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Determination of elements in blood of White New Zealand rabbits by NAA

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Abstract The determination of elemental concentrations for Br, Ca, Cl, Fe, K, Mg, Na, S, Sr, and Zn in blood samples from White New Zealand rabbits was performed applying the NAA technique. Twenty whole blood samples (12 male and 8 female) collected in research centers from Brazil (Aggeu Magalhães in Recife and Butantan Institute in São Paulo) were investigated, using the IEA-R1 nuclear reactor at IPEN/CNEN-SP-Brazil. These data can be used as references to perform biochemistry analyses in veterinary medicine using small quantities of whole blood (100–400 μL), simplifying the collection and the preparation of biological samples (it is not necessary to perform the serum separation nor to use specific reactants). Furthermore, the knowledge of the biochemical values in blood allows us to check the similarities with the blood estimations in human beings, which is an important condition for selecting laboratory animals. Finally, these data suggest a great similarity of the inorganic tissue profile of rabbits (White New Zealand) and humans.

Keywords NAA · Blood · White New Zealand rabbit · Reference values

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Introduction

Rabbits of all varieties are used in laboratory research, particularly in studies of bacteriology, physiology, nutrition, hormones investigation and also for dental tests and osseous prosthesis. Particularly, the White New Zealand species is heavily used in Brazil (Instituto Butantan) as an animal model in the immunological field, mainly for testing new vaccines and antibodies that could have medical or veterinary applications in the future.

Initially, White New Zealand rabbits were bred for domestic use and for human diet (due to its tender meat). The first litter of White New Zealand rabbits was bred in the United States in the early 20th century, but the original breeds that were used are unknown; it is believed that the Angora species has played some part in its development. Since them, the White New Zealand species has been used as an animal model mainly because of their resistance to allergies when compared to many other animals and their life expectancy of about 5–7 years when kept indoors and well-cared for. Also, they are easy to create and offer a fast procreation [1–3].

These laboratory animals require specific conditions for their adequate growth and reproduction because they are sensitive to sudden changes in temperature and to the excess or lack of daylight. The gestation period for rabbits is 27–32 days. Their development occurs at a fast pace, so much so that at 4 days of age, they already have hair and at 20 days are eating the same food given to the mothers. At 45 days old they can start the weaning process (separation of mothers from their babies).

According to Brazilian legislation, when the use of animals is necessary, it should be restricted to the smallest number possible [4]. Furthermore, the use of alternative methods that contribute to animal welfare are encouraged.

In recent years, the viability of using the NAA technique to perform biochemist analysis, using small quantities of whole blood (few μL), has allowed the investigation of several elements of clinical relevance in small size animal models [5–10], without sacrificing the animals and minimizing the stress during blood collection. In this study, we extended the use of INAA for determination of elements in whole blood samples of White New Zealand rabbits. Both males and females were used in this laboratory research and analyses were made on the base of gender, since known physiological variations are crucial for the appropriate application of the animal model.

Prior to collection of blood samples from the rabbits, reference material (IAEA-A13 Blood Animal) was analyzed to verify the accuracy and precision of the method.

Experimental procedure

The biological samples came from Aggeu Magalhães research center and Butantan Institute, both from Brazil. The animals were not sacrificed for this experiment; the blood samples were collected only from adult animals. Blood was collected through an incision in the ears of twenty adult rabbits (12 male and 8 female). For analyses requiring short irradiation times (minutes), aliquots of 100 μL (±0.5%) were transferred to Whatman (No. 41) filter paper (in duplicate) and dried for a few minutes using an infrared lamp. For analyses requiring long irradiation times (hours), samples of 400 μL (±0.5%) were stored in plastic cylinders (in duplicate) in preparation for analyses. Standard solutions obtained from high purity metals and salts were prepared following the same procedure. Each set (sample and standard) was irradiated in the IEA-R1 nuclear reactor at IPEN-CNEN/SP (IEA-R1, 3.5–4.0 MW, pool type). The short (2–5 min) and long (8 h) irradiation times were performed in the pneumatic facility and in the core of nuclear reactor, respectively. For ^{38}Cl ($T_{1/2}$ = 37 min, E_γ = 1642 keV), ^{24}Na ($T_{1/2}$ = 15 h, E_γ = 1368 keV) and ^{37}S ($T_{1/2}$ = 5 min, E_γ = 3104 keV) an irradiation time of 2 min and 5 min of counting were used. For ^{80}Br ($T_{1/2}$ ~ 16 min, E_γ = 616 keV), ^{49}Ca ($T_{1/2}$ ~ 9 min, $E\gamma$ = 3098 keV), ^{27}Mg ($T_{1/2}$ ~ 9 min, $E\gamma$ = 884 and 1012 keV), ^{42}K ($T_{1/2}$ ~ 12 h, E_γ = 1525 keV) and ^{87}Sr ($T_{1/2}$ ~ 2.8 h, $E\gamma$ = 388 keV) each set was irradiated for 5 min and after a decay time of 60 s they were counted for 15 min for Br, Ca and Mg determination, followed by 2 h of counting for K and Sr. For ^{59}Fe ($T_{1/2}$ ~ 44.5d, E_γ = 1099 and 1291 keV) and ^{65}Zn ($T_{1/2}$ ~ 244d, E_γ = 1116 keV) the samples and standards were irradiated for 8 h and, after a decay time of several days, they were gamma counted for 4 h. The elements Br and Ca were also determined following the 8 h long irradiation using ^{81}Br ($T_{1/2}$ ~ 35 h, E_γ = 776 keV) and ^{47}Ca ($T_{1/2}$ ~ 4.5 d, E_γ = 1297 keV) isotopes after decay times of 4 and 7 days, respectively.

The elements Ca, Cl, Fe, K, Mg and Na were selected due their clinical relevance for evaluating electrolyte diseases; also Zn was determined although its evaluation is not a common component of these biochemical studies. Br was selected because bromides are present in the Brazilian diet (mainly sea food) and also in medicinal drugs, both highly consumed by the Brazilian population. Sulfur was selected due its use as a fertilizer in Brazilian farms [11] which can increase the levels of sulfur in the body of these animals through their diet. Sr as well as Ca and Mg were also monitored due their relevance in studies involving tests of dental implants and osseous prosthesis usually performed using this animal model. Particularly, the clinical consequences after dental implants (bacterial infiltration) can affect the white blood cells causing electrolyte disorders.

A gamma spectrometer system with a semiconductor detector connected to an ADCAM multichannel analyzer and a PC computer was used to measure the induced gamma-ray activities. The detector was a HPGe of high resolution (FWHM = 1.89 keV), calibrated for energy through the measurements of standard sources of $Co^{56,60}$ and Eu^{152}. All gamma spectral analyses evaluations were performed using the IDF computer code [12] and the elemental concentrations were determined using in–house software [13].

Results and discussion

The certified values and those determined in this work for the quality material, as well as the *Z*-score values are presented in Table 1. The *Z*-score values obtained indicated that our results are satisfactory considering 95% confidence level. The concentration of the elements in blood samples of White New Zealand rabbits are shown in Table 2 and Fig. 1. In this table we include: the arithmetic mean (gL^{-1}), the standard deviation (1SD), the minimum and maximum values determined (gL^{-1}), the normal range (gL^{-1}) for a confidence interval of 95% (adopted for clinical practice), the detection limit (DL) as well as the range from human blood (literature) for checking the similarities. To better visualize these data, Br concentration results are shown in Fig. 2. In this figure, the data is presented as a function of gender, in descending order, including ±1SD and ±2SD range. Moreover, the minimum (0.0017 ± 0.0002 gL^{-1}) and maximum value (0.0070 ± 0.0009 gL^{-1}) can be seen. The normal range was obtained from a mean of the twenty analyzed samples.

According to Table 2, no significant difference was observed between male and female animals, although for

Table 1 Element concentrations obtained in the analysis of IAEA-A-13 animal blood reference material

Element	Our results mean ± 1SD	IAEA-A-13*	RSD (%)	Er (%)	*Z*-Score
Br (mg kg^{-1})	19.7 ± 2.5	22.0 ± 2.4	12.7	−10.4	−0.95
Ca (mg kg^{-1})	263 ± 43	286 ± 54	16.4	8.0	−0.43
Fe (mg kg^{-1})	2488 ± 214	2400 ± 144	8.6	3.7	0.61
Mg (mg kg^{-1})	109 ± 21	99 ± 29	19.3	10.1	0.36
K (g kg^{-1})	2.83 ± 0.22	2.50 ± 0.35	7.8	13.2	0.94
Na (g kg^{-1})	13.31 ± 0.55	12.60 ± 1.01	4.1	5.6	0.70
S (g kg^{-1})	6.91 ± 0.67	6.50 ± 0.52	9.7	6.3	0.79
Zn (mg kg^{-1})	14.83 ± 3.44	13.00 ± 1.04	23.2	14.1	1.76

* Certified values

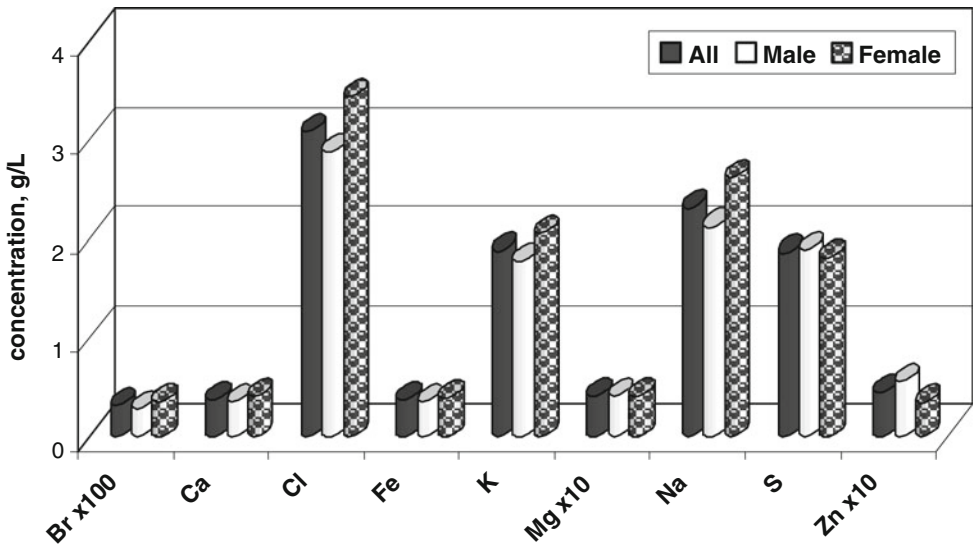

Fig. 1 Average elemental concentration in whole blood as a function of gender

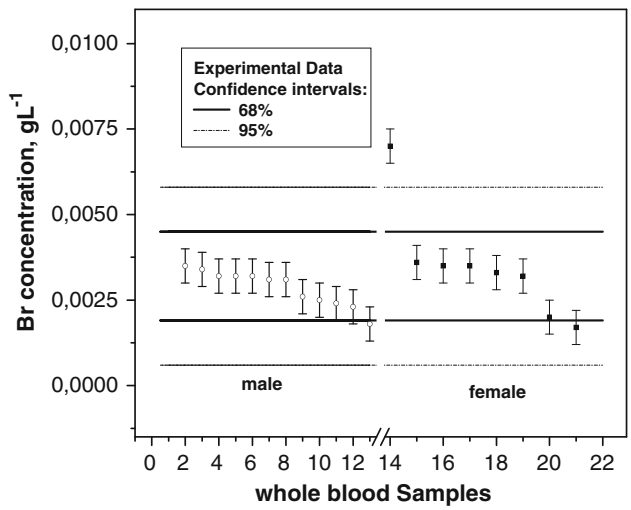

Fig. 2 Br concentration in whole blood as a function of gender

Br, Ca, Cl, Fe, K, and Na the mean value is slightly higher in females. For Zn this behavior is reversed.

The comparison of data with human blood estimation (see Table 2) reveals that there is similarity for Ca, Cl, K, Fe, Mg, Na, and Zn considering 68% of confidence interval (±1SD). However, the human range for K (1.09–1.53 gL^{-1} and Na (1.24–1.60 gL^{-1}) is near the lower limits for rabbits (1.19 gL^{-1} for K and 1.56 gL^{-1} for Na), suggesting that these elements must be carefully monitored in blood because small variations in these major elements in blood can be lethal for the organism. No significant differences in the concentration of Br in blood samples of rabbits and estimations for humans. The Br range (0.0020–0.0044 gL^{-1}) is within the human estimation (0.0024–0.0096 gL^{-1}). This large concentration variation for Br (in human) may be related to diet, mainly fish and seafood (rich in bromides).

The interval for S (1.26–2.46 gL^{-1}) presents a significant difference as compared to the human blood estimation (0.19–0.59 gL^{-1}); the similarity can be only observed considering ±3SD (0.06–3.66 gL^{-1}). A probable explanation is the diet. Recently, an investigation performed for mineral characterization of the ration administrated to equines used in the anti-venom production at Butantan Institute, indicated the presence of S at high level (factor 100 above nominal specification) [18] probably due the use of elemental-S as a fertilizer in tropical soils for agricultural production [11]. Thus, the high value for S in blood of

Table 2 Elements concentration in blood of White New Zealand rabbits

Elements	Mean	±1SD	Minimum value	Maximum value	Range [human]	DL
Br (g L^{-1})	0.0032	0.0012	0.0017	0.007	0.0020–0.0044	0.0016
	0.0028 M	0.0005			[0.0024–0.0096][a]	
	0.0035 F	0.0019				
Ca (g L^{-1})	0.371	0.12	0.24	0.496	0.251–0.491	0.07
	0.346 M	0.129			[0.150–0.316][b]	
	0.412 F	0.17			[0.116–0.246][c]	
Cl (g L^{-1})	3.09	1.07	1.78	4.08	2.02–4.16	0.02
	2.88 M	1.2			[2.34–3.00][a]	
	3.45 F	1.37				
Fe (g L^{-1})	0.363	0.117	0.24	0.496	0.279–0.453	0.1
	0.346 M	0.129			[0.19–0.41][c]	
	0.391 F	0.165				
K (g L^{-1})	1.88	0.69	0.94	2.9	1.19–2.57	0.59
	1.77 M	0.77			[1.09–1.53][a]	
	2.06 F	0.86				
Mg (g L^{-1})	0.041	0.018	0.024	0.067	0.023–0.059	0.011
	0.041 M	0.019			[0.040–0.074][b]	
	0.041 F	0.021				
Na (g L^{-1})	2.31	0.75	1.58	3.2	1.56–3.06	0.02
	2.12 M	0.82			[1.24–1.60][a]	
	2.62 F	1.03				
S (g L^{-1})	1.86	0.6	1.26	2.69	1.26–2.46	0.94
	1.89 M	0.68			[0.19–0.59][c]	
	1.82 F	0.69				
Sr (mg L^{-1})	47 M	15	33	53	32–62	12
					[0.013 0.028][d]	
Zn (g L^{-1})	0.0045	0.0014	0.0031	0.0068	0.0031–0.0059	0.0011
	0.0056 M	0.0025			[0.0042–0.0068][c]	
	0.0034 F	0.0014				

[a] Human values from Ref. [14] for Br, Cl, K and Na

[b] Human values from Ref. [15] for Ca and Mg

[c] Human values from Ref. [16] for Ca, Fe, S and Zn

[d] Human values from Ref. [17] for Sr

DL Detection limit

M Male

F Female

rabbits may be related to the diet. Of course a specific investigation in the ration adopted for rabbits must be conducted to confirm this.

Sr was determined only for male animals although it has been observed in female with poor statistics (uncertainty $\sim$70%) suggesting that the concentration of Sr in male $>$ Sr in female. This concentration results are high compared to human being blood estimation so for investigations involving tests of dental implants or osseous prosthesis, the use of males may not be appropriate. In the same way, iodine was identified in male and female samples but it was not determined due to poor statistics. We intend to continue these analyses in urine, complementing the blood investigation. Particular efforts will be made to perform measurements that allow the determination of iodine because its variation is related to metabolic dysfunction (hyper and hypothyroidism) as well as Sr in females for checking the similarities with human beings.

Conclusion

Multi-elemental blood analysis is an important tool in assessing the health of animals, helping to identify clinical and sub-clinical symptoms of several diseases. The results of this investigation are of great use to researchers who adopt rabbits as animal models because they can optimize the biochemical analysis using small quantities of blood, an advantage when the biological material is restricted. The data can also be used in other areas that adopt this rabbit species as an animal model.

Furthermore, the knowledge of these biochemical values allows checking the similarities between male and female animals as well as with human beings, an important condition for selecting laboratory animals. Specifically, the data obtained suggest no significant differences related to gender and a great similarity of inorganic tissue profile of rabbits (White New Zealand) and humans.

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