

# EFFECT OF NICKELIDE TITANIUM IMPLANT ON THE DEHYDRONENASE ACTIVITY OF LYMPHOCYTES OF REGIONAL LYMPHATIC NODE

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**ABSTRACT** — The dehydrogenase activity of lymphocytes of lymphatic node has been studied under the intraosseal nickelide titanium implantation. Obtained data show nickelide titanium implantation has caused the changes of energetic status of lymphocytes with essential differences in activity of redox ferment systems. The moderate increase of SDG activity and decrease of NAD-dependent dehydrogenases have manifested a disturbance of the main path of energy providing of cells; the activity of shuttle system ( $\alpha$ -GPDG) coordinating interaction between processes of respiration and glycolysis is increased; a tendency of anaerobic path of energy production through activation of glycolysis (LDG) is observed. Immune processes have the central place in biocompatibility problem so observed change of energetic metabolism of lymphocytes may be regarded as a sign of immune reconstruction of organism.

**KEYWORDS** — lymphatic node, lymphocyte dehydrogenases, nickelide titanium implantation

The study of mechanisms of interaction between construction materials and biological media is actual. This problem is very important because of dental implantation methods are used in practice. Successes of the last years connecting with the creation of new implantation materials on the base of nickelide titanium [4] demand their verification in the tissue structures. The lymphologic development permits to appreciate the effect of implantation material on organism with a new methodological approach. The interest of biologists and clinicians to the fermentative activity of intracellular metabolism of lymphocyte is explained, from the one side, phylogenetically fastened lymphocyte ability to react to any homeostasis changes, and, from the other side, the fact that the change of lymphocyte ferment activity is appeared earlier than the quantitative changes of leukocyte formula, blood serum proteins etc.

The study of R.P. Narcissov and collaborators (1969–1980) has showed the lymphocyte is an “enzyme mirror” reflecting the state of metabolic processes in internal organs which can’t be studied directly. So R.P. Narcissov [5] has named the cytological analysis of lymphocyte ferment status an “indirect biopsy”



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permitting to estimate the state of metabolic processes of organs and systems. Lymphocyte cytoenzymology gives notion about quantitative changes in lymphocyte populations, subpopulation reconstructions, prognosis and character, hardness and affectivity of disease treatment, dynamics of adaptative process [8, 9].

The aim of this work is to study the effect of intraosseal nickelide titanium implantation on the change of dehydrogenase activity of lymphocytes of regional lymphatic node in experiment.

## MATERIAL AND METHODS

100 Wistar breed white rat males with body mass 180–200 g grown in the vivarium of Scientific Research Institute of Cytology and Genetics of SB RAMS have been used. Animals have been divided into two groups: the first group of pseudooperated animals is control; the second group with nickelide titanium implant is experimental. Implantation bed has been formed in the alveolar process of lower jaw in the molar teeth region with ball-shaped dental drill under ether narcosis. Nickelide titanium alloy (NT-10) has been introduced. Operative wound is stapled.

Pseudooperated animal group has been included animals with formed implantation bed without introduced nickelide titanium. Lymphocytes have been studied on smears-impressions made from incision of surfaced neck lymphatic nodes located on the upper pole of the lower jaw salivary gland on the 7, 14,

30, 60 and 120<sup>th</sup> days after implantation. The same smears-impressions of pseudooperated animals have been used as controls. Animals have been decapitated under the ether narcosis. Not less than 10 animals in each group have been used.

The cytochemical indexes of activity of important intracellular ferments such as lactate dehydrogenase [L-lactate *NAD*-oxidoreductase, (*KP* 1.1.1.27), LDG] catalyzing the final stage of glycolysis namely reverse reaction of pyruvate reduction into lactate; succinate dehydrogenase [succinate; (acceptor) – oxidoreductase, (*KP* 1.3.99), SDG ] catalyzing the reaction of succinate oxidation into fumarate in the Crebs' cycle: *NADH*- diaforase (*KP* 1.6.99.1), *NADH-R*); mitochondrial glycerophosphate dehydrogenase [L-glycerol-3-phosphate:acceptor]-oxidoreductase, (*KP* 1.1.99.5),  $\alpha$ -GPDG(m)], cytoplasmatic glycerophosphate dehydrogenase [L-glycerol-3-phosphate: *NAD*-oxidoreductase, (*KP* 1.1.1.8)  $\alpha$ -GPDG(c) have been estimated as key ferments of electron carrier.

The ferment activity has been estimated with quantitative method of R.P. Narcissov [5] in M.V. Robinson's modification [9] based on the counting of visible formase granules formed in 30–50 lymphocytes after dyeing with n-nitrotetrasole violet. Reliability of results has been estimated with Student's t-criterion.

## RESULTS AND DISCUSSION

This study has shown the specific change of energetic metabolism of lymphocytes of regional lymphatic node under endosteal implantation of nickelige titanium in various period of a study.

### SDG

Nickelige titanium implantation has lead to the change of ferment activity of SDG lymphocytes of regional lymphatic node (fig.1). At the 7<sup>th</sup> day of study SDG activity hasn't reliably differed from control value. At the 14<sup>th</sup> day ferment activity has increased up to  $16,68 \pm 1,01$ , that is more than control value on 25,03%. At the 30<sup>th</sup> day SDG activity has increased up to  $17,33 \pm 0,58$  and is reliably more than control value on 28,45%. At the 60<sup>th</sup> day SDG activity has decreased to  $15,21 \pm 0,54$ , but this value is reliably more than control value of corresponding period of study on 16,92%. Dynamics of decrease of SDG activity has remained at the 120<sup>th</sup> day of study. Ferment activity is  $14,92 \pm 0,55$ , that is reliably more than control value on 9,79%. It should be note that SDG activity is increased during the study period reaching the maximal value at the 30<sup>th</sup> day. SDG is metabolically important because it is supposed that lactate isn't accumulated in the cells rich in this ferment and the complete oxidation of glucose may be expected through the cycle of

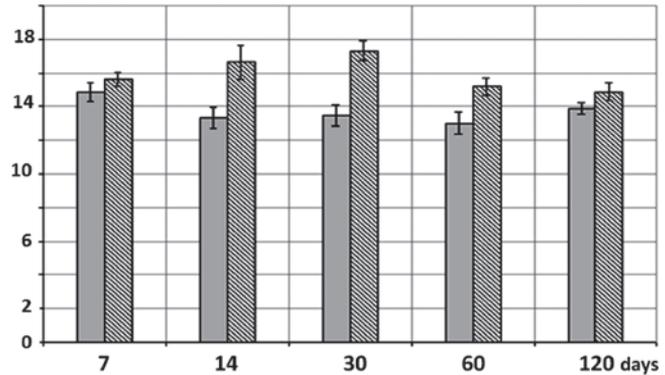


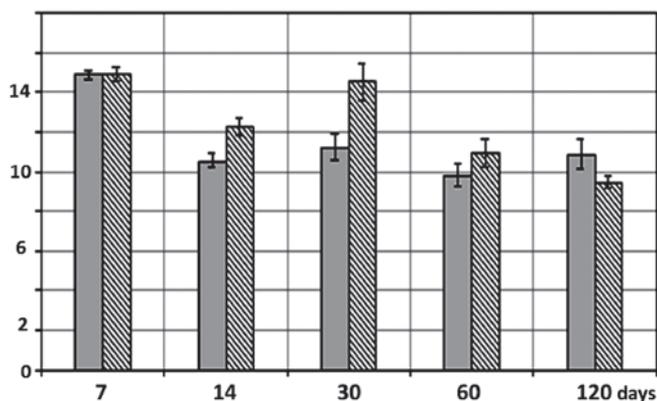
Fig. 1. The change of SDG activity of lymphocytes of regional lymphatic node at experimental implantation with nickelige titanium (the left column is pseudooperated animals; the right column is implantation with nickelige titanium. On the vertical axis shows the value of the ratio of granules at one lymphocyte)

tricarbons acids with big quantity energy release under the main aerobic type of lymphocyte metabolism [9, 10]. And the increase of SDG activity is exactly accounted for this fact. The increase of energetic potential of cell through the SDG activity increase is possible to be a manifestation of adaptive reaction of cell to the nickelige titanium implant and its biologic action. Decreased SDG activity at the 60<sup>th</sup> and 120<sup>th</sup> day may be explained with passive action of alloy observed after 30 days [2].

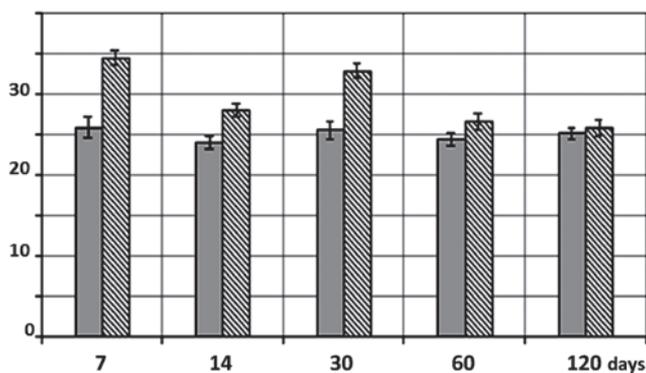
### LGD

The change of LDG activity has phase character during the experiment: maximal ferment activities have been observed at 7<sup>th</sup> and 30<sup>th</sup> day ( $14,88 \pm 0,35$  and  $14,5 \pm 0,92$ ), accordingly, minimal ferment activities have been observed at 60 and 120<sup>th</sup> day ( $10,9 \pm 0,73$  and  $9,43 \pm 0,31$ ). LDG activity in experimental group is higher than in control group during the first 60 days and lower than control value at the 120<sup>th</sup> day of experiment (fig. 2).

*NADH-R* activity is different in various periods of study (fig.3). Minimal *NADH-R* activity has been observed at the 7<sup>th</sup> day is  $18,42 \pm 0,63$  (control value is  $22,82 \pm 1,48$ ). At the 14<sup>th</sup> day activity is increased on 16,42% and is  $21,50 \pm 0,84$  (control value is  $24,32 \pm 0,76$ ). At the 30<sup>th</sup> day ferment activity in the experimental group is increased on  $24,32 \pm 0,76$  in comparison with 14<sup>th</sup> day of experiment. Because of this dynamics the statistically relevant differences of *NADH-R* activity in control and experimental groups haven't been observed at the 30<sup>th</sup> day of experiment ( $26,36 \pm 1,56$  и  $25,67 \pm 1,05$ ), accordingly. At the 60<sup>th</sup> day ferment activity is increased still more on 3,47% in animal group with nickelige titanium implant and



**Fig. 2.** The change of LDG activity of lymphocytes of regional lymphatic node at experimental implantation with nickelide titanium (the left column is pseudooperated animals; the right column is implantation with nickelide titanium. On the vertical axis shows the value of the ratio of granules at one lymphocyte)



**Fig. 3.** The change of GPDG (mitochondrial) activity of lymphocytes of regional lymphatic node at experimental implantation with nickelide titanium (the left column is pseudooperated animals; the right column is implantation with nickelide titanium. On the vertical axis shows the value of the ratio of granules at one lymphocyte)

decreased on 3,2% in control group and is  $26,64 \pm 0,65$  and  $25,54 \pm 1,23$ , accordingly. At the 120<sup>th</sup> day the change of *NADH-R* activity is observed as a tendency: increase in the group of pseudooperated animals, decrease in the group with nickelide titanium implant. Because of this dynamics at the 120<sup>th</sup> day of experiment the statistically relevant differences of *NADH-R* activity in control and experimental groups haven't been observed. *NADH-R* together with SDG play the most important role among the flavine dependent dehydrogenases. They catalyze the carrier of *NADH* electrons to acceptor, one of protein of respiratory chain containing nongemine iron. Flavoproteids play the role of intermediate carrier of electrons between dehydrogenases and cytochromes. Molecules being the carrier

of electrons are grouped into supramolecular structures so named respiratory ensembles. These ensembles containing strictly defined number of molecules of each carrier of electrons are included in the structure of inner mitochondrial membrane. The dynamics of *NADH-R* activity change permits to analyze the state of mechanisms providing the dynamic balance of redox reactions in lymphocytes under implantation. Respiratory accept looks like a cascade device bringing free energy portions convenient for cell. Reactions of oxidation phosphorylation preserve about 40% energy releasing in the process of electron carrier.

The study of dynamics of *NADH-R* activity of lymphocytes of regional lymphatic node under conditions of experimental nickelide titanium implantation permits to note the decrease of ferment activity at the 7<sup>th</sup> and 14<sup>th</sup> day of experiment. Statistically relevant differences of *NADH-R* activity in control and experimental groups haven't been observed at the 30<sup>th</sup>, 60<sup>th</sup> and 120<sup>th</sup> day of experiment. Intramitochondrial *ATF* and *ADF* pool is separated from cytoplasmatic pool, but exchange between them is possible through the carrier. The very complicate exchange of tricarbon acids cycle products and phosphate takes also place between cytoplasm and intramitochondrial compartment. Reduction equivalents of cytoplasmatic *NADH* may indirectly introduce into mitochondria with the help of glycerophosphate shuttle mechanism. Because of existence of this mechanism electrons releasing at the oxidation stage of glycolysis in cytoplasm are included in electron carrier in mitochondria that leads to the form of pyruvate as a final product of anaerobe reactions. There are special shuttle mechanisms carrying the reduction equivalents from mitochondria (where they formed) into cytoplasm. Glycerophosphate shuttle mechanism is the most important.

#### *α*-GPDG (mitochondrial)

*α*-GPDG mitochondrial activity is reliably higher than in control group during the experiment (fig. 4). Maximal ferment activities at the 7<sup>th</sup> and 30<sup>th</sup> days are higher than control values on 32,95% and 28,74%, accordingly, at the 14<sup>th</sup> day – on 16,43%, at the 60<sup>th</sup> day – on 9,17%, at the 120<sup>th</sup> day a tendency of increase of *α*-GPDG mitochondrial activity has been observed. High amplitude change of *α*-GPDG mitochondrial activity under the nickelide titanium implantation is unfavorable prognostic sign which significantly overtakes the other possible immune and clinic manifestations [6].

#### *α*-GPDG (cytoplasmatic)

The most high *α*-GPDG cytoplasmatic activity observed at the 7<sup>th</sup> day ( $30,83 \pm 1,2$ ) is higher than control value on 24,51%. At the 14<sup>th</sup> day activity is decreased to  $26,48 \pm 0,89$ , that is higher than control value on

14,03%. From 14<sup>th</sup> to 30<sup>th</sup> days it is observed the following decrease of  $\alpha$ -GPDG cytoplasmic activity, and at the 30<sup>th</sup> and 60<sup>th</sup> day statistically reliable differences haven't been observed. At the 120<sup>th</sup> day  $\alpha$ -GPDG cytoplasmic activity is increased once more up to  $25,91 \pm 0,58$  that reliably is higher than control value on 11,7% (fig.5). Described dynamics shows the intensification of coordinating role of glycerophosphate shunt and intensive cooperation of various ferments for energy providing of cell that permits cell to use protein catalysts more economically [1, 7].

## CONCLUSION

Experimental nickelde titanium implantation has caused the change of energetic status of lymphocytes of regional lymphatic node. Essential differences in dynamics of oxidative-reductive ferment systems have been observed. The moderate increase of SDG activity and decrease of *NAD*-dependent dehydrogenases have manifested a disturbance of the main path of energy providing of cells. At those conditions the significance of reserve paths is increased: a tendency of anaerobic path of energy production [activation of glycolysis (LDG)] is observed, and activity of shuttle systems ( $\alpha$ -GPDG) coordinating interaction between processes of respiration and glycolysis is also increased.

Immune processes have the central place in biocompatibility problem so observed change of energetic metabolism of lymphocytes may be regard as a sign of immune reconstruction of organism. Obtained data show expediency of correction of energetic metabolism of immune competent cells being in the state of energetic tension under the nickelde titanium implantation.

## REFERENCES

1. **BULYGIN G. V.** Metabolic status of peripheral blood lymphocytes in mechanisms of adaptation of man to new ecologic conditions // Thesis of dissertation of DM. – Tomsk, 1992. – 34 p. (In Russia)
2. **GOZAYA L.D.** Allergic diseases in orthopedic stomatology. M., – 1988. (In Russia)
3. **LENINER A.** The Basises of biochemistry: translation from English. – M., 1985. (In Russia)
4. Medical materials and implants with form memory / Gyunter V.E., Dambaev G.C., Sysolyatin P.G. et al. – Tomsk, 1998. (In Russia)
5. **NARCISSOV R.P.** The use of n-nitrotetrasole violet for quantitative cytochemistry of dehydrogenases of lymphocytes of man // Archive of anatomy. – 1969. – № 5. – P. 55–91. (In Russia)
6. **NARCISSOV R.P.** Prognostic abilities of clinic cytochemistry // Soviet pediatrics. Instl. 2. – M., 1984. – P.267–275. (In Russia)
7. **POLONSKAYA M.G.** The study of dynamics of correlative connections between physiologic parameters on the different stages of pathologic process // Thesis of dissertation of Phd. of medicine. – Krasnoyarsk, 1992. – 18 p. (In Russia)
8. **ROBINSON M.V.** Morphology and metabolism of lymphocytes / M.V. Robinson, L.B. Toporkova, V.A. Trufakin. – Novosibirsk, 1986. – 76 p. (In Russia)
8. **ROBINSON M.V.** Morphocytochemical features of lymphocytes in norm under destabilizing influences and autoimmune processes and diseases // Thesis of dissertation of DM. – Novosibirsk, 1994. – 58 p. (In Russia)
9. **SOKOLOV V.V.** Cytochemistry of ferments in prophylactic pathology / V.V. Sokolov, R.P. Narcissov, L.A. Ivanova – M., 1975. – 123 p. (In Russia)

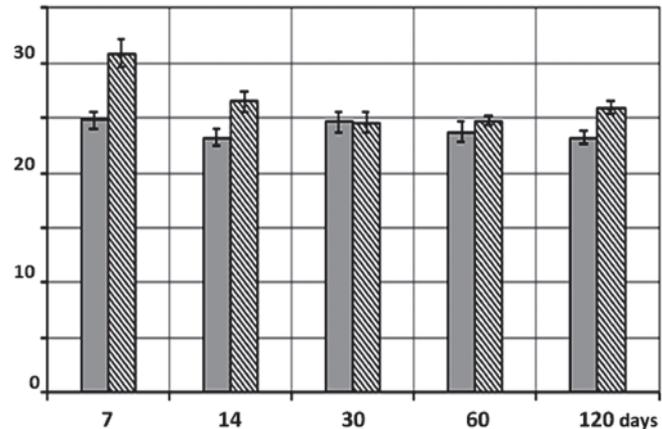


Fig. 4. The change of GPDG (cytoplasmic) activity of lymphocytes of regional lymphatic node at experimental implantation with nickelde titanium (the left column is pseudooperated animals; the right column is implantation with nickelde titanium. On the vertical axis shows the value of the ratio of granules at one lymphocyte)

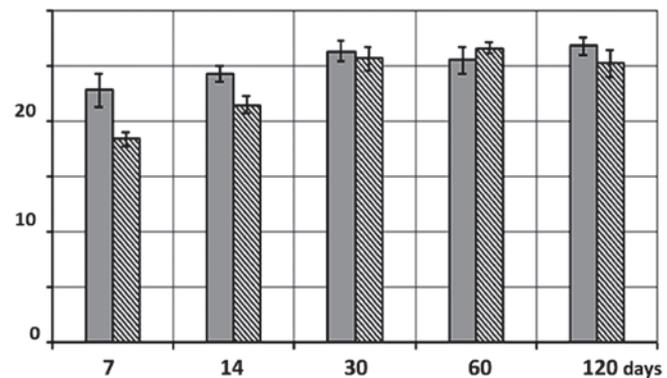


Fig. 5. The change of NAD-reduced form activity of lymphocytes of regional lymphatic node at experimental implantation with nickelde titanium (the left column is pseudooperated animals; the right column is implantation with nickelde titanium. On the vertical axis shows the value of the ratio of granules at one lymphocyte)