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THE CHANGES OF ACTIVITY OF SEPARATE CARBOHYDRATE METABOLISM ENZYMES IN BLOOD ERYTHROCYTES OF PREGNANT RATS UNDER VANADIUM CITRATE

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The data on the effect of vanadium citrate, which is organic, on carbohydrate metabolism and the content of estradiol in the blood of rats, as well as the relationship between these indicators are missing. Therefore, the aim of the work was to study the effect of this compound on the activity of glucose-6-phosphate dehydrogenase, lactate dehydrogenase, estradiol content and to establish correlations between the activity of the studied enzymes and the estradiol content in pregnant female rats under the effect of vanadium compound. The study was carried out on 25 female Wistar rats Rattus norvegicus, divided into five groups: group I - non-pregnant females, group II - pregnant females consuming pure water without additives, groups III, IV, V - females which during the mating and pregnancy periods received the solution of vanadium citrate at the concentrations of 3.75, 15.63 and 62.5 μgV/kg of body weight, respectively. Our studies have shown that glucose-6-phosphate dehydrogenase, lactate dehydrogenase and estradiol content increase in pregnant females of group II compared to group I of non-pregnant animals. The increase in the activity of carbohydrate metabolism enzymes in pregnant females of group II may be due to the activation of the pentosophosphate pathway and the intensification of glycolysis in erythrocytes. In pregnant females, the effect of vanadium citrate resulted in the decrease in the activity of glucose-6-phosphate dehydrogenase and lactate dehydrogenase in the blood of animals in groups IV and V, and the content of estradiol in the animals of all three experimental groups (III, IV, V), compared to group II of pregnant females. It has also been established that under the effect of this compound between glucose-6-phosphate dehydrogenase and lactate dehydrogenase activity in pregnant females there is a strong reverse correlation (r = -0.92), between lactate dehydrogenase activity and estradiol content – a weak correlation (r = -0.42), while between the content of estradiol and glucose-6-phosphate dehydrogenase activity there is a strong direct correlation (r = 0.74). The values of glucose-6-phosphate dehydrogenase activity in the animals of group IV, which received the solution of vanadium citrate at the concentration of 15.63 μgV/kg of body weight, was similar to that of the animals in group I. This may indicate the normalizing effect of vanadium citrate on the activity of glucose-6-phosphate dehydrogenase at the indicated concentration, which is probably due to the ability of the vanadium microelement to inhibit the activity of this enzyme. The decrease in lactate dehydrogenase activity in groups IV and V, compared to group II, may also be due to the effect of this compound. Vanadium citrate at the concentration of 15.63 μgV/kg of body weight caused the recovery of the enzyme activity to the values of the activity in group I. At this concentration, the most remarkable positive effect on the enzyme activity of carbohydrate metabolism and the content of estradiol is observed. Our studies allow to consider this vanadium compound as a potential dietary supplement for pregnant women.

Keywords: correlation, glucose-6-phosphate dehydrogenase, lactate dehydrogenase, estradiol, vanadium citrate, rats, blood.

Introduction. Endocrine and metabolic changes are the result of physiological processes that occur in the maternal and fetal organisms. A disorder of carbohydrate metabolism in blood is often the reason for abnormalities during the pregnancy course and fetal development (Kuliszkiewicz-Janus M., Zimny A., 2003). It is known that the activity of carbohydrate metabolism enzymes in placenta is low, which is the basis for searching new therapeutic agents as additional dietary supplements for the pregnancy relief (Burton G.J., Jauniaux E., 2004).

In the period of pregnancy, the prooxidant processes increase in the organism, which is manifested by the rise in metabolism activity of placental mitochondria and depletion of the antioxidant system. These processes lead to a prooxidant-antioxidant imbalance, which is accompanied by the appearance of oxidative stress (Bizerea T.O. et. al., 2018). In the normal course of pregnancy, the regulation of oxidative stress is provided by compensatory antioxidant mechanisms, but with the intensification of prooxidant processes, the regulation of these pathways is violated (Toescu V. et al., 2002). Glucose-6-phosphate dehydrogenase is one of the most important enzymes that protect cells from oxidative stress (Kuliszkiewicz-Janus M., Zimny A., 2003). It is capable of detoxifying reactive oxygen species produced endogenously or exogenously.

As energy-consuming processes in the mother's body increase during the pregnancy period, the high activity of glucose-6-phosphate dehydrogenase revealed in the study can be a consequence of the intensification of these processes. Moreover, it is known that glucose-6-phosphate dehydrogenase is involved in the processes of NADP recovery which is used in the processes of the synthesis of fatty acids, steroids and is oxidized by enzymes. (Frosal S. et al., 2004). The benefit of determining the concentration of estradiol is due to the regulatory influence of female sex hormones on the carbohydrate metabolism. At the same time, LDH is a good diagnostic indicator of the clinical condition of the body during the pregnancy period.

To improve therapeutic efficiency, many new vanadium compounds are synthesized. These compounds are included in active and functional enzyme centers, and are also important for tyrosine phosphorylation and cellular signaling. In addition, various forms of vanadium have a significant effect as cofactors that inhibit the function of many enzymes (glyceraldehyde-3-phosphate dehydrogenase, lipoprotein lipase, tyrosine phosphorylase, glucose-6-phosphate dehydrogenase, glycogen synthase, adenylate cyclase and cytochrome oxidase) and stimulate the function of others (Na^+/K^+-ATPases, H^+/K^+-ATPases, myosin-ATPases, dinein, adenylyl kinase, phosphofructokinase and cholinesterase) (Korbecki J. et al., 2012). There are a lot of data about normoglycemic property of vanadium, its ability to regulate the carbohydrate metabolism in total, its participation in immunoregulatory mechanisms (immunosuppression, inflammation) (Crans D.C. et al., 2004; Zwolak I., 2014).

This trace element affects the reproductive function of the organism. In particular, the authors Wang Y.X. et al. noticed the relationship between the vanadium content in the plasma of seminal fluid and the estradiol content (Wang Y.X. et al., 2018). However, the information about the effect of this trace element on the course of pregnancy is limited.

Therefore, the purpose of our work was to study the correlation changes of carbohydrate metabolism enzymes activity - glucose-6-phosphate dehydrogenase (G-6-PDH) and lactate dehydrogenase (LDH), as well as the estradiol content in the blood of pregnant female rats under the effect of vanadium citrate.

Materials and methods. The investigation was carried out on 25 laboratory female rats *Rattus norvegicus* of the Wistar line with the body weight of 140-160 g, which were divided into five groups: group I - non-pregnant females, group II - pregnant females, which consumed pure water without additives, rats of groups III, IV and V - in the period of mating and pregnancy received the solution of vanadium citrate in concentrations of 0.03, 0.125 and 0.5 μg V/ml of water respectively, which equals 3.75; 15.63 and 62.5 μg V/kg of body weight. Females were kept under vivarium conditions on a standard diet for laboratory animals. The consumption of water (in groups I and II) and vanadium citrate solution (in groups III, IV and V) was carried out at the rate of 20 ml per animal per day. Daily fixation of drinking water was carried out. Animals received solutions of vanadium citrate during 40 days. Erythrocytes and plasma of female rats were under study, in which the activity of G-6-PDH, LDH and the level of estradiol were determined. Red blood cells were prepared by centrifugation (1000 g during 15 min) and washed in a physiological solution of 0.85 N NaCl. The activity of G-6-PDH and LDH was determined by spectrophotometric method based on the oxidation-reduction of nicotinamide coenzymes (Vlizlo V.V. et al., 2012). The estradiol level was determined by ELISA method. The statistical justification of research results was carried out using the computer programs «*Statistika*» and «*Excel*». The statistic analysis of the average values was performed according to Student's criterion. Correlation analysis was performed according to Pearson criteria.

Results and discussion. Erythrocyte G-6-PDH is the most sensitive to the intense endocrine changes in the organism during pregnancy. This enzyme is responsible for the condition of red blood cells, their functioning and their life spam (Gulersen M. et al., 2016). The results indicate the central role of the G-6-PDH activity in antioxidant defenses. G-6-PDH provides the formation of NADPH necessary for the transformation of the oxidized form of glutathione into the reduced. Due to the increase in the content of reduced glutathione, the regulation of oxidation-reduction processes in the cell is provided. The revealed growth in the G-6-PDH activity indicates an increase in energy expenditure in the cell (Frosali S., et al., 2004).

The G-6-PDH activity increased by 9.51% in pregnant animals of the group II, compared to the group I of non-pregnant females (fig.1). Since this enzyme is vital for normal embryonic development, the increase in its activity in erythrocytes can indicate the activation of pentose phosphate pathway, where NADPH is produced. (Catanzerite V.A. et al., 1995).

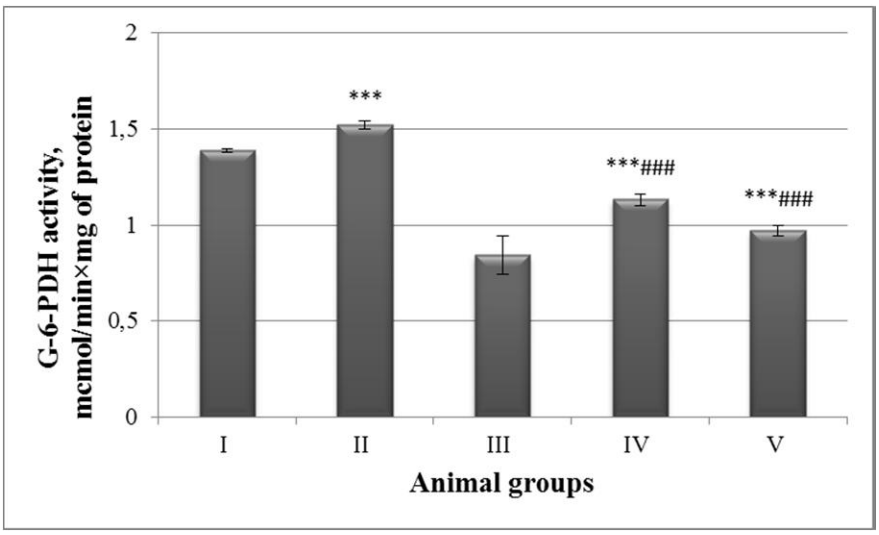

Fig. 1. The activity of glucose-6-phosphate dehydrogenase in erythrocytes, mcmol/min×mg of protein
There and forward: I –non-pregnant females; II – pregnant females, which consume pure water; III – pregnant females which consume the solution of vanadium citrate in the concentration of 0.03 mcg V/ml; IV - pregnant females which consume the solution of vanadium citrate in the concentration of 0.125 mcg V/ml; V - pregnant females which consume the solution of vanadium citrate in the concentration of 0.5 mcg V/ml.

*Note: * - p<0.05; ** - p<0.01; *** - p<0.001, as compared to I group; # - p<0.05; ## - p<0.01; ### - p<0.001, as compared to II group.*

Determination of activity LDH is important for pregnancy state diagnostic, for identifying possible problems with this physiological state and timely correction of them. In erythrocytes of the group I animals, it made up 0.335±0.03 μmol/min×mg of protein (fig.2). However, in the blood of the group II animals, the increase in the activity of this enzyme by 68% was observed, compared to the group I. As it is known, the LDH promotes the transformation of sugars into energy at the cellular level. Therefore, the growth of its activity causes the intensification of glycolysis. (Butt A.A. et al., 2002; Catanzerite V.A. et al., 1995).

Estradiol and progesterone are produced by placenta and regulate the initial stages of pregnancy during luteal-placental shift. They are the main hormones of pregnancy. According to our research, the estradiol content increased almost in three times in females of the group II, compared to non-pregnant females of the group I, which indicates the normal course of pregnancy (fig. 3) (Golbasi H. et al., 2018).

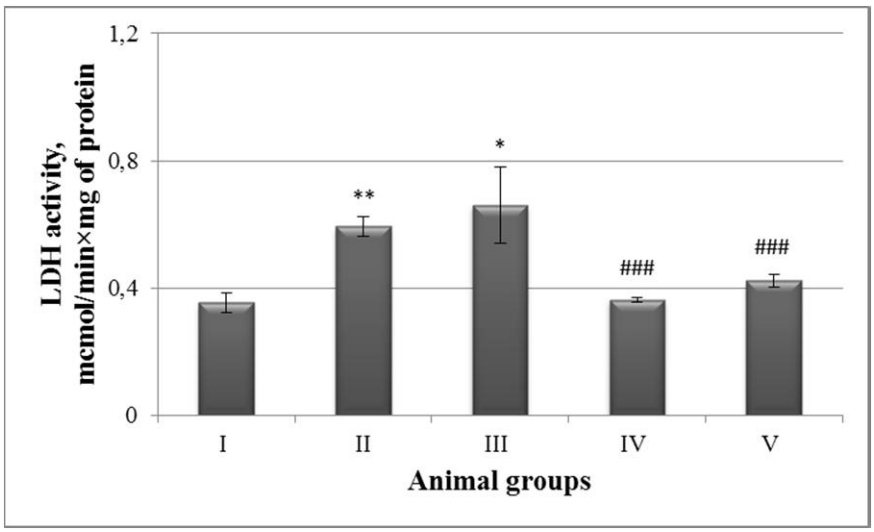

Fig. 2. The activity of lactate dehydrogenase in erythrocytes, mcmol/min×mg of protein

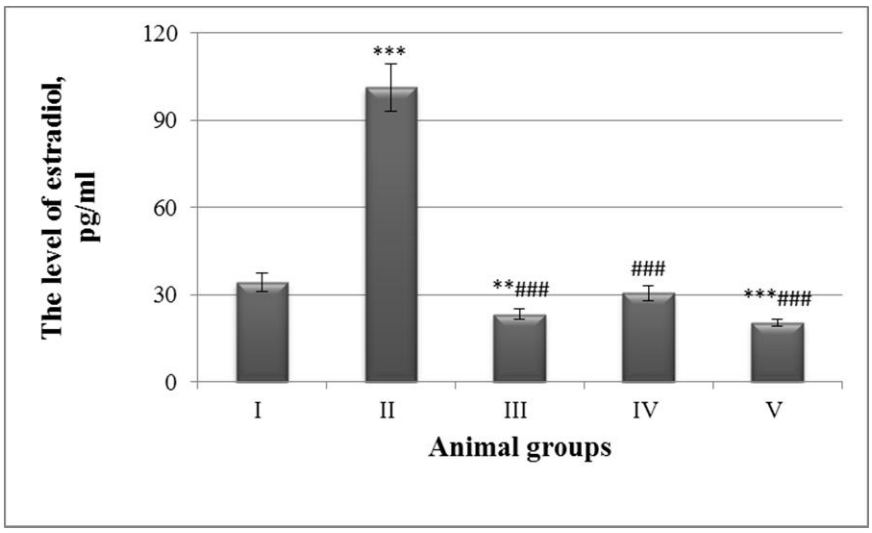

Fig. 3. The level of estradiol in blood plasma, pg/ml

Females of the groups IV and V receiving vanadium citrate showed a decrease in the activity of G-6-PDH by 25.53 and 36.18 %, respectively, compared to the group II of pregnant females. Vanadium is known to be able to influence the activity of many enzymes. In the form of vanadate it is similar to phosphate due to the similarity of structure and charge. Therefore, this trace element, like phosphate, is able to form ether bonds, which affects its biological properties, in particular, it has an inhibitory effect on the activity of certain enzymes, including G-6-PDH (Zizic M. et al., 2014). It is also known that under the effect of vanadium, the genes expression and enzyme activity, namely, G-6-Phase, fructose-2,6-bifosfatase, pyruvate kinase, and probably G-6-PDH, are normalized. In our studies, the value of enzyme activity in animals of the group IV in concentrations of vanadium citrate of 15.63 mcg/kg of body weight was similar to that in animals of the group I (Kioseoglou E. et al., 2015).

Under the condition of watering vanadium citrate to pregnant females in the groups IV and V, the LDH activity decreased by 39.06 and 28.79%, compared to the females of the group II. Since LDH is a glycolytic enzyme, the influence of vanadium suppresses glycolysis. As a consequence, vanadium citrate has a stabilizing effect on the activity of this enzyme, its approaching in the mentioned groups to the values of non-pregnant females of the group I (Preet A. et al., 2005).

The estradiol content in pregnant females which consumed vanadium citrate decreased: in the group III- by 77.02%, in the group IV- by 69.82%, and in the group V- by 83.92%, compared to pregnant females of the group II. Such a decrease in the estradiol content may be due to the inhibition of vanadium citrate activity of steroidogenic enzymes (Chandra A.K. et al., 2007). Observed, estradiol content decrease occurred within the limits of the physiological norm, the effect of the studied compound on this hormone can be considered positive.

The relationship between the activity of G-6-PDH, LDH and estradiol level in the experimental groups (III, IV and V), which were watered vanadium citrate in different concentrations, was determined. It was established that a strong inverse correlation was found (r=-0.92) between the activity of G-6-PDH and LDH under the effect of vanadium citrate in erythrocytes of pregnant females (fig.4). A weak inverse correlation (r=-0.42) was established between LDH activity and estradiol content (fig.5), while a strong direct correlation (r=0.74) was established between the estradiol content and G-6-PDH activity (fig.6). The data obtained indicate a similar dynamics of the studied indicators.

Conclusions. According to the research results, the activity of carbohydrate metabolism enzymes in erythrocytes and estradiol content in plasma of pregnant female rats increased. Changes of G-6-PDH activity may be due to activation of pentose phosphate pathway in pregnant animals, increasing of LDH activity may be due to the intensification of glycolysis, while high level of estradiol is normal for them.

Vanadium citrate at concentrations of 15.63 and 62.5 μg V/kg of body weight normalizes the activity of carbohydrate metabolism enzymes and estradiol content, which enables to regard this compound as a potential dietary supplementation during pregnancy.

The obtained data indicate the correlation between the activity of carbohydrate metabolism enzymes and estradiol content in the blood of pregnant females of rats under the effect of vanadium citrate at various concentrations.

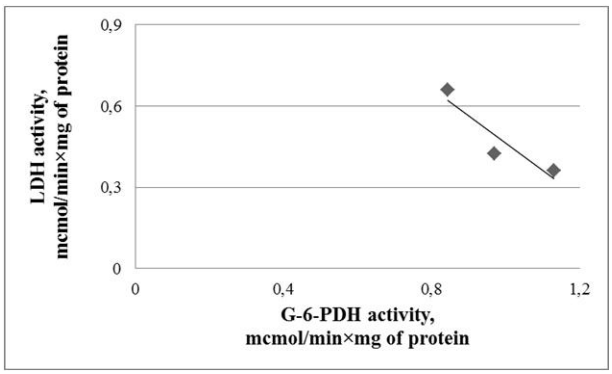

Fig. 4. Correlation between the activity of G-6-PDH and LDH under the effect of vanadium citrate in erythrocytes of pregnant females (r=-0.92)

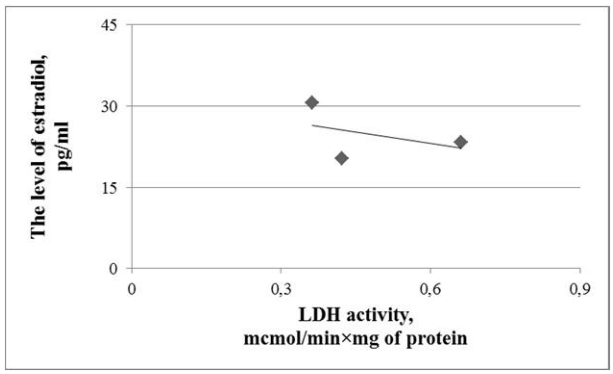

Fig. 5. Correlation between the activity of LDH and estradiol content under the effect of vanadium citrate in erythrocytes of pregnant females (r=-0.42)

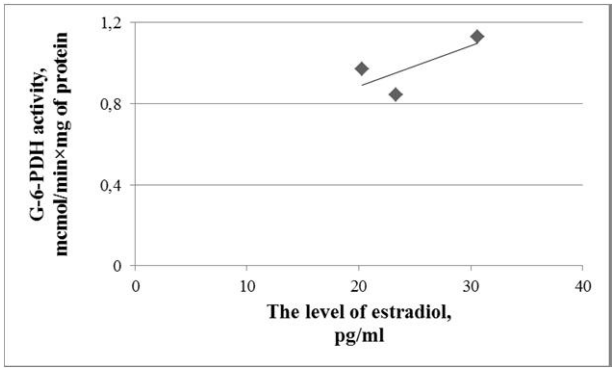

Fig.6. Correlation between the activity of G-6-PDH and estradiol content under the effect of vanadium citrate in erythrocytes of pregnant females (r=0.74)

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ЗМІНИ ПОКАЗНИКІВ ВУГЛЕВОДНОГО ОБМІНУ В КРОВІ ВАГІТНИХ САМОК ЩУРІВ ЗА ДІЇ ЦИТРАТУ ВАНАДІЮ

Г. В. Климець

Дані про вплив цитрату ванадію, який є органічним, на показники вуглеводного обміну та вміст естрадіолу в крові щурів, а також зв'язок між цими показниками відсутні. Тому метою роботи було вивчити вплив цієї сполуки на активність глюкозо-6-фосфатдегідрогенази, лактатдегідрогенази, вміст естрадіолу та встановити кореляційні зв'язки між активністю досліджуваних ензимів та вмістом естрадіолу у вагітних самок щурів за дії сполуки Ванадію. Дослідження проведені на 25 самках Rattus norvegicus лінії Wistar, які були поділені на п'ять груп: I група – невагітні самки, II – вагітні самки, що споживали чисту воду без добавок, III, IV, V – самки, які в період спаровування і вагітності отримували розчин цитрату ванадію в концентраціях 3,75, 15,63 та 62,5 мкгV/кг маси тіла відповідно. Наші дослідження показали, що у вагітних самок II групи відбувається зростання активності глюкозо-6-фосфатдегідрогенази, лактатдегідрогенази та вмісту естрадіолу, порівняно із I групою невагітних тварин. Зростання активності ензимів вуглеводного обміну у вагітних самок II групи можливо зумовлено активацією пентозофосфатного шляху та інтенсифікацією гліколізу в еритроцитах. У вагітних самок за впливу цитрату ванадію відбувалось зниження активності глюкозо-6-фосфатдегідрогенази та лактатдегідрогенази в крові тварин IV та V груп, та вміст естрадіолу у тварин всіх трьох дослідних груп (III, IV, V), порівняно із II групою вагітних самок. Також встановлено, що за впливу цієї сполуки між активністю глюкозо-6-фосфатдегідрогенази та лактатдегідрогенази у вагітних самок спостерігається сильна обернена кореляція (r=-0,92), між активністю лактатдегідрогенази та вмістом естрадіолу – слабка обернена кореляція (r=-0,42), в той час як між вмістом естрадіолу та активністю глюкозо-6-фосфатдегідрогенази – сильна пряма кореляція (r=0,74). Значення активності глюкозо-6-фосфатдегідрогенази у тварин IV групи, яким випоювали розчин цитрату ванадію у концентрації 15,63 мкгV/кг маси тіла були подібними до значень у тварин I групи. Це може вказувати на нормалізуючий ефект цитрату ванадію на активність глюкозо-6-фосфатдегідрогенази у вказаній концентрації, який ймовірно зумовлений здатністю мікроелементу Ванадію інгібувати активність даного ензиму. Зниження активності лактатдегідрогенази у IV та V груп, порівняно зі II групою, також може бути обумовлено ефектом цієї сполуки.. Цитрат ванадію у концентрації 15,63 мкгV/кг маси тіла спричиняв відновлення активності досліджуваних ензимів до значень активності у I групі. За цієї концентрації спостерігається найбільш виражений позитивний ефект на активність ензимів вуглеводного обміну та вміст естрадіолу. Наші дослідження дозволяють розглядати цю сполуку ванадію як потенційний дієтичний препарат для вагітних.

Ключові слова: кореляція, глюкозо-6-фосфатдегідрогеназа, лактатдегідрогеназа, естрадіол, цитрат ванадію, щури, кров.

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