

## URANIUM INCORPORATION INVESTIGATION IN WISTAR RATS BONE

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### ABSTRACT

To study anomalies in bone provoked by ingestion of natural uranium, an experiment with Wistar rats was executed at the facilities of UNESP. Six groups of rats, with two animals each, were fed with uranyl nitrate at the different uranium concentrations during two months. The uranium concentration and other elements (As, Ca, Fe, K, Na Rb, Sr and Zn) identified in each bone group, in function of the uranium ingestion, were evaluated by neutron activation analysis. The uranium concentration uptake in bone samples was determined and these results in function of intake were investigated.

### I. INTRODUCTION

The transfer of radionuclides from food to human or animal body, particularly the uranium ingestion, is not well described because experimental data are scarce. Many studies have shown that most of the uranium absorbed in the human system is accumulated in the organs, bones, tissues, blood, etc. As a contribution this paper describes an experiment which the uranium incorporation is investigated in rats bones. The motivation to perform this experiment came from the fact that the sources of calcium used in animal feeding supplement, of various brands commercialized in Brazil, present high concentration of uranium (from 2 to 200 ppm) [1]. Since humans are consumers of animals products it is important to human health to investigate the uranium incorporation, via the diet. Based on this fact, in this experiment the aim is to evaluate the uranium transfer coefficient for rats bone and extrapolate it to human bones by means of the general multiple compartments model.

### II. MATERIAL AND METHODOLOGY

To perform this experiment Wistar rats have been selected as a convenient animal in terms of the low cost and ease of handling. According to Tandon *et al.* [2] the bone composition consists of 35% of mineral salts (chiefly

calcium and phosphorus), 20% of organic matrix (of which 95% is collagen) and 45% of water. The inorganic fraction is made of crystals forming hexagonal plates, chiefly hydroxyapatite, but they also contain carbonate, citrate and small amounts of sodium, potassium, chloride, fluoride and a number of trace elements.

The Wistar rats used in this study were inbred in the facilities of UNESP Laboratory. The experiment was performed with 15 male rats, 15 days old, assembled in groups with 2 animals each, and a control group with 3 animals. During the experiment they were kept in a temperature and air-controlled room. The animals were fed during 60 days with chow doped with uranyl nitrate (except for the control group), at the following ppm concentrations per group: 0.5, 2, 10, 20, 50 and 100. The control and measurements of the amount of ingested food and animal weight were carried out daily during the experiment, after that the animals were sacrificed and dissected. The samples of bones (femurs) were calcinated, ground and homogenized and the ashes were analysed by neutron activation analysis (NAA).

### III. IRRADIATION AND MEASUREMENTS

To identify and to determine the concentrations of the elements present in the bone ashes samples, ~100 mg were weighed and sealed in the polyethylene bags. Aliquots of

standards the elements of interested, with well know concentrations, were pipetted onto 1 cm<sup>2</sup> pieces of Whatman N<sup>o</sup>.40 filter paper, evaporated to dryness under and infrared lamp, and were also sealed into polyethylene bags. Sample and standards were irradiated in the IEA-R1m nuclear reactor of IPEN/SP. To identify half live isotopes, the samples were irradiated using a pneumatic system at a thermal neutron flux of 5. 10<sup>12</sup> n/ cm<sup>2</sup>.s for 3 minutes and in the central core at a flux of 10<sup>12</sup> n/cm<sup>2</sup>.s for 8 hrs, to identify long half live isotopes. For uranium analysis, samples and standard were irradiated inside a Cadmium capsule. After the irradiation each sample was analyzed with gamma-spectrometer with Ge detector of high resolution (FWHM<1.87 keV at 1.32 MeV of <sup>60</sup>Co), mounted inside a lead shield hood in order to reduce the background radiation. The detector was operated with 671 ORTEC amplifier, in pile-up rejection mode. The sample and standard were  $\gamma$  - counting and the area of the select  $\gamma$  transitions were calculated and compared. For P determinations, the beta particles of <sup>32</sup>P were measured using a Geiger Muller detector.

#### IV. RESULTS AND DISCUSSION

In the mammals most of calcium and phosphorus are in the bone and although the bone may seem static, it suffer continuous renovation to adapt to mechanical efforts and to supply Ca and P to the metabolism. Since the bone has about 98% Ca and 85 % P of the body, wide variation (deficiency or excess) of these elements may affect the bone structure. According to the *table 1* the concentrations of Ca and P as well as of U in each bone group, were performed by neutron activation analysis (NAA) and checking of these results indicated no variations of Ca and P in function of the uranium ingestion. The concentration of the As, Fe, K, Na, Rb, Sr and Zn, for 20, 50 and 100 ppm, were also determined by NAA and the results are present in *Table 2*. The concentrations of the elements Cl, Mn and Mg were not determined but a relative evaluation, between the control group with the others, have been done in function of uranium ingestion and the results are present in the Fig. 1

TABLE 1. Concentration of Ca, P and U, in Function of the Uranium Dosage.

Element [ $\mu\text{g/Kg}$ ]	samples						
	control	0.5 ppm U	2 ppm U	10 ppmU	20 ppm U	50 ppmU	100 ppmU
Ca*	32.2(21)	31.6(21)	33.9(24)	33.3(23)	33.8(24)	31.1(21)	30.8(21)
U	192.7(108)	126.5(142)	184.0(179)	311.9(169)	314.5(289)	794.3(445)	1524.8(490)
P*	20.4(3)	20.1(3)	17.4(3)	16.4(2)	19.2(3)	18.2(3)	18.6(3)

\* amount in (%)

TABLE 2. Concentration of As, Fe, K, Na, Rb, Sr and Zn, for the Doses of 20, 50 and 100 ppm of U .

Element [ $\mu\text{g/kg}$ ]	Sample			
	Control	20 ppm U	50 ppmU	100 ppmU
As	59.2(39)	48.6(55)	84.6(80)	63.8(127)
Fe	176(18)	118(13)	167(16)	163(15)
K	7039(79)	5045(147)	11049(340)	4536(136)
Na	3103(16)	2534(13)	4371(22)	3965(36)
Rb	5.8(6)	3.2(3)	4.1(3)	6.0(2)
Sr	436.1(91)	408.3(66)	400.4(91)	390.8(56)
Zn	447.2(57)	402.7(88)	435.1(57)	416.9(53)

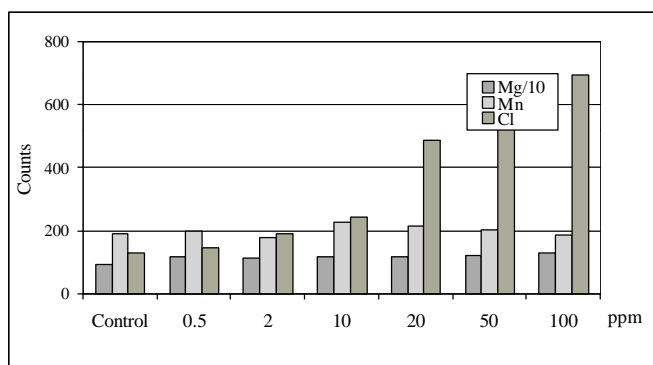


Figure 1. Relative Evaluation of the Elements Cl, Mn and Mg Present in the Bone Samples Related to the Uranium Ingestion. The Control Group had Been Used as a Comparison Standard

According to the IAEA report [3], the total concentration of uranium for human bone is  $\sim 0.02$  mg/kg. In other hand, the absorption of actinide nitrates in rats, have been reported  $\sim 1.5\%$ , respect to the dosage (intake) [4]. In the table 3 are presented the uranium measured in ash-weight amounts.

Table 3. Uranium Concentration in Rat Bones.

Dosage [ mg/kg]	K [mg/kg]. $10^{-3}$	% uranium uptake/intake
control	193(11)	-
0.5	127(14)	25.4(28)
2	184(18)	9.2(9)
10	312(17)	3.12(17)
20	315(29)	1.60(15)
50	794(45)	1.60(9)
100	1524(49)	1.52(5)

According to this table for low uranium ingestion it not possible to observed the organ saturation but the factor  $\sim 1.5$ , estimated in this experiment, for high uranium concentration ( $<20$ ppm-U) agree with Sullivan *et al* [4].

The transfer coefficient of uranium ( $f$ ) was determined by  $f=k/p.c$ , where  $k$  is the amount of uranium measured in a bone (see table 1),  $p$  is its weight and  $c$  is the amount of uranium daily intake by the animal. The results are showed in Table 4

Table 4. Transfer coefficient from diet to bone tissue.

Dosage [ $\mu$ g/ml]	Daily intake [g/d]	wet weight [Kg] $10^{-3}$	K [g]. $10^{-10}$	$f$ [d/kg]. $10^{-3}$
control	-	3.06977	192.7(108)	-
0.5	$2.4 \cdot 10^{-5}$	9.31726	126.5 (142)	56.57(635)
2	$1 \cdot 10^{-4}$	9.02294	184.0(179)	20.39(198)
10	$5.3 \cdot 10^{-4}$	6.91609	311.9(169)	8.51(46)
20	$1.11 \cdot 10^{-3}$	7.74646	314.5(289)	3.11(1)
50	$2.8 \cdot 10^{-3}$	10.05411	794.3(445)	2.64(16)
100	$5.2 \cdot 10^{-3}$	9.95194	1524.8(490)	2.88(9)

The transfer coefficient of rat bone, as function of the uranium concentration is presented in fig. 2. In the spite of increment dosage, only 0.3% (per kg of weigh of bone),

of the daily uranium concentration, is absorbed by animal. This could indicate that uranium behavior is typical of an essential element, like was showed by Arruda *et al* [1].

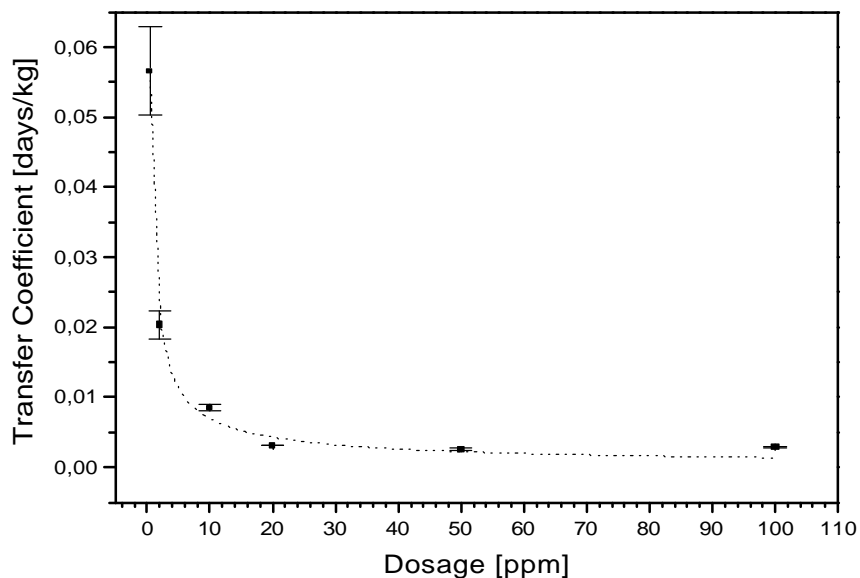


Figure 2. Transfer Coefficient of Rat Bone, as Function of the Uranium Concentration (in ppm) in the Animals Food.

Based in this pilot study, an other experiment with superior animals (Beagles dog) is in progress at the UNISA facilities. Particularly these animals were chosen because 90% of their physiological characteristics are similar to those in humans. The idea is to check the conditions established in the present experiment and also to estimate the uranium transfer coefficient, to the humans, in more realistic condition.

Financial Support: FAPESP and UNISA

#### ACKNOWLEDGMENTS

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