A Simple Method for Acid-extraction of Cadmium from Tissue

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Determination of cadmium in tissues by atomic absorption or colorimetric methods requires that the element should be brought into suitable solution prior to analysis. The two most widely used methods are wet-ashing with a combination of nitric acid, sulfuric acid or perchloric acid (PETERING et al. 1971), or dry-ashing in a muffle furnace (PISCATOR and LIND, 1972). These methods are time consuming or need frequent attention of a technician. In order to try out several methods 180-200 g rats were injected with 109-Cd labeled $CdCl_2$ prepared in 0.9 % NaCl. The isotope was purchased from The Radiochemical Centre, Amersham, U.K. and adjusted to a specific activity of 100 µCi/mg with non-radioactive $CdCl_2$. Two doses were tried; 0.2 mg Cd/kg and 0.05 mg Cd/kg both given ip. The rats were killed by decapitation 7 days after injection. Liver, kidney and heart were rapidly dissected out and kept ice-cold until further processing the same day. Hair was taken with a sharp scissor from the back of the animal. Each organ was divided into portions of 0.5-1.0 g and chopped with a sharp razor blade. The specimens were then transferred to small pieces of plastic foil and placed in standard polyethylene counting vials (NEN NEF-938). The 109-Cd content was measured in a Nuclear Enterprices NE 8312 γ-spectrometer. The specimens were then quantitatively transferred

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to suitable test-tubes or flasks, dried over-night at 100 °C, and treated in three different ways:

Dry-ashing. The tubes were placed in a 110 °C drying cabinet over-night. They were then placed in a muffle furnace at 450 °C for 48 hours. The completely ashed tissue was dissolved in 5 ml 1 N HNO_3.

Wet-ashing. The samples were transferred to 100 ml flasks and 5 ml of 2:5 (v/v) concentrated sulfuric acid:nitric acid was added. The flasks were placed on an electric plate and heated until the sulfuric acid began to condense on the glass walls. Occasionally a few drops of conc. nitric acid was added until all organic material had become completely mineralized. The flasks were cooled to room temperature and 5 ml 10 % H_2O_2 was added. Then the flasks were again heated to boiling.

Acid extraction. One ml concentrated nitric acid was added to each sample. The test tubes were covered and placed in a 50 °C drying cabinet over-night. Four ml distilled water was added and the content mixed well.

The supernatants prepared according to the three methods were now ready for atomic absorption measurement, either directly or after formation of a cadmium complex and extraction of this complex into an organic solvent (YEAGER et al, 1973). For this study a small volume was taken from each sample and counted for 109-Cd in the γ-spectrometer.

The recovery was calculated as (cpm in supernatant/cpm in tissue)x100 and thus gives the yield of cadmium as percent of expected value. The results are given in the table. The dose which determine the content of cadmium in tissue, does not affect the yield of cadmium for any of the three methods. The yield of cadmium as determined with one method is the same for all tissues

TABLE

Comparison of percent recovery for three tissue-mineralization methods. 109-Cd labelled $CdCl_2$ was given ip to rats; dose A 0.2 mg/kg, dose B 0.05 mg/kg. Each value represents the mean of four determinations with range in brackets.

Dose A	Wet-ashing	Dry-ashing	Acid extraction
Heart	56 (53-59)	76 (72-78)	81 (79-83)
Liver	57 (54-60)	77 (74-78)	85 (82-86)
Kidney	56 (55-57)	77 (73-78)	84 (83-85)
Hair	55 (52-57)	75 (73-78)	85 (84-87)
Mean value	56	76	84
Dose B			
Heart	54 (52-57)	76 (73-79)	82 (80-84)
Liver	55 (50-59)	78 (76-80)	85 (81-90)
Kidney	57 (54-60)	75 (70-80)	85 (82-88)
Hair	56 (53-59)	75 (71-79)	85 (80-90)
Mean value	55	76	84

investigated. The wet-ashing method shows the lowest yield. This low yield could be the result of either adsorption of cadmium to the glass vessels, the formation of some volatile compound or other during mineralization. To proove this the glassware was thoroughly washed with acid after mineralization, but no cadmium could be eluted from the glass by this procedure. It is therefore reasonable to believe that the main loss of cadmium is by evaporation of some volatile cadmium compound produced during the boiling procedure.

The dry-ashing was performed at 450 ^{o}C which is above

the melting point of cadmium metal (320.9 °C). Cadmium would be present as Cd^{2+} in biological material, but at such an elevated temperature which is maintained for 48 hours one would expect some loss due to vaporization.

With the acid extraction method the samples are heated to 50 °C only and vaporization of cadmium at this temperature is not very likely. The loss of cadmium which occurs might result from losses due to transfer of sample and adsorption of cadmium to glassware. These types of losses would apply to all three methods. But nevertheless, in the acid extraction method recovery is 30 % higher than in the wet-ashing procedure. Acid extraction is a simple method with good reproducibility and gives the best recovery of the methods tried. As recovery is not quantitative further studies should be directed toward obtaining 100 % recovery. Cadmium is present in very small amounts in our environment, and it is therefore important to optimize the methods to the highest accuracy and precision possible.

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