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Cadmium Concentrations in Human Renal Cortex Tissue (Necropsies)

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Cadmium is toxic to most living organisms. It occurs as part of different types of rocks, sedimentation sludges, coals and mineral oils; in minerals, cadmium (Cd) is frequently associated with zinc. Its world wide presence and considerable industrial use has given rise to an increase in its content in trophic food chains, which contribute mainly to human exposure. Oral absorption is relatively low and is influenced by the solubility of the compound, type of diet, and individual nutritional state. Interest in Cd contamination began after the outbreak of itai-itai disease in Japan. Evaluation of Cd contamination has been carried out in all the countries of the European Economic Community, and it has been estimated that in Spain emissions to the atmosphere and water are respectively 6.89 and 3.79% of total emissions in the European Communities (ECC 1992).

When critical body concentration is reached, renal malfunction and damage are produced, proteinuria being the first sign, with increased urinary excretion of low molecular weight proteins such as β_2-microglobulin, lysozyme, retinol binding proteins and immunoglobulin chains (Elinder et al. 1985). After exposure, the kidney is the organ which contains the highest concentrations of Cd and retains it longest. Moreover, studies carried out on humans not occupationally exposed to Cd reveal that 50% of the body burden is found in the kidneys (Kjellström 1979).

In recent years much research has focused on determining factors contributing to the level of exposure and degree of accumulation of Cd in renal cortex (Spicket and Lazner 1979; Blanusa et al. 1985; Scott et al. 1987). Cd in the renal cortex increases with age, reaching a maximum between 40-50 years (Elinder 1985). Differences found among populations have been associated with daily intake in the diet and smoking habits (Friberg and Vahter 1983; Baselt and Cravey 1990).

Taking into consideration the lack of studies on factors

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influencing the Cd burden in renal cortex in our country, the aim of the present paper was to find out the levels of Cd in renal cortex samples obtained from necropsies (n=77) of inhabitants of Andalusia, Spain, and compare them with levels in other populations not occupationally exposed to the element; also to investigate the influence of individual factors, such as sex, age and drug addiction on said Cd levels.

MATERIALS AND METHODS

Samples (n=77) of renal cortex tissue were obtained from necropsies of residents of Andalusia, Spain. Of these, 55 were male, and 20 female, with ages ranging from 1.5 to 80 years. According to the chemical-toxicological reports, the cause of death in 33 cases (42.86%) was drug abuse (heroin, cocaine, etc.). It was not possible to obtain information on tobacco-smoking habits.

Kidney samples were stored at -15°C until analysis. After defreezing cortices were separated from medullae and wet and dry weights were determined, although all the results were expressed in micrograms of Cd per gram of wet weight, this system being the most usual. Approximately 0.5 g of tissue was digested with 2 mL nitric acid in a closed polytetrafluoroethylene (PTFE) vessel with pressure valves, in a microwave oven (model MDS-81D, CEM Corporation) operated at 30% power for 10 minutes. In order to complete the digestion, the treatment was repeated after addition of 2 mL hydrogen peroxide 30% v/v. The digested material was made up to 25 mL with deionized water.

All the water used was purified in a Milli-Q system (Millipore Corporation) at a resistance of 18 megaohms/cm. Laboratory glassware was kept overnight in 5% nitric acid and then washed with deionized water and dried in a dust-free atmosphere. Reagents and Cd standards were all from Merck (Suprapur quality).

Analyses were carried out by Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) and atomic absorption flame spectrometry (AAFS). A sequential spectrometer, model Plasma 40, Perkin Elmer, was used (wavelength 214.438 nm), attached to a PC XT IBM Model 286 computer and EPSON FX-800 printer. The instrument used a source of radio frequency of 40.78 MHz, Potency 1.1 Kw, detachable lamp, double tube nebulizing chamber (Scott type) and cross flow nebulizer. The nebulizing chamber and nebulizer were from Ryton (Philips Petroleum Co.). A peristaltic pump carried solutions to be measured into the nebulizer.

Analyses by AAFS were carried out using a Perkin-Elmer model 2380 spectrophotometer, with air-acetylene flame, 228.8 nm resonance line and 0.7 mm slit. The calibration curve was linear up to 10 μg/L. In order to evaluate the accuracy of

the procedure the results obtained from both techniques were compared. For 15 renal cortices, the linear regression equation was Y(ICP-AES)= (0.934 ± 0.05) x (AAFS) + (5.40 ± 2.98), with a confidence level of 95% (R^2=0.9914). 0.05 and 2.98 are the standard deviations for the slope and intercept, respectively.

It is clear that if each sample yielded an identical result with both ICP-AES and AAFS the regression line would have a zero intercept and a slope of 1. Deviations from this ideal situation will occur even if systematic errors are absent. Thus, for testing if the intercept differed significantly from zero and if the slope differed significantly from unity a Student-t test was performed in each case by determining the confidence limits of the parameter at the 95% confidence level (Thompson 1990). The results obtained indicated that the calculated slope and intercept did not differ significantly from the ideal values 1 and 0 respectively which showed a good agreement between the results obtained by ICP-AES and AAFS.

Bovine liver of National Bureau of Standards (NBS, 1577) was the reference sample, submitted to the same digestion procedure as test samples. The value obtained by our procedure was 0.29 ± 0.01 μg/g, in excellent agreement with the certified value 0.27 ± 0.04 μg/g.

Once established that the frequency curve of Cd concentrations did not fit a Normal distribution non parametric statistical methods where used for further significance tests:

Dean and Dixon's test was used for assessing suspect measurements (possible outliers) (Meier and Zünd 1993). In order to establish the significant sources of factors of variation in the Cd concentration of samples, conventional ANOVA and non-parametric ANOVA (Kruskal-Wallis test) (Miller and Miller 1993) were performed. For testing significant differences in the Cd level for two sets or groups of data, both non parametric Mann-Whitney and Kolmogorov-Smirnof tests were applied (Miller and Miller 1993).

Many of these computations were performed using the CSS.STATISTICA™ programme from StatSoft™, with an IBM AT personal computer with an 80386 processor, mathematic coprocessor, and Hewlett Packard Laser Jet IV printer.

RESULTS AND DISCUSSION

The frequency curve of cadmium concentrations fitted to a Gamma distribution (Figure 1) (Feller 1957). Dean and Dixon's test was used to reject possible outliers. According to this procedure two cases were eliminated, leaving a total of 75 cases.

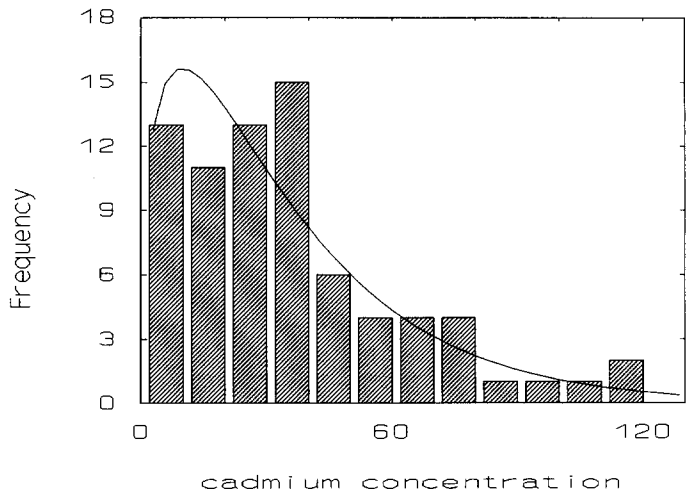

Figure 1. Frequency distribution of Cd concentrations (μg/g) in renal cortex

The arithmetic mean was 35.18±26.44 μg/g and extreme values ranged from 0.45 to 119.09 μg/g. Having used non parametric methods for the statistical analysis, the median was chosen as the best measure of centralization, being 30.87 μg/g.

Our results compared favorably to those found in Europe and the USA in people not occupationally exposed to Cd (Ellis et al. 1984) (Summer et al. 1986). In a previous paper (López-Artiguez et al. 1993) we found that Cd intake in the population of Seville was 18.18 μg Cd/day/person less than the Provisional Tolerable Weekly Intake (PTWI) of 0.4-0.5 mg/week/person established in 1973 by FAO-WHO. According to the intake of Cd in our population, studied in the forementioned paper, the values found in renal cortex were as expected. Our results were very much lower than the values in the Japanese population with an intake of 50-100 mg/kg; but rather higher than European values (Friberg et al. 1986; Ewers et al. 1990). In the Health Criteria Document for Cd (WHO 1992), 200 mg/kg wet weight in renal cortex was established as the critical concentration for producing renal damage. Our results indicate that all the samples contained concentrations well below this value.

Simultaneously the relationship between Cd concentrations and three individual factors (age, sex, and drug addiction) were studied. A preliminary analysis of variance indicated that of the three proposed factors, only age represented a significant influence, confirmed by the non-parametric technique of Kruskal-Wallis. Sex and drug addiction effects

were not significant (Table 1).

Table 1. Concentration of Cd ($\mu g/g$) in renal cortex according to sex, drug abuse and age.

Factor		N	Median	Minimum	Maximum
Sex:	Male	55	32.55	0.45	119.09
	Female	20	27.35	1.99	91.74
Drugs:	Non-Addict	41	31.03	0.45	119.09
	Addict	34	29.45	0.48	112.75
Age: Years	0-20	3	2.98	0.45	8.09
	21-30	34	29.45	0.48	119.09
	31-40	15	46.32	6.67	112.75
	41-50	7	25.46	1.99	73.44
	>50	16	22.92	5.84	102.97

Table 2. Non-parametric significance tests of the distribution of Cd levels according to age groups.

Test	Groups (p values)			
Contrast	1:2	2:3	3:4	4:5
Mann-Whitney	0.012	0.015	0.341	0.548
Kolmogorov-Smirnof	$p<0.05$	$p<0.01$	n.s*	n.s*

* non significant

The profile of Cd burden in the kidney with age displays slightly different characteristics depending on the study. For some studies maximum Cd accumulation was found at approximately 50 years (ECC 1992) while for others (Ewers et al. 1990) it ranged between 30 and 50 years; finally others concluded that the decrease starts as from 60 years (Pandya et al. 1985). The maximum accumulation of Cd (as median) in kidney was found in the 31-40 age group, diminishing thereafter (Table 2). Significant differences appeared in Cd content in the first three age groups, variations corresponding to groups over 40 years of age not being significant (Table 2).

There is disagreement in the conclusions of the different studies carried out on the influence of sex on Cd content in kidney: some authors (Friberg and Vahter 1983; Scott et al.

1987) found higher concentrations in women, others (Gretz and Laugel 1982) in men, although in all cases the differences were not significant.

Consequently, these first data on the Cd burden in kidney in the Andalusian population agree with those from other European populations. From the 3 factors considered, significant correlation of Cd levels has not been found either with sex or with addictive drugs; the correlation of Cd levels with age was confirmed, with maximum concentrations at 40 years of age.

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