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APPLICATION OF NEUTRON ACTIVATION ANALYSIS FOR TRACE ELEMENT DETERMINATION IN PLASMA AND EGG YOLK SAMPLES OF *Bothrops jararaca*

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ABSTRACT

In this work, neutron activation analysis was applied in the determination of trace elements in plasma and egg yolk samples. These determinations are of great interest to identify the elements that could be used in the study of nutrient transport through the plasma to the egg yolk. Plasma and egg yolk samples were obtained at the Laboratório de Herpentologia of the Instituto Butantan, SP, from pregnant female *Bothrops jararaca* snakes originated from several locates of São Paulo State, Brazil. For the analyses the samples were first freeze-dried and then irradiated along with elemental standards at the IEA-R1 nuclear research reactor. Samples and standards were irradiated for 16 hours under thermal neutron flux of $5x10^{12}$ n cm^{-2} s^{-1}. After adequate decay times, the gamma-ray measurements were carried out using a hyperpure Ge detector coupled a gamma-ray spectrometer. Concentrations of Br, Ca, Fe, Na, R, Se and Zn were determined in these samples. These findings suggest the use of neutron activation analysis of egg yolk and plasma samples to investigate the nutrient transport from the yolk to the embryo or across the placenta for *Bothrops jararaca* snake species. For quality control of the results NIST 1566b Oyster Tissue certified reference material was analyzed using the same experimental conditions adopted in the analyses of plasma and egg yolk samples.

1. INTRODUCTION

Among vertebrates, reptiles possess many species that are letally affected by environmental alterations. Venomous snakes are important members of the ecosystem, occupying the top of many food chains. The control of sylvatic rodent population by snakes is a well-known role of snakes in the wild. The population’s increase of those mammals, which are natural reservoirs of many viruses (like the Hanta virus), leads to human epidemics on areas where snakes are scarce due to massive killing. Due to this factor, Brazil recently adopted strict legal measures to avoid rodent predator capture and killing.

Nevertheless, many of the predatory snakes are venomous to human beings. The only known treatment for a venomous snakebite is the use of species-specific antisera produced in horses after injection of purified venom, obtained by milking several individuals. The most common snake accidents in Brazil are caused by species *Bothrops jararaca* (~90% of all reported cases).

Since these animals are important in maintaining the animal species system in equilibrium it becomes vitally important to study the reproductive physiology of this viviparous snake for its preservation.

There is a great interest to understand the evolution of snake viviparity from neotropical areas, since published studies in this field are scarce and the results sometimes contradictory. Lourdais et al [1] studied climate effects on embryonic development in a viviparous snake, *Vipera aspis* and concluded that environmental thermal conditions influence the female body temperature and gestation length, embryo viability, and offspring phenotypes. Thompson and Speak [2] presented a review with recent information on the yolk contents of a variety of squamate reptiles in order to know how the nutrients are transported from yolk to embryo or across the placenta. Thompson et al [3] measured the contents of ion, energy, lipid, nitrogen and fat-soluble vitamins of the freshly ovulated eggs and neonates or viviparous lizard *Niveoscincus metallicus* to quantify uptake of nutrients across the placenta.

In this study, neutron activation analysis was applied to determine elements present in plasma and egg yolk samples of the *Bothrops jararaca* snake species to select elements that could be used in the nutrient transport study through the placenta during snake embryonic development.

2. EXPERIMENTAL

2.1. Plasma and Egg Samples of the *Bothrops jararaca* Snake Species

Plasma and egg yolk samples of the *Bothrops jararaca* snake species were obtained at the Laboratório de Herpentologia of the Instituto Butantan, SP, from pregnant female *Bothrops jararaca* snakes originated from several locates of São Paulo State, Brazil. For the analyses the samples were first freeze-dried and then aliquots of about 200 mg were weighed in polyethylene involucres.

2.2. Procedure for Neutron Activation Analysis

The synthetic elemental standards were prepared by pippeting 50 μL of the elemental standard solutions onto sheets of Whatman No. 40 filter paper. These solutions containing one or more elements were prepared using certified standard solutions provided by Spex Certiprep Chemical, USA. These filter sheets were dried at room temperature inside a desiccator and, then were placed into clean polyethylene involucres. In these standards the quantities of each element, in μg (in parentheses) were the following: Br (5.2), Ca (1000), Fe (280), Na (500.0), Rb (4.0), Se (40) and Zn (35.0).

The samples were irradiated in the IEA-R1 nuclear reactor along with the synthetic standards of the elements. They were irradiated for 16 hours under thermal neutron flux of $5x10^{12}$ n cm^{-2} s^{-1}. After adequate decay times, the irradiated samples and standards were measured by a hyperpure Ge detector Model GX2020 coupled to Model 1510 Integrated Signal Processor, being both from Canberra. The resolution (FWHM) of the system was 0.90 keV for 122 keV gamma-ray peak of ^{57}Co and 1.87 keV for 1332 keV gamma ray of ^{60}Co. Each sample and standard were measured at least twice for different decay times. The radioisotopes measured were identified according to their half-lives and gamma- ray energies. The concentrations of elements were calculated by a comparative method. The radioisotopes used in serum analyses were: ^{82}Br, ^{47}Ca, ^{59}Fe, ^{24}Na, ^{86}Rb, and ^{65}Zn

The quality assurance of the analytical results was evaluated by analyzing certified reference material, NIST 1566b Oyster Tissue provided by the National Institute of Standards and Technology (NIST), USA. This reference material was analyzed by applying the same experimental conditions used in serum analyses. The element concentrations of reference material were evaluated on a dry weight basis, as recommended in the certificate. A moisture weight loss of 5.2% was found to correct the final results.

3. RESULTS AND DISCUSSION

Results obtained for certified reference material NIST 1566b Oyster Tissue showed good precision and good agreement with the certified values [4] for all the elements analyzed (Table 1). The relative standard deviations of the results were lower than 5.9 % and relative errors varied from 0.36 to 4.6 %. The standardized difference or Z score values [5] obtained for the elements analyzed were $|Z| < 1$, indicating that our results are satisfactory and in agreement with the certified values.

Results obtained in the analysis of plasma samples and their respective egg yolks presented in Table 2. The most prominent changes in yolk are seen in calcium content per egg/oocyte. The amount of calcium increases slightly in the samples analyzed and is maximum in the fertilized egg without a visible embryo (Animal 3; Table 2). When embryos are visible, there is an abrupt fall in yolk calcium content (Animal 4; Table 2). The same profile is observed for the other elements analyzed. This is consistent with the idea that yolk products are used for embryo growth. These elements are probably bound to yolk proteins that are transferred to the embryo. The concentration of bromine in plasma samples obtained from the same animals used for yolk analysis increases steadily and is maximal in the individuals bearing eggs with embryos. All other element concentrations are approximately constant in the samples, with the exception of calcium that shows the highest values in animals with embryonated eggs. This could be an indication that calcium is being transferred from plasma to oocytes, but more results are needed to support this supposition. The amount of sodium determined in the plasma is compatible with the one determined for other reptiles (that vary from 1.58 mg/ml to 5.54 mg/ml).

Table 1. Elemental concentrations obtained for NIST 1566b Oyster Tissue certified reference material

Elements	This work				Certified values [4]
	Mean ± SD[a]	RSD[b], %	Er[c], %	Z score	
Br, $\mu g\ g^{-1}$	51 ± 3	5.9	-	-	-
Ca, $\mu g\ g^{-1}$	841 ± 25	3.0	0.36	+ 0.09	838 ± 20
Fe, $\mu g\ g^{-1}$	196.3 ± 6.3	3.2	4.6	- 1.02	205.8 ± 6.8
Na, $\mu g\ g^{-1}$	3155 ± 151	4.8	4.3	- 0.89	3297 ± 53
Rb, $\mu g\ g^{-1}$	3.150 ± 0.021	0.7	3.4	- 0.08	3.262 ± 0.145
Se, $\mu g\ g^{-1}$	2.08 ± 0.05	2.4	0.97	+ 0.8	2.06 ± 0.15
Zn, $\mu g\ g^{-1}$	1386 ± 23	1.6	2.7	- 0.74	1424 ± 46

a. SD= standard deviation; b. RSD= relative standard deviation; c. Er = Relative error

4. CONCLUSIONS

In conclusion, this study revealed that the experimental procedures used could be successfully applied element determination in plasma and egg yolk from *Bothrops jararaca* snake species. These preliminary data encourage further study of nutrient transport through the placenta during embryonic development

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Table 2. Element concentrations obtained in plasma and yolk samples of the snake *Bothrops jararaca*

Elements	Yolk samples (μg/egg)			
	Animal 1	Animal 2	Animal 3	Animal 4
Br	20.88	27.74	33.06	10.63
Ca	41,470.00	38,640.00	47,220.00	8,410.00
Fe	121.57	168.10	136.42	18.68
Na	100.28	6.93	5.31	1.72
Rb	20.51	37.01	4.53	12.17
Se	1.51	1.75	2.72	0.35
Zn	149.69	137.98	171.03	34.7
Elements	Plasma samples (μg/ml)			
	Animal 1	Animal 2	Animal 3	Animal 4
Br	4.82	7.87	11.93	17.45
Ca	300.00	150.00	180.0	330.00
Fe	1.00	0.92	0.82	0.97
Na	2,420.00	3,230.00	3,350.00	2,640.00
Rb	0.34	0.71	0.65	0.82
Se	0.04	0.10	0.07	0.09
Zn	3.69	4.57	5.47	4.53

Animal 1=oocytes with 3.17 cm diameter; **Animal 2**=Oocytes with 3.90 cm; **Animal 3** = fertilized eggs (without embryos); **Animal 4** = fertilized eggs (with embryos)