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BLOOD BIOCHEMISTRY EVALUATION IN HORSES HYPERIMMUNE SERA PRODUCTION

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ABSTRACT

In the present study the biochemical values for Cl, K and Na in whole blood from health equine used for immunization process were determined using nuclear methodology. 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 value for Cl, K and Na in whole blood of equines to perform clinical practical.

1. INTRODUCTION

In the last years we have performed several investigations in veterinary medicine using a nuclear procedure, namely the semi-parametric Neutron Activation Analysis technique [1]. This procedure was chosen because it can be considered an efficient method to perform quantitative analysis of some elements, important for clinical practical, simultaneously [1-6].

The principle of this analytic technique is the irradiation of the selected material with neutrons, together with the activation detectors (Au foils) used for measurement of the flux distribution, followed by the measurement of the γ-ray activities induced in the sample, where the elements in the sample can be identified by the energy associated to the emitted γ-ray and quantified by using their nuclear properties.

This methodology has been applied with success for clinical practical which a differential, i. é, biochemistry analysis using whole blood. Particularly, in veterinary medicine, this kind of investigation have been done in beagles, hamsters, rabbits, rats and also in several mouse strains [1-5] as well as in human beings [6].
Now, we extended this application to determine of the concentration of Na, K and Cl in equines used for sera production. These data are very important because these elements are majority in blood consequently small variation in their concentration are an important indicator for the control of the health status of theses animals during the evolution of the hyperimmunization process in serum production [7].

2. EXPERIMENTAL PROCEDURE

For this study twenty equines (race not defined) with 12-36 moths -old, (13) females and (7) males animals, from São Joaquim Farm at Butantan Institute (São Paulo, Brasil), were used. All the samples were collected from animals not submitted to the hyperimmunization process yet. About 1ml of whole blood was collect by strangle puncture in tubes "Vacutainer" (vacuum plastic tubing) although anticoagulants, using needles of 25 x 8mm.

To determine the concentration of the elements in blood, aliquots of 100 μl (prepared in duplicate) were transferred to the filter paper (Whatman N^{o} 42) and dried for few minutes using an infrared lamp. Each biological sample, together with both the bare and Cadmium-covered Au samples (two small Au foils, ~1mg each), were then sealed into individual polyethylene bag and irradiated in the nuclear reactor at IPEN/SP (IEA-R1, 2-4MW, pool type), allowing the simultaneous activation of these materials (i.e, sample and Au detectors). After that, we obtained the gamma spectra for both Au activation detectors as well as the biological sample in order to determine the neutron flux and the concentration of the activated elements in the sample under the exact same irradiation conditions.

An HPGe detector connected to an ADCAM multichannel analyzer and to a PC computer was used to measure the induced gamma-ray activity. The area of the peak corresponding to the 411 keV transition of ^{198}Au was evaluated (for both, i.e, the bare and Cadmium-covered) to perform the neutron flux distribution. The same way, each biological sample was also gamma-counted, and the areas of the peaks corresponding to gamma transitions related to the nuclides of interest were evaluated i. é, ^{38}Cl ($T_{1/2}$=37 min, E_γ =1642 keV), ^{42}K ($T_{1/2}$=12h, E_γ =1525 keV) and ^{24}Na ($T_{1/2}$=15h, E_γ =1368 keV). All gamma spectra analysis evaluations were performed using the IDF computer code [8] and the concentration of each element was then obtained by using an in-house software [9].

The irradiation time of 2 minutes; counting time of 15 seconds for Au detectors and 10 minutes for the biological sample and BG radiation allowed us to conclude the analysis of each sample in about 30 minutes or less.

3. RESULTS

The concentrations of the Cl, K and Na in whole blood samples of equines are shown in Table 1. All the results are a mean of duplicate analyses and the associated errors represent one standard deviation. In figures 1, 2 and 3 the concentration's results in blood for Cl, K and Na, respectively, are shown and the indicative interval for the reference values were included as well as the human being whole blood estimation, for comparison.

Table 1. The Concentration of Cl, K and Na in whole blood samples of equines

Elements	Mean	SD (68%)	Minimum Value	Maximum Value	Indicative Interval
Cl, g l^{-1}	2.41	0.26	1.94	2.99	2.15 - 2.67
K, g l^{-1}	1.63	0.34	1.10	2.13	1.29 – 1.97
Na, g l^{-1}	1.96	0.24	1.56	2.41	1.72 – 2.30

M: mean
SD: standard deviation

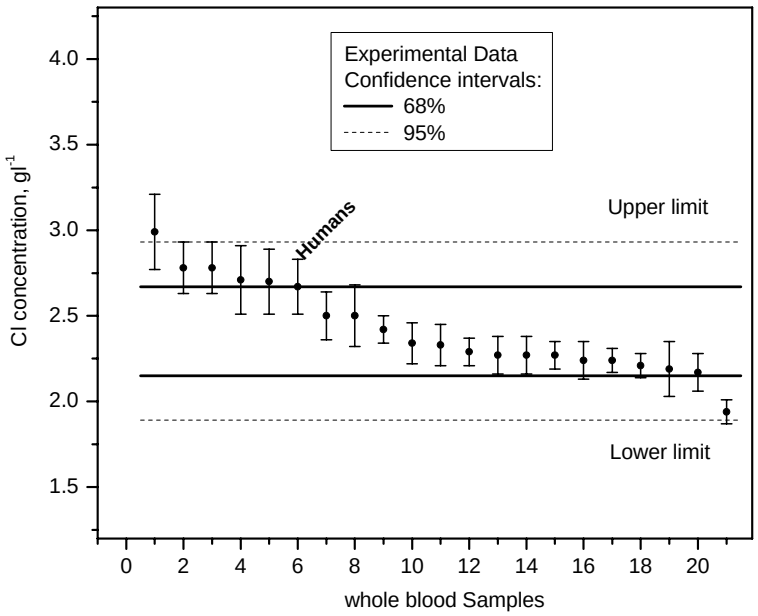

Figure 1. Chlorine concentration results in whole blood samples of equines; the horizontal lines represent the indicative intervals. The values are arranged by decreasing concentration.

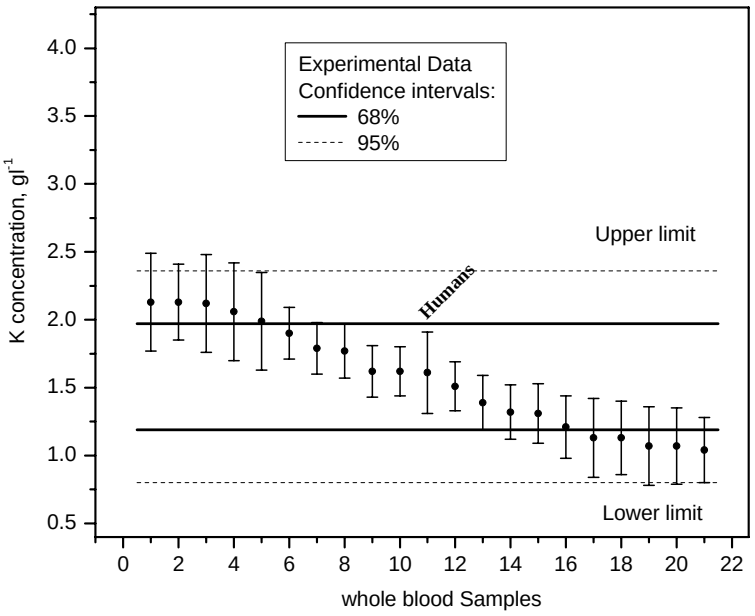

Figure 2. Potassium concentration results in whole blood samples of equines; the horizontal lines represent the indicative intervals. The values are arranged by decreasing concentration.

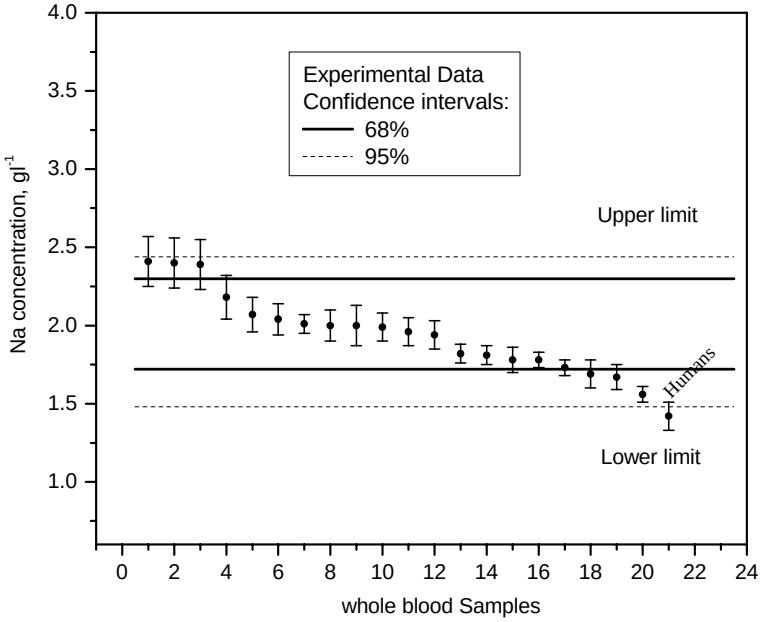

Figure 3. Sodium concentration results in whole blood samples of equines; the horizontal lines represent the indicative intervals. The values are arranged by decreasing concentration.

4. CONCLUSION

The biochemical values for Cl, K and Na in whole blood of equines were obtained aiming their utilization, in the future, for clinical practical.

The results of neutron activation analysis indicated that the occurrences of elements evaluated were similarly distributed for the equines and human being, suggesting the choice of this animal is an adequate experimental model for serum production.

The knowledge of the elemental composition of whole blood using NAA is an agile procedure that can be used for monitoring the health status of equines. Besides, this tool will be very important for checking the evolution of the hyperimmunization process in serum production.

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