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# Total and Metallothionein-Bound Cadmium in the Liver and the Kidney of a Population in Barcelona (Spain)

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Since the beginning of XXth century environmental pollution by cadmium has been increasing in most European countries due to industrial and agricultural emissions ( Drasch G.A. 1983). Among the heavy metals of environmental concern, cadmium is one of the most widespread with variable but significant concentrations normally found in tobacco smoke, food, drinking water and soils (Schroeder et al. 1967, Hallenbeck W.H. 1984, Watanabe et al. 1987).

Cadmium has a broad spectrum of toxic effects in man including nephrotoxicity, hypertension and osteomalacia ( Mitchell et al. 1983, Hallenbeck 1984, Roels et al. 1990 ), while its possible status as a carcinogen remains controversial.

After ingestion and absorption a significant fraction of the metal accumulates in tissues with an estimated half-life of about 10 years in humans ( Lauwerys et al. 1984, Benn et al. 1988). Kidney is the main target organ of both accumulation and toxicity with nephrotoxic effects being better of understood after several classic studies with workers occupationally exposed to cadmium were reported ( Roels et al. 1981, Roels et al. 1983). In recent years however major concern has been devoted to the possible nephrotoxic effects of cadmium at low concentrations on the general population, specially after Lauwerys et al. 1992 reported significant renal effects in some general populations of Belgium.

Toxic effects of cadmium on renal function cause increased proteinuria, aminoaciuria, glucosuria and decreased phosphate tubular reabsorption. Early signs of renal toxicity include an increase in the urinary excretion of low-molecular-weight ( LMWP ) proteins which are normally reabsorbed by tubular cells (Friberg 1983). Since nephrotoxicity is in most cases an irreversible process, great efforts are actually being made to develop biological markers for the early detection of structural or functional changes at various sites of the renal parenchyma ( Lauwerys et al. 1992).

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Since no information was available in Spain concerning either the daily intake of cadmium or the accumulation in tissues we initiated a study to evaluate the concentration of cadmium in the kidney and the liver in the urban general population of Barcelona.

Cadmium bound to metallothionein (MT) was also assessed in both tissues since it is well known that this protein plays a crucial role in cadmium kinetics and toxicity. The metallothionein is a low molecular weight protein with high cysteine and metal content. It serves a homeostatic function for the essential metals, and also a detoxification function for metals such as cadmium and mercury.

## MATERIALS AND METHODS

40 specimens of the liver and the kidney ( cortex and medulla ) were obtained from necropsies at the " Instituto Anatómico Forense" of Barcelona (Spain) during 1991. Samples from tissues with pathological abnormalities were not included and those corresponding to individuals with a possible occupational exposure to cadmium were rejected for the present study. Tissues of individuals who have died of other causes than traumatic accidents and with possible kidney or liver disease were also not included. Ages ranged from 18 to 80 years. Samples were obtained before 48h post mortem and frozen at -20° C until analysis.

0.5 g of fresh tissue was digested with conc. $HNO_3$ ( suprapur MERCK 4371 ) at 70° C for 24h. The digest was diluted with variable volumes of ultrapure distilled water and the cadmium concentration was analyzed by graphite furnace atomic absorption spectrometry ( FLAAS ) with a PERKIN ELMER Zeeman 3030 spectrometer, HGA-600 furnace and AS-60 automatic sampler. L'VOV platform, Zeeman background correction and other specifications of S.T.P.F. ( Stabilized temperature platform furnace ) concept were followed.

Digested samples were mixed with a matrix modifier of diammonium hydrogen phosphate ( 0.5 g/100 mL), magnesium nitrate ( 0.24 g/100 mL) and Triton X-100 ( 0.1 % m/v).Cadmium concentration was calculated using a calibration curve with aqueous standards (1, 5 and 10 µg/L from a stock solution of 1000 µg/ mL Merck Titrisol ® 9960 ) treated with the matrix modifier, after an agreement with the conventional internal addition methods was confirmed. The accuracy of the results was assessed with a simultaneous analysis of a reference pig kidney (cadmium certified value = 2.71 µg/g (N :5 ; M ± SD: 2.6 ± 0.29; CV: 11.3 %); Promochem CRM186) and bovine liver (Cd= 298 ng/g (N :5 ; M ± SD: 282± 20; CV: 12 %); Promochem CRM185).

Metallothionein ( MT ) content in the liver and the kidney was analyzed

by gel filtration column chromatography. Tissues samples (about 0.5 g) were homogenized ( Polytron PCU, Kinematica 6271 ) with 3 mL of Tris-HCl buffer ( 30 mM, pH=7.4 ) and centrifuged at 100,000 x g for 5 minutes. Supernatant was heated for 3 min at 100° C and centrifuged again at 8000 x g to precipitate heat-label proteins and increase chromatografic resolution ( Herbert et al. 1985 )

A Sephadex G-75 column ( 50 x2 cm) was used with an elution flow-rate of 0.5 mL/min. and Cd concentration was determined by FLAAS in each fraction . MT content was calculated according to a molecular weight of 6,600 Dalton and the molar ratio 7 moles Cd/ mol of MT. The presence of the complex Cd-thionein in the eluates was confirmed at 250 nm with a Hewlett-Packard 8452 A diode array spectrophotometer ( Kagi et al. 1960).

## RESULTS AND DISCUSSION

Cadmium concentrations found in liver, kidney cortex and kidney medulla are shown in Table 1.

Our results are similar to those found in other European countries (Angerer et al. 1988, Heilmaier et al. 1987 ) except in some Scandinavian areas were much lower concentrations have been reported ( Skerfving et al. 1992). The results are also clearly lower than those found in several areas of Japan ( Iwao et al. 1983; Honda et al. 1987) and in some cadmium- polluted areas of Belgium ( Lauwerys et al. 1984). Table 2.

Classic epidemiological studies performed on occupationally exposed workers showed that impairment of the tubular reabsorption of LWMP occurred when cadmium concentration in urine exceeded 10-15 $\mu$g/g creatinine. Such excretion of cadmium occurs when its average concentration in renal cortex amounts to approximately 200 $\mu$g/g (Roels et al. 1983). But more recent studies have shown that when more sensible renal markers are assessed ( e.g. sialic acid, N-acetyl-ß-D-glucosaminidase, Retinol Binding Protein (RBP)...) the threshold for the appearance of early nephrotoxic effects may be as low as 2 $\mu$g of cadmium /g creatinine. This concentration would represent an average concentration of cadmium in renal cortex of about 50 $\mu$g/g ( Lauwerys et al. 1992). In our population we have not found any value above this virtual " safe limit" ( maximum value found was 31 $\mu$g/g) suggesting that the appearance of cadmium-induced nephrotoxicitty in the general population of Barcelona is unlikely.

Cadmium concentration in renal cortex correlated positively up to an age 60 years ( $r= 0.79$, $p< 0.001$) followed by a marked decrease in older

| Tissue | M ± SD (a) | Range (a) |
|---|---|---|
| Liver | 0.98 ± 0.5 | 0.32-2.32 |
| Kidney cortex | 14.6 ± 5.9 | 2.40-31.0 |
| Kidney medulla | 8.60 ± 4.3 | 1.45-16.7 |

Table 1. Cadmium concentration in tissues of the human population in Barcelona . ( a ) 40 determinations; Unit μg/g wet tissue.

| | Kidney cortex | Liver |
|---|---|---|
| Iwao et al. 1983 ( Japan, Smokers) | 45.5 ± 32 .0 μg/g | 2.9 0± 2.4 μg/g |
| Iwao et al. 1983 ( Japan ) ( Non smokers) | 64.8 ± 42.7 μg/g | 5.20 ± 3.2 μg/g |
| Honda et al. 1987 (Ishikawa) | 32.5 ± 2.0 μg/g | 68.5 ± 1.8 μg/g |
| Angerer et al. 1988 ( Munich) | 27. 3 ± 29.7 μg/g | 1.10 ± 0.5 μg/g |
| Heilmaier et al. 1987 ( Munich) | 30 μg/g | 1.0 μg/g |
| Skerfving et al. 1992 ( Sweden ) | 5.4 μg/g | |

Table 2. Cadmium concentration in tissues in the human population. Results reported for the other authors.

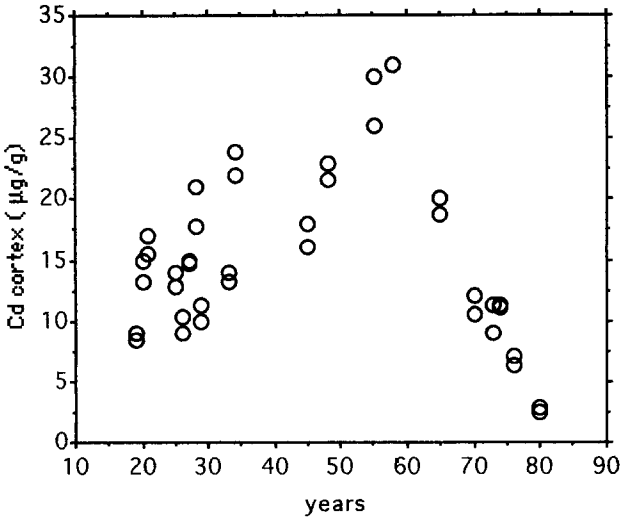


Figure 1. Correlation between cadmium concentration in renal cortex and age.

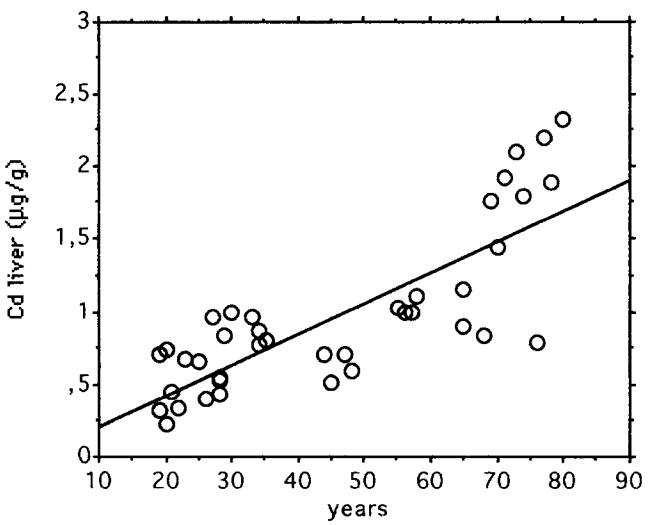


Figure 2. Correlation between cadmium concentration in liver and age.

subjects ( r= 0.98, p < 0.001 ), Figure 1. This phenomenon has been described by other authors ( Syversen et al. 1976, Iwao et al. 1983, Honda et al. 1987), but no clear explanation for this late decrease has been found to date.

On the other hand, cadmium accumulation in liver increased and correlated with age in all the ranges evaluated y =0.02 x + 3.27 E -4, r= 0.77 ( Figure 2). Cadmium concentration in liver showed a three fold increase older subjects ( 80 years) relative younger subjects ( 18 years). Liver showed a different accumulation pattern compared to the kidney cortex suggesting a very different role of liver in cadmium kinetics and age-depenent deposition.

Cadmium bound to metallothionein (Cd-MT) was estimated in liver renal cortex and renal medulla. Mean value of renal cortex Cd bound to MT was 10.9±6.0 μg/g (M±S.D. N=20) while the values in renal medulla and liver were 5.1 ± 3.4 μg/g and 0.56 ± 0.34 μg/g respectively ( M±S.D. N=20). MT-bound Cd and total Cd correlated positively in the liver ( r = 0.91, p<0.001) and the kidney ( r = 0.83, p< 0.001) in all cases evaluated. This results showed that over 50% of cadmium accumulated in the human tissues under study is bound to metallothionein, which agree with other human reports and confirms the decisive role of this cystein-rich protein in humans ( Webb M. et al. 1982 ). The potential risk of the remaining non- MT cadmium may be also significant and its assessment require further investigation.

REFERENCES

Angerer P., Kessel R., Bencze K., Tewordt M., Manermayer R., Friesen A. (1988 ). The cadmium content of human tissues from biopsies. Zentralbl. Bakteriol. Mikrobiol. 187 : 18-30.

Benn E.M., Pitorwski J.K., Sobezak- Kozlowska M., Dmuchowski, C. (1988). Cadmium, zinc, copper and metallothionein levels in human liver. Int. Arch. Occup. Environ. Health. 60 : 413-417.

Drasch G. A. ( 1983). An increase of cadmium body burden for this century. An investigation on human tissues . Sci. Total Environ. 26 : 111-119.

Friberg L. (1983). Cadmium. Ann. Rev. Publ. Health . 4: 367-373.

Hallenbeck,W.H. (1984). Human health effects of exposure to cadmium. Experientia 40 :136-142.

Heilmaier H., Drasch G., Kreschmer E., Summer,K. (1987). Metallothionein, cadmium, copper and zinc levels of humans and rats tissues. Toxicol. Lett. 38 : 205-211 .

Herbert E.; Heilmaier H.; Karl H.;Summer K. (1985).Metallothionein content and zinc status in various tissues of rats treated with iodoacetic

acid and zinc ". Arch. Toxicol. 56: 247-251.
Honda R., Nogawa K. ( 1987).Cadmium, zinc and copper relationships in kidney and liver of human exposed to environmental cadmium. Arch. Toxicol. 59 : 437-440.
Iwao S., Kenzaburo T., Sugita M. (1983).Variation of cadmium accumulation among Japanese. Arch. Environ. Health. 38 : 156-162.
Kagi J.H.R., Vallée B.L. (1960). Metallothionein : a cadmium and zinc-containing protein from equine renal cortex J. Biol. Chem. 235: 3460-3465.
Lauwerys R., Hardy R., Job M., Buchet J.P., Roels H., Bruaux P., Roudia O. ( 1984). Environmental pollution by cadmium and cadmium body burden : an autopsy study. Toxicol. Lett. 23 : 287 -289.
Lauwerys R., Bernard A., Cardenas, A. ( 1992). Monitoring of early nephrotoxic effects of industrial chemicals . Toxicology Lett. 64 : 33-42.
Mitchell Perry H., Kopp S.J. (1983). Does cadmium contribute to human hypertension ?. Sci. Total Environ. 26: 223-232.
Roels H. A., Lauwerys R.R., Buchet J. P., Bernard A., Chettle, Harvey, T.C, Al Haddad I.J. ( 1981 ). In vivo measurement of liver and kidney cadmium in workers exposed to this metal : Its significance with respect to cadmium in blood and urine. Environ Res. 26 : 217-240.
Roels H., Lauwerys R., Dardevine A.N. (1983). The critical level of cadmium in human renal cortex. Toxicology Lett .15 : 357-360.
Roels H.A., Lauwerys R.R., Buchet J.P., Bernard A.M., Lijnen P., Van Honte G. (1990 ). Urinary kallikrein activity in workers exposed to cadmium, lead or mercury vapor. Br. J. Ind. Med. 47 : 331-337.
Syversen L.M., Stray T.K., Syversen G.B., Ofstad J. (1976) " Cadmium and zinc in human liver and kidney." Scand. J. Clin. Lab. Invest. 36 : 251-256.
Schroeder H.A , Nason A.P , Tipton I.H , Balassa J.J. (1967) Essential trace metals in man : zinc. Relation to environmental cadmium. J. Chronic Dis.20 :179 - 210.
Skerfving Staffan, Ulf Nilsson. (1992) Assessment of accumulation body burden of metals. Toxicol. Lett. 64/65: 17-24.
Watanabe T., Cha C.W., Song D.,Ikeda M. (1987). Pb and Cd levels 195.
Webb M., Cain K. ( 1982 ). Functions of metallothionein. Biochem. Pharmacol. 31 : 137-142.