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Parasitology and Helminthology

Arginase in the Organs of Water Snake (*Natrix tessellata*, Laurenti, 1768) and Cestode (*Ophiothaenia europaea*, Odening, 1963) Parasitizing in it

Ketevan Nikolaishvili*, Tsitsino Lomidze*, Lali Murvanidze*

* *Institute of Zoology, Ilia State University, Tbilisi*

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ABSTRACT. The activity of arginase in the organs of water snake (*Natrix tessellata*) and cestode (*Ophiothaenia europaea*) inhabiting its intestine was determined. pH optimums are established. It was shown that the greatest activity of arginase in water snake was noted in kidneys and the least activity was in the liver. No arginase was detected in the intestine. Considerable activity of the arginase of the parasite is established.

The role of biochemical adaptation of arginase in the system parasite-host is discussed. *© 2012 Bull. Georg. Natl. Acad. Sci.*

Key words: *Natrix tessellata, Ophiothaenia europaea, arginase.*

Studies of adaptational reactions in parasite worms to the habitation medium, have always interested the researchers dealing with biochemistry of helminths [1-3]. Helminths of warm-blooded and cold-blooded animals have often been the object of study. However, these processes have hardly been investigated in the endogeneous parasites of snakes and their hosts. Detection of the peculiarities of metabolism in the above animals is very important for comparative biochemistry, biochemistry of helminths and system parasite-host formation. We focused attention on arginase enzyme. A number of studies are devoted to arginase from different tissues of vertebrate animals. It is also found in the body of helminths of different classes and some researches connect the role of this enzyme with adaptation to parasitizing [2-5].

Proceeding from the integral system parasite-host we considered it to be necessary to determine the activity of arginase of both components using the example of water snake (*Natrix tessellata*, Laurenti, 1768) and cestode (*Ophiothaenia europaea*, Odening, 1963) parasitizing in its intestine. We aimed to find the values of enzyme activity in the organs of host and parasite. The peculiarities revealed are very important for a deeper understanding of interrelations in the system parasite-host.

Materials and Methods. The liver, kidneys and intestines of water snakes and cestodes *O.europaea*

served as material for the investigation. 5 exemplars of water snakes were brought from the territory close to Tbilisi water reservoir and 3 exemplars from the environs of Rustavi.

The animals caught near the water reservoir were all infected with cestodes (invasion intensity is 3-9 pieces), and animals brought from the environs of Rustavi were free of cestodes.

Out of fresh-frozen organs of water snakes and cestodes 2.5% and 5% water homogenates were prepared. After extraction in cold conditions homogenates were centrifuged at 3000 rev/min for 10 min. Sediment was thrown away and supernatant served as a source of enzyme.

The activity of arginase was determined according to the method of Khramov and Galaev [6]. The calculation was done by a calibration curve, constructed on dissolving the standard solution of urea. Specific activity was expressed in μg of urea per mg of protein for 1 hour at 37 °C.

Protein was determined by Lowry [7].

Results and Discussion. In homogenates of tissues of water snake and cestodes *O.europaea* arginase activity was revealed. Arginase helps to decay arginine to ornithine and urea. Enzyme activity is evaluated by the quantity of the latter. The results of evaluation are presented in Table 1. They show that maximal activity was detected in kidneys of the water snake (197.5±13.84 μg of urea/mg of protein/hour).

Arginase was absent in the intestine and its activity in the liver was quite low, totaling 7.31±0.89 μg of urea/mg protein/hour. Comparing the values of enzyme activity in the liver and kidneys of the snake one may say that high-active arginase is located in the kidneys. Abundant blood circulation ensures both their excretory function and intensive metabolism. Low activity of arginase in liver of the water snake can be explained by the fact that snakes and birds have no system of ureogenesis. Adaptation of these animals to the surface life led to the creation of uricotelism and formation of uric acid as the final product of protein metabolism.

Table 1. Activity of arginase in the organs of water snake and cestode *Ophiothaenia europaea* (at pH 9.5)

Object of investigation	Activity of arginase in μg of urea/mg protein/hour
Kidney	197.5±13.84
Intenstine	not revealed
Liver	7.31±0.89
O.europaea	35.95±6.24

Low activity value of arginase in the liver of water snake agrees with the data for arginase of chicken's liver obtained by Sokhina and Koloskova [2]. It should be assumed that liver arginase N.*tessellata* refers to the type of non-ureotelic arginases, which take part in the process of rendering ammonia harmless. Such arginase is an evolutionarily more ancient enzyme with a function independent of ureotelism. Some authors consider that while establishing the "ureotelism" in the liver of amphibians the specific ureotelic arginase is induced, which functions together with the present philogenetically more ancient non-ureotelic arginase [8,9]. Comparing the values of the activity of arginase of liver of the water snake and snake's cestode with the data for arginase of the chicken's liver and her parasite *Ascaridia galli* [2], we noted that these values are very close to each other (Table 2).

From this example we can see that arginine decay in the liver of birds and snakes occurs almost with equal intensity and their arginases possibly fulfill analogous functions. Adaptation of these animals to the medium with limited water content occurred in similar conditions and was reflected on nitrous me-

Table 2. Arginase activity in the liver of water snake and chicken, nematodes *Ascaridia galli* and cestodes *Ophiothaenia europaea*

Object of investigation	Activity of arginase in μg of urea/mg protein/hour
Liver of water snake	7.31±0.89
*Liver of chicken	8.62
Ophiothaenia europaea	57.05±7.97
**Ascaridia galli*	50.51

* According to Sokhina and Koloskova [2]

tabolism. Parallelly to this the process of biochemical adaptation of intestine helminths parasitizing in these animals took place. The above mentioned arginase activity and uric acid as the final product of nitrous metabolism can serve as confirmation of this. The latter is produced as a result of purine decay in cestodes [18].

The study of the influence of the concentration of hydrogen ions on the activity of arginase of the water snake organs and *O.europaea* allowed to establish pH optimum of enzymes. For liver arginase it was equal to 7.5 (activity18.39 μg of urea per mg protein/hour). A similar value of pH optimum was determined for one of the isoforms of arginase plerocercoid *Ligula intestinalis* from fishes [11]. It is known that pH optimum range of arginase can vary from 9 to 10.5. For kidney arginase of water snake it was 9.5 (activity 197.5 μg urea/mg protein/hour).

This value is close to the values of pH optimum of arginases of vertebrate animals [12-14] and some other helminths [11, 15-17]. Arginase activity of cestodes at pH 9.5 was 5.5 times lower than the activity of kidney enzyme and at the same time it almost 5 times increased the level of arginase activity in the liver of water snake. Unlike arginase of liver and kidneys of water snake pH arginase optimum *O.europaea* shifted to more alkalinity and was equal to 10.5, and activity totaled already 57.05 μg urea/mg protein/hour. Such difference in activity at different values of pH, probably points to the change of ionization state of different ionizing enzyme groups, which then influence on catalytic center of the latter.

Comparing enzyme activity in the organs of water snake at optimum value pH, it is shown that high-active arginase is localized in kidneys. It exceeds 27 times the liver enzyme activity. Thus, it is evident that intensity of arginine decay in different organs of water snake differs, taking into account intestines. Products of protein hydrolysis and aminoacids are actively absorbed by mucous membrane of the intestine, then they pass into blood taking part in metabolism of those organs and tissues which have arginase. Adaptation to the medium with abundant quantity of food substances in cestodes inhabiting intestine found reflection not only on their morphology (absence of digestive system) but on metabolism as well. Arginine is easily accessible for *O.europaea.* Arginase activity in hosts intestine was not revealed. Parasite's own arginase activity was significant (Table 1) and almost 8 times exceeded the enzyme activity of water snake's liver. It should be noted that earlier we have revealed acid and alkaline phosphatases in homogenates of water snake cestode. Their activity three times exceeded the level of activity of those enzymes in tapeworm of African hieroglyph python [10]. We suppose that high activity of phosphatase and arginase in *O. europaea* is the reflection of intensity of metabolic processes characterizing the given type of cestode and adaptation to habitat.

It is quite difficult to determine the role of the investigated enzymes, as non-ureotelic arginases have many other functions. They participate in the regulation of a definite quantity of urea, arginine and other guanidine compounds, in proline formation. The latter is involved in biosynthesis of polyamines necessary for division and differentiation of cells [19]. Similar synthetic processes may take place in hosts and their parasites. According to the data by Lee [20] and Brand [21] the function of the arginase of parasite worms is to provide them with proline which is used in collagen cuticle formation. Polyamine synthesis in helminths does not occur and requirements in these substances are completely fulfilled on the host's account [22]. Their fast growth, reproduction system development and great fecundity are probably provided in this way.

At arginine hydrolysis in water snake kidneys and cestode body both in host and parasite considerable quantity of urea and ornithine must be accumulated. It is known that in reptiles and birds osmoregulation occurs via uric acid. Urea as low molecular substance in helminths inhabiting the intestines of sea fishes performs an important function of osmoregulation on cellular level [3,4]. We are not quite

sure about it as to *O. europaea* and water snake, but we suppose that the formed urea might be an agent additional to uric acid, which strengthens osmoregulation process in both host and parasite.

Arginase also takes an active part in adaptation processes in both vertebrate animals (tailless amphibians) at some stages of ontogenetic development [23,24] and in helminths with formation of arginase isoenzymes [5, 25]. Correlation between kinetic and thermodynamic parameters of arginases in nematodes and their hosts [2,3], temperature optimums and thermostability of fishes cestode arginase with temperature homeostasis of host [2,26,27] is also an evidence of adaptation.

On the basis of the above-said we can conclude that non-ureotelic arginases play an important and diverse role in metabolism emerging as an adaptive factor.

The results of our work showed that arginase activity of water snake is connected with the intensity of arginine decay in the organs in which they are localized. At the same time the level of activity of cestode enzyme attests to the high level of biochemical adaptation of *O. europaea* metabolism to the conditions in the water snake intestine ensuring metabolic balance in the interrelationship of parasite and host.

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პარაზიტოლოგია და ჰელმინთოლოგია

წყლის ანკარას (*Natrix tessellata*, Laurenti,1768) ორგანოებში და ცესტოდაში (*Ophiothaenia europaea*, Odening, 1963) ფერმენტ არგინაზას აქტივობის შესახებ

ქ. ნიკოლაიშვილი*, ც. ლომიძე*, ლ. მურვანიძე*

*ილიას სახელმწიფო უნივერსიტეტი, ზოოლოგიის ინსტიტუტი, თბილისი

(წარმოდგენილია აკადემიის წევრის ი.ელიავას მიერ)

წყლის ანკარას ორგანოებში და მის ნაწლავში მობინადრე ცესტოდაში განსაზღვრულ იქნა ფერმენტ არგინაზას აქტივობა და დადგინდა pH ოპტიმუმი. ნაჩვენებია, რომ წყლის ანკარას თირკმელებში ფერმენტი აქვლენს მაღალ აქტივობას, ღვიძლში – შედარებით დაბალს. ნაწლავში არგინაზა არ იქნა გამოვლენილი. აღინიშნა აგრეთვე პარაზიტის არგინაზას მნიშვნელოვანი აქტივობა. განხილულია მოცემულ პარაზიტ-მასპინძლის სისტემაში ფერმენტ არგინაზას ბიოქიმიური ადაპტაციის როლი.

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