #1248, (X) monkey #1259; 3 ppm group, (●) monkey #1244, (X) monkey #1249; 30 ppm group, (●) monkey #1202, (X) monkey #1247, (O) monkey #1258; 300 ppm group, (●) monkey #1213, (X) monkey #1243, (O) #1253.

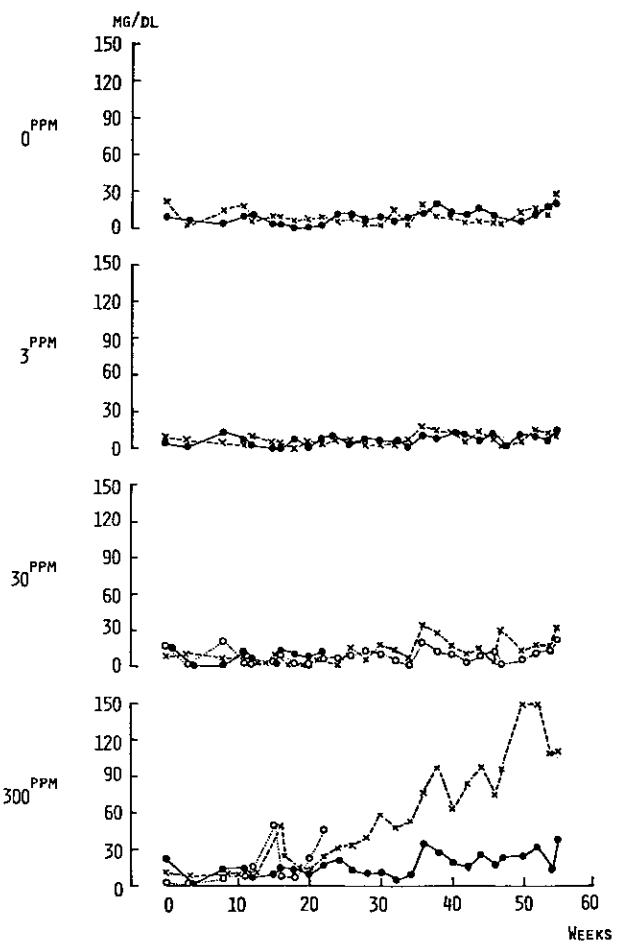

FIGURE 2. Urine protein (quantitative). Symbols as in Figure 1.

of the 300 ppm group indicated an increase in urine protein concentration after 15th week. These monkeys also exhibited glycosuria 1 week later. The urine protein concentration of the third monkey of the 300 ppm group increased after the 36th week; glycosuria was also present. Two of three monkeys of the 300 ppm group showed first a sharp transient increase after 15 weeks, followed large increases after 20 weeks.

Urine Glucose (Qualitative). Qualitative tests for glycosuria in the 0 and 3 ppm group monkeys were generally negative and only occasionally slightly positive (Fig. 3). The monkeys in the 30 ppm group were negative to the qualitative test for glycosuria over a period of 55 weeks. Two of three monkeys of the 300 ppm group monkeys showed glycosuria after 16 weeks of the experiment. Glycosuria was generally detected intermittently in the first stage and persistently in the later stage.

Urine Amino Acids. The urine amino acid concentration of the 0, 3, and 30 ppm groups remained at a normal level 5.8 ± 1.6 *mM* (average and one standard deviation of 10 untreated monkeys) over a period of 55 weeks (Fig. 4). Two of three monkeys of the 300 ppm group showed an increase in urine amino acid concentration (12–20 *mM*) after 15 or 16 weeks. The last monkey of the 300 ppm group showed a gradual increase in urine amino acid concentration after 44 weeks. However, it should be noticed here that the amino acid concentration of the 300 ppm group was not statistically different from that of the 0 ppm group.

Amino Acid Analysis of Urine. One monkey each of the 30 and 300 ppm groups at 24 weeks and 8 other monkeys of the 0, 3, 30 and 300 ppm group at 55 weeks were subjected to amino acid analysis of urine. In the 300 ppm group, proline was the amino acid present in highest concentration; this was followed by hydroxyproline, threonine, serine,asparagine, citruline, alanine, lysine, and histidine (Table 1). These data suggest that the monkeys suffered from panaminoaciduria.

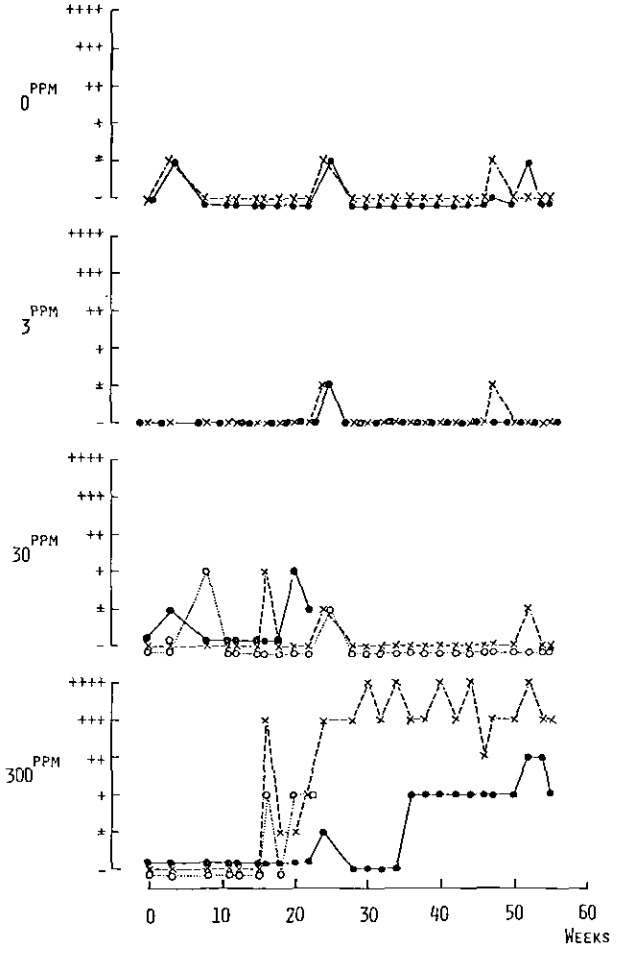

FIGURE 3. Urine glucose (qualitative). Symbols as in Figure 1.

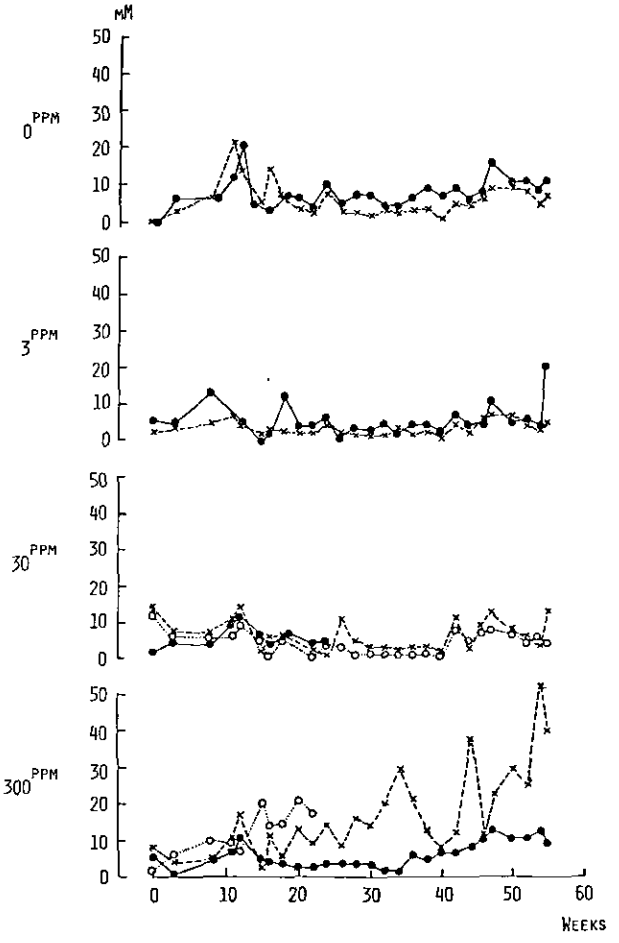

FIGURE 4. Urine amino acid. Symbols as in Figure 1.

Urine β_2-Microglobulin-like Protein. The monkeys excreted two kinds of β_2-microglobulin-like protein of different molecular weights but with electric mobilities almost the same as that of human β_2-microglobulin (*16*) (Fig. 5). Furthermore, human and monkey β_2-microglobulins possess common antigens (*16*), and monkey urine β_2-microglobulin-like protein could be determined by a micro-Ouchterlony method with the use of human antisera at a detection limit of 0.71 mg/dl human β_2-microglobulin.

In monkeys of the 30 ppm group, urine β_2-microglobulin-like protein was not detectable over a period of 55 weeks. One of two monkeys of the 300 ppm group indicated a positive immunological test to β_2-microglobulin-like protein after the 30th week. The protein concentration increased gradually to 5.68 mg/dl at 52 weeks of experiment, although it fluctuated from day to day.

Urinary Protein Similar to Retinol-Binding Protein. As reported by Nomiyama and Yotoriyama (*16*), monkeys excrete protein of the same molecular weight, electric mobility, and antigeneity as human retinol-binding protein (RBP). Urine RBP-like protein could be determined with the use of human antisera by a micro-Ouchterlony method and cross immunoelectrophoresis at detection limits of 0.31 and 0.04 mg/dl, respectively.

In the 30 ppm group, RBP-like protein was not detectable in urine over a period of 55 weeks (Fig. 6). In the 300 ppm group, the qualitative test by the cross immunoelectrophoresis for RBP-like protein became positive in one of two monkeys after 12 weeks. After 24 weeks it became positive by the micro-Ouchterlony method. Urine RBP-like protein concentration increased gradually with time of exposure. In another monkey receiving 300 ppm, RBP-like protein became positive after 54 weeks.

Plasama Urea Nitrogen

Plasma urea nitrogen of untreated monkeys was 23.1 ± 4.6 mg/dl (average ± standard deviation). No specific changes were observed in the 0 and 3 ppm groups. The 30 and 300 ppm groups showed relatively high plasma urea nitrogen concentration after the 36th week.

Creatinine Clearance (C_{cr})

Average creatinine clearances of the 0, 3, and 30 ppm groups were 12.2 ± 2.2 ml/min (Fig. 7), and no effects of Cd were observed during 55 weeks experiment. On the other hand, the creatinine clearance of the 300 ppm group decreased to 8 ml/min (63% of normal level) after the 47th week.

Tubular Reabsorption of Phosphate (%TRP)

Average tubular reabsorption of phosphate was 99.6% in the 0, 3, and 30 ppm groups (Fig. 8). No decrease was observed over a period of 55 weeks. One of two monkeys of the 300 ppm group showed a decrease in tubular reabsorption of phosphate after 28 weeks. Another monkey of the 300 ppm group showed a slight decrease in tubular reabsorption of phosphate after the 36th week. Tubular phosphate reabsorption in these monkeys fluctuated from day to day, but gradually fell to values of 96.7 and 96.1% after 55 weeks.

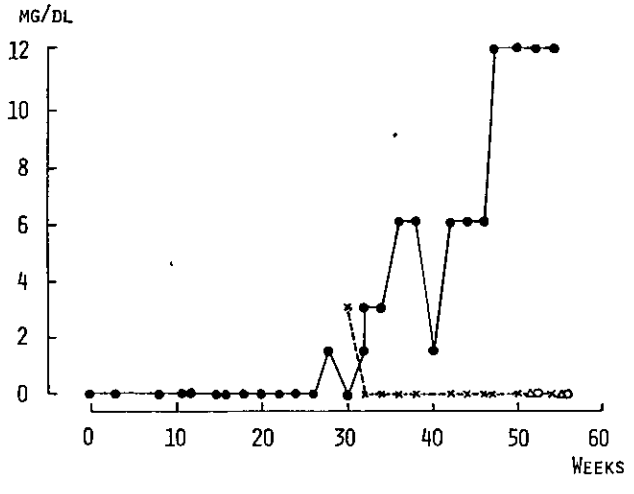

FIGURE 5. Urine β_2-microglobulin-like protein: (●, X) 300 ppm; (○, △) 30 ppm.

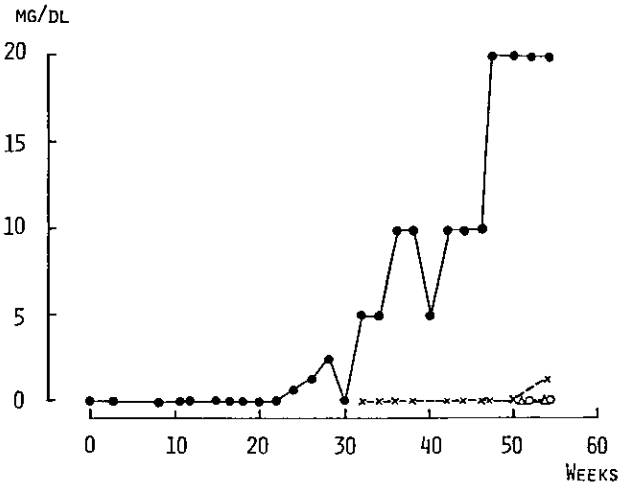

FIGURE 6. Urine retinol-binding protein. Symbols as in Figure 5.

Blood

Blood Glucose. The blood glucose of untreated monkeys was 87 ± 13 mg/dl. None of the 0, 3, 30, and 300 ppm group monkeys showed any changes in blood glucose.

Erythrocyte Count. Erythrocyte counts of the 0, 3, and 30 ppm group monkeys remained at 580 ± 22 × 10^4 /mm^3 throughout all 55 weeks. The erythrocyte count of the 300 ppm group monkeys after 16 weeks decreased gradually with cadmium administration to 290 × 10^4/mm^3 (48% of normal count) at the 55th week.

Leucocyte Count. There were no changes of leucocyte count which could be attributed to cadmium administration. The leucocyte count of untreated monkeys was 15,400 ± 3,400/mm^3, but showed large individual differences.

Hemoglobin Content. The hemoglobin level in blood of untreated monkeys was 13.7 ± 0.8 g/dl. (Fig. 9). Hemoglobin contents of the 3 and 30 ppm groups did not change following cadmium administration. The hemoglobin content for the 300 ppm group monkeys after 20 weeks began to decrease to 7.3 ± 0.7 g/dl (53.2% of untreated monkeys) at the 55th week.

Hematocrit (Ht). The hematocrit of untreated monkeys was 41.3 ± 2.1%. The 3 and 30 ppm group monkeys did not show any effects of cadmium on hematocrit. The hematocrit of the 300 ppm group monkeys decreased to 20.0 ± 1.4% (48.4% of normal level) at the 55th week.

Hemogram. Eosinophils constituted 3.0 ± 2.2% of leucocytes of untreated monkeys. None of the 3, 30, and 300 ppm group monkeys showed consistent alterations due to cadmium administration.

Stab-form leucocytes of untreated monkeys constituted 0.7 ± 0.8% of total leucocytes. No cadmium effects were observed in any monkeys.

Segmented leucocytes in untreated monkeys with two lobules constituted 14.8 ± 10.6% of total leucocytes, those with three lobules 10.5 ± 7.4%, four lobules 1.4 ± 1.1% and over five lobules 0%. No cadmium effect was observed in the 3 and 30 ppm group monkeys. Stab-form leucocytes and segmented leucocytes with two and three lobules in the

Table 1. Amino acid analysis of urine of monkeys given oral cadmium administration.

	Amino acid level, *mM*									
	0 ppm group		3 ppm group		30 ppm group			300 ppm group		
Amino Acids	#1248	#1259	#1244	#1249	#1202[a]	#1247	#1258	#1253[a]	#1213	#1243
Phosphoserine								0.11		
Taurine		4.43		2.98	1.10		2.83	0.78	0.80	0.98
Hydroxyproline								0.35		1.70
Aspartic acid	0.08	0.08	0.20				0.08		0.25	0.85
Threonine	0.07		0.30		0.04				0.90	4.63
Serine			0.25		0.03			0.82	0.35	3.88
Asparagine			0.20			0.10		1.66	1.03	4.70
Proline								1.44	1.20	11.1
Glutamic acid	0.25	0.30	0.40	0.20		0.93			1.13	
Citrulline	0.63	0.53	1.0	0.28		1.50			0.88	2.78
Glycine		5.95		5.0	1.37		1.55			
Alanine					0.03			0.77	0.68	2.23
Valine								0.25		0.83
Cystine								0.26	0.15	0.53
Cystathionine					0.03			0.06		0.05
Methionine								0.07		
Isoleucine								0.10		0.08
Leucine								0.12		0.28
Tyrosine								0.41		1.33
Phenylalanine					0.04			0.29		
γ-Amino-*n*-butyric acid	0.85	0.38	2.2	0.98	0.18	0.78	0.35	0.10	0.18	0.63
Ornithine					0.05	0.20		0.54	0.48	1.70
Ethanolamine	0.45				0.22			0.44		
Ammonia	1.35	0.83	1.73	0.83	0.49	1.75	0.75		2.5	6.75
Lysine	0.18		0.58	1.33	0.07		0.28		1.75	2.48
1-Methylhistidine					0.97			0.05		
Histidine					0.02			0.89	1.03	4.43
3-Methylhistidine								0.08	0.08	
Arginine								0.03		0.35
Total amino acids (except for ammonia)	2.50	11.67	5.13	10.77	4.14	3.51	5.09	9.67	10.9	45.5

[a] Sacrificed at 24 weeks of the experiment.

300 ppm group monkeys showed relatively higher ratios: stab-form leucocytes 2.0 ± 1.2%, two lobule-segmented leucocytes 14.7 ± 4.8% and three lobule-segmented leucocytes 15.8 ± 2.7%. A tendency for a shift of the hemogram to the left was observed.

Monocytes of untreated monkeys constituted 0.1 ± 0.3% of total leucocytes. The 3, 30, and 300 ppm group monkeys did not show any cadmium effects.

The lymphocyte count of untreated monkeys was 69.0 ± 16.5% of total leucocytes. Lymphocytes of the 300 ppm group was found a little smaller than those of other groups.

No atypical leucocytes were observed in control monkeys. On the other hand, atypical leucocytes were intermittently observed in the 30 and 300 ppm group monkeys.

Plasma Protein, Plasma Enzymes, and Plasma Electrolytes

Plasma Protein Concentration The plasma protein concentration of control monkeys was 8.3 ± 0.3 g/dl. No cadmium effects were observed.

Electrophoresis of Plasma Protein. The plasma albumin/globulin ratio (A/G) in untreated monkeys was 1.71 ± 0.30. In the 300 ppm group the A/G ratio was somewhat lower over a period of 55 weeks, but no change was observed during the experiment (1.58 ± 0.31).

The plasma albumin fraction of control monkeys was 62.7 ± 4.6%. The plasma albumin of the 300 ppm group monkeys was slightly low although they were not significantly different from that of control monkeys.

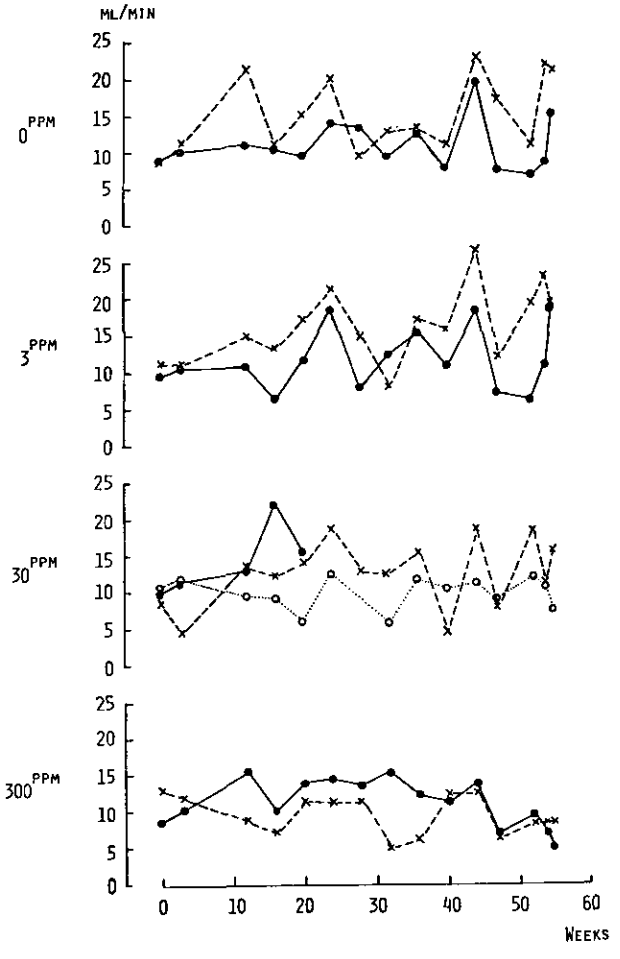

FIGURE 7. Creatinine clearance. Symbols as in Figure 1.

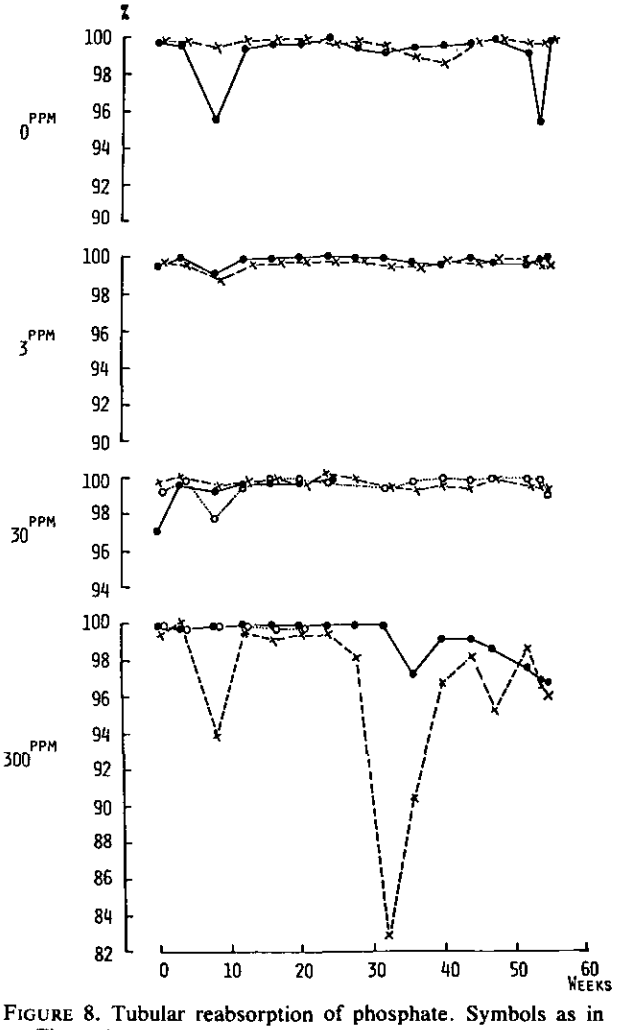

FIGURE 8. Tubular reabsorption of phosphate. Symbols as in Figure 1.

The plasma α_1-globulin of untreated monkeys was 4.7 ± 1.4% of total protein. No significant change was observed in cadmium treated monkeys.

The plasma α_2-globulin fraction of control monkeys was 5.5 ± 1.1% of total protein. No change was observed due to cadmium.

The plasma β-globulin of control monkeys was 12.7 ± 3.2%. No cadmium effect was observed.

The γ-globulin fraction of plasma of control monkeys was 13.9 ± 2.4%. No cadmium effects were observed during the experiment.

Plasma GOT. Plasma GOT of the 0 ppm group was 34 ± 3 units (Fig. 10). In the 0, 3, and 30 ppm group monkeys no change with time was observed. GOT of all three monkeys of the 300 ppm group increased to 60 ± 13 units after 12 weeks.

Plasma GPT. Plasma GPT of untreated monkeys was 31 ± 3 units (Fig. 11). The GPT of the 0, 3, and 30 ppm group monkeys showed no cadmium effect, while the GPT of the 300 ppm group monkeys increased to 66 ± 10 units after 12 weeks.

Plasma LDH. The plasma LDH of untreated monkeys was 521 ± 30 units (Fig. 12). The 0, 3, and 30 ppm group monkeys did not show any change in plasma LDH due to cadmium administration. Plasma LDH of the 300 ppm group monkeys increased significantly to 848 ± 112 units after 12 weeks.

Plasma Alkaline Phosphatase. Alkaline phosphatase of untreated monkeys was 51 ± 9 units. None of the three groups of monkeys (0, 3, and 30 ppm groups) showed changes due to cadmium administration. One of two animals in the 300 ppm group showed an elevated alkaline phosphatase level.

Plasma Alkaline Phosphatase Isoenzyme. In monkey plasma, a single band of alkaline phosphatase was detected just corresponding to human alkaline phosphatase$_3$ (ALP_3) on electrophoresis. This monkey alkaline phosphatase was different from osseous alkaline phosphatase in heat tolerance test. The alkaline phosphatase isoenzyme pattern was not changed by cadmium over a period of 55 weeks.

Plasma Cholinesterase. The cholinesterase

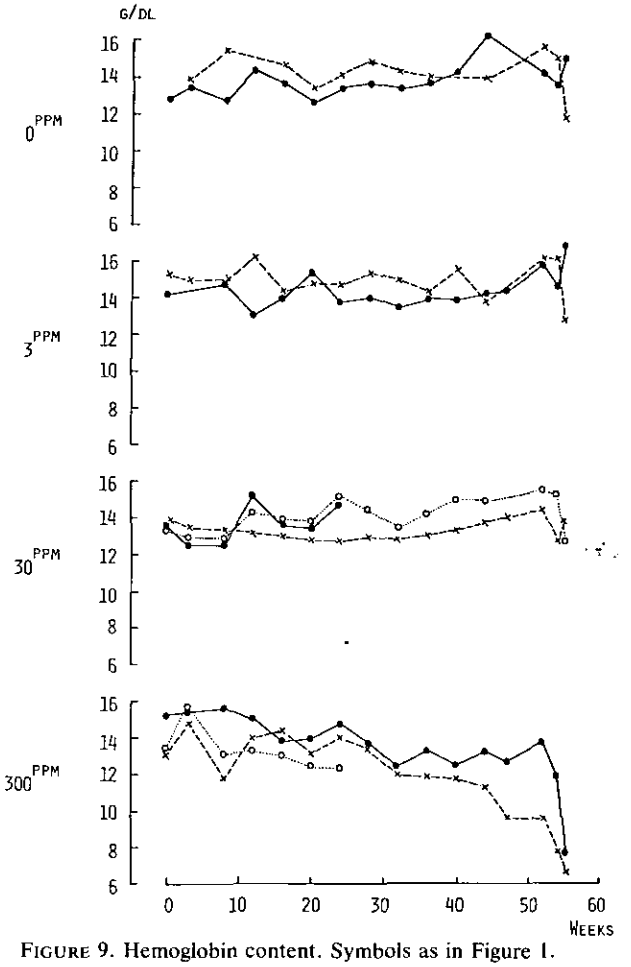

FIGURE 9. Hemoglobin content. Symbols as in Figure 1.

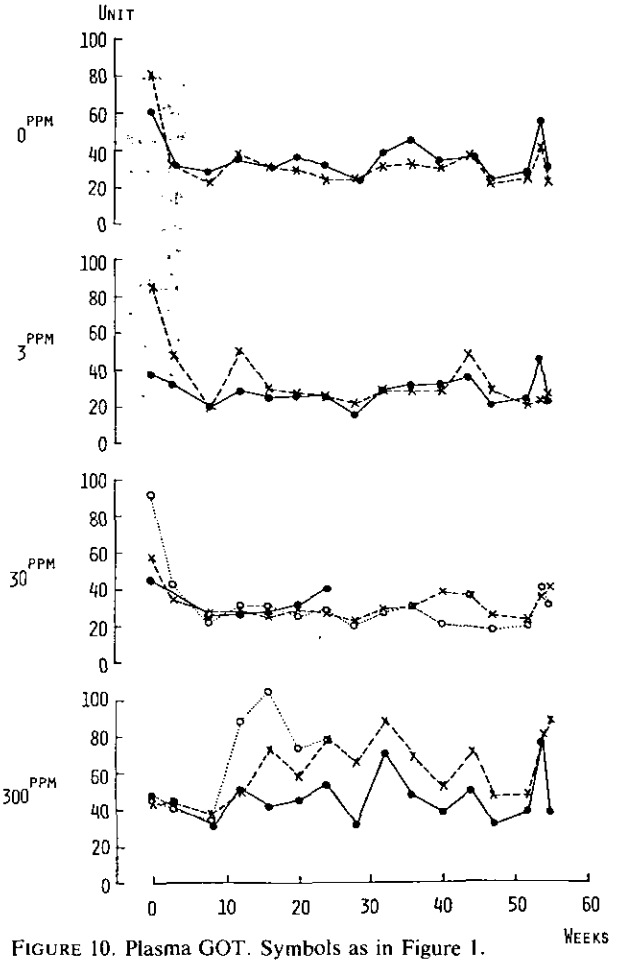

FIGURE 10. Plasma GOT. Symbols as in Figure 1.

level of untreated monkeys was 0.96 ± 0.14 units. Cadmium did not change cholinesterase in the 0, 3, and 30 ppm group monkeys, but 300 ppm cadmium depressed cholinesterase to 0.70 units (72.9% of normal level) at 54-55th week. The significance of this is uncertain.

Plasma Leucine Aminopeptidase (LAP). Leucine aminopeptidase of untreated monkeys was 54.1 ± 7.4 units. Leucine aminopeptidase of all monkeys in the 0, 3, 30, and 300 ppm groups decreased throughout the experiment. The average activity was 19.5 ± 2.2 units (36.0% of normal level) at 51 weeks. No dose-response relationships were observed between cadmium level and decreases in leucine aminopeptidase.

Plasma Leucine Aminopeptidase Isoenzyme. There was a single peak or several peaks of plasma leucine aminopeptidase isoenzymes corresponding to human α_1-globulin on electrophoresis. However, dose-response relationships were not observed in the pattern over a period of 55 weeks of experiment.

Plasma Monoamine Oxidase (MAO). Plasma monoamine oxidase was measured at the 55th week (Table 2). Monoamine oxidase levels of the 0, 3, and 30 ppm group monkeys were 369 ± 16 units, while those of the 300 ppm group monkeys were 31 and 272 units.

Plasma Sodium. The plasma sodium of untreated monkeys was 144 ± 3 meq/l. Cadmium had no effect on plasma sodium.

Plasma Potassium. The plasma potassium of untreated monkeys was 4.6 ± 0.7 meq/l. All animals showed a tendency to decrease this level to 3.0 ± 0.1 meq/l (65% of normal level) at 55th week. No dose-response relationships between cadmium dose level and plasma potassium were observed.

Plasma Chlorine. Plasma chlorine was determined at intervals, and no significant change was observed following cadmium administration.

Plasma Calcium. Plasma calcium was also determined at intervals. Plasma calcium of control monkeys was 10.3 ± 0.2 meq/l. No dose-response relationships between cadmium dose level and

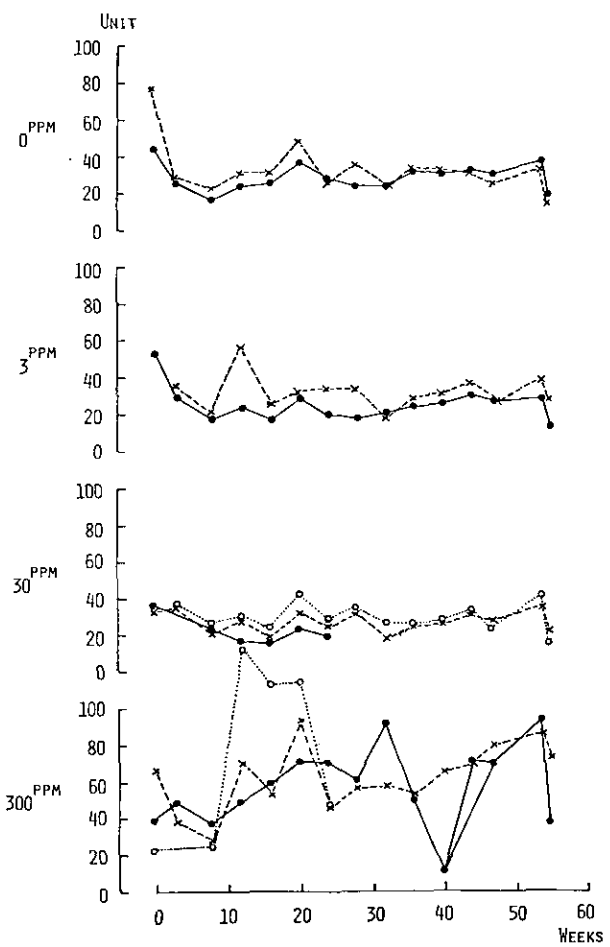

FIGURE 11. Plasma GPF. Symbols as in Figure 1.

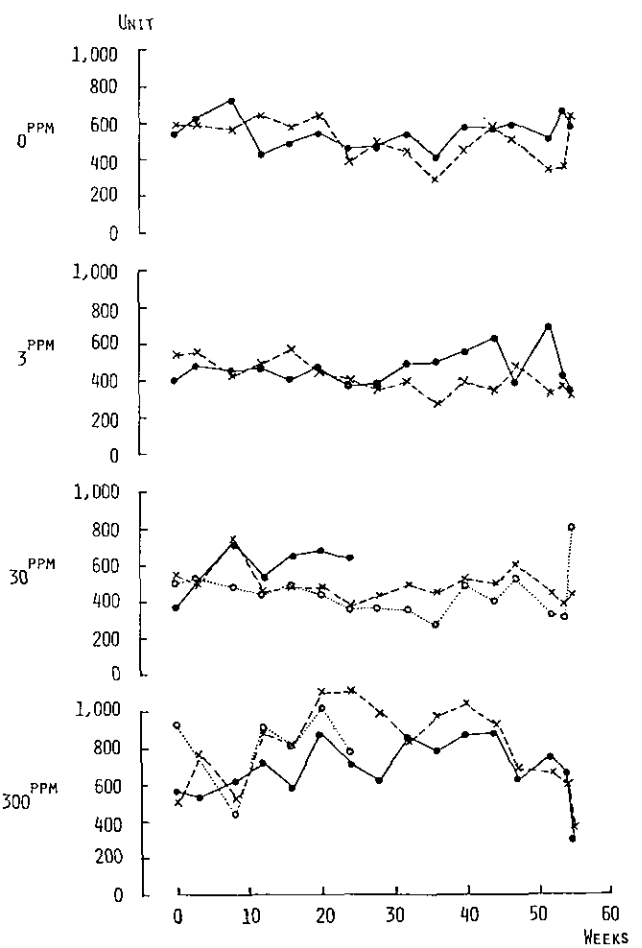

FIGURE 12. Plasma LDH. Symbols as in Figure 1.

plasma calcium were observed.

Plasma Phosphate. Plasma phosphate of untreated monkeys was 6.60 ± 0.88 meq/l. (Fig. 13). The 0 ppm group monkeys showed no change in plasma phosphate during the experiment, and almost no change was also observed in the 30 ppm group monkeys. Plasma phosphate decreased markedly to 1.63 meq/l. (26.8% of normal level) in the 300 ppm group monkeys at 55th week.

Electrocardiogram and Blood Pressure

As indicated above, electrocardiograms were recorded twice after 44 and 52 weeks of the experiment. No effects of cadmium were recognized on these two electrocardiograms (Fig. 14). Two monkeys of the 300 ppm group showed relatively low voltage in QRS; this however was also frequently observed in normal monkeys. Maximum blood pressures of all animals were between 110 and 140 mm Hg. No cadmium effect, such as elevation in blood pressure, was observed. Blood pressure was measured by the direct method at the time of autopsy, and two monkeys of the 0 ppm group showed a higher blood pressure (152 and 176 mm Hg) than did the cadmium exposed animals.

Urine Cadmium

Urine cadmium concentration and urinary excretion of cadmium of eight untreated monkeys were 1.33 ± 1.09 μg/l. (average ± one standard deviation); and 0.29 ± 0.16 μg/day; of nine animals one showed values of 13.3 μg/l. and 1.26 μg/day and was omitted.

The urine cadmium concentration of the 3 ppm group monkeys fell into the range of 1–10 μg/l. (Fig. 15). The urine cadmium concentration of the 30 and 300 ppm group monkeys increased right after cadmium administration was begun. The urine cadmium level of two of three monkeys of the 30 ppm group was between 20 and 60 μg/l. at 4–8 weeks, and the last monkey of the 30 ppm group reached the same level at 28 weeks. Two of three monkeys of the 300 ppm group reached the maximum level at the 16th week and remained almost at the same concentration afterwards; the cadmium concentration of monkey #1213 was between 200 and 300 μg/l. and that of monkey #1243 was between 700 and 1000 μg/l. The urine cadmium concentration of monkey #1243 of the 300 ppm group was as high as 2000 μg/l. in the 55th week of experiment. In one of two monkeys of the 0 ppm group (monkey #1248), urine cadmium concentration was high before the experiment. Though it decreased with the beginning of the experiment, it was still significantly higher than that of untreated monkeys.

Excessive cadmium was excreted in the 0 ppm group. Urinary excretion of cadmium of the 3 ppm group increased to 1–5 μg/day until 11 weeks. The urinary excretion of cadmium of the 30 ppm group increased to 5-15 μg/day up to week 22-28 and remained at that level afterwards. Urinary excretion

Table 2. Effects of dietary cadmium on serum MAÓ.

Cadmium level, ppm	Animal	Serum MAÓ	
		54 Weeks	55 Weeks
300	1213	56	31
	1243	28	272
30	1247	220	378
	1258	846	338
3	1244	663	381
	1249	408	365
0	1248	64	377
	1259	907	374
Untreated	1201	433	
	1254	524	

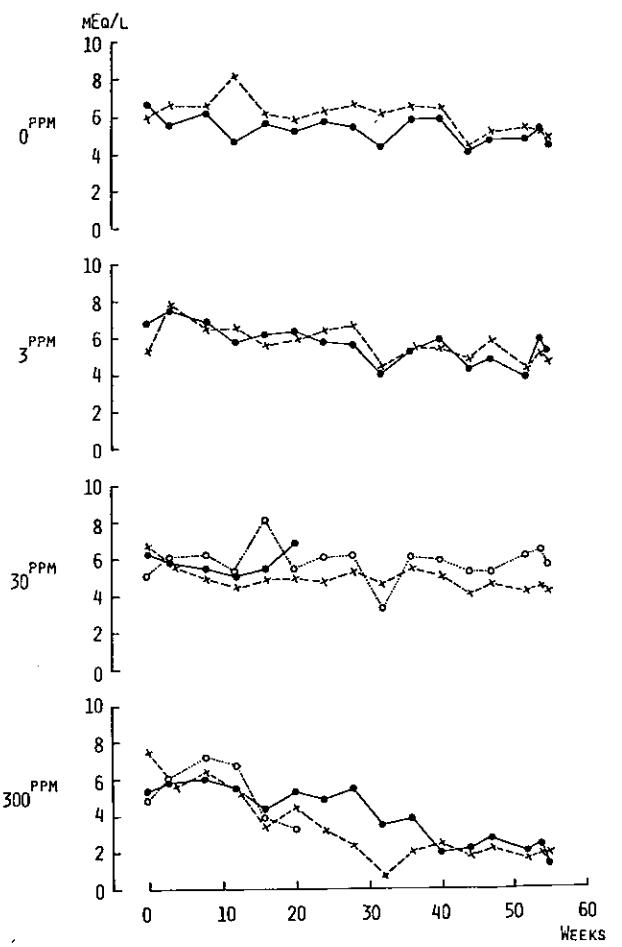

FIGURE 13. Plasma phosphate. Symbols as in Figure 1.

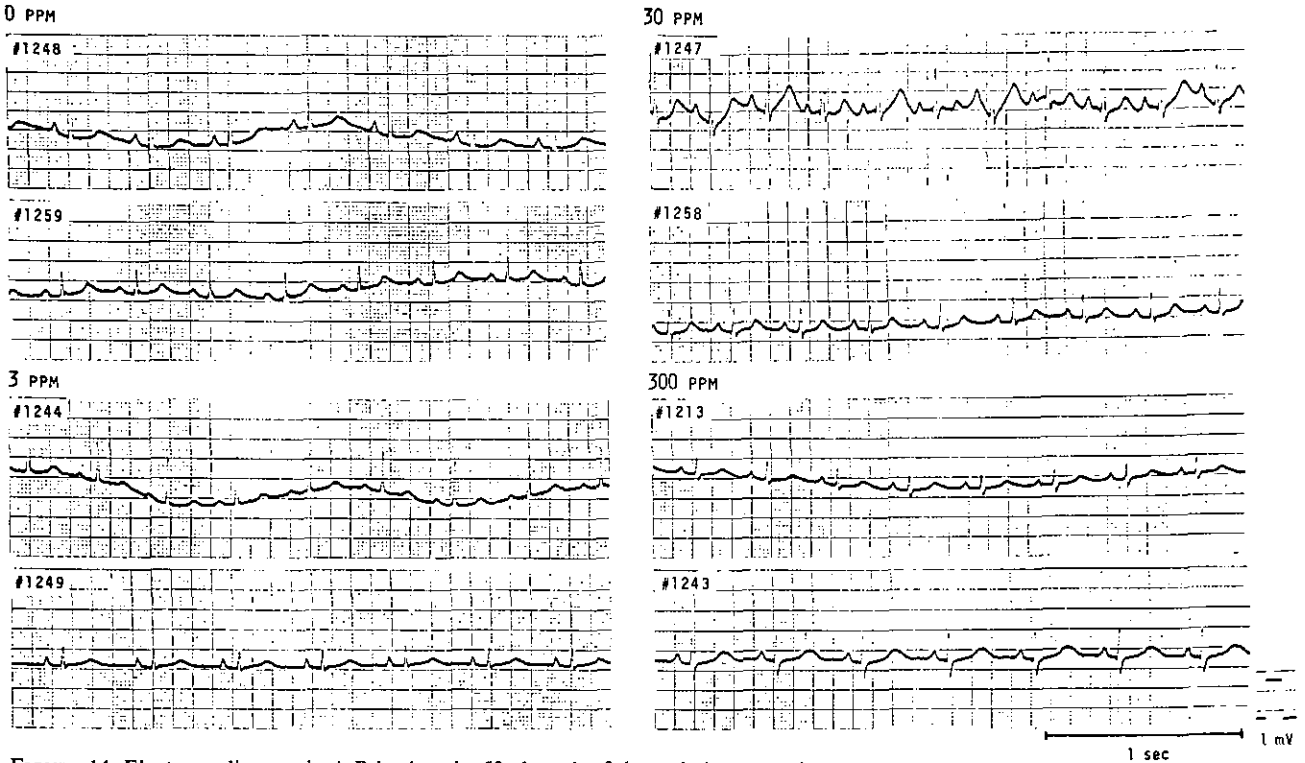

FIGURE 14. Electrocardiogram in A-B lead at the 52nd week of the cadmium experiment.

of cadmium of the 300 ppm group increased till the 16-18th week and remained between 100 and 350 μg/day. One monkey of the 300 ppm group excreted 680 μg/day (2.3% of dietary cadmium 30 mg/day) into urine at 55th week of experiment.

Fecal Cadmium

The cadmium concentration in feces of untreated monkeys was 70 ± 37 μg/g, except 210 μg/g in monkey #1213. Fecal cadmium increased right after the cadmium administration and remained at the same level, depending on the feeding level throughout the experimental period; 10-80, 30-100, and 600-1000 μg/g for the 3, 30, and 300 ppm groups, respectively. Only monkey #1248 of the 0 ppm group showed a large variation of feces cadmium ranging from 1 to 70 μg/g.

Organ Weight

On autopsy the following organs were weighed; spleen, pancreas, liver, kidney (right and left, and cortex and medulla, separately), testis (right and left), adrenal glands (right and left), thymus (right and left), submandibular glands (right and left), lung, heart, thyroid (right and left), parotid (right and left), brain. Results are shown in Table 3. No specific changes due to cadmium were observed.

Tissue Cadmium

Tissue cadmium concentrations are listed in Table 4. A higher tissue cadmium concentration was detected in monkeys at the higher doses. The liver to renal cortex ratio was higher in the higher cadmium dose groups. Independently of dose level, the highest tissue cadmium concentration was observed in the renal cortex and the liver. Other tissues with high cadmium concentrations were renal medulla, pancreas, adrenal glands, spleen, submandibular gland, and parotids. Cadmium concentration was low in cerebrum, cerebellum, and skull, but higher in ribs.

As for cadmium content in the organs (Table 5), the largest cadmium reservoir was the liver, and the higher cadmium accumulation rate was observed in the liver especially for a higher cadmium dose level group. The other organs with large cadmium content were kidneys, pancreas, submandibular glands, parotids and heart.

Pathological Findings

All monkeys were necropsied after drawing whole blood under Ketamine hydrochloride anesthesia.

The detailed information is contained in Table 6, and only the main topics will be described here.

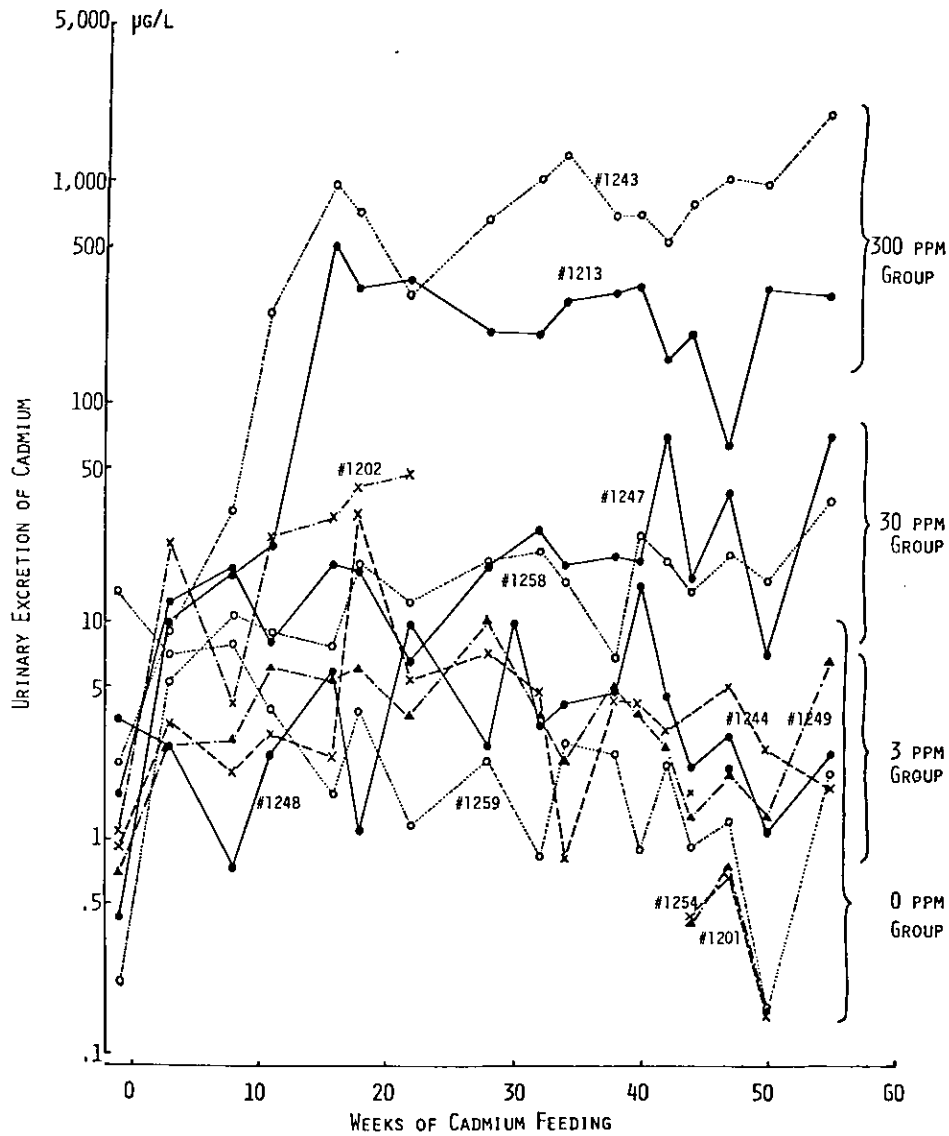

FIGURE 15. Urine cadmium.

There were vacuolar degeneration and small necrotic foci in the peripheral zone of hepatic lobules of the 300 ppm group monkeys (Fig. 16).

Slight swelling and faint disintegration of brush borders of renal tubular epithelium were observed in the 3 and 30 ppm group.

In the 300 ppm group monkeys, there were the area of degeneration to necrosis and regeneration of epithelium of renal proximal tubules with distinct mitotic figures.

No pathological changes were observed in osseous tissues.

No significant lesion in other organs was proved, regardless of any dose level of cadmium.

Discussion

Biological Responses to Cadmium

In the present experiment, monkeys which were given pelleted food containing 300 ppm cadmium were found to suffer from anemia and renal and hepatic dysfunction after 12 weeks. There have been very few other reports, however, on clinical picture of animals given cadmium for more than 1 year (*18, 19*). Nomiyama et al. (*18*) reported that rabbits fed 300 ppm cadmium over a period of 54 weeks showed aminoaciduria after 16 weeks and proteinuria and glycosuria after 40 weeks. Nomiyama and Nomiyama (*19*) also reported on

Table 3. Tissue weight of rhesus monkeys.

	Tissue weights, g									
	300 ppm			30 ppm			3 ppm		0 ppm	
	#1253[a]	#1213[b]	#1243[b]	#1202[a]	#1247[b]	#1258[b]	#1244[b]	#1249[b]	#1248[b]	#1259[b]
Body weight	3650	4630	4010	4080	4360	6130	5170	4970	4830	5280
Spleen	5.3	3.3	3.4	4.6	4.6	4.8	4.8	5.2	6.7	3.6
Pancreas	9.2	12.0	9.3	—	11.4	13.7	14.6	9.6	13.7	13.5
Liver	78.5	104.0	96.5	91.3	106.9	133.4	97.5	97.9	102	103
Kidneys										
Right	16.7	11.4	9.2	9.2	11.5	14.5	9.2	11.0	9.6	12.4
Left	15.1	11.5	8.9	9.2	10.1	14.7	9.4	10.5	10.4	11.5
Testis										
Right	2.2	11.7	14.5	1.6	—	15.2	4.4	10.6	22.6	13.7
Left	1.7	11.0	16.2	1.6	—	14.8	3.5	11.2	21.2	12.6
Adrenals										
Right	0.28	0.44	0.3	0.29	—	0.29	0.30	0.32	0.3	0.42
Left	0.35	0.47	0.3	0.26	—	0.50	0.40	0.31	0.53	0.53
Thymus	3.3	3.1	1.2	3.8	10.0	7.3	9.0	5.1	—	13.8
Brain	89.6	95.0	78.5	90.5	96.9	106.1	91.0	87.7	97.5	92.0
Renal cortex	—	—	7.94		8.34	—	7.67	9.30	—	8.82
Renal medulla	—	—	0.91		0.85	—	0.74	0.83	—	0.93
Lungs	38.6	—	—	28.8	—	—	—	—	—	—
Heart	17.9	16.4	17.5	15.6	38.5	—	17.8	22.4	21.7	18.7
Thyroids										
Right	0.42	0.32	0.30	0.45	0.42	0.4	0.37	0.49	0.16	0.18
Left	0.37	0.27	0.26	0.63	0.7	0.53	0.48	0.49	0.29	0.28
Mandibular glands										
Right	1.67	2.5	1.88	1.34	6.5	3.4	—	1.7	—	3.08
Left	1.70	2.5	2.52	1.31	6.3	5.5	—	1.9	—	4.08
Parotids										
Right	—	8.4	11.7	—	1.9	12.3	—	4.1	—	9.1
Left	—	5.8	10.6	—	1.7	11.4	—	4.3	—	7.2

[a] After 24 weeks.
[b] After 55 weeks.

Table 4. Tissue cadmium of rhesus monkeys fed $CdCl_2$.

	Tissue concentration of Cd, μg/g wet weight									
	300 ppm			30 ppm			3 ppm		0 ppm	
Tissue	#1253[a]	#1213[b]	#1243[b]	#1202[a]	#1247[b]	#1258[b]	#1244[b]	#1249[b]	#1248[b]	#1259[b]
Spleen	33.6	50.4	26.7	—	10.9	9.5	2.2	2.8	2.5	1.2
Pancreas	—	150.0	79.6	—	—	23.7	5.6	6.5	3.7	2.1
Liver	426.5	630.4	409.8	55.8	161.4	111.9	40.8	36.0	36.2	16.8
Renal cortex	756.5	582.7	350.2	299.5	727.3	464.8	160.7	243.0	180.2	145.1
Renal medulla	—	154.9	128.2	—	69.1	72.8	—	21.6	29.6	25.2
Testis	—	18.9	7.7	2.6	6.0	1.7	0.7	0.6	0.3	0.2
Adrenal	72.1	125.9	62.7	14.2	31.6	24.7	5.2	5.2	4.0	1.7
Thymus	20.3	28.3	29.8	1.1	2.2	1.9	0.5	0.9	0.6	0.2
Lung		17.8	11.7	1.2	3.6	0.1	1.3	0.7	0.6	0.4
Heart	24.2	43.6	23.4	2.3	5.2	3.9	0.4	0.6	0.4	0.3
Thyroid	—	13.4	18.6	0.0	3.9	3.3	1.9	2.4	1.0	0.3
Mandibular gland	44.3	52.0	43.2	6.2	13.4	8.9	1.9	2.5	1.8	1.0
Parotids	—	45.4	21.1	—	6.6	5.1	2.1	1.5	1.7	1.1
Rib	9.1	20.32	10.93	0.9	2.3	1.68	0.48	0.76	0.34	0.25
Skull	—	4.3	3.9	—	2.0	0.7	1.8	1.7	0.2	0.1
Cerebrum	—	1.5	1.1	0.0	0.4	0.3	0.1	0.2	0.1	0.1
Cerebellum	—	1.9	1.2	0.0	0.5	0.3	0.1	0.1	0.1	0.0
Intestine	—	—	30.6		8.5	12.5	6.6	5.2	2.5	1.1
Serosa	40.5	—	—	5.8	—	—	—	—	—	—
Mucosa	41.5	—	—	—	—	—	—	—	—	—
Cecum	—	—	81.1	16.7	8.8	1.5	1.6	1.3	1.3	0.4
Colon	—	—	—	—	—	1.9	1.7	0.7	1.1	0.5
Muscle	—	5.20	4.03	0.35	1.02	0.56	0.37	0.28	0.11	0.09
Bile	0.00	1.32	3.80	0.00	0.56	—	0.03	0.05	0.03	0.00

[a] After 24 weeks.
[b] After 55 weeks.

Table 5. Cadmium concentration in rhesus monkey organs.

	300 ppm			30 ppm			3 ppm		0 ppm	
	#1253	#1283	#1243	#1202	#1247	#1258	#1244	#1249	#1248	#1259
Body weight, kg	3.65	4.63	4.01	4.08	4.36	6.13	5.17	4.97	4.83	5.28
Cadmium content in organs, μg										
Spleen	178.1	166.3	90.8	—	50.1	45.6	10.6	14.6	16.8	4.6
Pancreas	—	1800	740	—	—	325	82	62	51	28
Liver	33,480	65,562	39,546	5,095	17,254	14,928	3,978	3,524	3,692	1,730
Kidney										
Total	—	—	6,126	—	1,847	—	—	4,834	—	3,195
Cortex	—	—	5,920	—	1,7	—	2,726	4,796	—	3,137
Medulla	—	—	306.2	—	138.1	—	—	38.1	—	57.4
Testis (R&L)	—	429.0	236.4	8.3		51.0	5.5	13.1	13.1	5.3
Adrenals (R&L)	45.4	114.6	37.6	7.8		19.5	3.6	3.3	3.3	1.6
Thymus	67.0	87.7	35.8	4.2	22.0	13.9	4.5	4.8		2.8
Lung	—	—	—	34.6	—	—	—	—	—	—
Heart	433.2	715.0	409.5	35.9	200.2		7.1	13.4	8.7	5.6
Mandibular glands	149.3	260.0	190.1	16.40	171.5	79.2	—	9.0	—	7.2
Parotid glands	—	644.7	468.8	—	23.8	120.9	—	12.6	—	17.9
Parotid glands	—	7.91	10.42	0.00	4.37	3.07	1.62	2.35	0.45	0.14
Parotid glands	—	644.7	468.8	—	23.8	120.9	—	12.6	—	17.9
Thyroid	—	7.91	10.42	0.00	4.37	3.07	1.62	2.35	0.45	0.14

another group of rabbits given 300 ppm of cadmium over a period of 54 weeks. Animals stopped gaining weight after 10 weeks, and exhibited aminoaciduria after 21 weeks, and proteinuria after 27 weeks. In these studies, however, liver dysfunction and low molecular weight proteinuria were not observed. These results are, therefore, difficult to compare with the present experiment.

In the present experiment on cadmium health effects in monkeys, proteinuria, glycosuria, and aminoaciduria were detected intermittently at first and then continuously during cadmium intoxication. This result agrees well with reports on cadmium health effects in rabbits (*18, 20*); it also brings to mind a report on itai-itai disease patients with renal failure, who showed intermittent proteinuria and/or glycosuria in the early stage of itai-itai disease and persistent proteinuria, glycosuria, and aminoaciduria in the later stage (*21*). Therefore, in order to detect health effects of cadmium in residents of cadmium-polluted areas at an early stage, proteinuria, glycosuria, and aminoaciduria should be looked for repeatedly at suitable intervals.

It was found that low molecular weight proteinuria, especially retinol-binding proteinuria, is detectable by the use of cross-immunoelectrophoresis earlier than other symptoms of intoxication, such as proteinuria, glycosuria, and/or aminoaciduria. The antiserum to human retinol-binding protein was used in the present experiment. The antigenicity of monkey retinol-binding protein was almost the same as that of human beings (*16*), and an effect of cadmium in monkeys was detectable with the use of human anti-retinol-binding protein sera earlier than with other tests. On the other hand, monkeys and humans appear to have common antigens for β_2-microglobulin (*16*), and monkey β_2-microglobulin was detectable with low sensitivity also by a micro-Ouchterlony method with the use of human antisera (Biochemical Industry, Tokyo). Urine β_2-microglobulin concentration of monkey #1243 in the 300 ppm group was found to increase after 30 weeks of experiment. Specific antisera for monkey β_2-microglobulin and cross-immunoelectrophoresis might enhance the detection limit, and β_2-microglobulin as well as retinol-binding protein shall become sensitive indices for early diagnosis in the future.

Slight general proteinuria, glycosuria, aminoaciduria, and renal dysfunctions were detected later than low molecular weight proteinuria. The 300 ppm group monkeys showed slightly decreased tubular reabsorption of phosphate after 28-36 weeks, increased plasma urea nitrogen after 30 weeks, and decreased creatinine clearance after 54 weeks. The above results suggested that renal failure appeared after long exposure to excessive dose of cadmium. There have been very few reports on renal functions of animals exposed to cadmium for a long period. The authors feel strongly it necessary to study cadmium toxicity on animals after long-term exposure to cadmium as in the present study.

In the present experiment on monkeys, hepatic dysfunction appeared at an early stage. It should be noticed here that the elevations of plasma enzymes such as GOT, GPT and LDH appeared earlier than renal dysfunctions. Therefore, hepatic dysfunctions seemed a sensitive index for cadmium effects. Axelsson and Piscator (*22*) observed the elevation of serum GOT after 17 weeks in rabbits injected with 0.25 mg Cd/kg-day. At the 17th week, hepatic cadmium concentration was 450 μg/g. Kimura (*23*) also reported increased plasma GOT and GPT in rabbits after 2 weeks subcutaneous Cd injection of 2

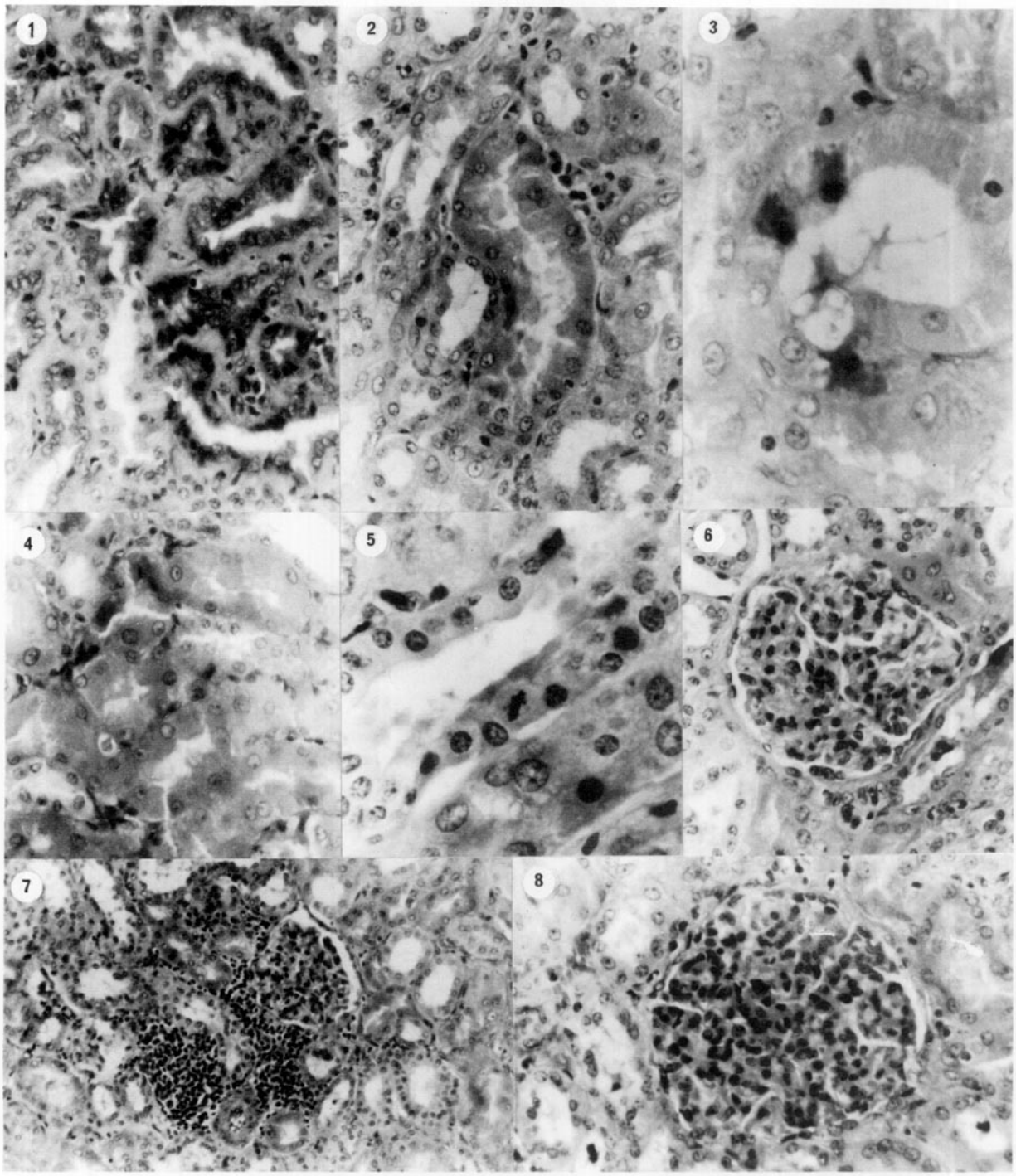

FIGURE 16. Pathological changes at 300 ppm Cd (*1*) Histology of the renal cortex of animal #1253 showing atrophy in the proximal portion of nephron with abundant degenerative epithelium, ×300; (*2*) Animal #1243 occasional swollen eosinophilic large tubule are seen, oblique section, ×300; (*3*) Animal #1243, dark stained degenerative cells and vivid intact epithelium detected in the same proximal convolution, ×600; (*4*) Animal #1253, a group of swollen eosinophilic large tubules, Cross section, ×300; (*5*) Animal #1243, a few mitotic figures are clearly seen in the proximal portion. ×600. Animal (*6*) #1243 thickened basement membrane and swelling of epithelial lining of Bowman's capsule, ×300; (*7*) Animal #1243, small round cell infiltration in the interstitial tissue of the cortex, ×300; (*8*) Animal #1253, glomerulus containing increased number of nuclei and enlarged moderately, ×300.

Table 6. Pathological findings.

Organ	Findings	300 ppm			30 ppm			3 ppm		0 ppm	
		#1253	#1243	#1213	#1202	#1247	#1258	#1244	#1249	#1248	#1259
Heart	Focal myocarditis	±					+		±	+	
	Intimal thickening of coronary arteriole								+		
Aorta	Edema of intima		+								
	Intimal thickening	+	+								
	Sparse and edematous change of inner media	+									
Spleen	Follicles with germinal center	+	+		+	+	+	+	+	+	+
	Swelling of capillary wall in the follicle	+	+	+		+			±	±	±
	Hemosiderosis	+	+								
Lung	Lung mites		+		+						
	Golden brown-black pigmentation	+	+	+	+		+	+	+	+	+
	Localized bronchiolitis and peribronchiolitis	+	+		+		+	+	+	+	+
	Focal pneumonia	+	+		+		+	+			
	Bronchioloectasia		+		+						
	Emphysema	+		+	+	+	+	+	+		
	Perivascular cell infiltration							+		+	+
	Lymphoid foci		+		+		+	+			
	Adhesive pleurisy	+			+				+	+	
Tongue	Small round cell infiltration			+			±			±	±
Salivary glands	Small round cell infiltration				+		+				+
	Fibrosis and stasis of secretion				+						
Tonsila	Follicles with germinal center		+	+		+	+	+	+	+	+
	Hemorrhage						+				
Esophagus	Small round cell infiltration		+	+			+	+	+		
	Lymphoid foci		+	+							
	Gongylonemiasis	+					+	+			
Stomach	Small round cell infiltration	+	+	+	+		+		+	+	+
	Lymphoid foci	+	+	+			+		+	+	+
	Eosinophilic infiltration	+			+						
	Adenomatous polyp (Nochtia nochti infection)	+									
Duodenum	Desquamation of epithelial cells	+	+	+	+	+	+	+	+		
	Fusing of villi	+	+	+							
	Necrotic changes of villi	+		+	+						
	Small round cell infiltration			+	+	+	+	+	+	+	+
	Lymphoid foci			+	+	+	+	+	+	+	+
	Hemosiderosis				+	+		+			
Jejunum-ileum	Increase of goblet cells					+		+	+		
	Small round cell infiltration			+			+	+	+	+	+
	Eosinophilic infiltration									+	
	Hyperplasia of Peyer's palates		+	+		+					
Large intestine	Trichuriasis	+									
	Oesophagostomiasis	+			+						
	Diverticulosis of the large intestine	+									
	Small round cell infiltration	+	+	+			+			+	
	Eosinophilic infiltration		+								
	Hyperplasia of lymph nodules			+		+	+	+			
Mesenteric nodes	Atrophy of follicles								+	+	
	Follicles with germinal center						+				+
	Swelling of capillary wall in the follicle								+		
	Sinus catarrh						+			+	
	Hemosiderosis and erythrophagia						+		+	+	
Pancreas	Large islets of Langerhans		+						+		+
	Small round cell infiltration						+		+		

Table 6 Continued

Organ	Findings	300 ppm		30 ppm			3 ppm		0 ppm		
		#1253	#1243	#1213	#1202	#1247	#1258	#1244	#1249	#1248	#1259	
Liver	Centrolobular swelling	+	+	+		+	+	+	+	+	+
	Diffuse swelling				+						
	Peripheral vacuole degeneration	+	+								
	Activation of Kupffer cells			+			+				
	Small necrotic foci	+	+			+			+		
	Cholangiolar hyperplasia						+				
	Cell infiltration in the portal areas	+	+							+	+
Gallbladder	Thickening of mucous membrane			+	+						
Kidney	Proximal convoluted tubules:										
	Swelling and disintegration of brush border	+	+	+	±	±	±	±	±		
	Atrophy and dilatation	+	+	+							
	Proteinous fluid in the tubules	+	+	+	+	+				±	
	Focal area of degeneration to necrosis	+	+	+							
	Desquamative changes	+	+	+							
	Swollen eosinophilic large tubules	+	+	+							
	Regeneration of tubular epithelium	+	+	+							
	Mitosis in the tubular linings	+	+	+							
	Renal corpuscles:										
	Thick basement membrane of Bowman's capsule	+	+	+	+						
	Swelling of the lining of Bowman's capsule		+	+							
	Dilatation of Bowman's space	+	+	+							
	Proteinous substance in the Bowman's space	+	+	+							
	Thick basement membrane of glomerular tufts	+								+		+
	Increase of glomerular nuclei	+	+	+	+						
	Interstitial tissue and lower nephron:										
	Small round cell infiltration		+	+	+			+	+	+	
	Calcification									+	
	Proteinous fluid in the collecting tubules		+	+					[a]		
	Eosinophilic abscess in the cortex	+									
Urinary bladder	Hyperplastic change of epithelium									+		+
	Vacuole formation in the epithelial cells					+					
	Small round cell infiltration	+	+	±	+			+	+	+	±	
	Edema in the proprial to submucosal area				+						
	Urinary calculi		+								
Testis and epididymis	Development stage	±	++	++	±	±	+	+	+	++	++
	Spermatogenesis	−	+	+	−	−	+	±	+	+	+
Prostate	Development stage	±	+	+	±	+	+	+	+	+	+
	Small round cell infiltration						+	+	+	+	
Pituitary	Decrease of acidophile cells		+								
	Cysts in the anterior lobe						+		−		
Thyroid	Small round cell infiltration			+			+				
Thymus	Regressive change	+	+++	+	+	+	++	++	++	++	+++
Adrenals	Cortical thickening	+	+			+	+	+	+	+	
	Increase of lipid in the cortical cells			+	+		+	+	+		
	Degenerative changes of Z. reticularis		+								
	Pigmentation or histiocytic infiltration	+			+						
Brain		OB[b]	OB	OB	OB	OB	OB	[c]	OB	[d]	OB	OB
Bone		OB	OB	OB	OB	OB	OB	OB	OB	OB	OB	OB
Bone marrow		OB	OB	OB	OB	OB	OB	OB	OB	OB	OB	OB

[a] Collecting tubules contained desquamated epithelial cells.
[b] OB = no remarkable lesion.
[c] Hemorrhages observed around IV ventricle.
[d] Subependymal edema and edema of Virchow-Robin space.

mg/kg-day. It is very necessary to study liver function in cadmium intoxication further. Furthermore, although Nomiyama et al. (*24*) reported a marked decrease in the albumin to globulin ratio in plasma of rabbits given subcutaneous injections of cadmium, no significant changes were observed in the present experiment.

There have been many reports on anemia in animals given cadmium. In the present experiment, anemia appeared at an early stage in the 300 ppm group monkeys. Anemia is, as is well known, caused by many etiologies. Therefore, it is very difficult to say definitely whether cadmium pollution causes anemia in the general population. However, it might be possible to employ anemia as index for health effects of cadmium in human beings. As to the etiology of cadmium anemia, iron deficiency was thought to be one of the etiological agents, because iron administration was effective for anemia in cadmium poisoning (*25*). Another suspected etiology was an increased destruction of erythrocytes (*26, 27*). In the present experiment a slight shift to the left of leucocytes in the hemogram was observed, but there was no decrease in leucocyte counts especially in neutrophilic leucocytes. Therefore, anemia presumably is not related to bone marrow dysfunction. Neutrophils of the 300 ppm group monkeys, furthermore, increased during the experiment. This fact suggested acute hemopoietic reactions against cadmium.

No relationships were observed between cadmium dose and plasma calcium. The 300 ppm group monkeys showed markedly decreased plasma phosphate right after cadmium administration and slightly depressed tubular reabsorption of phosphate after 28 weeks, but no increase in plasma alkaline phosphatase on a group basis. These results suggest some cadmium effects on bone in rhesus monkeys. There were no significant changes in electrocardiogram and blood pressure due to cadmium in the present experiment. However, there might be many abnormalities in the electrocardiogram at the beginning of experiment. As was already shown by Perry et al. (*28*), the blood pressure was unnecessary to indicate high blood pressure levels throughout the cadmium administration period. Therefore, electrocardiogram and blood pressures should be checked consecutively throughout the experiment and compared with the results before the experiment.

Dose-Response Relationships and Critical Concentration of Cadmium

In the present experiment, monkeys were given cadmium orally at dose levels of 0, 0.3, 3, and 30 mg/day per animal, that is, 100 g pelleted food containing 0, 3, 30, and 300 ppm cadmium as mentioned above.

In the 3 ppm group, no other health effects were observed than (1) one monkey gained little weight during the experiment, (2) there was a gradual decrease of plasma phosphate with time of experiment, and (3) renal pathological changes such as swelling of renal tubular epithelium occurred.

In the 30 ppm group, there was one monkey which showed reduced gain in body weight, increased urine protein concentration, and pathological swelling and uneven brush border of renal tubular cells. The urine cadmium was 20–60 μg/l., and cadmium concentrations in renal cortex of two monkeys were 460 and 730 μg/g. These data suggest that the critical concentration of cadmium is below 460 μg/g.

In the 300 ppm group marked effects were seen. In particular, retinol-binding proteinuria and hepatic dysfunction were detected first on 12 weeks of experiment; at 24 weeks urine cadmium was 200–1000 μg/l., and the cadmium concentration in the renal cortex and the liver were 757 and 427 μg/g, respectively. The above data suggested that the critical concentrations of cadmium in the renal cortex and the liver were below 379 and 214 μg/g, respectively. (If cadmium were accumulated in organs at a constant rate, the cadmium concentration in renal cortex at 12 weeks would be 757 μg/g $\times$ 12/24 = 379 μg/g; the cadmium concentration in liver at 12 weeks would be 427 μg/g $\times$ 12/24 = 214 μg/g). Proteinuria, glycosuria, and aminoaciduria appeared at 15–16 weeks, and the critical concentration of cadmium in renal cortex for proteinuria, glycosuria, and aminoaciduria was estimated as 473 μg/g, in agreement with the estimated critical concentration for the 30 ppm group below 460 μg/g. Furthermore at 55 weeks, very slight pathological changes in renal tubules were observed in the renal cortex containing 300–465 μg/g cadmium, and significant pathological changes were recognized in the renal cortex containing 410–630 μg/g. The estimated critical concentration based on histopathology data agreed well with the estimate based on clinical pathology data.

The above results suggest a minimum effective oral dose of cadmium for male rhesus monkeys of 2520 mg (equivalent to 720 mg/kg), that is, 30 mg Cd/day per animal for 12 weeks. The critical concentration of cadmium in the renal cortex was 380 μg/g for low molecular weight proteinuria, and 470 μg/g for proteinuria, glycosuria, and aminoaciduria. Furthermore, symptoms of cadmium intoxication appeared when urine cadmium concentration exceeded 20 μg/l. The critical cadmium concentration in the liver was estimated at 210 μg/g.

Table 7. Comparative analysis of tissue cadmium.

Cadmium feeding level, ppm	Period, week	Animal	Cd in liver, μg/g wet weight			Cd in renal cortex, μg/g wet weight		
			Jichi	Kobe	Keio	Jichi	Kobe	Keio
300	24	1253	406.8 446.2	390	51.7	763.0 750.0	672	121.0
	55	1213	630.4 630.4	502	550.9	587.2 578.1	452	461.7
	55	1243	407.8 411.8	303	345.3	365.7 334.6	298	326.3
30	24	1202	54.1 57.5	49.6	7.5	303.6 295.3	311	63.5
	55	1247	158.2 164.5	131	131.6	728.4 726.1	588	540.6
	55	1258	110.6 113.2	113	107.1	468.2 461.4	458	402.0
3	55	1244	41.9 39.7	26.8	37.4	163.7 157.7	99	161.5
	55	1249	34.3 37.7	28.7	30.9	229.4 256.6	174	181.9
0	55	1248	35.4 36.9	26.5	35.2	181.0 179.3	124	125.9
	55	1259	16.5 17.0	14.4	14.0	143.4 146.8	114	132.0

Tissue Cadmium

As already mentioned in the results section, as dose of tissue cadmium increased, a larger portion of the cadmium accumulated in the liver. This might cause hepatic dysfunction at an early stage of cadmium intoxication in monkeys. Cadmium also accumulated in endocrine organs such as pancreas, submandibular glands, and parotids. Therefore, it is necessary to study endocrine organs in future cadmium intoxication studies. Simultaneous and comparative determinations of tissue cadmium were performed at Jichi Medical School, Kobe University and Keio University at 24 weeks. Most results agreed well with each other as shown in Table 7. Analytical results of Jichi Medical School, where tissue cadmium was determined with the use of 1 g samples weighed at time of sampling were significantly higher than those obtained at Kobe University, where tissue cadmiun was determined with the use of samples of smaller pieces weighed some time after sampling. Both Jichi Medical School and Kobe University determined cadmium by correcting the background with a deuterium lamp. Therefore, it is not clear what caused the difference in the results. Duplicate determinations at Jichi Medical School agreed well with each other. Based on the above reason, tissue cadmium in the present paper is discussed on the basis of analytical data from Jichi Medical School.

In the present experiment, no significant differences were observed in cadmium concentration in the renal cortex and the liver between the 0 ppm group and 3 ppm group. Specifically, the liver to renal cortex ratio of cadmium of monkey #1248 was 0.20, which was almost the same as that of two monkeys of the 3 ppm group (0.25 and 0.16). This suggested that monkey #1248 of the 0 ppm group was exposed to cadmium. Feces cadmium of monkey #1248 was sometimes extraordinarily high. Urine cadmium concentration on monkey #1248 was also occasionally higher than that of the 3 ppm group monkeys. From monkey food powder left in the food box of monkey #1248, 3 ppm cadmium was detected. It was impossible for a monkey to take food from the next monkey, because each monkey cage was situated about 5 cm away from the next cage. It is possible, therefore, that inadvertently cadmium was fed several times to monkey #1248 of the 0 ppm group. Furthermore, feces cadmium concentrations of most monkeys were very high in the untreated period, but feces cadmium of monkey #1259 of the 0 ppm group decreased when pelleted food was changed from Oriental Co. to CLEA. This

Table 8. Cadmium level in untreated monkey tissues.

Animal	Institution	Cd level, μg/g	
		Liver	Renal Cortex
1256	Jichi	1.75	16.01
		3.89	16.27
35	Azabu	0.27	6.26
		0.35	5.04
		0.34	5.53

Table 9. Calculation of apparent biological half-time of cadmium in rhesus monkeys at sacrifice at 55 weeks.

	300 ppm		30 ppm		3 ppm		0 ppm	
	#1213	#1243	#1247	#1258	#1244	#1249	#1248	#1259
Body weight at sacrifice, kg	4.63	4.01	4.36	6.13	5.17	4.97	4.83	5.28
Total body burden of Cd, mg	163	65.4	34.4	30.9	5.60	7.05	6.12	4.10
Avg body concn, μg/g	35.2	16.3	7.89	5.04	1.08	1.42	1.27	0.78
Intestinal absorption, %	3.0	1.8	3.9	3.6	6.5	8.2	6.9	4.7
Cd uptake/day, μg	771	453	100	91.5	16.7	21.2	16.6	11.3
Fecal Cd excretion/day, μg	350	280	9.5	10.1	2.0	2.6	0.46	0.40
Avg Cd deposit/day, μg	421	173	90.5	81.4	14.7	18.6	16.1	10.9
Liver and kidney Cd, mg	59.6	33.0	24.2	23.2	3.87	5.35	4.23	3.29
Ratio of total body burden the liver and kidney Cd	2.76	1.98	1.42	1.33	1.45	1.32	1.45	1.25
Apparent biological half-time at sacrifice, yr.	0.88	0.44	6.9	5.8	5.3	5.1	25.3	19.5

suggests unfortunately that there was some cadmium exposure of monkeys before the experiment. Based on the above reasons, tissue cadmium was determined on two further untreated monkeys. As shown in Table 8, tissue cadmium levels were low, with liver to renal cortex ratios of cadmium as low as 0.17 and 0.06, respectively. These data support the suggestion that monkey #1248 of the 0 ppm group was given cadmium because of feeding errors. In spite of this probable error, no health effects were observed in monkey #1248. This suggests that occasional administrations of cadmium at a high level increases cadmium accumulation in the body, but that no health effects appear before tissue cadmium reaches the critical concentration.

Apparent Biological Half-Time of Cadmium at Sacrificed Stage

Cadmium is accumulated in the body, but little is excreted into urine and feces. Cadmium is transported from the liver to the kidneys. Because of the toxicodynamic features of cadmium, the biological half-time of cadmium should not be calculated on a one-compartment model. However, to compare the biological half-time by dose level the apparent biological half-time of cadmium at sacrificed stage in Table 9 was, tentatively, calculated from the following equation:

k = ln 2/biological half-time
= cadmium excretion (per day)/total body burden of cadmium

The average apparent biological half-time of the 0 ppm group was 22.4 years, of the 3 ppm group, 5.2 years; of the 30 ppm group, 6.4 years; and of the 300 ppm group, 0.66 years. These results suggest that the more cadmium taken into the body, the shorter is the apparent biological half-time. Most metal toxicology researchers believe the idea that the apparent "biological half time of cadmium" is quite long, regardless of the exposure level (*29–31*); this is not borne out by our data presented here. However, it should be noticed that the results here agree well with our previous data on the apparent biological half-time of rabbit plasma cadmium (*32*) and cadmium of rabbit renal cortex and liver (*33*) as well as cadmium of mouse kidney and liver (*34*). The apparent biological half-time in all the above experiments became shorter as animals were given more cadmium. It seems quite natural from the viewpoint of biology that an animal excretes more poisonous metals from the body when excessive dose of poisonous metals are given.

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