ANALYSIS OF MAJOR TRACE ELEMENTS IN ALBINO RABBITS BY NEUTRON ACTIVATION ANALYSIS: REFERENCE DATA

Cibele B. ZAMBONI, Sabrina METAIRON and Frederico A. GENEZINI
Instituto de Pesquisas Energéticas e Nucleares - IPEN/CNEN –, P.O.BOX Caixa Postal 11049, 05422-970, São Paulo - SP, Brazil
czamboni@ipen.br

The Br, Cl, K, and Na concentrations were determined in whole blood samples of Albino Rabbit using the semi-parametric NAA technique. The results were compared with human being whole blood estimation permitting a discussion about the similarities between the medium values and the reference intervals taken ± 1 and ± 2 SD (Standard Deviation). We intend to use these data as interval values (normal range) in whole blood to perform clinical analyses.

Keywords: whole blood, albino rabbits , biochemical values, clinical analysis

INTRODUCTION

In health area investigations of new medicines, vaccines, antibodies as well as researches in immunobiological field for serum production of venomous, are first tested in laboratory animals (mainly hamster, mice and rabbits) in function of the facilities related to medico-legal implications for, after, to be tested in human being. Usually in these medical investigations the animals must be constantly submitted to the clinical analyses for checking their health status. One important evaluation is the blood analyses because this body fluid represents the most important biological referential of circulatory system condition and alterations in its ion concentration represents an important tool for medical investigation. Biochemistry analyses are performed in serum and, particularly, when small size animals are involved the biological material can be scarce.

In the last years the semi- parametric Neutron Activation Analysis (NAA), using Au as neutron flux monitor [1] , has been successfully applied by us for investigation of several elements in blood and urine of the small sized animals (Hamster and Mice) [2-6] as well as in human being [7], resulting in an efficiency procedure for clinical practice. The advantage in using whole blood is relate to the fact that this nuclear procedure needs small quantity of biological material (100µl of whole blood) when compared with the conventional analyses (~0.5ml of serum) [8,9] . But, for

using whole blood to perform these biochemistry analyses it is essential to establish the reference value in blood for the species or animal.

Based on it, in this study we intend to use the semi- parametric NAA technique for evaluation of the normal range of Br, Cl, K and Na in whole blood samples of Albino Rabbits of New Zealand to monitor the clinical status of this animal as well as to check the similarities with the human. The elements Cl, K and Na were selected in function of the clinical relevance for evaluation of electrolyte disorders and Br because bromide are usually present in medicines and it can also be present in antidotes (for example, in serum production of venomous) so it is very important also to check its level in blood.

This study is part of a project entitled: "Determination of reference values for concentrations of trace elements in whole blood using nuclear methodology", nowadays in development at Instituto de Pesquisas Energéticas e Nucleares (IPEN - CNEN/SP) in collaboration with several research centers as well as with blood banks and hematological laboratories and from different regions of Brazil. The data from the Albino Rabbits will contribute for applications in veterinary medicine related to biochemistry of whole blood.

EXPERIMENTAL PROCEDURE

In this study the biological samples came from Centro de Pesquisas Aggeu Magalhães at Recife city. For sample preparation, the whole blood was collected by twelve male adult rabbits. To determine the concentration of the elements in the biological samples the Cd ratio technique was used for the measurement of thermal flux distribution [1]. In this technique, Au foils (~1mg), both bare and Cd covered (1mm thick), are irradiated together with the biological sample (100μl) in the IEA-R1 nuclear reactor at IPEN/SP (IEA-R1, 2-4MW, pool type), for 2 minutes, allowing the simultaneous activation of these materials under the exact same irradiation conditions. A γ-spectrometer system with a semiconductor detector connected to an ADCAM multichannel analyzer and to a PC computer were then used to measure the induced gamma-ray activity. The detector was a HPGe of high resolution (FWHM=1.85 keV) calibrated for energy and efficiency through the measurements of standard sources of Co^{56} and Eu^{152}. All gamma spectra analysis evaluations were performed using the IDF computer code [10].

Using this procedure it is possible to quantify simultaneously the following radioactive nuclides: Br ($T_{1/2}$=16 min, E_γ 616 keV), ^{38}Cl ($T_{1/2}$=37 min, $E\gamma$ =1642 keV),^{42}K ($T_{1/2}$=12h, E_γ =1525 keV) and ^{24}Na ($T_{1/2}$=15h, E_γ =1368 keV) using in-house software [11].

RESULTS AND DISCUSSION

The concentration of the elements in whole blood samples are shown in Table I. Considering that all the analysis were performed in duplicate, the results are the mean value and the associated errors are represented by one standard deviation (68%). These results were compared to the human being estimation [7] and the behavior of these data can be observed in Fig. 1, 2, 3 and 4 for Br, Cl, K, and Na, respectively.

Table 1. Indicative interval for the reference values of the elements Br, Cl, K and Na in Albino rabbits whole blood samples by using NAA .

Elements (g l^{-1})	Mean	SD	Minimum Value	Maximum Value
Br	0.0011	0.0004	0.0031	0.0175
Cl	3.15	0.13	2.67	3.47
K	1.59	0.19	1.32	1.93
Na	2.28	0.20	1.83	2.45

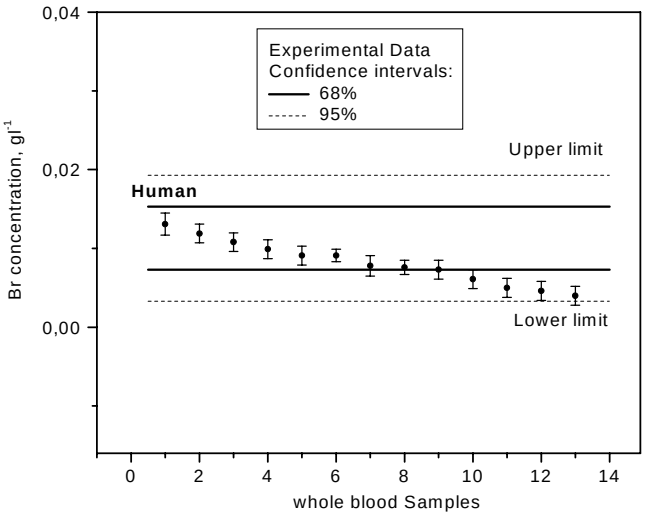

Figure 1. Bromine concentration results in whole blood samples of Albino Rabbits; the horizontal lines represent the indicative intervals. The values are arranged by decreasing concentration.

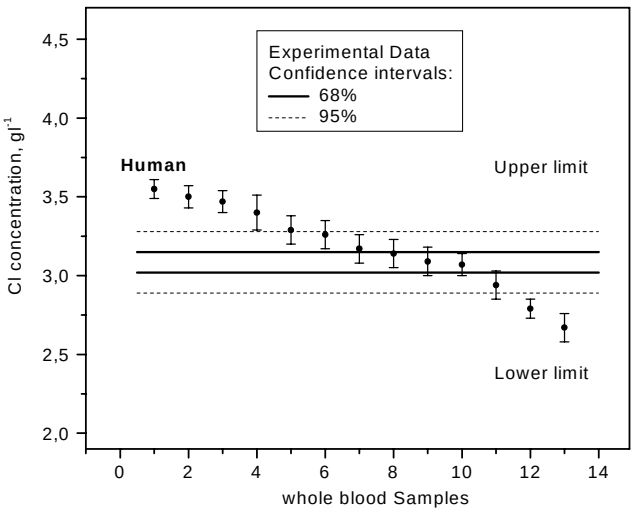

Figure 2. Chlorine concentration results in whole blood samples of Albino Rabbits; the horizontal lines represent the indicative intervals. The values are arranged by decreasing concentration.

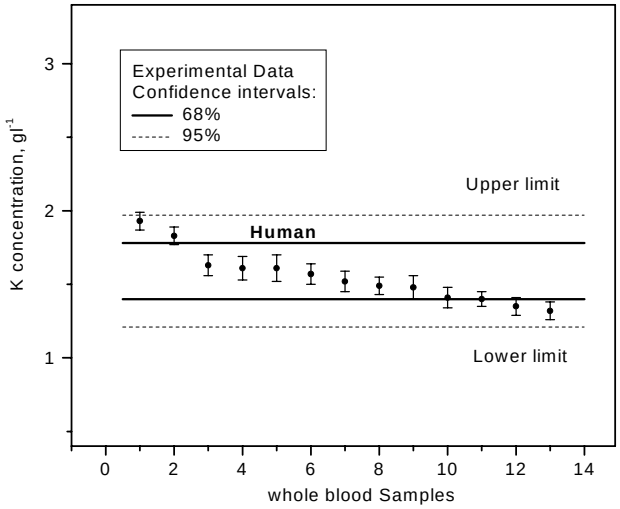

Figure 3. Potassium concentration results in whole blood samples of Albino Rabbits; the horizontal lines represent the indicative intervals. The values are arranged by decreasing concentration.

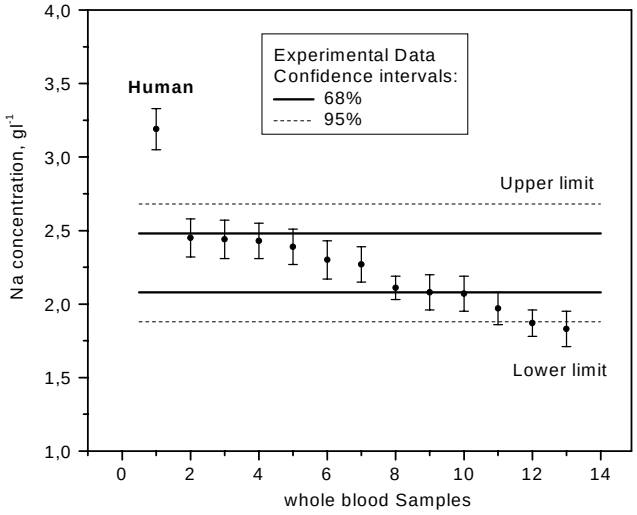

Figure 4. Sodium concentration results in whole blood samples of Albino Rabbits; the horizontal lines represent the indicative intervals. The values are arranged by decreasing concentration.

According to this comparative analyses there is compatibility for Br and K considering 95% of confidence interval (±2SD) but, for Cl and Na the levels are altered in relation to human whole blood estimation values, suggesting physiologic differences . Of course, more statistical is essential

for better definition of reference values for these elements in blood but the median values proposal in this study can help the check the health status of them .

CONCLUSION

The data obtained in this investigation will allow researchers to optimize their studies in terms of time (simultaneous evaluation of the elements) and also to perform biochemical analyses using whole blood an advantage when the biological material is restrict. Besides, the use of whole blood reduces the stress of the animals due the collection of small quantity of biological material (100μl) in comparison with the conventional analyses (at least 0.5ml). Finally, the knowledge of the biochemical values permit to check the similarities with the estimations for Br, Cl, K and Na in human being an important condition for selecting laboratory animals.

REFERENCES

1. W. R. Filho, C. B. Zamboni. ***Determination of the thermal neutron flux in a Nuclear Reactor.*** XXVIII Reunião de Trabalho sobre Física Nuclear no Brasil, *de 07 a 11 de setembro, Guarujá - SP, Brasil, 2005.*

2. L. C. OLIVEIRA, C. B. ZAMBONI, F. A. GENEZINI, A. M. G. FIGUEIREDO, G. S. ZAHN J. of Radioanal. and Nucl. Chem. 263, 3(2005)783.

3. . B. Zamboni, I. M. M. A. Medeiros, J.A. G. Medeiros, O.A. Sant'Anna. ***Differences in trace element concentrations in whole blood of small sized animals using ANAA***. International Nuclear Atlantic Conference, INAC, de 28 de agosto a 02 de setembro, Santos - SP, 2005.

4. C. B Zamboni, GS Zahn, OA Sant'anna. **Trace elements at whole blood of distinct mouse lines by using NAA**. *AIP*, **884**, pp.507 (2007).

5. M. F. Suzuki, C. B. Zamboni, M. D. F. Carvalho and O. A. Sant'Anna**. Differences in trace element concentrations in whole blood of SJL/j mice using NAA**. INAC Santos, SP, Brazil, September 29 to October 5, 2007.

6. . R. Aguiar , C. B. Zamboni , and José A. G. Medeiros. **Trace elements at whole blood of Hamster Golden using semi parametric NAA technique.** INAC Santos, SP, Brazil, September 29 to October 5, 2007.

7. L. C. Oliveira, CB Zamboni, J Mesa. **Quantitative estimation of Br, Cl, K and Na in sample blood by NAA**. *Journal of Radioanalitical and Nuclear Chemistry*. **269**, pp.541 (2006).

8. FRÉJAVILLE, J. P.; KAMOUN, P. (eds). *Guide des examens de laboratoire*. Paris, Flammarion, 1981.

9. RAPHAEL, S. S. (ed). *Lynch's medical laboratory technology*. 4.ed. Philadelphia: Saunders, 1983.

10. P. GOUFFON, *Manual do Programa Idefix,* IFUSP, São Paulo, Brasil (1987).

11. J. A. G. MEDEIROS, C. B. ZAMBONI, G. S. ZAHN, L. C. OLIVEIRA, L. DALAQUA Jr. ***Software para realização de análises hematológicas utilizando processo radioanalítico.*** 39º Congresso Brasileiro de Patologia Clinica / Medicina Laboratorial, de 19 a 22 de outubro, São Paulo - SP, Brasil, 2005.