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The use of Cerenkov Radiation in the Measurement of ^{115m}Cd in Blood and other Tissues

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INTRODUCTION

Cadmium has been acknowledged as one of most hazardous environmental pollutants. In this century cadmium has been used increasingly by the industry, causing a sharp increase in the environmental contamination. Both inhalation and dietary intake result in the accumulation of cadmium in the human body. The possible cadmium effects on the organism were discussed by FRIBERG et al. (1971). Metabolism of cadmium has been usually investigated on animals exposed to cadmium compounds labelled with radioactive ^{109}Cd or ^{115m}Cd (PARIZEK et al., 1969; PIOTROWSKI et al., 1974 a, 1975 b; BRYAN and HIDALGO, 1976; MAGOSH and WEBB, 1976a, 1976b).

Cadmium ^{115m}Cd used in the present work is known as high energy β emitter (E_{max}=1.63 MeV). Such emitters, especially ^{32}P (PARKER, 1974; PLESMUS and BUNCH, 1971; FRIC and PALOVCIKOVA, 1975; SMITH et al., 1972) are recently measured in the biological samples by using CERENKOV radiation.

CERENKOV radiation is observed when charged particles are passing through transparent medium at speed higher than light velocity in the same medium. In water, CERENKOV radiation is created when energy of β particles is greater than 0.26 MeV. This radiation can be easily detected using modern liquid scintillation counting equipment.

It is known that detection efficiency for CERENKOV radiation is usually lower (40-60 % for ^{32}P) in comparison with classical scintillation technique. This method, however, has several advantages making CERENKOV counting technique very attractive. The cost of analysis is significantly lower, because no scintillators are required and pure water is used as solvent. Preparation of sample is very simple, chemical quenching is eliminated and the maximum sample volume is limited by vial capacity only. The absence of chemical quenching,

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even in strong acid or base solutions, makes this method extremely attractive for the activity measurement of high energy β emitters in mineralized or solubilized tissues.

This paper deals with determination of ^{115m}Cd in blood and other mineralized tissues by CERENKOV counting.

MATERIALS AND METHOD

Chemicals and equipment: All chemicals were analaR grade. Rat tissues were mineralized in the counting vials. Solution 0.2 g/l of 4-methylumbelliferone (4-MU) in 10 % (v/v) ethanol in water was used for the activity determination (ROSS, 1971). Counting was performed with an automatic liquid scintillation spectrometer Intertechnique SL-30 at screw-top vials.

Sample preparation: Samples were prepared for analysis by two methods. In the first one, as proposed by MAHIN and LOFBERG (1966), tissue was mineralized by treating with 60 % perchloric acid and 30 % hydrogen peroxide solutions. In the second method samples were solubilized according HERBERG's (1960) procedure, which has been modified by us as follows: 0.5 M ethanolic sodium hydroxide solution has been used instead of potasium hydroxide as proposed by HERBERG. Before mineralization 0.1 ml of $^{115m}CdCl_2$, activity 142 200 dpm, was added to each sample. The absolute cadmium activity was determined by comparison with ^{32}P standard[x]. For this determination scintillation solution has been prepared by dissolving 8 g of Buthyl-PBD and 100 g of naphtalene in 1 liter of dioxane.

Mineralization according to the first method was performed as follows: to an aliquot of 0.5 ml of blood (or plasma) in counting vials 0.5 ml of 60 % perchloric acid was added and mixed thoroughly. 1 ml of 30 % H_2O_2 was than placed into vials and the content swirled again. Caps were screwed tightly into the vials which were next warmed at 70° to 80°C for 2.5 hours and occasionally mixed. Smaples of soft tissues and bone weighing 0.2 g were used and treated with 0.4 ml of 60 % perchloric acid followed by 0.8 ml hydrogen peroxide. After mineralization some samples were yellow (especially blood). This colour remained despite further adding of 30 % hydrogen peroxide. Samples in vials were cooled to room temperature and finally 10 ml of 4-MU were added.

In the second method solubilization was also carried out in counting vials. 5 ml of 0.5 M ethanolic sodium hydroxide solution was mixed in the vial with 0.5 ml of blood (or plasma) or with 0.2 g of soft tissue or bone. Samples were then heated at 50° to 60°C for 24 hours. After the tissue has been completely dissolved solution was cooled to room temperature and 2-2.5 ml of 30 % hydrogen peroxide so-

[x] Provided by Ośrodek Produkcji i Dystrybucji Izotopów OPIDI , Poland.

lution was added dropwise to reduce yellow colour of samples. Finally 5 ml of 4-MU solution was added.

RESULTS AND DISCUSSION

The optimum counting conditions were established on the basis of pulse height spectra of ^{115m}Cd in 4-MU solutions and determined for colourless and yellow samples. For comparison the similar spectrum was obtained also for standard ^{32}P solutions. All spectra are shown in Fig. 1.

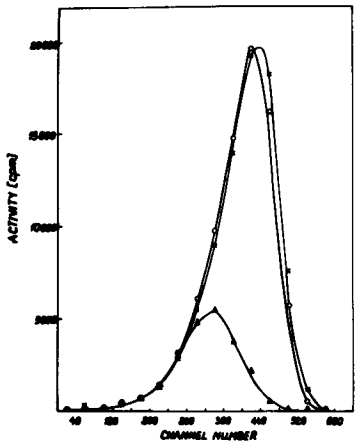

Fig. 1. Pulse height spectra of CERENKOV photons: o - o ^{115m}Cd in 4-MU colourless solution, Δ — Δ ^{115m}Cd in 4-MU yellow solution, x - x ^{32}P in 4-MU colourless solution.

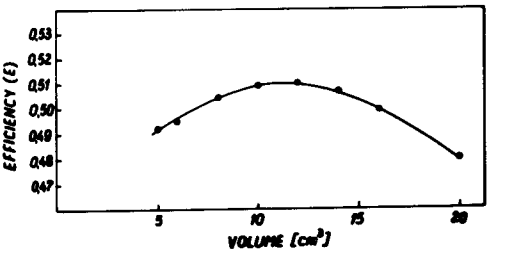

Fig. 2. The dependence of counting efficiency E on the solution volume

It is seen that in the case of colourless samples both spectra are very similar. For yellow solutions, however, significant spectrum shift is observed accompanied by marked decrease in counting efficiency.

During sample mineralization different volumes of hydrogen peroxide were used resulting in the final volume of sample variable in limits from 11.2 to 13 ml. For this reason the dependence of counting efficiency for ^{115m}Cd on the sample volume was investigated. It can be seen from fig. 2, that within the volume range of 10-15 ml the detection efficiency was almost constant.

The variable colour intensity of the samples following mineralization required proper standarization. We have found that the automatic external standarization method, based on the activity measurement of ^{137}Cs source in two channels of the SL-30 counter, cannot be used. Instead measurement of the activity ratio of the sample in two selected channels

appeared suitable for our determinations. Two channels, A 0-600 and B 0-300, were selected on the basis of pulse height spectra presented in fig. 1. Fig. 3 illustrates the relation between counting efficiency E and the channels ratio R of the samples.

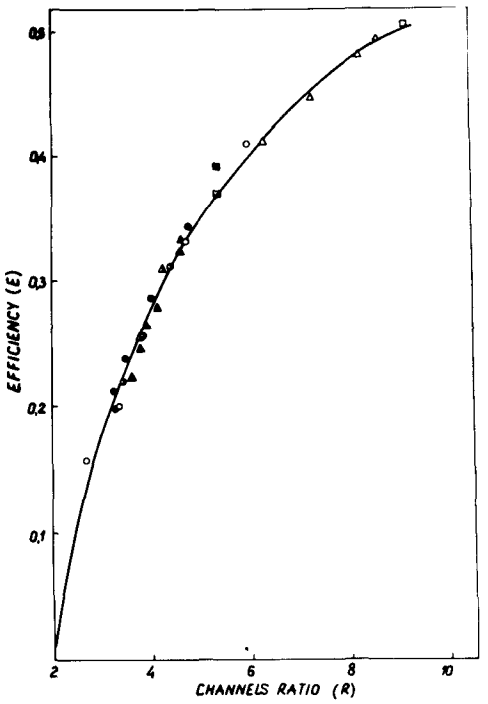

Fig. 3. The relation between counting efficiency E of ^{115m}Cd and the channels ratio R.
o yellow
Δ red
□ colourless
} 10 ml 4-MU solutions
● yellow
▲ red
■ colourless
} 5 ml 4-MU+ 5 ml 0.5 M C_2H_5ONa solutions
● yellow
▲ red
■ colourless
} 10 ml 4-MU+ 0.5 ml 60 % $HClO_4$+ 1 ml 30 % H_2O_2
The curve is drown according to the equation (1).

Our results obtained for colourless, red and yellow solutions (coloured with varying intensity) fitted well the curve given by the equation:

$$E = -0{,}230 + 0{,}164\ R - 0{,}0094\ R^2 \qquad (1)$$

The coefficients of this equation were calculated by the least squares method. The relative error caused by using calibration curve from fig. 3 did not exceed 5 % for efficiency $E > 0{,}20$. The approximation of the calibration curve by a quadratic function is useful for the automatic calculation of absolute activity.

The results of the activity measurement of different tissues mineralized by MAHIN's and LOFBERG's (1966) method are shown in the table 1. The minimum detectable activity (MDA) of ^{115m}Cd, which can be measured by this method was calculated as: $MDA\ (pCi) = 3 \cdot \sqrt{I_t / t} / 2.22 \cdot E$ where: I_t - the background 49 cpm, t- time of the measurement of the sample activity (10 minutes), E - the absolute counting efficiency.

Table 2 shows the results of activity measurement for the sample solubilized by modified HERBERG's (1960) method.

TABLE I

The mineralization with perchloric acid and hydrogen peroxide

Tissue	Activity in channel A (cpm) I_{0-600}	Channel ratio $R=\frac{I_{0-600}}{I_{0-300}}$	Efficiency E	Absolute activity (dpm) $A=\frac{I_{0-600}}{E}$	Recovery (%)	MDA (pCi)
blood	46576	4.91	0.350	133074	93,6	8.53
plasma	51456	5.11	0.362	142144	100.0	8.25
liver	53819	5.27	0.371	145065	102.0	8.05
liver×	48370	4.76	0.340	142265	100.0	8.78
kidney	51457	5.37	0.370	137585	96.7	7.98
spleen	50925	5.03	0.356	143048	100.5	8.39
bone	49768	4.91	0.350	142192	100.0	8.53

× Solution after mineralization was neutralized with NaOH before adding 4-MU.

TABLE II

The solubilization with ethanolic solution of sodium hydroxide

Tissue	Activity in channel A (cpm) I_{0-600}	Channel ratio $R=\frac{I_{0-600}}{I_{0-300}}$	Efficiency E	Absolute activity (dpm) $A=\frac{I_{0-600}}{E}$	Recovery (%)	MDA (pCi)
blood	21855	3.00	0.170	128559	90.4	17.56
plasma	35310	3.69	0.250	141240	99.3	11.95
liver	33155	3.69	0.250	132620	93.3	11.95
kidney	38284	4.14	0.296	126338	90.9	10.06
spleen	19173	2.95	0.163	117626	82.7	18.32
bone	29665	3.48	0.209	130110	91.5	14.29

The results given in both tables show that the CERENKOV counting technique is suitable for the measurement of ^{115m}Cd in the mineralized biological samples. For the samples mineralized by MAHIN's and LOFBERG's (1966) method the lowest counting efficiency was 0.34 and the cadmium recovery was found to be in the range 93.6-102 %. As the way of mineralization excludes any loss of activity, the dispersion in the recovery is mainly caused by the application of the calibration curve.

The samples solubilized by HERBERG's (1960) method were measured with lower counting efficiency (0.16-0.30) due to unremovable, stronger than in MAHIN's method, yellow colour. In this range of counting efficiency the calibration curve shows a great slope, resulting in a greater error in the calculation of absolute activity.

Both mineralization techniques used in this work allowed us to obtain higher values of cadmium recovery than in other more commonly applied methods compared lately by SYVERSEN and SYVERSEN (1975). The recoveries of ^{109}Cd reported by these authors were: 56 % for wet mineralization with H_2SO_4 and HNO_3 (2:5), 76 % for 48 hrs dry ashing at 450°C and 84 % in the case of 12 hours extraction with HNO_3.

Our results show that the mineralization with perchloric acid and hydrogen peroxide is the best method for tissue preparation for ^{115m}Cd measurement by CERENKOV technique. The main advantages of this method are: great speed and simplicity of sample preparation, good counting efficiency for ^{115m}Cd in strong acid solutions and the possibility for automatic measurement in liquid scintillation counters.

SUMMARY

A new method for the activity measurement of ^{115m}Cd in biological samples was proposed. After tissues mineralization with 60 % perchloric acid and 30 % hydrogen peroxide (I) or with 0.5 M ethanolic sodium hydroxide (II) the solutions were measured in automatic liquid scintillation counter SL-30 by CERENKOV technique. The channels ratio method was successfully applied for standardization.

The recoveries of ^{115m}Cd varied in the ranges: 93.6-102 % and 82.7-99.3 % for method I and II, respectively.

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