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THE INFLUENCE OF ELECTROLYTES OF THE IONS AND THE INHIBITOR ON THE ACTIVITY OF TOTAL ATPASE OF ERYTHROCYTES OF BROILER CHICKENS

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Abstract. The total ATPase activity of erythrocytes of broiler chickens depends on the concentration of ions Na^+ and K^+. The ATPase activity at 76.5% due to magnesium ions, 96.5% of the sodium ions and 47.6% of potassium ions. Maximum ATPase activity was observed in incubation medium containing ions: Na^+ 120 mmol/ml, K^+ 20 mmol/ml; Mg^{2+} - 3.0 mmol/ml and was of 9,24±0,23 nmol •mg protein^{-1}•min^{-1}. The ATPase activity has no effect of the specific inhibitor – strophanthin-K in the concentration range of 0-100 mg•l^{-1} in the medium containing ions Na^+ and K^+, and in an environment without them.
Key words. ATPase, ions of electrolytes, ATPase inhibitor, broiler chickens

One of the main properties of living cells is the selective transport of substances and energy into the cell from the external environment, in which the leading role plays the active transport carried out by enzyme systems of membranes (ion pumps), integral components of which are ATPases. ATP-dependent ion pumps, representing a complex of enzymes that provide both primary transport of cations (H^+, Na^+, K^+, Ca^{2+}) and anions (Cl^-, $HCO3^-$) against their electrochemical gradients, and secondary active transport through the membrane into the cell sugars, amino acids, organic acids, due to the energy of transmembrane concentration gradient of Na^+ ions. With the work of ATPase also linked the generation of the currents, transmembrane potential and the transmission of nerve impulses, the processes of conjugation of oxidative phosphorylation. Topical is studying the effect of different ions on the ATPase activity [1-8].

In connection with the above topic of our work was to study the effect on the total ATPase activity of erythrocytes of broiler chickens ions of electrolytes and strophanthin-K.

The study was carried out on chickens-broilers cross "ISA". Conditions of keeping and feeding of animals in conformity with applicable regulations. Blood for research was taken from the veins of the neck. As an anticoagulant used environment Elswere. Stable the blood was placed in the flask in melting ice (+4 ^{0}C) and 20-30 min were delivered to the lab for research. Separation of erythrocytes from plasma was performed by centrifugation in a refrigerated centrifuge (+4 ^{0}C) for 30 min at 1000 rpm after separation of Erythrocytes from plasma was twice washed with physiological solution (pH of 7.4). ATPase activity is determined by the increase in concentration of inorganic phosphate after incubation at 37^{0}C for 45 minutes and expressed as nanomoles of inorganic phosphate (Pi), 1 mg of protein cleaved per minute. Inorganic phosphate and protein in the homogenate was determined by spectrophotometric methods [9-13].

We conducted a series of experiments to identify the optimal concentration and ratio of ions of the electrolytes in the incubation medium for determination of total ATPase activity of erythrocytes of birds, the effects of inhibitors on the activity of ATPase (Fig. 1-3, tab. 1). In result of the conducted researches it was established, ions of sodium, potassium and magnesium exerted an activating effect on ATPase activity in different concentration ranges, so the maximum ATPase activity is seen when concentrations of ions: sodium – 115-145 (Fig. 4) of potassium ions is 19-28 (Fig. 5), magnesium ions is 2.0 – 4.0 mmol•ml^{-1} (Fig. 6).

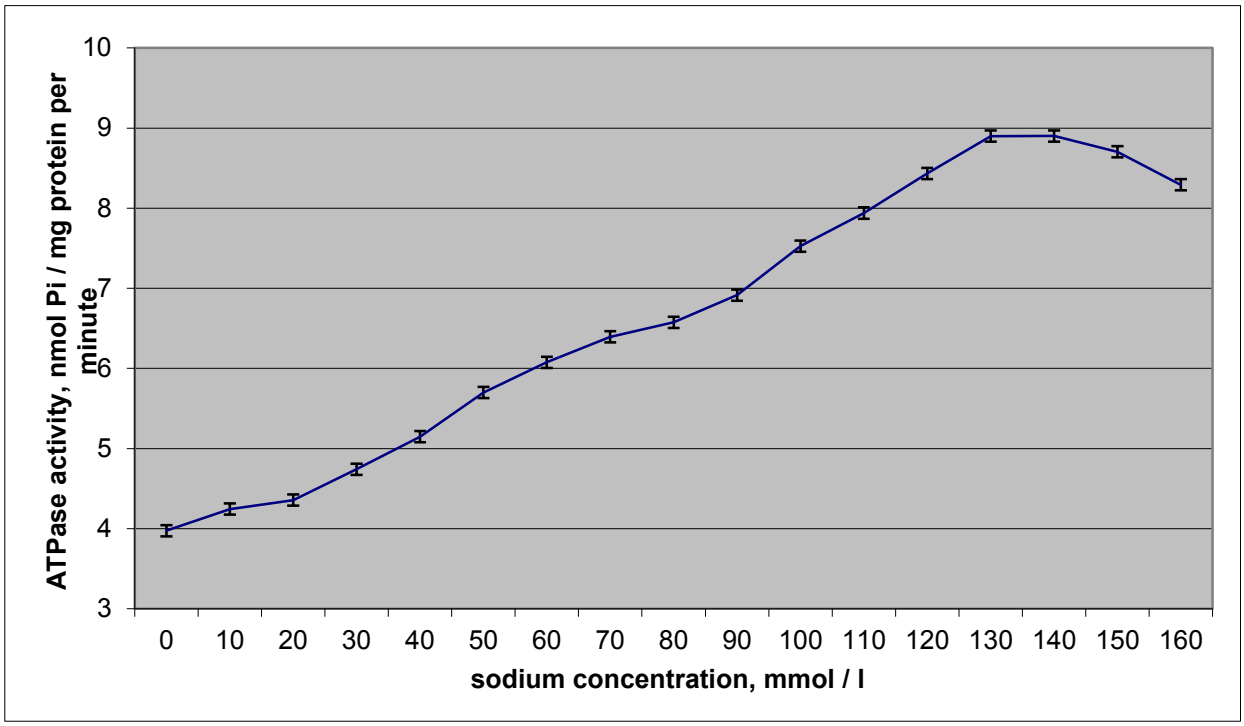

Figure 1. The effect of sodium ions on the activity of ATPase, (n=10), P≤0,05.

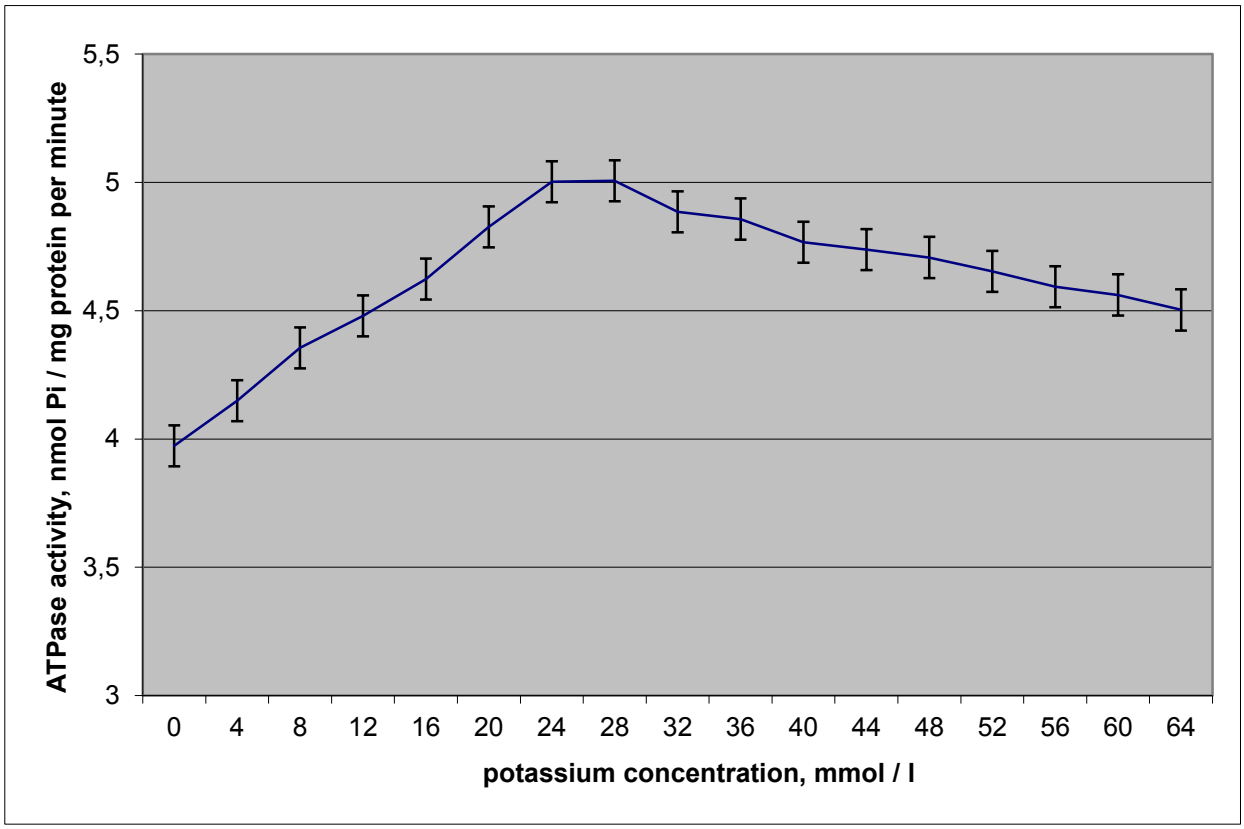

Figure 2. The influence of potassium ions on the ATPase activity, (n=10), P≤0,05.

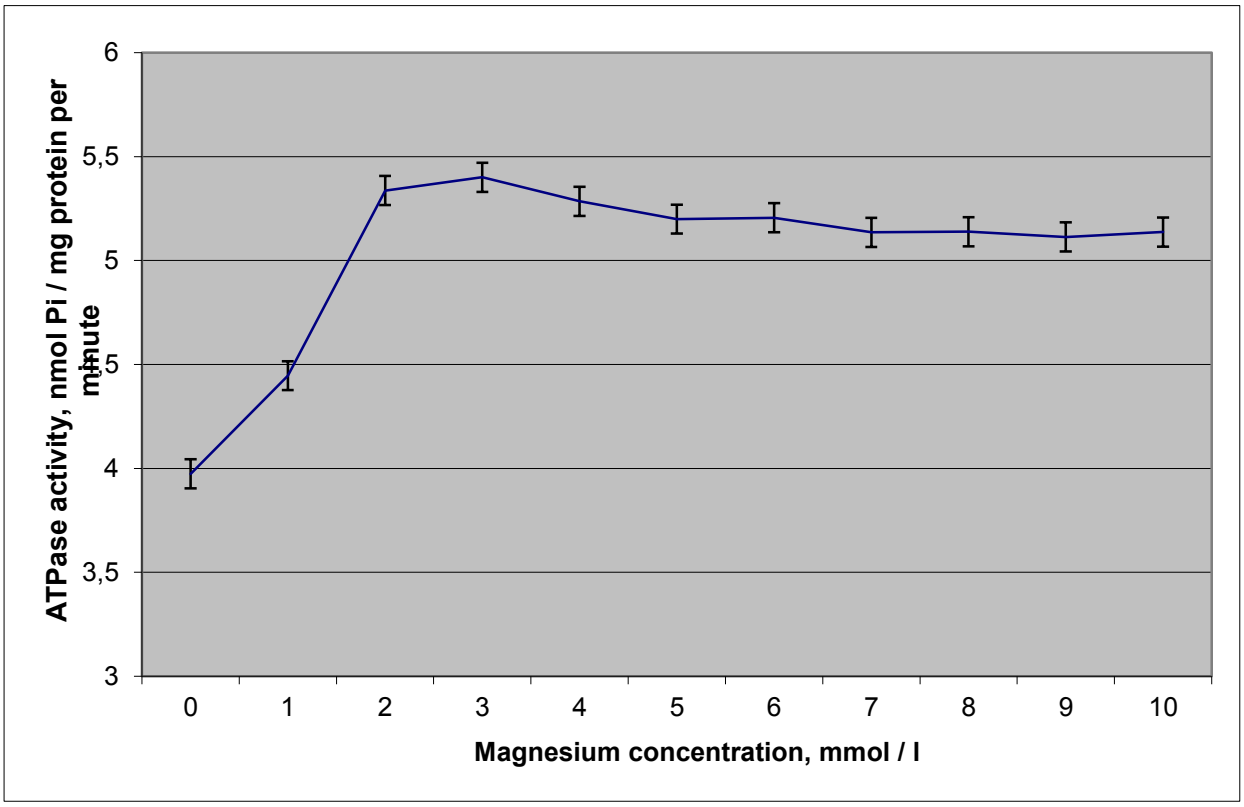

Figure 3. Influence of magnesium ions on the activity of ATPase, (n=10), P≤0,05.

To establish the degree of influence of the ions Na^+, K^+ and Mg^{2+} on the activity of ATPase was conducted by one-way ANOVA. As independent variable was defined as the activity of ATPase in the presence in the incubation medium of the corresponding ion. For a zero reference point taken was the activity of the enzyme in the medium without addition of ions. The studies were established the coefficients of determination of the enzyme activity from independent factors.

The ATPase activity was at 76.5% due to magnesium ions, 96.5% of the sodium ions and 47.6% of potassium ions. The study of optimal combinations of concentrations of these ions showed that the maximal ATPase activity was observed in incubation medium containing Na^+ 120 mmol•ml, K^+ 20 mmol•ml; Mg^{2+} - 3.0 mmol•ml (table. 1) and was of 9.24± 0,23 nmol •mg protein^{-1}•min^{-1}.

Table 1.

The effect of combinations of ion composition on the activity of ATPase, (n=10)

Number environment	Jonah incubation medium	The activity of ATPase, Pi nmol • mg protein^{-1} • min^{-1}
1	Na^+, K^+, Mg^{2+}	9,33±0,07***
2	Na^+, Mg^{2+}	8,42±0,07***
3	K^+, Mg^{2+}	7,18±0,09***
4	Mg^{2+}	5,40±0,06***
5	-	3,97±0,08

*** - P≤0.001 compared to the ATPase activity in the medium No. 5

To evaluate the influence of combinations of ions on ATPase activity was performed one-way ANOVA. As the independent variable was determined by the ionic composition of the incubation medium. For a zero reference point taken was the activity of the enzyme in the medium without addition of ions. The studies were established the coefficients of determination of the enzyme activity from independent factors. The ATPase activity of 92% deterministic (P≤0.05) added to the incubation medium of the ions Mg^{2+}, 98% addition of K^+ ions, Mg^{2+} and 99% ions Na^+ , Mg^{2+} and Na^+, K^+, Mg^{2+}. The obtained results indicate that sodium ions play a major regulatory role in enzyme activity. Due to the

fact that the true substrate ATPase is a complex of Mg^{2+}-ATP magnesium ions also had a significant impact on the activity of ATPase. At the same time, potassium ions exhibit the least effect on enzyme activity.

The analysis of these data suggests that the magnitude of the transport of substances, and, consequently, the ATPase activity significantly depends on the quantity of sodium and magnesium in plasma. Thus, the excess or deficiency of these ions in the feed of broiler chickens can affect the level of intracellular metabolism, causing significant violations. Due to the fact that that the use of ouabain (strofantina G) [10] does not substantially suppress the ATPase activity of avian erythrocytes, we have studied the influence strofantina present-K ATPase.Strophanthin -G and -K – alkaloids, isolated from the seeds of tropical vines of Strophanthus gratus and Strophanthus kombe, respectively. Strophanthin-K is different in chemical structure from strophanthin-G carbohydrate portion, respectively b-D-glucose (or b-D-Cimarosa) and α-L-rhamnose. Strophanthin-K in our studies did not exert statistically significant (P≤0.05) effect on the activity of ATPase of erythrocytes in a medium containing sodium and potassium ions, and environments without them (Fig. 4).

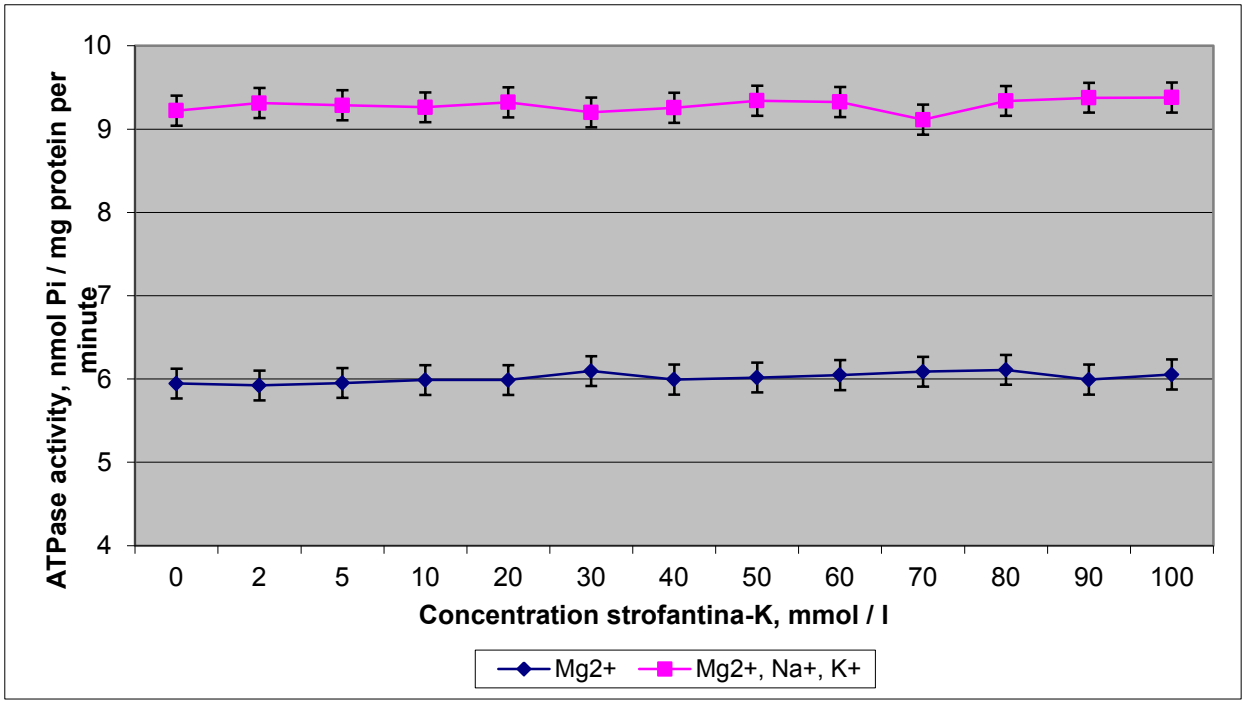

Figure 4. The effect of strophanthin-K on the activity of ATPase in the absence of Na^+ ions and K^+ in the medium containing these ions in optimum concentration (n=10), P≤0,05.

This is confirmed by one-way ANOVA analysis of the data, the coefficient of determination of the effect of strophanthin-K in the environment without ions on the activity of ATPase was 3,9% (P>0,05), in the environment with ions Na+, K+, Mg2+ and 2,5% (P≤0,05). Regression analysis showed that the equations of the ATPase activity of linear and almost parallel to the x-axis, which confirms the lack of dependence of enzyme activity on the concentration of the inhibitor in medium containing ions Na+ and K+, and without them (Fig. 5 and 6).

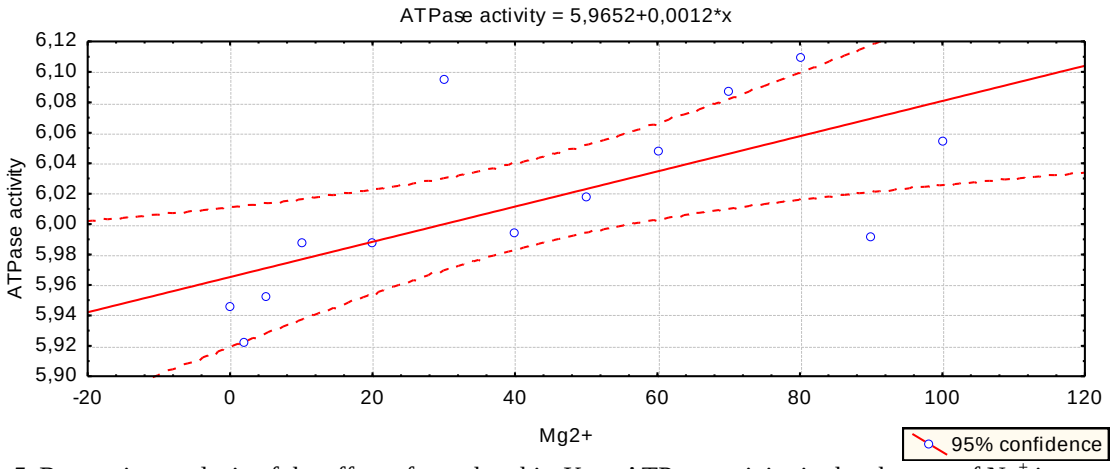

Figure 5. Regression analysis of the effect of strophanthin-K on ATPase activity in the absence of Na^+ ions and K^+.

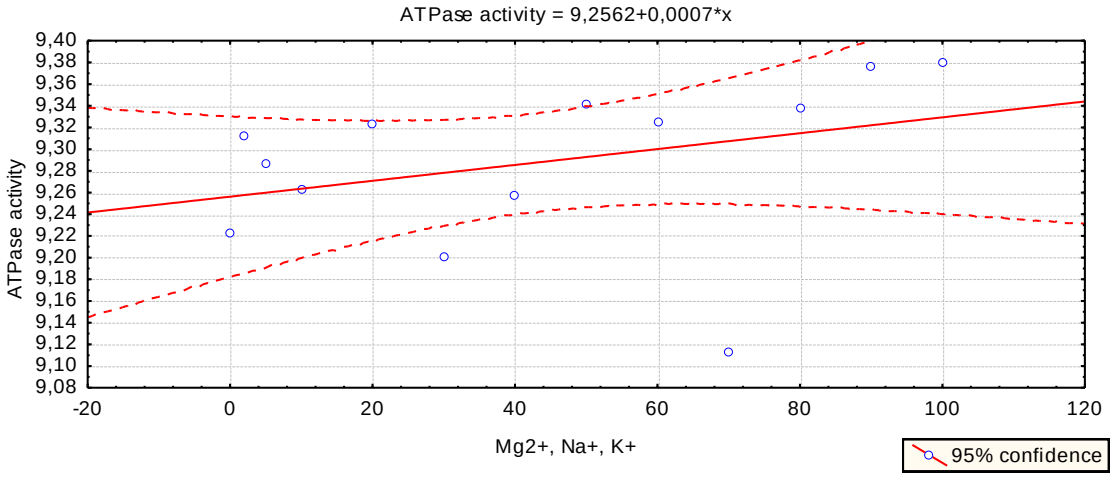

Figure 6. Regression analysis of the effect of strophanthin-K on ATPase activity in the medium containing ions Na^+ and K^+.

Thus, studies have shown that the activity of ATPase of erythrocytes of broiler chickens against specific concentrations of ions sodium, potassium and magnesium. Thus, the greatest activating effect on the ATPase activity seen at concentrations of ions: sodium – 115-145, potassium ions – 19-28, of magnesium ions is 2.0 – 4.0 mmol•ml-1. The activity of the ATPase at 76.5% due to magnesium ions, 96.5% of the sodium ions and 47.6% of potassium ions. Maximum ATPase activity was observed in incubation medium containing ions: Na^+ 120 mmol•ml, K^+ 20 mmol•ml; Mg^{2+} - 3.0 mmol•ml and was of 9.24±Fn 0,23 nmol •mg $protein^{-1}$•min^{-1}. The ATPase activity has no effect of the specific inhibitor – strophanthin-K in the concentration range of 0-100 mg•l^{-1} in the medium containing ions Na^+ and K^+, and in an environment without them.

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