Relationship between the Distribution of Phosphorus Compounds in the Tissues of the Bivalve Anodonta cygnea and Environmental Phosphorus Level

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The chemical composition of surface waters, including their phosphorus content, is subject to great diversity conditioned by geological and soil conditions and by the influence of man's activity [1, 3, 4].

The observable fluctuations in the chemical composition of the aquatic environment are of considerable importance in the life of hydrobionts, in which the absorption of mineral matter directly from the water occupies a key place in the general balance. It has been established that a high ability to concentrate salts, including phosphorus, is a feature of invertebrates [10, 12, 13, 17]. It should be noted that the rate of absorption of mineral salts by invertebrates is appreciably dependent on environmental content [11, 15], and that the distribution of ions absorbed from the water in the body is not equal in different tissues [9].

Consequently, published data indicate the high capacity of invertebrates, including mollusks, to utilize mineral matter from the water. However, information on the interrelationship between the environmental level of phosphorus and the trend of its metabolism in the invertebrate organism is very scanty. In this connection we investigated the effect of an increased level of inorganic phosphorus in the water on some of the indices of phosphorus metabolism in bivalve tissues.

Material and research technique. The experiments were conducted in aquaria on tissues of the bivalve Anodonta cygnea.

We investigated the effect of raised concentrations of inorganic phosphorus in the water (0.3 and 0.6 mg/l). Phosphorus content in the control aquarium was 0.06 mg/l. Use was made in the experiment of mollusks with a mean mass of 48.1 g. Water temperature was 18 - 20°C, oxygen content 5.56 - 6.92 mg/l, CO_2 content 7.86 - 8.36 mg/l, pH 7.75 - 8.10. An increased phosphorus concentration in the experimental aquaria was created by adding suitable amounts of disodium phosphate. After the mollusks had spent 1, 3 and 7 days in the experimental medium a study was made in the mantle, hepatopancreas and gills of total phosphorus content, using the standard procedure [6], of inorganic phosphorus by the method of Lowrie and Lopez as modified by Skulachev [8], and of the adenine nucleotides of ATP, ADP and AMP by paper electrophoresis [16]. The Na^+ and K^+ activity of activated adenosine triphosphatase, and the Mg^{++} activity of combined adenosine

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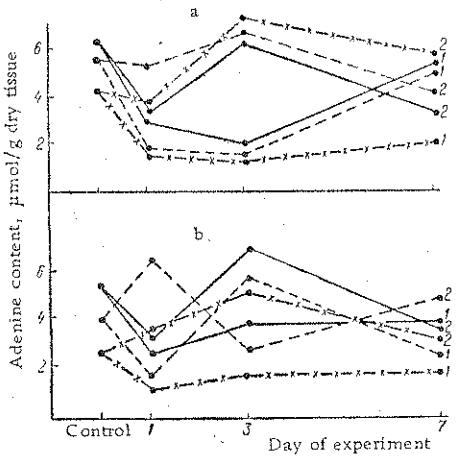
Table 1

Effect of increased phosphorus concentration in water on total phosphorus content in tissues of mollusks, mg% of dry tissue.

Day	Control	Phosphorus concentration in water, mg/l			
		0.3		0.6	
	$M \pm m$	$M \pm m$	p	$M \pm m$	p
		Mantle			
1st	1877,9±67,95	1777,50±66,95	>0,05	3074,70±136,8	<0,001
3rd	2100,70±178,50	3738,20±160,24	<0,001	2216,58±325,58	>0,05
7th	1989,30±79,01	4166,70±178,0	<0,001	3393,90±315,20	<0,001
		Hepatopancreas			
1st	2573,50±199,3	1955,90±58,9	<0,05	3593,80±181,40	>0,05
3rd	3253,40±185,80	3494,60±215,4	>0,05	3768,90±247,0	>0,05
7th	2913,60±480,30	2907,60±119,0	>0,05	3484,40±367,5	>0,05
		Gills			
1st	5386,00±257,9	6461,30±269,0	<0,05	5915,70±178,5	>0,05
3rd	6371,00±263,30	5118,40±180,0	>0,05	5252,20±357,00	>0,05
7th	5878,50±384,50	4950,52±241,0	>0,05	6370,50±166,30	>0,05

Note. Here and in Tables 2 and 3 invariably n = 5.



Effect of various phosphorus concentrations in the medium on ATP, ADP and AMP content in the tissues of mollusks:

A, mantle; B, hepatopancreas; 1, 2, phosphorus concentrations in water respectively 0.3 and 0.6 mg/l; C, control; _____ ATP: - - - ADP; - X - X AMP.

triphosphatase were assessed from the increase of inorganic phosphorus in the incubation medium. The data were statistically processed [5].

Table 2

Effect of increased phosphorus concentration in water on inorganic phosphorus content in the tissues of mollusks, mg% dry tissue.

Days	Control	Phosphorus concentration in water, mg/l			
		0,3		0,6	
	$M \pm m$	$M \pm m$	P	$M \pm m$	P
		Mantle			
1st	260,55±12,52	106,25±15,0	<0,001	442,53±32,18	<0,001
3rd	300,60±26,83	221,70±29,25	>0,05	292,45±6,79	>0,05
7th	220,49±21,12	649,76±24,52	<0,001	212,12±12,12	>0,05
		Hepatopancreas			
1st	334,70±7,84	161,80±23,52	<0,001	336,00±28,20	—
3rd	342,50±27,40	354,84±39,79	>0,05	487,85±25,0	<0,05
7th	326,92±20,50	682,60±80,00	<0,001	359,37±25,0	>0,05
		Gills			
1st	525,74±10,63	707,74±10,63	<0,001	409,88±22,09	<0,05
3rd	312,90±40,00	273,68±12,63	>0,05	582,28±30,38	<0,001
7th	447,18±43,66	797,03±7,93	<0,001	358,43±14,45	<0,05

RESEARCH RESULTS AND DISCUSSION

The experiments showed that an increase in the level of phosphorus in the water appreciably influenced its entry into the organism of the mollusks and its distribution there. A typical tissue reaction of their glandular organs to an increase of phosphates in the water was established. Thus, total phosphorus in the mantle of the mollusks was appreciably increased from the third day of the research when there was 0.3 mg/l of phosphorus in the medium (Table 1). The rate of total phosphorus accumulation in the mantle increased with the time spent by the mollusks in such a medium. It should be noted that when phosphate content in the water was increased to 0.6 mg/l the amount of total phosphorus in the mantle began to increase on the very first day under these conditions.

No significant changes of total phosphorus in the hepatopancreas of the mollusks were observed throughout the research period when phosphorus concentration in the medium was 0.3 and 0.6 mg/l, with the exception of the first day of the research. Total phosphorus content in the gill tissue rose considerably (by up to 20%) only on the first day spent by the mollusks in water with a phosphorus concentration of 0.3 mg/l; thereafter it was close to the control value.

An increase of organic phosphorus content in the water to an amount 5 - 10 times its content in natural water has an appreciable effect on the trend of bioenergetic processes in the tissues of the mollusks (Fig.). This effect was most marked in the mantle. Thus, ATP content in the mantle of Anodonta kept in a medium containing 0.3 mg/l of phosphorus declined by practically 68% in the first three days. When phosphorus concentration in the water was increased to 0.6 mg/l, the ATP level in the mantle also declined from the very first days, remaining at a low level in the subsequent seven day period of adaptation. When phosphorus concentration in the water was 0.3 mg/l the tissue ATP and AMP content in the mantle was also below content in the control. Conversely, ATP content in the hepatopancreas declined only on the first day of the experiment for the above concentrations of phosphorus in the water. On other days the difference in the amount of ATP was statistically insignificant. The dynamics of tissue distribution of ADP and inorganic phosphorus justifies the assumption that there was a switch of the processes of oxidative phosphorylation and glycolysis in the

Table 3

Effect of increased phosphorus concentration in water on activity of Na^+, K^+ and Mg^{++}-ATP-ase in tissues of mollusks, μg P/mg of protein in 1h.

Days	Control	Phosphorus concentration in water, mg/l			
		0.3		0.6	
	$M \pm m$	$M \pm m$	P	$M \pm m$	P
Mantle					
1st	0,75±0,15	2,70±0,30	<0,001	—	
3rd	0,60±0,09	1,08±0,24	>0,05	0,33±0,06	>0,05
7th	0,90±0,18	1,80±0,15	<0,05	3,25±0,04	<0,001
Hepatopancreas					
1st	1,22±0,09	1,48±0,06	<0,05	0,54±0,16	>0,05
3rd	1,24±0,09	0,54±0,08	<0,001	0,23±0,03	<0,001
7th	1,23±0,09	2,02±0,19	<0,05	1,65±0,04	<0,05
Gills					
1st	0,97±0,25	2,70±0,15	<0,001	4,24±0,34	<0,001
3rd	1,08±0,12	1,27±0,09	>0,05	1,07±0,03	>0,05
7th	0,86±0,18	5,84±0,33	<0,001	1,10±0,07	>0,05

hepatopancreas when the mollusks found themselves in a medium with a high phosphorus content.

Thus, with 0.3 mg/l of phosphorus in the water the amount of inorganic phosphorus in the hepatopancreas of the mollusks (Table 2) increased appreciably (almost three-fold) by comparison with its level in the controls, which is one essential factor for the switching of oxidation processes to glycolytic processes, but only after the animals had spent seven days under these conditions. With 0.6 mg/l of phosphorus in the medium glycolytic processes predominated over oxidation processes after three days of adaptation. ADP level in the hepatopancreas was lower during these periods than in the control.

The effect of increased inorganic phosphorus concentrations in the water on the content of macroergic phosphorus compounds is less manifested in gill tissue. Thus, the amount of ATP in the gills altered only when they were in the medium containing 0.3 mg/l of phosphorus. It is of interest to note that whereas ATP content in the gills declined during the initial period of adaptation to 0.3 mg/l, it was above the control value after a longer period (three days). A tendency toward increase is to be noted in the dynamics of inorganic phosphorus in the glandular tissue of the gills of mollusks kept in a medium containing 0.3 mg/l of phosphorus throughout the seven day adaptation period. The highest values for inorganic phosphorus in the gill tissue were reached at the end of the research (80% above the control). When phosphorus concentration in the water was raised to 0.6 mg/l the content of inorganic phosphorus in the gills was increased only with a three day adaptation period. ADP level was little affected, and was below the control only during the first days of the experiment at 0.3 mg/l of phosphorus in the medium.

Under the influence of increased phosphorus content in the environment of the fresh water mollusk Anodonta cygnea appreciable changes are also to be noted in the activity of Na^+, K^+, Mg^{++}-ATP-ase (Table 3). The nature of these changes in the tissues was dependent on phosphorus level in the water. Thus, the activity of the enzyme in the mantle was raised throughout a seven day period of their adaptation to 0.3 mg/l of phosphorus. However, when phosphorus content in the water was higher (0.6 mg/l) ATP-ase activity increased only at the end of the

research. Activity of Na^+, K^+, Mg^{++}-ATP-ase declined sharply with a three day adaptation period to 0.3 mg/l of phosphorus.

Nevertheless, when the time spent by the invertebrates in the medium containing 0.3 mg/l of phosphorus was increased, the activity of the enzyme rose far above the control level (by 60%). Phosphorus content in the water, 0.6 mg/l, immediately caused a reduction of adenosine triphosphatase activity in the hepatopancreas. Although the activity of the enzyme was increased by the end of the research, the increase was less than in animals from the medium with 0.3 mg/l of phosphorus. Conversely, ATP-ase activity in the gills of the experimental mollusks was raised only when there was a daily increase of phosphorus concentration in the water, and on the seventh day at 0.3 mg/l. For other periods the activity of the enzyme was similar to the activity in the control animals. The alterations of adenosine triphosphatase activity noted are an indication of the intensified functional activity of the mantle and hepatopancreas under the influence of increased environmental phosphorus content.

Consequently, the research has established the dependence of the metabolism of phosphorus compounds, including macroergic compounds, in the bivalve Anodonta cygnea on environmental phosphorus concentration. It must be noted that, as has been shown by previous research [7], the mollusks tolerate wide ranges of variation in the calcium content of the water thanks to their ability to utilize it. However, they exhibit appreciably higher reactivity to a relatively small increase of environmental phosphorus. Appreciable differences were found in the tissue reactions of the mantle, hepatopancreas and gills. The most significant changes in the distribution of the phosphorus compounds were found in the mantle tissue. Thus, alongside increased total phosphorus content in it, a clearly expressed fall was noted in tissue ATP content against the background of appreciable activation of Na^+, K^+ and Mg^{++}-ATP-ase. The observable changes in the metabolism of phosphorus compounds in the mantle may be explained by the role of phosphorus in the metabolism of mineral substances, including phosphorus. Such a conclusion is in agreement with the data of those researchers who have noted increased mantle function in ion absorption [14]. It should be noted that the nature of the changes in the metabolic processes of the organs investigated during the first days of adaptation to increased phosphorus level in the water and after a longer period (seven days) differed appreciably.

REFERENCES

1. Almazov, A. M., A. I. Denisova and Yu. G. Maystrenko. 1967. Gidrokhimiya Dnepra i ego vodokhranilishch. (The hydrochemistry of the Dnieper and its reservoirs). Kiev, Nauk. dumka Press, 316 pp.
2. Asatiani, V. S. 1953. Biokhimicheskiy analiz. (Biochemical analysis), part 1, Tbilisi, Gruzmedgiz, 942 pp.
3. Astapovich, I. G. 1968. The effect of the intensification of fish management on the hydrochemical regime, primary production and fish productivity of ponds in Byelorussia. Author's abstract of candidature thesis, Moscow, 26 pp.
4. Konenko, A. D. 1952. Gidrokhimicheskaya kharakteristika malykh rek USSR. (A hydrochemical description of the small rivers of the Ukraine). Kiev, Ukrainian Acad. Sci. Press, 171 pp.
5. Mountsevichyute-Eringene, Ye. V. 1964. Simplified mathematical statistical methods in medical research. Patolog. fiziologiya i eksperim. terapiya, No. 4, pp. 71 - 74.
6. Petrun'kina, A. M. 1961. Fraktichеskaya biokhimiya. (Practical biochemistry). Moscow, Medgiz, 425 pp.

7. Romanenko, V. D., O. M. Arsan, V. L. Tsiomatina, S. P. Vesel'skiy, V. F. Pryadko and N. Yu. Yevtushenko. 1977. The role of calcium ions in the metabolism of Anodonta cygnea. Gidrobiol. zhurn., 13, No. 5, pp. 115 - 119.
8. Skulachev, V. P. and L. L. Kiselev. 1960. Oxidation with and without phosphorylation, repeated uptake. Biokhimiya, 25, No. 1, pp. 90 - 96.
9. Allen, J. A. 1970. Experiments of the uptake of radioactive phosphorus by bivalves and its subsequent distribution within the body. Comp. Biochem. and Physiol., 36, No. 1, pp. 131 - 141.
10. Coffin, C. C., F. R. Hayes, L. H. Jordey and S. G. Whitenway. 1949. Exchange of materials in a lane studied by the addition of radioactive phosphorus. Can. J. Res., No. 1, pp. 27 - 32.
11. Greenaway, P. 1971. Calcium regulation in the freshwater mollusk Limnea stagnalis (L.). (Gastropoda. Pulmonata). J. Exp. Biol., 54, No. 3, pp. 609 - 620.
12. Fletcher, G. L. 1971. Accumulation of yellow phosphorus by several marine invertebrates and seaweed. J. Fish Res. Board. Can, 28, No. 5, pp. 793 - 796.
13. Nduku, W. K. and A. D. Harrison. 1976. Calcium as a limiting factor in the biology of Biomphalaria preifferi. Hydrobiologia, 49, No. 5, pp, 143 - 170.
14. Rumsey, T. J. 1972. Osmotic and ionic regulation in a terrestrial snoril Pomatiaselegans (Gastropoda. Prosobranchia) with a note on some tropical Pomatiasidae. J. Exp. Biol., 57, No. 1, pp. 205 - 215.
15. Thomas, J. D. and G. Louph. 1974. The effect of external calcium concentration on the rate of uptake of this ion by Biomphalaria glabrata (Say). J. Anim. Ecol., No. 3, pp. 861 - 871.
16. Stransky, L. 1963. Determination of the adenine nucleotides by paper electrophoresis. J. of Chromatography, 10, No. 4, pp. 456 - 462.
17. Van der Borght O. and S. Van Puymbrolck. 1966. Calcium metabolism in freshwater mollusk: quantitative importance of water and food as supply for calcium during growth. Nature, 210, No. 5038, pp. 791 - 793.

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