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# STAGES OF DEVELOPMENT OF STREPTOZOTOCIN-INDUCED EXPERIMENTAL DIABETES MELLITUS

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**Abstract**

The article presents data about the stages of experimental diabetes mellitus under conditions of the streptozotocin administration. It is noted that in the early development of experimental diabetes, changes in the kidneys proceeds in three stages: physiological adaptation; increasing morphological and functional changes and maladaptation; pathophysiological stage. The data related to changes in glomerular filtration rate, acid excretion, individual morphological alterations, etc. are given. The indicated changes in the opinion of the authors indicate an adaptive reaction of the system of regulation of water-salt metabolism and its main effector organ - the kidneys - to hyperosmia, caused by hyperglycemia with the subsequent formation of diabetic nephropathy.

**Key words: diabetes mellitus, streptozotocin, kidneys, hyperglycemia, glomerular filtration.**

**Introduction.** Nowadays diabetes mellitus (DM) is one of the most common chronic diseases, characterized by universal metabolic disorders. The effect of chronic hyperglycemia

is the generally acknowledged factor in the development of vascular complications. Scientists suggest that this effect is mediated by two non-enzymatic metabolic processes – proteins glycosylation with the formation of terminal products of glycosylation and lipids peroxidation [2, 4, 6].

Diabetes associated renal failure causes death in 10-20% of patients with diabetes. It also increases the risk of developing myocardial infarction and stroke. Among patients with diabetes, the risk of death is twice as high as in those who do not suffer from this illness [3, 5]. That is why scientists around the world face the challenge of working out certain measures to prevent the development of diabetes and its complications. In order to determine the features of the course of this disease and its complications, scientists apply a variety of experimental models using experimental animals.

Today in the arsenal of researchers there are many genetic and non-genetic models of experimental diabetes mellitus of both the first (insulin-dependent) and second (non-insulin-dependent) types [1, 7].

Famous models used for scientific research are the following: pancreatic DM (removal of 9/10 of pancreas in the experimental animals (mainly in dogs) (Mering and Minkovskyi, 1889)); aloxane-induced DM (animal administration of aloxane – a substance that selectively damages b-cells of pancreatic islets); streptozotocin-induced DM (introduction to experimental animals of streptozotocin – an antibiotic that selectively affects the β-cells of the pancreatic islets); virus-induced diabetes (caused by infection of experimental animals with viruses of certain strains); dithizone-induced diabetes (the introduction of dithizone to animals – a substance that binds zinc and, thus, disrupt insulin deposit and secretion); immune DM (introduction of antibodies to animals against insulin); metahypophyseal diabetes (prolonged administration to animals of the adenohypophysis hormones – somatotropic hormone, ACTH, etc.); metasteroid diabetes (prolonged glucocorticoids introduction to animals); genetic DM models (breeding of pureblooded mice and other animals with a hereditarily conditioned form of the disease) [8, 9, 10].

Among the above models, streptozotocin-induced one is especially popular, as it makes possible to simulate DM of both the first and second type, depending on the medication dose, the method and the frequency of its administration. However, despite the widespread use of this technique, the time dynamics and stages of development of streptozotocin-induced experimental DM at an early period of its formation still remain unexplored.

Therefore, the given paper **aims at** detecting the peculiarities of changes in the morpho-functional state of kidneys in the time dynamics of the development of experimental streptozotocin-induced diabetes mellitus.

**Material and methods.** The experiment was conducted on 32 sexually mature nonlinear white male rats, weighing from 0.17 to 0.20 kg. Animals were divided into four groups. The first (I) is the control group (n = 7), the rats in this group followed the standard feeding, lighting and housing regimen. Animals of the experimental groups (II – n = 8; III – n = 8 and IV – n = 7) received one dose (70 mg/kg) of streptozotocin intraperitoneally (Sigma, USA) [1]. In the 2nd group animals were slaughtered and appropriate studies were carried out 11 days after streptozotocin administration, the indices in the animals of the third group were examined after 21 days, IV - after 31 days, respectively. Animals with a glycemic level exceeding 10mmol/l were involved in the experiment.

To investigate the main indices, the slaughter of animals was carried out under light ethereal anaesthesia, conforming to the provisions of the EEC Directive No. 609 (1986) and the Order of the Ministry of Health of Ukraine No. 690 dated September 23, 2009 "On Measures for Further Improvement of the Organizational Standards of the Use of Experimental Animals". To evaluate the function of the vascular-glomerular apparatus of the kidney, the animals were loaded with tap water in the amount of 5% of body weight, and urine was collected 2 hours later. A quantitative estimation of protein status in histochemical preparations, stained with bromphenol blue according to Mikel Calvo, was performed by means of computer microspectrophotometry method based on the R/B coefficient.

Reliability of difference in the indices was determined using Student's t-test. In the tables the values of reliability ("p") are given only for the reliable (p=0.05 or less) differences in the indices, which were studied.

Computer microspectrophotometry method was performed in compliance with all standardization requirements [11]. Digitized copies of the images, obtained using the Delta Optical Evolution 100 (planar magnetic lenses) microscope and the digital camera Olympus SP-550UZ, were analyzed in the medium of the computer program ImageJ (1.48v, free license, W. Rasband, National Institute of Health, USA, 2015), in particular, by means of the probe method in the RGB color analysis system, the values "R" and "B" were received, on the basis of which the R/B coefficient was calculated. The arithmetic mean and its error were calculated, and the difference in the average tendencies was estimated using the odd Student's test (PC program PAST 3.10, free license, O.Hammer, 2015). The value of the R/B coefficient is interpreted in the following way – if it is greater than 1, then the carboxyl

groups predominate over the amino groups in the proteins, moreover, the larger this value, the more significant the predominance. If the value of the R/B coefficient is less than 1, then the amino groups predominate over the carboxyl groups in the proteins. The value of this index is that during the entire period of diabetes mellitus development the processes of oxidation and glycosylation of amino groups of proteins are activated from the very beginning, which leads to changes in the ratio between the carboxyl groups and the amino groups in the proteins [11, 12].

**Results and discussion.** In our opinion, the results of today's experimental works on the modeling of diabetes and its complication – diabetic nephropathy, mostly represent data that relate to the clinical manifestations of the disease that has already developed. We made an attempt to investigate the primary reaction of the kidneys, involved in the process of the body's response to the effects of hyperglycemia even before the corresponding clinical signs.

Analyzing the obtained results on the $11^{th}$, $21^{st}$ and $31^{st}$ day of the experiment, we came to the conclusion that changes in the indices of renal activity occur in three stages, in other words, the development of streptozotocin-induced diabetes has at least three consecutive stages: physiological adaptation; increasing morphological and functional changes and deadaptation; pathophysiological stage.

In the first stage – physiological adaptation – the reaction of the system of regulation of water-salt metabolism and kidneys to the phenomenon of hyperosmia, caused by hyperglycemia, is likely to occur. Studying the condition of these processes in this period, we have determined that on the $11^{th}$ day the glomerular filtration rate does not decrease, as it is described in most works on DM, but on the contrary, it significantly increases more than twice (Table 1). This is confirmed by calculations of endogenous creatinine clearance, the concentration of which increases in urine from 0.72±0.04 to 1.68±0.04 mmol/l on the $11^{th}$ day, 1.71±0.06 on the $21^{st}$ day and 2.03±0.11 mmol/l on the $31^{st}$ day, respectively. At the same time, the creatinine rate in the blood plasma is slightly reduced at the beginning of the observation and significantly reduced up to the $31^{st}$ day of the experiment.

The increased glomerular filtration rate indicates an effective excretion of the end products of nitrogen metabolism from the body (in this case, creatinine). Accordingly, the decreased diuresis is possible only with the growth of relative water reabsorption (Table 1).

Table 1

| Index | Groups of animals | | | |
|---|---|---|---|---|
| | Control | Diabetes 11th day | Diabetes 21st day | Diabetes 31st day |
| | (n=7) | (n=8) | (n=9) | (n=8) |
| Creatinine concentration in plasma, mmol/l | 57.23±3.27 | 47.38±1.28 p<0.02 | 33.89±2.37 p<0.01 | 28.63±1.61 p<0.01 |
| Creatinine concentration in urine, mmol/l | 0.72±0.04 | 1.68±0.04 p<0.001 | 1.71±0.06 p<0.001 | 2.03±0.11 p<0.01 |
| Ccr rate, mcl/min | 378.64±27.24 | 864.12±32.00 p<0.001 | 879.85±75.83 p<0.001 | 1125.21±80.21 p<0.001 |
| Relative water reabsorption, % | 91.81±0.77 | 97.17±0.09 p<0.001 | 99.99±0.02 p<0.05 | 98.55±0.13 p<0.05 |

**Notes:** p – reliability of the difference between the control and experimental groups; n – number of animals, Ccr – glomerular filtrate.

These adaptive changes in the initial period are particularly easily seen on the example of the acid-regulating function of the kidneys, as one of the most sensitive indicators of kidneys activity, since it essentially depends on the exchange of hydrogen ions for sodium ions, on the activity of secretion in the distal tubules and collecting ducts.

Our experiments revealed that during 11th-31st day of the streptozotocin-induced diabetes development, the phenomena of enhancement of acid-releasing activity of the kidneys take place, judging by the urine pH and the increase in excretion of hydrogen ions, ammonia and titrated acids (Table 2).

Table 2

| Index | Groups of animals | | | |
|---|---|---|---|---|
| | Control | Diabetes 11th day | Diabetes 21st day | Diabetes 31st day |
| | (n=7) | (n=8) | (n=9) | (n=8) |
| Urine pH, units | 7.05±0.11 | 6.25±0.10 p<0.001 | 6.19±0.10 | 6.14±0.12 p<0.05 |
| Excretion of hydrogen ions, mmol/100 mcl Ccr | 0.20 ± 0.19 | 0.50±0.18 p<0.05 | 0.53±0.18 p<0.05 | 0.62±0.13 p<0.01 |
| Excretion of titrated acids, mmol/100 mcl Ccr | 9.38±0.38 | 15.88±0.18 p<0.05 | 18.42±0.16 p<0.01 | 24.35±0.19 p<0.001 |
| Ammonia excretion, mmol/100 mcl Ccr | 18.34±2.82 | 35.66±0.67 p<0.05 | 37.63±7.15 p<0.01 | 44.83±6.25 p<0.01 |

**Notes:** p – reliability of the difference between the control and experimental groups; n – number of animals, Ccr – glomerular filtrate.

Activated acid regulating function cannot be said to fully manage the elimination of excess waste products and various acids. But the removal of active, titrated acids, ammonia, both in general and in terms of the active nephron unit, indicates that in this period the phenomena of metabolic acidosis begin to manifest in the body, because the kidneys are not so active in removing acids from the body, if there is no their surplus. This is confirmed by the fact that in this period of experimental DM metabolic disorders are present (Table 2).

The further development of diabetes leads to an increase in the processes of proteins glycosylation, even greater increase in the glomerular filtration rate, an increase in proximal reabsorption of sodium ions, and an increase in the release of active acids. Macrophages activation, which causes subsequent morphological rearrangements, is also characteristic for this stage.

First of all, it concerns mesangial matrix and mesangiocytes. The increase in GFR suggests that the level of glycosylation and other proteins increases, and hence the decrease in charge occurs. The decrease in charge causes changes in the glomeruli of the kidneys and later leads to changes in its vessels. First and foremost, the vessels of the renal cortex and later deeper layers, such as the cerebrospinal fluid, are affected (Table 3).

Table 3

| Index | Groups of animals | | | |
|---|---|---|---|---|
| | Control | Diabetes 11$^{th}$ day | Diabetes 21$^{st}$ day | Diabetes 31$^{st}$ day |
| | (n=7) | (n=8) | (n=9) | (n=8) |
| Mesangial matrix and mesangiocytes, R/B | 1.13±0.019 | 1.49±0.017 | 1.68±0.016 | 1.79±0.019 |
| Basal membranes of renal glomeruli with endothelial cells, R/B | 1.09±0.014 | 1.10±0.018 | 1.38±0.012 | 1.44±0.017 |
| Basal membranes of convoluted tubules, R/B | 1.10±0.018 | 1.10±0.022 | 1.34±0.021 | 1.43±0.018 |
| Epithelium cytoplasm of the convoluted tubules, R/B | 1.22±0.014 | 1.26±0.019 | 1.27±0.022 | 1.39±0.024 |

**Notes:** p – reliability of the difference between the control and experimental groups; n – number of animals.

In the basal membrane of the renal glomeruli tendency to changes appears on the 11$^{th}$ day, although significant changes are observed on the 21$^{st}$ day, and they continue to develop up to the 31$^{st}$ day. These changes may be responsible for the emergence of additional protein in the urine and the development of proteinuria in the future. Membranes of the convoluted

tubules also begin to react in the same period. Interestingly, the cytoplasm of the convoluted tubules epithelium responds only on the 31st day. It proves that vessels, basal membrane, structures that are in contact with blood suffer structurally in the first place, and not the cytoplasm. In renal glomeruli the pressure is higher, due to several factors, including the increase in glucose concentration. But in general, the cells and even connective tissue are not the first to be affected in cerebrospinal fluid, but endotheliocytes and blood vessels located here. Obviously, during the onset of diabetes, where hyperglycemia is the main acting mechanism, the actual amount of connective tissue in the kidney's cerebral fluid does not yet increase. Another important point is that the connective tissue replaces the dead nephrons. Therefore, we can assume that at this time there is no phenomenon of nephrons death yet.

The indicated changes in the functional and morphological state of the kidneys at the experimental DM are gradually increasing. With further influence of persistent hyperglycemia, processes become irreversible. Changes occur not only in the indices of essential renal functions, but also in the thickness and charge of the basal membrane, vascular endothelium and connective tissue, microalbuminuria development is observed, it leads to proteinuria, a decrease in the glomerular filtration rate and, eventually, to diabetic nephropathy. The general course of the initial response of the excretory system to hyperglycemia is shown in the diagram.

**Conclusion.** Our studies allow us to say the following - the changes in renal function described above in the first and second stages are not nephropathy yet. In the formation of the phenomena of metabolic acidosis, the kidneys are not only well, but intensely react to these processes (especially in terms of the unit of the active nephron). Changes in urine pH indicate that the activation of acid reflux occurs both at the level of proximal tubules (this is primarily titrated acids) and at the level of the distal tubules (increased ammonia excretion). Morphological changes are not vivid yet, which indicates the initial stage of the development of experimental streptozotocin-induced diabetes. However in the third period pathophysiological changes typical for diabetic nephropathy are formed.

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STREPTOZOTOCIN-INDUCED DIABETES DIAGRAM