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BIOCHEMICAL STUDIES ON THE NATURAL OBJECTS IN THE ORGANIZATION OF CHEMICAL EXPERIMENT AT SCHOOL

БИОХИМИЧЕСКИЕ ИССЛЕДОВАНИЯ НА ПРИРОДНЫХ ОБЪЕКТАХ ПРИ ОРГАНИЗАЦИИ ХИМИЧЕСКОГО ЭКСПЕРИМЕНТА В ШКОЛЕ

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Abstract. *This article describes the under-realized possibilities of using experimental research in the organization of research work in the school. The level of research work of students at the current stage of education development requires deep, well-founded and confirmed by concrete digital data results. Research work is one of the types of intellectual activity of students associated with the solution of creative and research tasks with the indication of the main stages of work. The teacher should deliberately direct their activities to the development and formation of students' research interests in chemistry and biology lessons, to create a common system of educational and educational work.*

Key words: *educational process, biochemical studies, chemical experiment.*

To achieve a high level of education and self-education, a great role should be given to the implementation of creative tasks, the development of scientific and educational projects, as well as research work, which form the practical orientation of learning and help to increase the cognitive activity of students. The teacher's possession of the methodology for organizing scientific research activities of students is the key element of improving school education. [1, 2].

For example, in grades 8-9, students study the composition of hemolymph using standard biochemical kits, and then in grades 10-11, they can also consider the composition of internal organs (hepatopancreas), as a result, students form a holistic idea of the metabolism occurring in the body. In the course of such work, students are not only study biochemistry, but also make conclusions about the regularities of metabolism in living organisms [3, 4].

The aim of the study is to substantiate the possibility of using biochemical methods for organizing research work of students at school on the example of studying biochemical parameters in the hemolymph and hepatopancreas of freshwater aquatic organisms.

When conducting an experiment, the teacher, together with the students, can change the goal and objectives of the planned research, while maintaining the basic structure of the experiment and the determined indicators, which allows students to work out the methods of experimental research and conduct them over several years, and then use the accumulated data for comparison. At the same time, the relevance, novelty, independence of research and a variety of topics are preserved using one object of research with the same determinable indicators. Examples of topics in

which the object of study is hemolymph and hepatopancreas, and the determined indicators of the content of total protein in hemolymph and hepatopancreas, and uric acid in hemolymph can be the following research works:

1. Influence of anthropogenic factors on the content of biochemical parameters in the tissues of pulmonary molluscs.

2 Content of key indicators of metabolism in the hemolymph of gastropods depending on the season of the year.

3 Influence of toxicants on biochemical parameters in the tissues of pulmonary molluscs, depending on the type of oxygen transport.

4. Comparative characteristics of the total protein content in mollusks living in water bodies of the Republic of Belarus.

5. Determination of the concentration of total protein in the hepatopancreas of pulmonary molluscs depending on the type of oxygen transport.

6. Influence of salts of heavy metals on the content of total protein in the hepatopancreas of gastropods depending on the season of the year.

Method for determining the concentration of uric acid in hemolymph.

Determination of the concentration of uric acid is carried out by the enzymatic method using standard biochemical kits, which include a buffer solution, an enzyme solution, a calibration solution with a uric acid concentration of 357 μmol / L.

Materials and methods: the hemolymph, buffer solution, enzyme solution, uric acid calibration solution, distilled water, a thermostat, a spectrophotometer (photoelectric calorimeter).

Experimental technique:

1. Prepare a working reagent by mixing reagent 1 (buffer solution) and reagent 2 (enzyme solution) in a ratio of 4: 1.

2. Measure and add 0.02 cm^3 of the hemolymph, calibrator and distilled water to 3 tubes for experimental, calibration and blank samples, respectively, then add 1 ml of working reagent to each tube.

3. Samples are mixed and incubated for 10 minutes in a thermostat at a temperature of + 37 ° C.

4. The optical density of the calibration and experimental samples is measured on a spectrophotometer or photocalorimeter at a wavelength of 510 nm against a blank sample.

5. The concentration of uric acid is calculated by the formula:

$$C_{op.} = (E_{op.}/E_{cal.}) \cdot 357$$

where, $C_{op.}$ – the concentration of uric acid in the studied hemolymph (μmol / l); $E_{op.}$ – optical density of the solution containing the investigated hemolymph; $E_{cal.}$ – - optical density of the solution containing the calibration solution; 357 – concentration of uric acid in the calibration solution (μmol / l).

Method for determining the concentration of total protein in the hemolymph.

The determination of the total protein concentration is carried out by the biuret method using standard biochemical kits, which include, a monoreagent, a calibration solution with a total protein concentration of 83 mg / ml.

Materials and methods: the hemolymph, monoreagent, total protein calibration

solution, distilled water, spectrophotometer (photoelectric calorimeter).

Experimental technique:

1. Measure and add 0.02 cm^3 of hemolymph, calibrator and distilled water to 3 test tubes for experimental, calibration and blank samples, then add 1 ml of monoreagent to each test tube.

2. Samples are mixed and incubated for 30 minutes at room temperature.

3. The optical density of the calibration and experimental samples is measured on a spectrophotometer or photocalorimeter at a wavelength of 540 nm against a blank sample.

4. The concentration of total protein is calculated by the formula:

$$C_{op.} = (E_{op.}/E_{cal.}) \cdot 83$$

where, $C_{op.}$ – the concentration of total protein in the studied hemolymph (g / l); $E_{op.}$ – optical density of the solution containing the investigated hemolymph; $E_{cal.}$ – optical density of the solution containing the calibration solution; 83 – concentration of total protein in the calibration solution (mg / ml).

Method for determining the concentration of total protein in the hepatopancreas

The method is based on measuring the intensity of the color that a protein solution gives in color reactions - biuret and Folin's reaction. When a protein interacts with an alkaline solution of copper sulfate ($CuSO_4$), complex compounds are formed (biuret reaction), which, with their tyrosine and cysteine radicals, reduce a mixture of phosphoric-tungstic and phosphoric-molybdic acids with the formation of a blue complex compound (Folin reaction). The color intensity of the complex, which depends on the amount of protein in the test sample, is measured on a photoelectric colorimeter with a red light filter.

***Equipment*:** tubes, graduated tubes, beakers, volumetric flasks for 50 and 100 cm3, pipettes for 1 and 2 cm^3 balance, centrifuge, spectrophotometer (photoelectric calorimeter).

Preparation of the reagents:

1. Solution A (2% Na2CO3 solution in 0.1 M NaOH solution).

2. 2% Na2CO3 solution in 0.1 M NaOH solution.

3.1% CuSO4 solution.

4.2% solution of sodium citric acid (Na3C6H5O7).

5. Solution B (prepared before determination) is a mixture of equal volumes of 1% $CuSO_4$ solution and 2% $Na_3C_6H_5O_7$ solution. Mix 25 cm^3 of a 31% solution of $CuSO_4$ and 25 cm^3 of a 2% solution of $Na_3C_6H_5O_7$.

6. Solution C (to be prepared before determination): add 1 cm3 of solution B to 50 cm3 of solution A.

7. Folin's reagent. The working solution of Folin's reagent is prepared by diluting the stock solution with distilled water in a 1:2 ratio (one part of Folin's reagent and two parts of distilled water). The working solution of Folin's reagent is stored in a sealed glass container at a temperature of 20 ° C for one week.

8. Standard solution of crystalline serum albumin 0.25 mg / ml in 0.1 M NaOH solution.

9. 1M NaOH solution.

10.6% solution of trichloroacetic acid (TCA).

The status of the work:

1. A sample of tissue weighing 100 mg is homogenized in a glass homogenizer or ground in a porcelain mortar with glass sand with a small amount of distilled water (about 1 cm^3).

3. Add distilled water so that the total volume of the homogenate is 10 cm3.

4. After 5 minutes, the homogenate is centrifuged for 10-15 minutes in an OPN-8 centrifuge at 5000-6000 rpm.

5. The centrifugate is poured into centrifuge tubes with a capacity of 20 ml.

6. For extraction and precipitation of proteins, an equal volume of 6% TCA solution is added to the centrifugate.

7. The solution is centrifuged in a CLN centrifuge for 10 minutes at 2500 rpm.

8. The protein precipitate is dissolved in 1-2 cm^3 of 1M NaOH.

9. The alkaline protein solution is transferred into graduated glass tubes, the volume is brought to 10 cm^3 with distilled water, mixed and used for the quantitative determination of the protein.

10. In chemical test tubes, place 0.1 cm3 of the test protein solution (2 parallel samples) and add 0.9 cm^3 of 30.1 M NaOH solution.

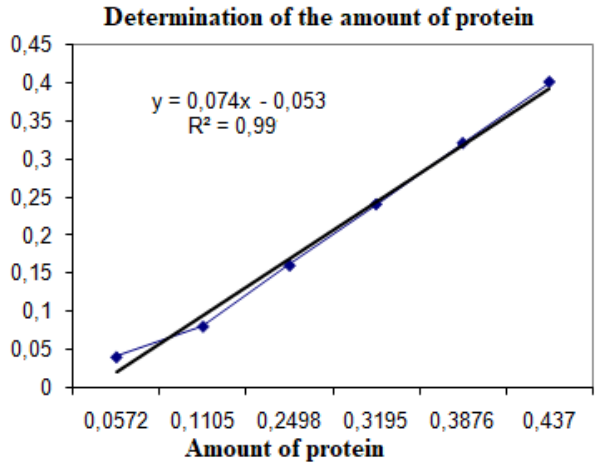
11. Add 0.1 cm^3 of distilled water and 0.9 cm^3 of 30.1 M NaOH solution to the blank sample.

12. In the experimental and blank test tubes, pour 2 cm^3 of the working solution C (a mixture of solutions A and B), mix well and leave for 10 minutes at room temperature.

13.Add 0.2 cm^3 of Folin's reagent and mix thoroughly immediately.

14. 30 minutes after the development of the color, the optical density (A) of the samples is measured on a spectrophotometer in a cuvette with a layer thickness of 1.0 cm at a wavelength of 750 nm, against a blank sample containing no protein.

15. Using the equation of the calibration dependence, calculate the iron content in this solution.



In the equation of the calibration graph for determining the protein content by the Lowry method y = 0.074x - 0.053, we substitute the obtained optical density results and calculate the protein concentration in the sample.

An example of a possible model experiment on the topic "The content of key indicators of metabolism in the tissues of pulmonary freshwater mollusks inhabiting water bodies of the Republic of Belarus"

The purpose of this work is to determine the content of total protein and uric acid in the tissues of freshwater lung molluscs living in the waters of the Republic of Belarus.

Materials and methods. Two types of freshwater pulmonary molluscs were used in the research - the common pond snail (*Lymnaea stagnalis*) and the horny coil (*Planorbarius corneus*). The studies were carried out on 144 freshwater lung molluscs, divided into two groups: 72 *Lymnaea stagnalis* and 72 *Planorbarius corneus*. Molluscs were collected from water bodies of four districts of the Vitebsk region (Table 1). Each research subgroup contained 9 molluscs. The collection was carried out in the autumn (September – October) and in the spring (April – May) period. Molluscs were collected manually.

Table 1

Places of collection of mollusks

Mollusk collection area	Place of collection	The name of the reservoir
Vitebsk region	c. Vitebsk	p. Vitsba
Dubrovensky region	v. Sheky	l. Afanasievskoe
Ushachsky region	v. Dubrovka	l. Dubrovskoe
Shumilinsky region	a/t Bashny	l. Budovest

An example of a possible model experiment on the topic "The content of key indicators of metabolism in the tissues of pulmonary freshwater mollusks inhabiting reservoirs of the Republic of Belarus"

To assess the state of the body, the key indicators of carbohydrate, nitrogen and lipid metabolism are determined and the rate of mobilization and utilization of energy substrates is studied under the influence of various anthropogenic factors.

The aim of the work is to determine the content of key biochemical parameters in the tissues of freshwater lung mollusks living in waters of the Republic of Belarus.

Material and methods. Two types of freshwater pulmonary molluscs were used in the research - the common pond snail (*Lymnaea stagnalis*) and the horny coil (*Planorbarius corneus*). The studies were carried out on 756 freshwater pulmonary molluscs, divided into two groups: 378 individuals of *Lymnaea stagnalis* and 378 individuals of *Planorbarius corneus*. Molluscs were collected from water bodies of four districts of the Vitebsk region (Table 1). Each research subgroup contained 9 molluscs. The collection was carried out in the autumn (September-October) and in the spring (April-May) period. Molluscs were collected manually.

Results and discussion. Mollusks from the Vitsba of the Vitebsk region are characterized by the following metabolic parameters (Table 2).

The concentration of uric acid is increased in the spring collection period by 1.5 times in the horny coil, and 2.9 times in the common pond snail compared to the autumn collection period. There were no statistical differences in the content of total protein in hemolymph in both types of mollusks. The total protein content in the hepatopancreas in the autumn period of collection exceeded the spring values of the

index by 1.4 times in *Pl. corneus* and 1.2 in *L. stagnalis*. Mollusks from lake Afanasyevskoe, Dubrovensky region, are characterized by the following metabolic parameters (Table 3).

Table 2

Metabolic indices in the hemolymph of *Planorbarius corneus* and *Lymnaea stagnalis* from the r. Vitsba, Vitebsk region (M ± m)

Indicators	Collection season	
	Spring (n=9)	Autumn (n=9)
Planorbarius corneus		
Total protein (hemolymph) (мg/мl)	37,04±0,52	33,31±0,46
Urine acid (μmol / l)	137,99±5,23[1]	92,14±2,02
Total protein (hepatopancreas) (мg/g)	189±7,1	256±8,2
Lymnaea stagnalis		
Total protein (hemolymph) (мg/мl)	14,03±0,22	15,87±0,25
Urine acid (μmol / l)	74,47±1,48[1]	25,46±0,64
Total protein (hepatopancreas) (мg/g)	271±7,6	323±21,7

Note – [1]p<0,05 compared to the autumn period of harvesting molluscs

Table 3

Metabolic parameters in the hemolymph and hepatopancreas of *Planorbarius corneus* and *Lymnaea stagnalis* from lake Afanasyevskoe, Dubrovensky region *(M±m)*

Indicators	Collection season	
	Spring (n=9)	Autumn (n=9)
Planorbarius corneus		
Total protein (hemolymph) (мg/мl)	33,40±0,63	31,24±0,65
Urine acid (μmol / l)	149,28±1,68[1]	82,46±2,16
Total protein (hepatopancreas) (мg/g)	123±5,2	139±8,6
Lymnaea stagnalis		
Total protein (hemolymph) (мg/мl)	13,14±0,33	14,14±0,17
Urine acid (μmol / l)	77,61±1,02[1]	35,31±0,49
Total protein (hepatopancreas) (мg/g)	196±4,7	228±7,8

Note – [1]p<0,05 compared to the autumn period of harvesting molluscs

Compared with the autumn collection period, the concentration of uric acid in the spring collection period is 1.8 times increased in the horny coil, and 2.2 times in the common pond snail. *Pl. corneus* and *L. stagnalis*, there were no statistically significant differences in the total protein content in hemolymph and hepatopancreas.

Mollusks from the lake Dubrovskoe, Ushachsky region, are characterized by the following indicators of metabolism (Table 4).

Pl. corneus, the uric acid content in the spring collection period increased by 1.5 times, and in *L. stagnalis* - by 2.5 times compared to the autumn period. No statistical differences were found when comparing two seasons of the year in terms of total

protein (hemolymph) in *L. stagnalis* and *Pl. corneus*. In the horny coil, the total protein content in the hepatopancreas in the autumn period of collection exceeded the values in the spring period by 1.4 times. No statistical differences were found when comparing two seasons of the year in the content of total protein (hepatopancreas) in *L. stagnalis*.

Table 4

Metabolic parameters in the hemolymph and hepatopancreas of *Planorbarius corneus* and *Lymnaea stagnalis* from Lake Dubrovskoe, Ushachsky region *(M±m)*

Indicators	Collection season	
	Spring (n=9)	Autumn (n=9)
Planorbarius corneus		
Total protein (hemolymph) (мg/мl)	35,36±0,95	35,14±0,60
Urine acid (μmol / l)	139,66±4,55[1]	96,36±2,36
Total protein (hepatopancreas) (мg/g)	150±7,3[1]	211±9,7
Lymnaea stagnalis		
Total protein (hemolymph) (мg/мl)	13,59±0,11	14,35±0,19
Urine acid (μmol / l)	72,58±1,30[1]	28,75±0,57
Total protein (hepatopancreas) (мg/g)	169±9,2	184±3,2

Note – [1]p<0,05 compared to the autumn period of harvesting molluscs

Molluscs from lake Budovest, Shumilinsky region, are characterized by the following metabolic parameters (Table 5).

Table 5

Metabolic parameters in the hemolymph and hepatopancreas of *Planorbarius corneus* and *Lymnaea stagnalis* from lake Budovest, Shumilinsky region *(M±m)*

Indicators	Collection season	
	Spring (n=9)	Autumn (n=9)
Planorbarius corneus		
Total protein (hemolymph) (мg/мl)	39,34±0,61	36,35±1,62
Urine acid (μmol / l)	157,82±4,52[1]	89,06±2,00
Total protein (hepatopancreas) (мg/g)	233±9,2[1]	205±7,5
Lymnaea stagnalis		
Total protein (hemolymph) (мg/мl)	14,48±0,28	14,93±0,24
Urine acid (μmol / l)	74,82±1,34[1]	30,36±0,76
Total protein (hepatopancreas) (мg/g)	164±6,0[1]	203±4,3

Note – [1]p<0,05 compared to the autumn period of harvesting molluscs

In *Pl. corneus*, the uric acid content in the spring collection period increased by 1.8 times, and in *L. stagnalis* by 2.5 times compared to the autumn period. There were no statistically significant differences in the content of total protein in hemolymph in *Pl. corneus* and *L. stagnalis* and in hepatopancass in *Pl. corneus*. The total protein level in the hepatopancreas in the spring period of collection exceeded the autumn values by 1.2 times in the pond snail.

Conclusion.

Changes in the content of key metabolic indicators in mollusks in the spring and autumn periods of collection can be associated with changes in the composition of the food supply, physical and physiological activity of organisms, and external influence of environmental factors. So the indicators in the autumn collection period are higher than in the spring due to the fact that in the fall the mollusks are preparing to fall into suspended animation and the body is actively accumulating nutrients. The low values of the studied parameters in spring are explained by the release of mollusks from suspended animation, during which aquatic organisms consumed the stored nutrients.

Based on the data obtained, an algorithm for establishing the ecological state of water can be created by analyzing simple and accessible methods for studying total protein, urea, and uric acid by three parameters - the season of analysis, habitat, and type of oxygen transport using two model organisms *Lymnaea stagnalis* and *Planorbarius corneus*.

Thus, our studies have shown that the content of total protein, urea, and uric acid in the hemolymph of two species of freshwater pulmonary molluscs, differing in the type of oxygen transport, naturally depends on the season and may differ due to the peculiarities of the chemical composition of the aquatic habitat.

Thus, pulmonary molluscs are convenient and most widely used objects for monitoring the biological state of aquatic ecosystems. In addition to their sensitivity to the actions of various physical, chemical and biological factors, one should also take into account the influence on the studied parameters of the season of the year and habitat. Changes in the metabolism of pulmonary freshwater molluscs are associated with the fact that they adapt to changing environmental conditions

The proposed experimental model contributes to the formation in students of a meaningful understanding of the essence of experiments, the sequence of work, the scheme for conducting experiments and compliance with their rules for safe conduct, predicting possible results, proving or refuting hypotheses, developing the ability to draw conclusions and generalize the material obtained. The use of a model experiment helps to develop the ability to observe facts and phenomena and explain their essence with the help of theories and laws, forms and improves experimental skills and abilities, instills the skills to plan their work and exercise self-control, contributes to general education, all-round development of the personality.

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Аннотация. *В данной статье рассказывается о недореализованных возможностях использования экспериментальных исследований при организации научно-исследовательской работы в школе. Уровень научно-исследовательских работ учащихся на современном этапе развития образования требует глубоких, обоснованных и подтверждённых конкретными цифровыми данными результатов. Научно-исследовательская работа является одной из видов интеллектуальной деятельности учащихся, связанной с решением творческой и исследовательской задач с указанием основных этапов работы. Учитель намеренно должен направляет свою деятельность на развитие и формирование исследовательских интересов учащихся на уроках химии и биологии, на создание общей системы учебной и воспитательной работы.*

Ключевые слова: *образовательный процесс, биохимические исследования, химический эксперимент.*

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