ANALYSIS OF INORGANIC ELEMENTS IN BIOLOGICAL SAMPLES OF C57BL/6J MOUSE STRAIN USING INAA

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ABSTRACT

The research for new medicine, vaccines and other products of interest in health area, for any disease, requires several *in vivo* tests using animal models on experiments for clinical analysis of actions in organism, focusing on the relation between these and the responses or reactions to their use, allowing or not their use in human being. The present investigation deals with the determination of elements concentration (Ca, Cl, K, Mg and Na) of clinical relevance in kidney and liver of C57BL/6J mice strain using the Instrumental Neutron Activation Analysis technique. Particularly, the C57BL/6J strain is one of the most widely used mice genetically modified for human disease studies. The biological samples were collected from 2 month old adult mice bred in the Biotherium (animal breeding) of UNIFESP (Federal University of São Paulo, Brasil) and at Human Genome Research Center (University of Sao Paulo, Brasil) and Biotechnology Center (IPEN, São Paulo, Brasil). The measurements were performed in the nuclear reactor IEA-R1 (3.5-4.5MW, pool type) at IPEN. These data will allow researchers to optimize their studies, both in terms of cost and time, by knowing the basal reference values in blood and organs of this strain. Additionally, this analytical procedure meets the needs of the world tendency that emphasizes the requirements to propose alternative methods for clinical research that contribute to animal welfare.

1. INTRODUCTION

In recent years the Nuclear Structure Laboratory at IPEN (SP, Brazil) has performed measurements related to the determination of metals and ions in whole blood in the inhabitants from Brazil as well as in several animals used for experimentation (animal model) using NAA[1-5]. This analytic procedure has been chosen because it presents advantages when compared with the conventional methods for biochemistry analyses mainly for investigation of small sized animal [3-5].

Particularly, the C57BL/6J is the most widely used mice genetically modified for use as a model of human disease. This mice strain has been used in a wide variety of research areas including cardiovascular biology, developmental biology, diabetes and obesity, genetics, immunology, neurobiology, and sensor neural research [6].

Recently we investigated blood, tibiae, quadriceps and heart of this strain [7, 8] and now we extended this investigation to kidney and liver. The determination of element concentrations in these biological organs may help to evaluate the efficacy of new treatments, as well as compare the advantages of different types of treatment schedules before performing tests in patients. Moreover, a profound knowledge of all physiological and pathological characteristics of the animal models is crucial for its reliable use.

In this study, we investigated elements of clinical relevance (Ca, Cl, K, Mg and Na), in kidney and liver of C57BL/6J mice strain using INAA. The knowledge of the element concentrations in these biological organs will allow researchers to optimize their studies, both in terms of cost and time, by knowing the basal reference values in blood and organs of this strain.

2. EXPERIMENTAL PROCEDURE

INAA was applied to conduct this investigation. The animal model C57BL/6J (7 males), were obtained from Biotherium (animal breeding) of UNIFESP (Federal University of São Paulo, Brasil) and from Human Genome Research Center (University of Sao Paulo, Brasil) and Biotechnology Center (IPEN, São Paulo, Brasil). These 2 month old mice were euthanized and the kidney and liver were collected. The organs were weighed and immediately frozen until they were analyzed. For irradiation each biological sample was weighed and sealed into a polyethylene capsule and irradiated at the IEA-R1 nuclear reactor from IPEN - CNEN/SP (3.5-4.5 MW, pool type) for four minutes (Ca, Cl, K, Mg and Na). The thermal neutron flux utilized ranged from $2.03 \cdot 10^{12}$ $cm^{-2} \cdot s^{-1}$ to $5.15 \cdot 10^{12}$ $cm^{-2} \cdot s^{-1}$. After neutron irradiation the samples were transferred to a clean container before counting procedure. Each sample was analyzed two times at least. Bovine Liver Powder (SRM 1557b) and Whole Blood Animal (IAEA A-13) were used as standards and for analytical quality control.

The measurements of the neutron induced activity of the samples were carried out using an ORTEC Model GEM-60195 detector and an ORTEC 671 amplifier coupled to a MCA ORTEC 919E. The background radiation was reduced by employing the iron shield and the concentration of each element in each biological sample was obtained by using the in-house software [9]. All the analyses were performed using PC computer.

3. RESULTS AND DISCUSSION

The certified values and the results determined in this work for the quality control, as well as the Z-score values are presented in Table 1 and they indicated that our results are satisfactory considering 95% confidence interval (Z-score < 2).

In the Tables 2 and 3 the element concentrations for kidney and liver, respectively, of C57BL/6J strain are presented as: the mean value from duplicate analyses, standard deviation (±1SD), minimum and maximum values, median and range for a confidence interval of 95%

(adopted for clinical practice). The data for tibia, whole blood, quadriceps and heart were showed in Table 4.

Table 1: Element concentrations obtained in the analysis of INAA in this work compared to the certified values

Elements	This work Mean ± 1 SD	Certified values Mean ± 1 SD	RSD, %	|Z Score|
Ca, mg.kg^{-1}	$0.123 \pm 0.014^{(1)}$ $263 \pm 44^{(2)}$	$0.116 \pm 0.004^{(1)}$ $286 \pm 54^{(2)}$	11.38 16.73	1.75 0.43
Cl, g.kg^{-1}	$2.76 \pm 0.19^{(1)}$	$2.78 \pm 0.06^{(1)}$	6.88	0.33
Mg, mg.kg^{-1}	$0.610 \pm 0.054^{(1)}$ $102 \pm 33^{(2)}$	$0.601 \pm 0.028^{(1)}$ $99 \pm 28^{(2)}$	8.85 32.35	0.32 0.11
Na, g.kg^{-1}	$2.5 \pm 0.15^{(1)}$ $13.1 \pm 0.31^{(2)}$	$2.42 \pm 0.06^{(1)}$ $12.6 \pm 1.01^{(2)}$	6.00 2.37	1.33 0.50
K, g.kg^{-1}	$2.03 \pm 0.43^{(2)}$	$2.50 \pm 0.35^{(2)}$	21.18	1.34

RSD: Relative Standard Deviation
$^{(1)}$ SRM 1577b Bovine Liver Powder
$^{(2)}$ IAEA A-13 Whole Blood Animal

Table 2: The Ca, Cl, K, Mg and Na concentrations in kidney of C57BL/6J mice

Elements, g.kg^{-1}	Mean ± 1 SD	Minimum Value	Maximum Value	Median	Range (95%)
Ca	0.285 ± 0.111	0.190	0.499	0.252	0.063 – 0.507
Cl	29.53 ± 1.92	25.90	31.40	30.10	25.69 – 33.37
K	9.81 ± 0.50	9.42	10.52	9.57	8.81 – 10.81
Mg	0.907 ± 0.111	0.771	1.136	0.891	0.685 – 1.129
Na	6.62 ± 1.69	5.39	9.99	6.10	3.24 – 10.00

Table 3: The Ca, Cl, K, Mg and Na concentrations in liver of C57BL/6J mice

Elements, g.kg^{-1}	Mean ± 1 SD	Minimum Value	Maximum Value	Median	Range (95%)
Ca	0.076 ± 0.016	0.058	0.099	0.073	0.044 – 0.108
Cl	4.63 ± 0.72	3.72	5.76	4.72	3.19 – 6.07
K	12.52 ± 0.62	11.18	12.98	12.75	11.28 – 13.76
Mg	0.495 ± 0.040	0.431	0.545	0.503	0.415 – 0.575
Na	3.17 ± 0.48	2.55	3.90	3.31	2.21 – 4.13

Table 4: The Ca, Cl, K, Mg and Na concentrations in biological tissues of C57BL/6J mice [7, 8]

Elements	Tibia g.kg^{-1}	Blood g.L^{-1}	Quadriceps g.kg^{-1}	Heart g.kg^{-1}
Ca	129.72 ± 6.28	0.289 ± 0.066	0.061 ± 0.016	0.035 ± 0.015
Cl	1.20 ± 0.13	3.13 ± 0.26	0.54 ± 0.05	1.33 ± 0.38
K	nd	2.45 ± 0.48	5.13 ± 0.48	2.82 ± 0.54
Mg	2.11 ± 0.28	0.027 ± 0.009	0.274 ± 0.022	0.163 ± 0.025
Na	3.50 ± 0.47	2.37 ± 0.25	0.59 ± 0.06	1.06 ± 0.28

nd: not applicable

To show in more details the results of Ca, Cl, K, Mg and Na concentrations for kidney and liver, Fig. 1 to 10 are showed considering a range for a confidence interval of 95% (adopted for clinical practice).
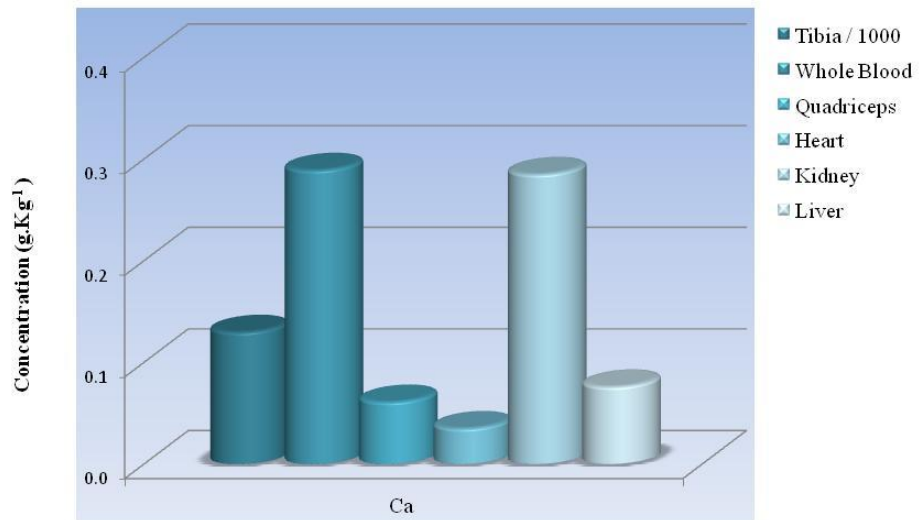
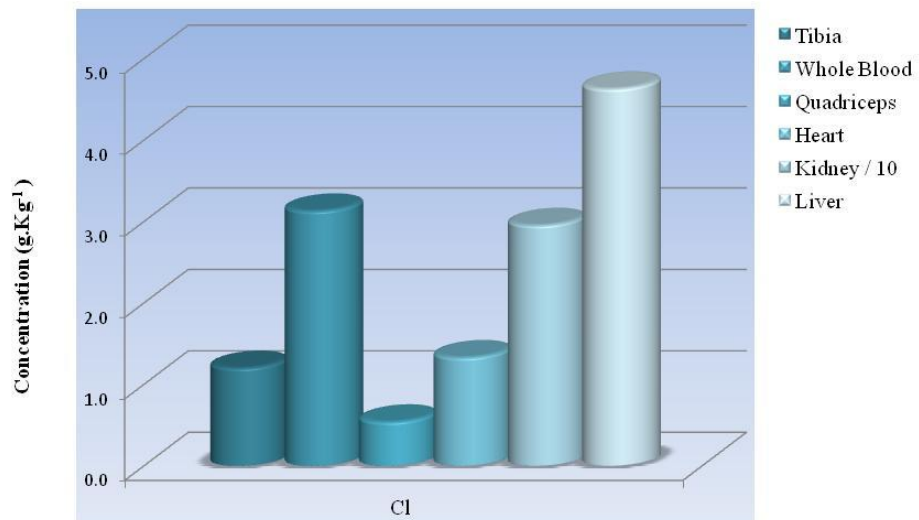**Figure 1: Ca concentration in biological tissues of C57BL/6J mice strain**

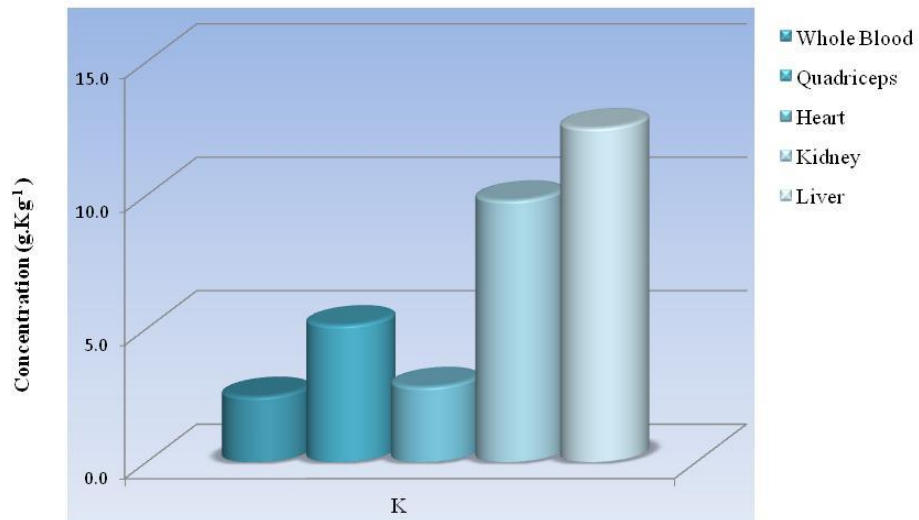**Figure 2: Cl concentration in biological tissues of C57BL/6J mice strain**

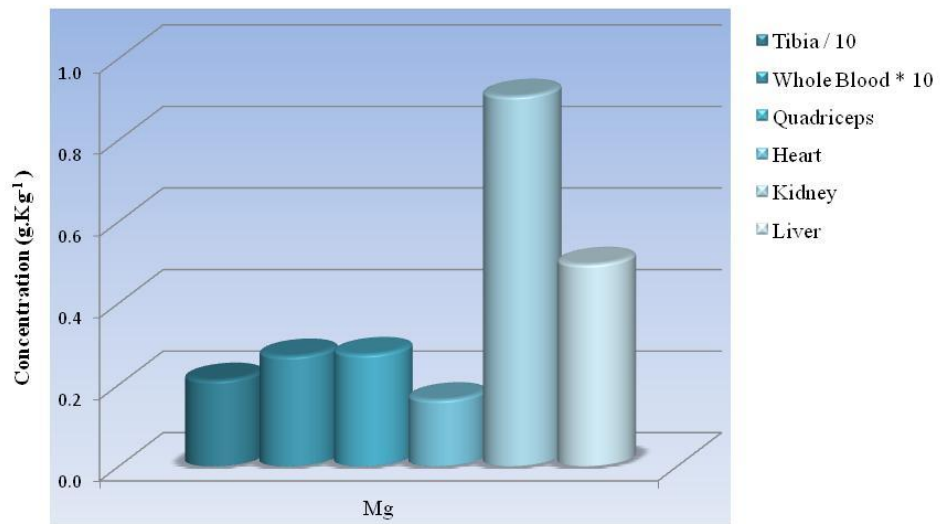**Figure 3: K concentration in biological tissues of C57BL/6J mice strain**

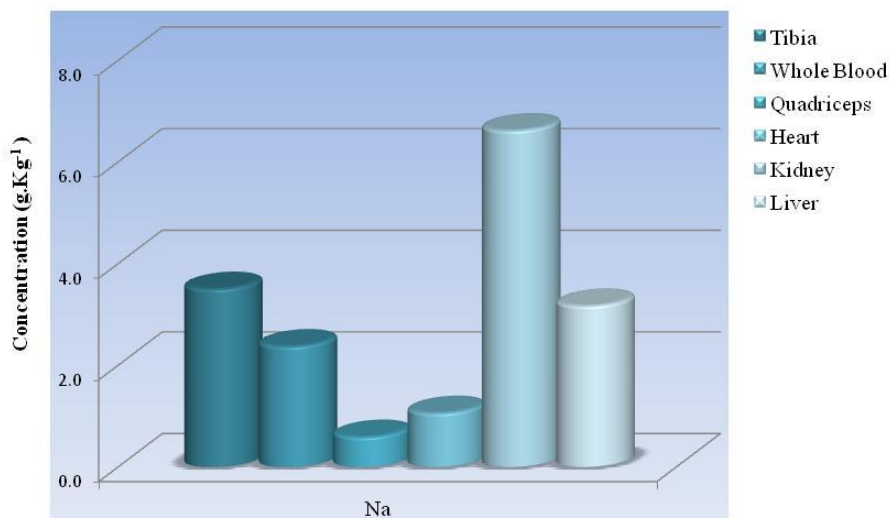**Figure 4: Mg concentration in biological tissues of C57BL/6J mice strain**

Figure 5: Na concentration in biological tissues of C57BL/6J mice strain

Related to Ca, Cl, K, Mg and Na concentrations for kidney and liver, the results show a consistency when compared with the control group mean value considering a range for a confidence interval of 95% (adopted for clinical practice).

These results can help to evaluate and to compare the advantages of different types of treatment schedule using this animal model, before performing tests in human, giving a better care and much better quality of life and survival to them. Moreover, a profound knowledge of all physiological and pathological characteristics of the animal models is crucial for its reliable use.

3. CONCLUSIONS

The determination of elements relevant for biochemical analysis in biological samples of C57BL/6J mice strain was evaluated by INAA. This research showed results that can help healthcare professionals to evaluate and conduct an appropriate diagnosis, treatment and care. Additionally, this analytical procedure meets the needs of the world tendency that emphasizes the requirements to propose alternative methods for clinical research that contribute to animal welfare.

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