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The Results of Study of Water-Soluble Proteins of Helminths of *Rana macrocnemis* Boul.

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ABSTRACT. Water-soluble proteins of three species of helminths *Rana macrocnemis* have been investigated by means of the electrophoresis method in PAAG. While comparing the protein spectra of *Haplometra brevicaca* (trematoda), *Nematotenia dispar* (cestoda) and *Pseudoacanthocephala caucasicus* (acanthocephala), both similarity of mobile fractions and some differences that allow us to make the conclusion about the specificity of types of water-soluble proteins and the proteinograms themselves, which can serve as an additional criterion when differentiating the types. *© 2007 Bull. Georg. Natl. Acad. Sci.*

Key words: *helminths, Rana macrocnemis, electrophoresis, water-soluble proteins.*

The helminthofauna of the Iranian longlegged wood frog *Rana macrocnemis* is very different. Representatives of various classes of parasitic worms revealed in this amphibian are substantially studied morphologically and the cycles of their evolution are determined [1-4]. However, helminths of *R.macrocnemis* have almost not been investigated biochemically. Some work was conducted in the aspect of host-parasite relations only in order to define the activity of phosphatase of monogenes and proboscis worms of *R.macrocnemis* [5]. We were interested in studying not only the enzyme systems of helminths but also protein spectra of parasitic worms for their species characteristics.

The aim of our work was to carry out comparative electrophoretic analysis of water-soluble proteins of helminths of *R.macrocnemis*.

The animals were captured on the territory the Pass of the Cross (the Great Caucasus) and in Lakes Bareti and Santa (Small Caucasus), and kindly presented by D.Tarkhnishvili, Doctor of Biological Sciences. Parasitological material was obtained by means of helminthological autopsy of the amphibian. Species composition of helminths was defined by senior research worker L.Petriashvili, Institute of Zoology.

In our study we used the most numerous species: trematodas – *Haplometra brevicaca* from the lungs, cestodes – *Nematotenia dispar* and acanthocephala species proteins *Pseudoacanthocephala caucasicus* from intestines.

The 5% water homogenates were prepared from several samples at each species to exclude individual differences of helminths. Extraction was carried out in cold for 20 min and then centrifuged for 5 min at 3 000 rev/min. After the concentration of the protein in supernatant was determined [6], the samples were mixed with a saccharose solution in ratio 1:1 and used for electrophoretic analysis.

Electrophoresis was conducted according to Davis [7] in the presence of sodium dodecyl sulfate in tris glycine buffer pH-8.3 in 7.5 % PAAG, in camera of Hungarian apparatus type "OE-107". Electrophoregrams were stained with Amido Black.

The results of the study show that spectra of proteins in these species of helminths differ (Fig.1). Most probably, it is presented by a mixture of easily soluble in water proteins extracted from tegument, skin-muscle sac, parenchyma, hypodermis and other tissues and organs of helminths.

On the electrophoregram of cestode (a) 8 protein fractions were revealed, while trematoda (c) counted about 12. It should be noted that lung trematodas were almost black. The latter points to the presence of frog's blood in their body. We assume that together with the own proteins, haemoglobin and other proteins of the host's blood are

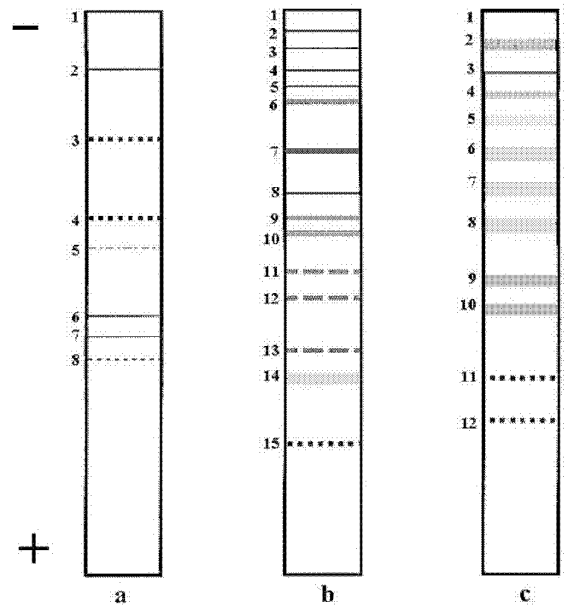

Fig. 1. Schemes of protein spectrum of helminths *R.macrocnemis* a - cestoda *N.dispar*, b - acanthocephala *P.caucasicus*, c - trematoda *H.brevicaca*.

also extracted from *H. brevicaca* and electrophoregram of trematodas is more multicomponent. The largest quantity of protein stripes was revealed in acanthocephala totalling about 15 (b). The obtained electrophoregram presents general spectra of proteins extracted from male and female specimens of acanthocephala. We suppose that the spectra of water-soluble proteins of helminths, revealed in our conditions make only a part of proteins extracted from the biomass of parasites. The total quantity of protein components is significantly higher. Taking into account great varietes of proteins in the body, we note that some of them may not be revealed on the electrophoregram of entire extracts, which is explained either by their insignificant concentration or their overlapping of the series of stripes due to the same charge.

Serious analysis and comparison showed that some protein fractions in different representatives coincided in mobility and concentration. It is the 1st fraction in all of them, the 2nd in cestodes, the 4th in acanthocephales and the 3rd in trematoda. The coincidence in mobility is noted in the central part of gels on the level of the 4th fraction in cestodes (*N.dispar*), the 9th in acanthocephalas (*P.caucasicus*) and the 8th in trematoda (*H.brevicaca*). The latter is represented by a wider stripe.

Despite these coincidences, there are more differences in spectra, which is seen in the rate of migration, concentration of protein in fractions and, as mentioned above, in the number of fractions.

The revealed differences, on the one hand, may be caused by the quantity of proteins extracted from the homogenate, on the other hand, they are caused by species differences of helminths, connected with their structure, cycle of development, environment, metabolism etc. There are proteins in the extracts specific to the given species or class. In this connection we must mention the work of L.Mikulikova [8] who compared the protein spectra of trematodas, cestodes and nematodes from different hosts and showed that for each taxonomic class a definite quantity of proteins was characteristic, having the same properties of migration. Each species of helminths has its own definite protein spectrum [9-11].

The obtained results give us the first idea about the spectrum of water-soluble proteins *N.dispar*, *P.caucasicus* and *H.brevicaca*. Differences between the investigated species originate on the molecular level. They are revealed in the spectrum of water-soluble proteins on electrophoregrams and can be used as an additional factor of relative species with the aim of differentiation.

პარაზიტოლოგია და ჰელმინთოლოგია

მცირეაზიური ბაყაყის Rana macrocnemis Boul. ჰელმინთების წყალში ხსნადი ცილების შესწავლა

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ნაშრომში ელექტროფორეზის მეთოდით პოლიაკრილამიდის გელში შესწავლილია მცირეაზიური ბაყაყის ჰელმინთების წყალში ხსნადი ცილები. ტრემატოდის (Haplometra brevicaca), ცესტოდის (Nematotenia

dispar) და აკანტოცეფალას (Pseudoacanthocephala caucasicus) ცილოვან სპექტრში გადაადგილების მიხედვით გამოვლენილია როგორც მსგავსი (თანმხვედრი), ასევე განსხვავებული ფრაქციები. მიღებული შედეგები ჰელმინთების ხსნადი ცილების სახეობრივი თავისებურებებით არის გამოწვეული. ამდენად, პროტეინოგრამები სახეობების დიფერენცირების დროს შეიძლება გამოყენებული იქნეს როგორც დამატებითი კრიტერიუმი.

REFERENCES

1. *А. Л. Калабеков* (1973). – В кн.: Сборник зоологических работ, Орджоникидзе, с. 19-32, 35-45.
2. *А. Л. Калабеков* (1975). – В кн.: Проблемы паразитологии ч.1. Материалы VIII научн. конф. паразитологии, Киев, с.205-207.
3. *Т. А.Гинецинская, А. А. Добровольский* (1978), Частная паразитология, ч.1, М.: с. 284-287.
4. *К. М. Рыжиков, В. П. Шарпило, Н. Н. Шевченко* (1980), В кн.: Гельминты амфибий фауны СССР. М., с. 255-256.
5. *ც. ლომძე, ქ.ნიკოლაიშვილი., ნ. მელაშვილი* (2005), მე-3 საერთაშორისო სამეცნიერო კონფერენცია — „ბიოლოგიისა და მედიცინის აქტუალური პრობლემები“. შრომათა კრებული, თბილისი: თსუ გამომცემლობა, გვ. 106-109.
Ts. Lomidze, K. Nikolaishvili, N. Melashvili (2005), 3rd International Scientific conference “Actual Problems of Biology and Medicine”, Tbilisi, 106-109 (Georgian).
6. *O. N. Lowry et al.* (1951), J. Biol. Chem. **193**, 265-275.
7. *B. Devis* (1964), J. Ann. N-Y. Acad. Sci., **121**, 404-427.
8. *L.* Mikulikova (1979), Folia parasitologica, **26**, 2, 111-112.
9. *В. В.Клименко* (1966), Материалы ВОГ, ч. 2, с.117-118.
10. *В. В. Клименко* (1967), Бюлл. Всес. ин-та гельминтол., вып.1, с. 61-64.
11. *В. В. Клименко, И. В. Величко* (1972), Паразитология, вып. 3, с. 291-296.

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