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HISTOENZYMIC DETERMINATION OF ACTIVITY OF ALKALINE PHOSPHATASE AND SUCCINATE DEHYDROGENASE IN THE RENAL TISSUE OF RATS AT NADP ADMINISTRATION ON THE BACKGROUND OF 31-DAYS EXPERIMENTAL DIABETES MELLITUS

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Abstract

The article presents data on the activity of some enzymes of rats' renal tissue in experimental diabetes mellitus in the early period of its formation. It was found that at 31 days of the experiment, the administration of NADP significantly increases the activity of ALP by more than twice, thereby improving the functioning of the proximal tubules in particular, and restores almost normal SDH activity, which allows us to assume that the positive metabolic effects of NADP are not only at the level of general metabolism, but also specifically at the organ level - the kidneys.

Key words: diabetes mellitus, streptozotocin, alkaline phosphatase, succinate dehydrogenase.

Introduction. Diabetes mellitus (DM) is a common non-epidemic disease of our time, which is widespread both in Ukraine and abroad. Its complications (micro- and

macroangiopathies) are quite varied and have early manifestations but kidney impairment with the development of diabetic nephropathy (DN) develops later than other ones [1].

Progressive development of DN leads to the that of chronic kidney disease (CKD) with the transition to chronic renal failure (CRF) [7,8,9]. Even though in various sources of literature there is a lot of data on the pathophysiological features of the development of diabetes, but still the main values of changes in the activity of the kidneys and their initial response to the manifestations of diabetes are not sufficiently described. The overwhelming majority of data refer to the description of a clinically developed form of the disease. In accordance with the classical representations of pathophysiology, the first response to the action of the pathogenic factor is the protective or adaptive reactions of the organism, which, in the case of prolonged pathogenic action, lead to the development of the corresponding pathology. However, in clinical conditions, it is not possible to follow the initial manifestations of DN with diabetes, therefore the study of the mechanism of development of diabetes and its complications in the experiment is relevant [10, 13, 14].

To study the functional state of cells in the renal tissue, studies of various enzymes are often used. At present, about 70 enzymes and isoenzymes have been found in urine, most of them are of the renal origin [3, 11, 12]. Alkaline phosphatase (ALP) and succinate dehydrogenase (SDG) are the leading ones among them. The diagnostic value of the activity of enzymes in urine is determined by their molecular weight, the selectivity of their reabsorption in the proximal tubules and the localization in the structures of the nephron. Biochemical methods of studying enzymes in biological fluids of an organism are the most common though the histochemical study of enzymes in biological tissues is used as well.

Scientists use different genetic and non-genetic models to reproduce the clinical picture of diabetes. One of the most common non-genetic chemical models is the administration of streptozotocin to experimental animals. The latter causes selective damage to β-cells and corresponds to type 1 diabetes in people [2, 4, 5].

Objective: to determine the activity of ALP and SDG enzymes in frozen sections of renal tissue in experimental animals with simulated diabetes mellitus against the backgroundof of NADP administration to establish the depth of damage to the structural and functional elements of the kidneys on the 31st day of the experiment.

Materials and methods. The study involved 32 sexually mature nonlinear males of white rats, weighing from 0.17 to 0.20 kg. The animals were divided into four groups. The first group (I) was a control one (n = 7), whose animals were on the standard feeding, lighting and maintenance. The experimental groups of animals (II–n=8; III–n=9) were administered

streptozotocin (Sigma, USA) at a single dose of 70 mg/kg intraperitoneally [2, 5]. The animals of the 2nd group were slaughtered and studied 31 day after the administration of streptozotocin, the rats of the 3rd group with DM were administered a NADP solution intraperitoneally at a dose of 30 mg/kg of body weight on isotonic sodium chloride solution. The experiment involved the animals whose glycemic level exceeded 10 mmol/l.

In order to investigate the necessary values, the slaughter of animals was carried out under a light etheric anesthesia, following the provisions of the EU Directive No. 609 (1986) and the Order of the Ministry of Health of Ukraine No. 690 of September 23, 2009 "On Measures for the Further Improvement of Organizational Norms of Work with the Use of Experimental Animals." The probability of difference of values was determined using the Student t-criteria. In the tables, the values of probability ("p") are given only for probable ($p = 0.05$ or less) differences of the studied values. Frozen sections of non-fixed tissue were used for histoenzymatic determination by the method of azo-coupling of alkaline phosphatase activity and determination of the succinate dehydrogenase activity by the tetrazolium method according to Z.Lojda [6].

In order to objectivize quantitative studies, the computer microdensitometry of specifically colored objects was performed in histoenzymatic specimens. To do this, they first received digital copies of the optical image of microscopic areas using the Olympus C-740UZ digital camera by means of the LUMAM-P8 microscope. Then, the digital copies of the image were analyzed using the GPL-licensed copy of the computer program GIMP version 2.0.4 (S.Kimpball & P.Mattis). In particular, the optical density of microscopic objects in units of optical density was measured.

Results and discussion. The activity of alkaline phosphatase (ALP) was investigated by the method of azo-coupling in the epithelium cytoplasm of the proximal tubules in experimental diabetes mellitus on the 31st day of its development. Alkaline phosphatase is a marker enzyme of the brush border and controls the processes of transmembrane transport. Being placed in the plasma membrane deeper than other enzymes of the brush border, it can be excreted in the urine with more severe damages to the tubules. A reliable ($p < 0.001$) reduction in the activity of the enzyme by 2.8 times was recorded in the animals of the experimental group II compared with the control values. When NADP was administered, the ALP activity was more than twice as high as in the rats that did not receive NADP, but by 10% less than in the control group (Table 1). That is, in this period of the development of diabetes the manifestations of severe lesions to the brush border of the renal tubules can be observed (Fig. 1).

Table 1

The staining optical density in determining the activity of alkaline phosphatase by the method of azo-coupling in the epithelium cytoplasm of the proximal tubules in experimental diabetes mellitus on the 31st day of the experiment and with the introduction of NADP (X±sx)

Groups of rats	The staining optical density
Control rats (n=7)	0,422±0,0019
1. Experimental group (31st day after streptozotocin administration) (n=8)	0,148±0,0017 p<0,001 p*<0,001
2. Experimental group (rats with DM+NADP) (n=9)	0,384±0,0020 p<0,01 p*<0,01

Note. n – number of animals in the group; p* – probability of difference between the experimental and the control groups of animals, p** - probability of difference between the experimental groups with DM on the 31st without NADP administration (according to Mann-Whitney criteria)

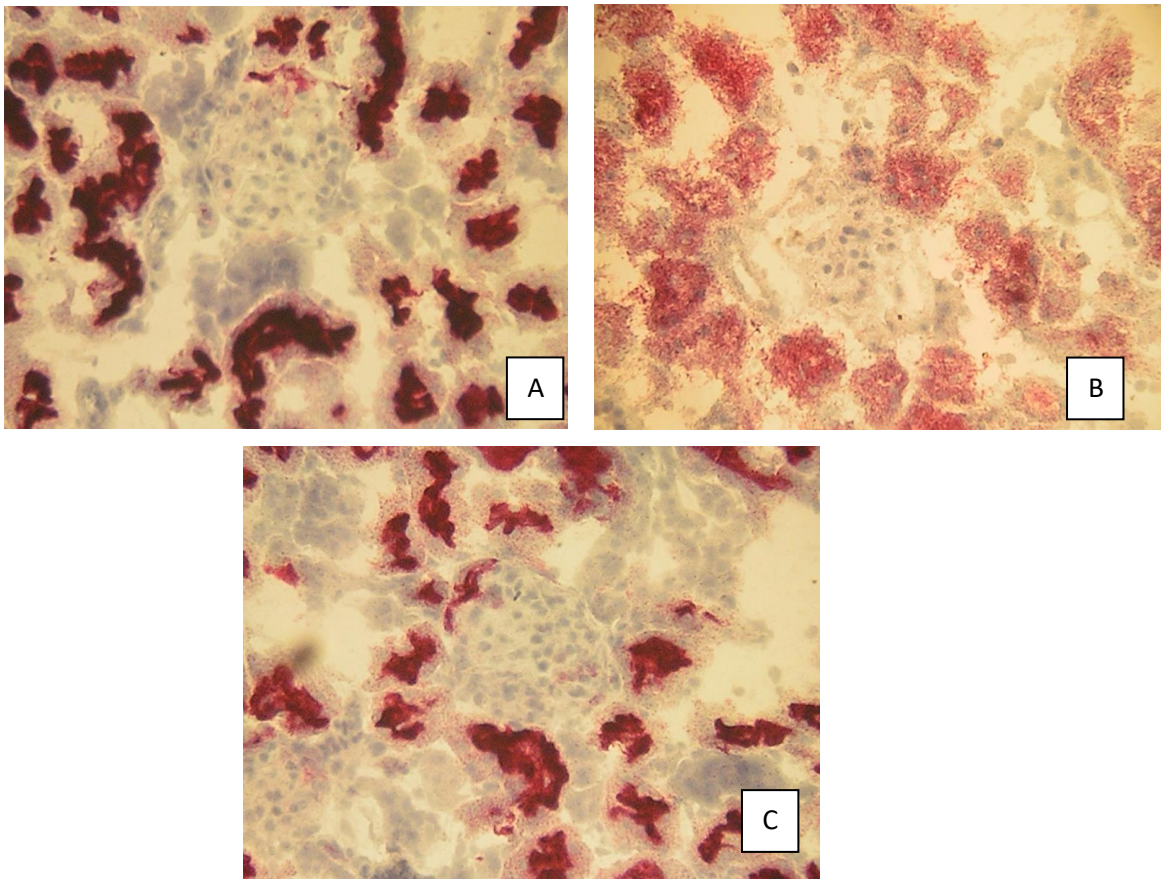

Fig. 1. Proximal tubules of the kidneys and renal glomerulus at experimental diabetes on the 31st day of observation. Histoenzymatic determination of alkaline phosphatase activity by the method of azo-coupling. Ob.40x. Oc.10x.

A) Control rat;
B) Experimental diabetes – 31st day;
C) Experimental diabetes (31st day) + NADP administration

We studied the staining optical density (in st.un of opt. dens.) while determining the activity of succinate dehydrogenase by the tetrazolium method in the epithelium cytoplasm of the convoluted tubules in experimental diabetes mellitus on day 31 of the experiment and with the introduction of NADP (Table 2).

Table 2

The staining optical density in determining the activity of succinate dehydrogenase by tetrazolium method in the epithelium cytoplasm of the proximal tubules in experimental diabetes mellitus on the 31st day of the experiment and with the introduction of NADP (X±sx)

Groups of rats	The staining optical density
Control rats (n=7)	0,244±0,0025
1. Experimental group (31st day after streptozotocin administration) (n=8)	0,229±0,026 p=0,005
2. Experimental group (rats with DM+NADP) (n=9)	0,238±0,0022 p=0,017; p*=0,011

Note. n – number of animals in the group; p* – probability of difference between the experimental and the control groups of animals, p** - probability of difference between the experimental groups with DM on the 31st without NADP administration (according to Mann-Whitney criteria)

Although the insulin deficiency is not constant, but partly compensated, insulin effects cause glucose metabolism disorders in cells of insulin-dependent organs, primarily in the muscle tissue, causing their impaired functioning. In turn, the kidneys, while in a functionally activated state, release a large number of these products. That is why we used nicotinamide adenine dinucleotide phosphate (NADP), whose administration blocks the processes of damage to mitochondrial oxidation due to the blockade of pyridine nucleotide breakdown.
This fact conforms with the earlier identified changes in tubular processes at this period. The changes of brush border and ALP activity can be explained by the violations of protein transport, unlike the SDG is an enzyme that is contained both in the cells of the proximal and especially the distal part of the nephron, and where the energy costs are higher.

Interestingly, the administration of NADP increases reliably the activity of ALP by more than twice (62%), thereby improving the functioning of the proximal tubules in particular, and restores almost normal SDG activity, which allows us to assume that the positive metabolic effects of NADP are not only at the level of general metabolism, but also specifically at the organ level, that is, the kidneys. Thus, under the influence of NADP there are positive changes in the morpho-functional state of the kidney brush border (Fig. 2).

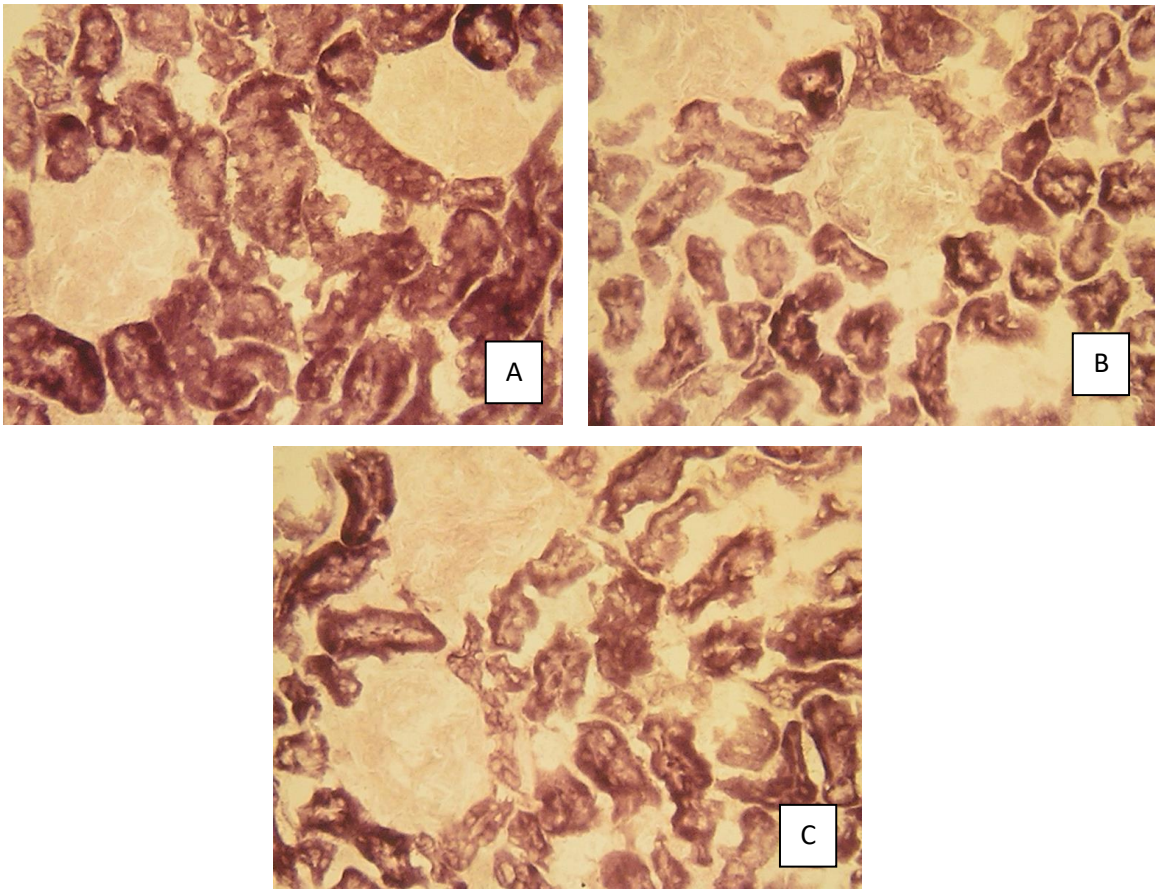

Fig. 2. Smooth renal tubules and renal glomerulus at experimental diabetes on the 31st day of observation. Histoenzymatic determination of succinate dehydrogenase activity by the tetrazolium method. Ob.40x. Oc.10x.

A) Control rat;
B) Experimental diabetes – 31st day;
C) Experimental diabetes (31st day) + NADP administration

Conclusion. The general conclusion on the results of conducted experiments is – in this period of experimental DM typical signs of this disease in animals are present, which are accompanied by a change in the morpho-functional state of the kidneys. Taking into account the obtained data, one can assert that changes in the activity of particular enzymes are caused by streptozotocin administration. The use of NADP partially restores the activity of succinate dehydrogenase and shows good results in alkaline phosphatase activity.

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