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# MORPHOFUNCTIONAL CHANGES OF RAT PANCREAS UNDER CONDITIONS OF NITRIC OXYDE EXCESS

***Summary.*** *The study was carried out on 40 male Wistar rats after daily intraperitoneal injection of donator of nitric oxide (NO) molecules sodium nitroprusside in a dose of 1.5 mg/kg in 1 day 1 (n = 6); 2 days (n = 6); 6 days (n = 6); 12 days (n = 6); 30 days (n = 6). It is established that an excessive NO intake leads to morphological changes in the pancreas: vasodilation with stasis of blood cells after 1 and 2 days, focal necrosis, destruction of acinar tissue, ductal dilatation with worsening outflow of pancreatic secretion after 6 days; fatty degeneration, segmental apoptosis in 12 days; compensated microcirculatory changes in the body, the formation of fibrous tissue in periductal and perivasal areas with penetration in the interlobular space in 30 days. In the blood serum we detected impaired exocrine pancreatic function: increased activity of pancreatic enzymes — α-amylase and trypsin up to 6 days, and in 30 days — they significantly reduced; increasing the levels of biochemical markers of fibrosis (protein-bound hydroxyproline and hexosamine); failure of endocrine function — increase of glucose level, that is, changes typical for chronic experimental pancreatitis.*

***Key words:*** *nitric oxide, sodium nitroprusside, chronic pancreatitis, fibrosis.*

## Background

Nitric oxide (NO) is a fat-soluble gas, a high-reactive and unstable compound, which is formed from L-arginine under the action of NO-synthase. NO easily inpours cell membranes, exists only for a few seconds and being subjected to oxidation transforms to nitrites and nitrates [1]. NO acts in all directions as a universal regulator of physiological functions and transmission of nerve impulses, a potent peripheral vasodilator and regulator of motor control and secretion. NO is a potent mediator of inflammation release in response to bacteria, viruses, proinflammatory cytokines [2].

Unlike classic primary messengers, which also regulates smooth muscle function, NO could induce those processes without interaction with receptors and plasma membrane by intracellular activation of cytosolic enzyme — soluble guanylate cyclase [3, 4].

Increased concentration of guanosine monophosphate activates GMP-dependent protein kinase and $Ca2^{+}$ ATPase, which is involved in dephosphorylation of myosin. This leads to the removal of $Ca2^{+}$ from the muscle cells and thus to vasodilation [5].

However, other physiological effects of NO, independent of the guanylate cyclase activation, is known, including posttranslational modification of proteins, lipids and other biomolecules. Possible targets of NO is soluble adenosine diphosphate (ADP)-ribosylating enzyme and transcription factors — through those NO may directly affect gene transcription and translation of the mRNA [6].

Despite the fact that many authors describes the protective role of NO, it is also capable of inducing cell damage. The effect of NO on cellular processes is highly relevant and pending problem [7].

Sodium nitroprusside, which is well known as simple NO-donor, can not be synthesized in cells and is an exogenous source of NO, which is traditionally used for experimental studies.

**Purpose:** to determine the morphofunctional characteristics of the rat pancreas under conditions of NO excess caused by the administration of sodium nitroprusside as a simple NO-donor.

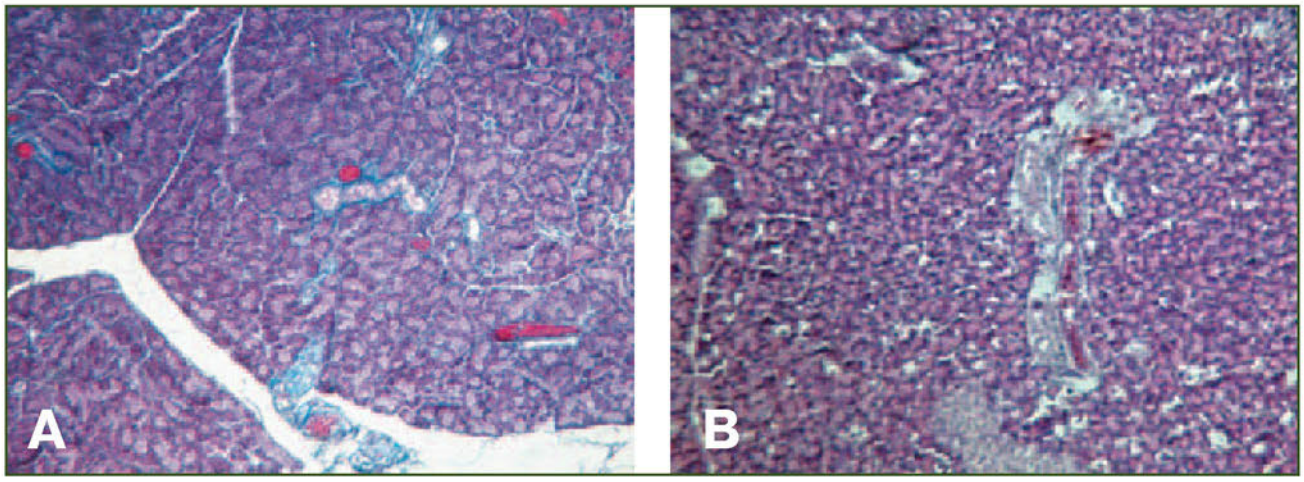


***Figure 1 — Rat pancreas after 24 hours (A) and 48 hours (B). Dilation of blood vessels and ducts, congestion and stagnation of secretion. Mallory's trich rome, ×100***

## Material and Methods

The study was conducted in 40 male Wistar rats weighted 180–230 g. Nitric oxide (NO) was administrated in the form of sodium nitroprusside by Reachem (Ukraine), at 1.5 mg/kg for 1 day (n = 6), 2 days (n = 6), 6 days (n = 6), 12 days (n = 6), and 30 days (n = 6). Rats were sacrificed by ketamine introduction in lethal dose of 200 mg/kg. The control group (n = 10) was formed of intact rats and received 0.9% NaCl for 1, 2, 6, 12 and 30 days.

The study was conducted following the standards of the European Convention of Bioethics (1997), European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, general ethical principles of animal experiments, approved in the law of Ukraine (№ 1759 — VI of 15.12.2009) «On protection of animals from cruel treatment».

To carry out biochemical determinations and histologic study, after excision, total pancreases were excised, trimmed of fat and lymph nodes. Some small pancreatic samples from each rat were immediately fixed in 10% formalin, paraffin-embedded sections were cut at 3–5 microns and mounted on glass slides. Sections were deparaffinized and stained with hematoxylin-eosin or Mallory's trichrome.

Biochemical process of fibrosis was evaluated on the content in the serum of free and protein-bonded hydroxyproline and hexosamines. NO production was determined by the total content of nitrite/nitrate in serum using Gris test. To estimate the exocrine function activity of pancreatic enzymes were measured in serum — α-amylase using set of Filisit-diagnosis and trypsin — using Erlanger test with modifications of Shaternikov.

State of the endocrine pancreatic function was evaluated by determining the serum levels of glucose set by «Phyllis-diagnosis» (glucose oxidase method).

## Results and Discussion

In 1 and 2 days after the experiment was started, in 100 % of rats dilation of blood vessels and ducts, congestion and stagnation of secretion were observed (Fig. 1). Severity of these symptoms ranged from mild to moderate.

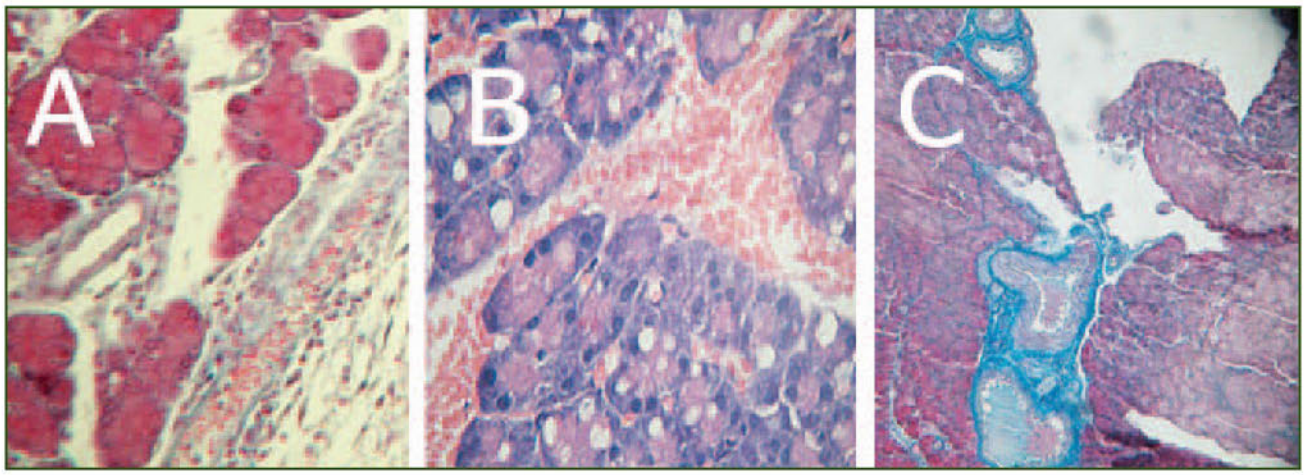


***Figure 2 — A) rat pancreas in 6 days after beginning of the experiment. Vasodilation and stasis of blood cells, inflammatory infiltration. Mallory's trichrome, ×200; B) rat pancreas in 12 days after beginning of the experiment. Severe dilatation of the vessels with stasis of blood cells, adipose degeneration, ×400; C) rat pancreas in 30 days after beginning of the experiment. Bands of fibrous tissue around the main ducts. Mallory's trichrome, ×100***

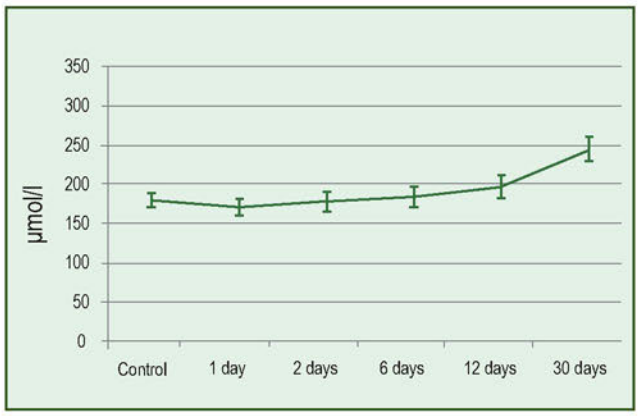


***Figure 3 — Concentration of protein-bonded hydroxyproline in the rat blood after administration of sodium nitroprusside***

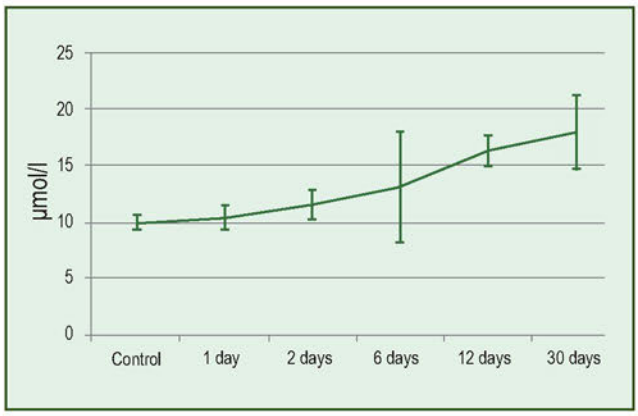


***Figure 4 — Concentration of free hydroxyproline in the rat blood after administration of sodium nitroprusside***

In 6 days of sodium nitroprusside intraperitoneal injections as NO-donor signs of acute pancreatitis were shown, the structural basis of which was inflammation — stromal infiltration by lymphocytes and leukocytes, dilation of blood vessels and intralobular ducts, stasis of blood cells. In 66.6 % of all cases big groups of hypersecretory acinar cells were found in parenchyma, in 75.0 % of those among aforementioned cells isolated small foci of necrosis was found.

12 days later the phenomenon of vessels plethora and stasis of blood cells became more pronounced. In 100 % of all cases stroma was diffusely infiltrated by inflammatory cells, excessive accumulation of secretion in the acinar cells was noted with segmental apoptosis of acinar tissue. Focal adipose degeneration was developed in 66.6 %.

Despite the pronounced inflammatory activity, which was observed after 12 days of the experiment, in all animals, sacrificed after 30 days of sodium nitroprusside daily administration, microcirculatory changes were almost entirely offset. Vessel diameter did not differ from the comparison group, infiltration was scarce or not observed. At the same time, in 83.3 % of all cases dilatation of the interlobular ducts and the development of intralobular connective tissue were observed. The tiny bands of fibrous tissue enveloped main ducts, major blood vessels and penetrates into the interlobular space (Fig. 2).

Morphological signs of pancreatitis were accompanied by the changes in biochemical parameters that characterizes metabolism of collagen. Processes of connective tissue anabolism on 30$^{th}$ day illustrated through content of protein-bonded hydroxyproline in blood — which was increased by 1.4 times from (179.28 ± 9.19) µmol/l (control group) to (243.81 ± 15.35) µmol/l ($p < 0.01$) and catabolism through content of free hydroxyproline — which was increased by 1.5 times (to 16.37 ± 1.39) µmol/l ($p < 0.01$) and 1.8 times (to 18.01 ± 3.27) µmol/l ($p < 0.05$) at 12$^{th}$ and 30$^{th}$ days, respectively. Compared to controls (9.96 ± 0.71) µmol/l those values indicated increased collagen synthesis and destruction (Fig. 3, 4). It ought to be noted that concentration of the protein-bonded hydroxyproline in the rat blood in 30 days was significantly higher than in one day ($p < 0.01$), 2 days ($p < 0.05$) and 6 days ($p < 0.05$).

Increased concentration of hexosamine (HA) in the blood indicates increased catabolism of carbohydrate-protein components of connective tissue, as HA forms part of the proteoglycans and its components — glycoproteins. Aside from that increasing of HA concentration is a factor that suggest inflammation, while long-term

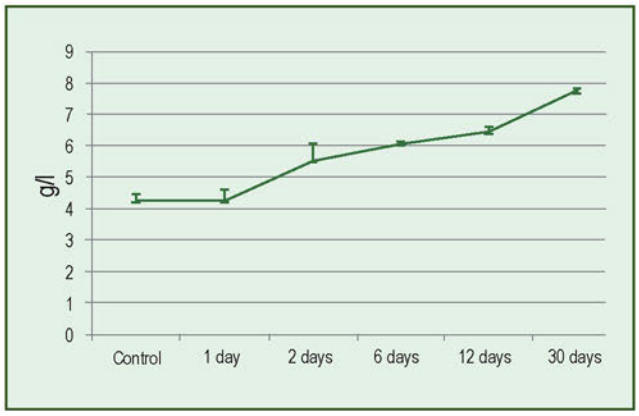


***Figure 5 — Concentration of hexosamine in the rat blood after administration of sodium nitroprusside***

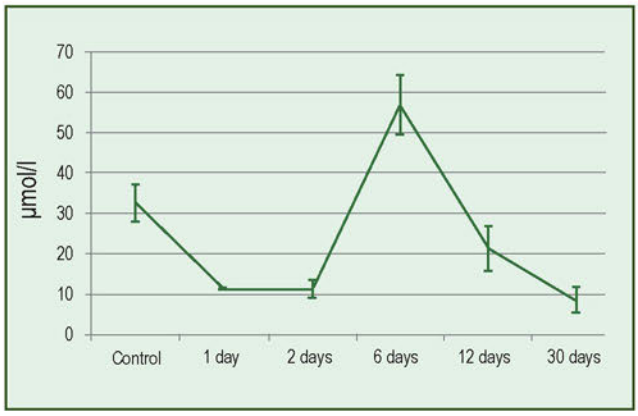


***Figure 6 — Concentration of nitrite/nitrate in the rat blood after administration of sodium nitroprusside***

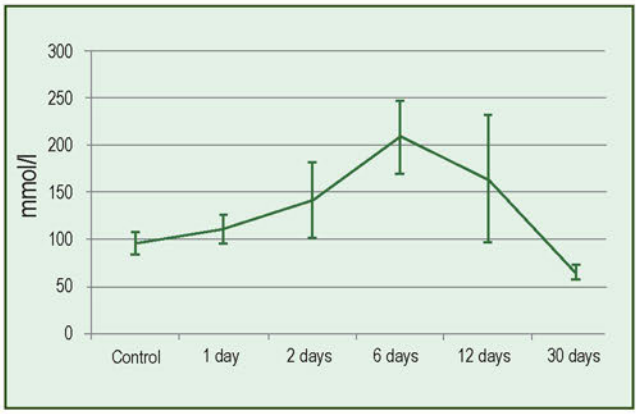


***Figure 7 — α-amylase activity in rats after administration of sodium nitroprusside***

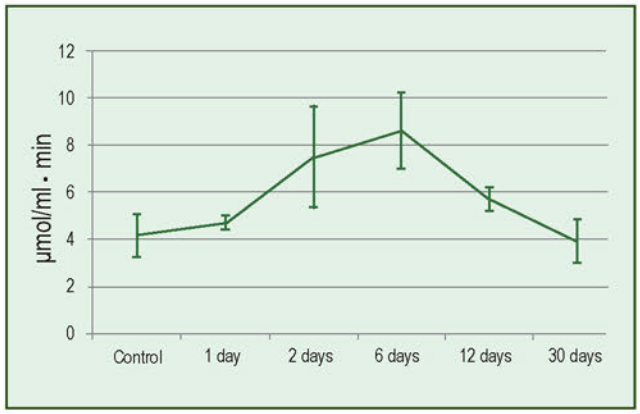


***Figure 8 — Trypsin activity in rats after administration of sodium nitroprusside***

inflammation of the pancreatic tissue may cause its destruction. Leading role in the pancreatic tissue destruction play proteolytic enzymes of polymorphonuclear leukocytes, which induce disintegration of macromolecular complexes containing HA. Probably HA may instigate processes of fibrosis and increasing of its concentration is first indication of changes among other parameters that characterize the functional state of the connective tissue. In this specific experiment, the content of HA in rat blood after 6 days of NG-nitro-L-arginine administration is increased by 1.4 times, to (6.05 ± 0.06) g/l ($p < 0.001$), after 12 days — by 1.5 times, to (6.42 ± ± 0.17) g/l ($p < 0.001$), and after 30 days — by 1.8 times, to (7.73 ± 0.06) g/l ($p < 0.001$) compared with the control group (4.27 ± 0.18) g/l mcM (Fig. 5). Concentration of the in the 30 days was significantly higher than in 1 day ($p < 0.001$), 2 days ($p < 0.01$), 6 days ($p < 0.001$) and 12 days ($p < 0.001$).

In 24 and 48 hours after the introduction of sodium nitroprusside significant decrease of nitrite/nitrate concentrations was observed in blood — by 2.9 times, to (11.34 ± ± 0.24) μmol/l ($p < 0.001$) and (11.21 ± 2.36) μmol/l ($p < 0.01$) in comparison with the control group (32.61 ± ± 4.55) μmol/l, whereas at 6th day there was a significant increase by 1.8 times, to (57.10 ± 7.28) μmol/l ($p < 0.05$). Here on those concentrations decreases smoothly till the 30th day by 3.7 times, to (8.75 ± 3.16) μmol/l ($p < 0.05$) (Fig. 6). The nitrite/nitrate concentration in the rat blood after 6 days of sodium nitroprusside administration was significantly higher than after 12 days ($p < 0.01$) and 30 days ($p < 0.001$).

After the first day of experiment slow increase in the activity of α-amylase was noted, with maximum at 6th day — progress by 2.6 times, from (96.02 ± ± 20.30) mg/s • l (control) to (209.41 ± 38.50) mg/s • l ($p < 0.05$). However, after 30 days was observed the significant reduction in the activity of this enzyme — by 1.5 times to (65.75 ± 8.03) mg/s • l ($p < 0.05$) (Fig. 7). Significant difference was observed in α-amylase activity at 6th day and at 30th day of sodium nitroprusside administration ($p < 0.05$).

Trypsin is the best marker for the detection of pancreas pathology, as it is specific to this organ. Activity of this enzyme was varied similarly to α-amylase activity: increasing after 6 days by 2.1 times from (4.19 ± 0.92) μmol/ml • min (control group) to (8.65 ± 1.64) μmol/ml • min ($p < 0.05$) with a following decrease to the levels of the control group at 30th day of the experiment (Fig. 8). Trypsin activity in the blood of the experimental animals after 30 days of sodium nitroprusside administration was significantly lower than in group which receives it only 6 days ($p < 0.05$).

The development of experimental pancreatitis under conditions of NO excess caused by the administration of sodium nitroprusside was accompanied by a gradual violation of glucose metabolism. There was a significant increase (by 1.4 times) of glucose concentration in blood from (3.18 ± 0.42) mmol/l (control group) to (4.34 ± 0.30) mmol/l ($p < 0.05$) and up to 1.7 times (5.37 ± 0.38) mmol/l ($p < 0.01$) in 12 and 30 days respectively (Fig. 9).

Thus, the development of pancreatitis was accompanied by the alteration of endocrine function (Fig. 9).

Hence, after 1 and 2 days of sodium nitroprusside administration there was a significant increase of NO metabolites in rat blood ($p < 0.001$), upward tendency in the activity of pancreatic enzymes — α-amylase and trypsin. Morphologically dilation of blood vessels and ducts were observed, alongside with accumulation of secretion in the pancreas.

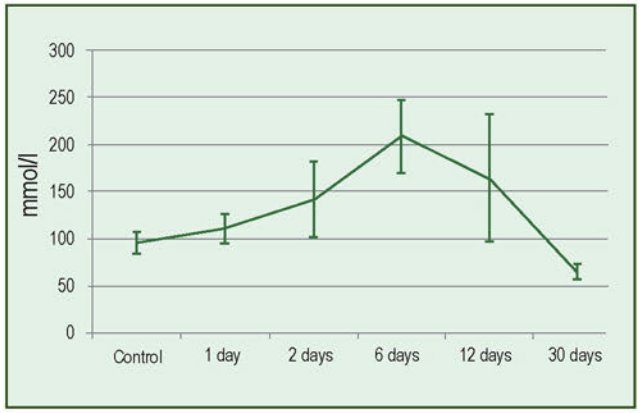


***Figure 9 — Glucose concentration in rat blood after administration of sodium nitroprusside***

After 6 days the maximum increase in serum enzyme activity — α-amylase ($p < 0.05$) and trypsin ($p < 0.05$), and of the NO metabolites concentration ($p < 0.05$) was noted, in addition to gradual increase of the HA concentration ($p < 0.001$). Morphological signs of acute pancreatitis were shown, the structural basis of which was inflammation — stromal infiltration by lymphocytes and leukocytes, dilation of blood vessels and intralobular ducts, stasis of blood cells, presence of hypersecretory acinar cells and isolated small foci of necrosis.

After 12 days there was a downward tendency in the serum activity of α-amylase and trypsin and significant reduction of the NO metabolites concentration compared to day 6 ($p < 0.01$). There was the significant increase of the protein-bonded hydroxyproline and free hydroxyproline concentration ($p < 0.01$) along with HA ($p < 0.001$). Morphological signs included stromal infiltration and vasodilation. Some acinar cells was in state of focal adipose degeneration or segmental apoptosis.

After 30 days the maximum levels of collagen synthesis was observed — highest concentration of protein-bonded hydroxyproline in the serum ($p < 0.01$), in parallel with catabolism — highest concentration of free hydroxyproline ($p < 0.05$) and HA ($p < 0.001$). Against this background functional failure of the pancreas was developing, which made itself evident in the sharp decrease of the pancreatic enzymes activity — namely α-amylase ($p < 0.05$) and trypsin ($p < 0.05$). Reduced concentration of NO metabolites (nitrite/nitrate) was observed after 1 ($p < 0.001$) and 2 ($p < 0.001$) days, with gradual increase occurred afterwards — maximum concentration after 6 days ($p < 0.05$), followed by a gradual decrease — minimum concentration after 30 days ($p < 0.001$). Following morphological changes after 30 days of sodium nitroprusside administration were observed: compensation of previously affected microcirculation (diameter of blood vessels did not differ from the comparison group); mild dilatation of the interlobular ducts and development of intralobular connective tissue. The tiny bands of fibrous tissue enveloped main ducts, major blood vessels and penetrates into the interlobular space zone, which is typical for chronic pancreatitis.

## Conclusions

1. NO excess caused by the intraperitoneal administration of sodium nitroprusside leads to following morphological changes in the pancreas: vasoditation with stasis of blood cells after 1 and 2 days; focal necrosis, destruction of acinar tissue, ductal dilatation and excessive accumulation of secretion after 6 days; adipose degeneration and segmental apoptosis after 12 days and morphologically compensated microcirculatory changes which accompanied the development of fibrous tissue around main ducts, major blood vessels and in the interlobular space after 30 days — changes typical for chronic pancreatitis.

2. Exocrine pancreatic function of rats responded to the excess of NO in ways of increasing pancreatic enzymes levels in blood serum — α-amylase and trypsin, which than significantly decrease after 30 days of experiment. The biochemical markers of fibrosis (free and protein-bonded hydroxyproline, hexosamine) also showed increase levels, accompanied with endocrine insufficiency. All this changes is peculiar to development of chronic experimental pancreatitis.

**Prospects for further research.** The results indicate the feasibility of continuing study for fibrous transformation in the rat pancreas under conditions of NO excess and opportunities for adenocarcinoma developing on this background.

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## МОРФОФУНКЦИОНАЛЬНЫЕ ИЗМЕНЕНИЯ ПОДЖЕЛУДОЧНОЙ ЖЕЛЕЗЫ КРЫС В УСЛОВИЯХ ИЗБЫТКА ОКСИДА АЗОТА

**Резюме.** Исследование проведено на 40 крысах-самцах линии Wistar после ежедневного внутрибрюшинного введения донатора готовых молекул оксида азота (NO) натрия нитропруссида в дозе 1,5 мг/кг через 1 сутки (n = 6); 2 суток (n = 6); 6 суток (n = 6); 12 суток (n = 6); 30 суток (n = 6). Установлено, что избыточное поступление NO приводит к морфологическим изменениям в поджелудочной железе: вазодилатации со стазом форменных элементов крови через 1 и 2 суток; очаговому некрозу, деструкции ацинарной ткани, дилатации протоков с ухудшением оттока панкреатического секрета через 6 суток; жировой дистрофии, сегментарному апоптозу через 12 суток; компенсированным микроциркуляторным изменениям в органе, формированию фиброзной ткани в перидуктулярной и перивазальной зонах с проникновением в междольковое пространство через 30 суток. В сыворотке крови отмечалось нарушение экзокринной функции поджелудочной железы: повышение активности панкреатических энзимов — α-амилазы и трипсина до 6 суток, а через 30 суток — их достоверное снижение; увеличение уровня биохимических маркеров фиброза (оксипролина белковосвязанного и гексозаминов); недостаточность инкреторной функции — повышение уровня глюкозы, то есть изменения, характерные для хронического экспериментального панкреатита.

**Ключевые слова:** оксид азота, нитропруссид натрия, хронический панкреатит, фиброз.

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## МОРФОФУНКЦІОНАЛЬНІ ЗМІНИ ПІДШЛУНКОВОЇ ЗАЛОЗИ ЩУРІВ В УМОВАХ НАДЛИШКУ ОКСИДУ АЗОТУ

**Резюме.** Дослідження проведено на 40 шурах-самцях лінії Wistar після щоденного внутрішньоочеревинного введення донатора готових молекул оксиду азоту (NO) натрію нітропрусиду в дозі 1,5 мг/кг через 1 добу (n = 6); 2 доби (n = 6); 6 діб (n = 6); 12 діб (n = 6); 30 діб (n = 6). Установлено, що надмірне надходження NO призводить до морфологічних змін у підшлунковій залозі: вазодилатації зі стазом формених елементів крові через 1 та 2 доби; осередкового некрозу, деструкції ацинарної тканини, дилатації протоків з погіршенням відтоку панкреатичного секрету через 6 діб; жирової дистрофії, сегментарному апоптозу через 12 діб; компенсованим мікроциркуляторним змінам в органі, формуванню фіброзної тканини в перидуктулярній та перивазальній зонах з проникненням у міждольковий простір через 30 діб. У сироватці крові відмічалося порушення екзокринної функції підшлункової залози: підвищення активності панкреатичних ензимів — α-амілази і трипсину до 6 діб, а через 30 діб — їх вірогідне зниження, збільшення рівня біохімічних маркерів фіброзу (оксипроліну білковозв'язаного та гексозамінів), недостатність інкреторної функції — підвищення рівня глюкози, тобто зміни, характерні для хронічного експериментального панкреатиту.

**Ключові слова:** оксид азоту, нітропрусид натрію, хронічний панкреатит, фіброз.