The study of pathohistological and morphofunctional changes in the endocrine pancreas is an integral part of experimental diabetology, which allows obtaining a significant amount of information on various aspects of the etiology and pathogenesis of diabetes and its complications using experimental animal models.

**Materials and methods.** The experiments were performed on 63 white non-linear adult male rats, experimental diabetes mellitus (EDM) was induced in 53 of them (10 intact rats served as control group). At 10, 20, 25, 30, 40 and 45 days after the administration of the diabetogenic substance, the experimental animals were withdrawn from the experiment, the pancreas was removed and serial sections were made, stained with hematoxylin and eosin according to the standard technique, as well as with aldehyde fuchsin – for the identification of β-cells of the islets of Langerhans. To quantify the endocrine part of the pancreas, the average diameter of the islets of Langerhans (μm), the average number of cells in the islets of Langerhans in the profile of the histological section, as well as the specific volume of the islets of Langerhans in the pancreatic tissue (%) were studied in serial histological sections.

**Results.** The course of alloxan-induced EDM was accompanied by significant destructive-degenerative changes in the pancreatic islets during all observation periods. On the 11th day after alloxan administration, most of the cells of the islets of Langerhans were in a state of necrosis with signs of karyopyknosis and karyorrhexis. As a result of autolysis processes, the number and size of islets of Langerhans, their specific volume in the pancreatic tissue, and the average number of cells in them were greatly reduced. No specific staining of β-cells with aldehyde fuchsin was detected. Moderate swelling of the pancreatic interstitium, focal dystrophic processes in the epitheliocytes of the exocrine pancreas, sometimes with pronounced microcystic formations, were found on the 11th day of the experiment.

On the 21st and 26th day after alloxan administration, the specific volume, the size of the pancreatic islets of Langerhans and their cellularity parameters continued to decrease reliably. The majority of Langerhans islets were in a state of necrosis, but signs of karyorrhexis, observed mainly in the center of the islets, were added to the previously existing signs of karyopyknosis and karyorrhexis. The cells of the exocrine pancreas showed no visible signs of alteration, indicating their recovery after the 11th day of the experiment.

In the 31-day EDM, all the main trends observed in the previous stages of the experiment were maintained, with the exception of necrotic changes in the cells of the islets of Langerhans – no necrotic insuloocytes were detected. However, no β-cells were detected in the pancreatic islets, similar to the situation on days 21 and 26 of the experiment. Similar histologic and morphometric results were obtained on day 41 of the experiment. The decrease in cytarity of the preserved islets was maximal in the 46-day EDM for the entire duration of the experiment, and aldehyde-fuchsin staining of histological sections did not reveal even single β-cells in the pancreatic islets of Langerhans.

**Conclusions.** A single intraperitoneal administration of alloxan solution at a dose of 160 mg/kg bw to experimental animals induced pathomorphologic changes in the islet part of the pancreas, which had a significant degenerative-destructive character already on the 11th day of the experiment, increased and persisted at all its stages. The diabetogenic cytotoxin alloxan caused severe dystrophic changes in the pancreatic islets of varying intensity at all stages of the experiment – from karyopyknosis/karyorrhexis beginning on the 11th day of observation to their burden with karyolysis beginning on the 21st day of observation. The necrobiotic processes led to a reliable decrease in the endocrine parenchyma and a decrease in the specific volume of the islets of Langerhans in the pancreatic tissue, a decrease in the average size of the islets of Langerhans with a significant reduction in their cellular composition. The beta-cells of the islets of Langerhans exceptionally served as a morphofunctional substrate for the pancreatotoxic effect of alloxan, while the cells of the exocrine pancreas did not show any significant signs of alteration already after the 11th day of the experiment. The absence of even single β-cells in the pancreatic islets at all time points of the experiment (when histological sections were stained with aldehyde-fuchsin) indicates the irreversibility of their damage and the absence of reparative regeneration. Alloxan-induced destruction of β-cells with subsequent loss of their secretory activity confirms the validity of the simulated experimental model for the development of decompensated diabetes in animals due to insulin deficiency.

**Key words:** Alloxan; Experimental Diabetes mellitus; Pancreas.
Despite a large number of comprehensive studies on the etiologic, pathogenetic, functional and morphologic aspects of diabetic complications, there are still open and controversial questions that require detailed investigation. In this regard, experimental diabetology is of great importance because a significant amount of information about various aspects of the etiology and pathogenesis of diabetes mellitus and its complications has been obtained precisely as a result of preclinical studies using experimental animal models [3].

Among the variety of experimental models of diabetes mellitus, non-genetic experimental models of diabetes using hydrophilic β-cell glucose analogues, such as alloxan, streptozotocin, chlorozotocin, cyproheptadine, etc., are considered the most easily reproducible [4-7]. In this category of DM models, the use of alloxan is the most widespread – it allows to unambiguously determine the type of DM induced in the experiment, since it has cytostatic properties with respect to pancreatic islet β-cells, thus providing an objective assessment of the processes studied [8-11].

There are a large number of modifications of alloxan diabetes, the severity of which varies depending on the method of alloxan administration and its dosage [12, 13]. At the same time, clinical and biochemical signs of diabetes (hyperglycemia, glucosuria, polyuria, polydipsia, weight loss, decrease in the concentration of C-peptide and insulin, increase in the concentration of glycated hemoglobin, etc.) do not allow to reliably assess the degree of damage to endocrinocytes of pancreatic islets [12, 13]. Meanwhile, the determination of pathohistological, morphofunctional changes in the endocrine pancreas has become an integral part of experimental diabetes research [14-16].

Considering that the objectives of the study were to perform a comprehensive evaluation of the histostructural peculiarities of the pancreas and the reparative potential of the affected pancreatic islets of Langerhans in rats with alloxan-induced experimental diabetes mellitus (EDM) of different duration.

Material and methods

The experiments were performed on 63 white non-linear adult male rats, weighing 0.18-0.20 kg, maintained under identical standard vivarium conditions. Experimental DM was simulated by intraperitoneal administration of alloxan solution (alloxan monohydrate, Acros Organics, Belgium) at a dose of 160 mg/kg to 53 animals after 12 h of food deprivation with maintained access to water ad libitum. At 10, 20, 25, 30, 40 and 45 days after administration of the diabetogenic substance, 53 animals with EDM and 10 (intact) rats of the control group were removed from the experiment – euthanasia was performed by decapitation under light ether anesthesia. The level of glucose in the blood samples taken at the moment of decapitation of the animals was determined and only those with persistent hyperglycemia exceeding 7.0 mmol/L were considered.

The pancreas was removed after the animals were decapitated. Paraffin-embedded material was serially sectioned at 5 μm thickness and stained with hematoxylin and eosin using standard techniques [17-19] and with aldehyde-fuchsin to identify beta cells of the islets of Langerhans [20-22]. To quantify the endocrine part of the pancreas, the mean diameter of the islets of Langerhans (μm), the number of cells in the islets of Langerhans in the profile of the histological section, as well as the specific volume of the islets of Langerhans in the pancreatic tissue (%) were studied in serial histological sections. Morphometric results were obtained using the open-source software ImageJ 1.52 (National Institutes of Health, USA).

The data obtained were statistically processed using the licensed software Statistica for Windows 8.0 (Statsoft. Inc., USA) with determination of the mean (X), standard error of the mean (Sx), interquartile range (Q25-Q75). Reliability of between-group differences was assessed using the nonparametric Mann-Whitney rank test [23].

The research was conducted in accordance with the provisions of the EU Directive No. 609 (1986) and the Order of the Ministry of Health of Ukraine No. 690 dated 23.09.2009 «On Measures for Further Improvement of Organizational Norms for Work with Experimental Animals».

Results

The results of our research showed no pathological changes in both the exocrine and endocrine pancreas of intact rats (Fig. 1A, 1B). The islets of Langerhans were mostly oval and round in shape, and only sometimes irregularly shaped. Very elongated islets were occasionally found, but not in every rat. The islets were clearly separated from the surrounding acinar parenchyma. Epitheliocytes in the islets were arranged in strands separated by capillaries. According to the literature, these characteristics of islet cells are typical for rats [11, 14, 16]. The cytoplasm of Langerhans islet cells was generally quite homogeneous, almost homogeneous, when stained with hematoxylin and eosin.

![Fig. 1. The pancreas of an intact rat. Islet of Langerhans: A – of maximal size, staining of the histological section with hematoxylin and eosin, Ob.20°. Oc.10°; B – of minimal size, staining of histological section with hematoxylin and eosin, Ob.20°. Oc.10°; C – staining with aldehyde fuchsins, Ob.40°. Oc.10°.](image-url)
The central part of the islets is densely and uniformly filled with β-cells (80-90% of the total number of insulocytes) and the periphery with α-cells. On average, 48.0±1.20 β-cells per islet were found in the islet portion of the intact rat pancreas. In the majority of islets, β-cells maintained a normal structure without dystrophy and signs of destruction, filling the islet evenly and maintaining the typical arrangement by strands. (Fig. 1B).

The course of alloxan-induced EDM was accompanied by significant destructive-degenerative changes in the pancreatic islets during all observation periods (Table). On the 11th day after alloxan administration, most of the cells of the islets of Langerhans were in a state of necrosis with signs of karyopyknosis and karyorrhexis (Fig. 2). As a result of the islets of Langerhans were greatly reduced. Their mean diameter was found to be twice lower in 11-day EDM than in controls, while the specific volume of Langerhans islets in pancreatic tissue was four times lower. The majority of the islets acquired an irregular shape, a clear border with the surrounding acinar parenchyma was often absent. The perimeter of islets mostly did not coincide with their cellularity – the average number of cells in the composition of islets of Langerhans, profiled by histological section, significantly decreased – by 68% in 11-day EDM in comparison with the index of intact animals. In some places the islets practically disappeared. The parameters of islets of Langerhans did not depend on their location in the pancreatic tissue.

**Table**

<table>
<thead>
<tr>
<th>Indices</th>
<th>Control, n=10</th>
<th>11-day EDM, n=10</th>
<th>21-day EDM, n=9</th>
<th>26-day EDM, n=8</th>
<th>31-day EDM, n=10</th>
<th>41-day EDM, n=8</th>
<th>46-day EDM, n=8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average number of cells in the islets of Langerhans in the profile of the histological section</td>
<td>128,0±9,24 [74.9; 181.1]</td>
<td>41,0±4,94 [20.1; 61.9] p&lt;0.001</td>
<td>25,0±3,22 [10.6; 39.4] p&lt;0.001</td>
<td>23,0±3,97 [7.2; 38.8] p&lt;0.001</td>
<td>26,0±4,16 [4.5; 47.5] p&lt;0.001</td>
<td>21,7±4,19 [4.0; 40.8] p&lt;0.001</td>
<td>20,3±4,31 [5.0; 38.7] p&lt;0.001</td>
</tr>
<tr>
<td>Average diameter of the islets of Langerhans, μm</td>
<td>447,1±27,0 [311.0; 583.0]</td>
<td>223,±23,53 [117.4; 328.6] p&lt;0.001</td>
<td>234±3,15 [8.0; 41.0] p&lt;0.001</td>
<td>22.4±3.74 [8.0; 40.1] p&lt;0.001</td>
<td>25.3±3.91 [8.5; 46.5] p&lt;0.001</td>
<td>21.1±3.25 [7.0; 38.0] p&lt;0.001</td>
<td>20.3±3.67 [7.7; 35.3] p&lt;0.001</td>
</tr>
<tr>
<td>Specific volume of the islets of Langerhans in the pancreatic tissue, %</td>
<td>0.989±0.0190 [0.902; 1.076]</td>
<td>0.247±0.0161 [0.159; 0.335] p&lt;0.001</td>
<td>0.121±0.0137 [0.056; 0.186] p&lt;0.001</td>
<td>0.122±0.0130 [0.063; 0.181] p&lt;0.001</td>
<td>0.123±0.0138 [0.055; 0.191] p&lt;0.001</td>
<td>0.120±0.0135 [0.062; 0.178] p&lt;0.001</td>
<td>0.118±0.0142 [0.061; 0.175] p&lt;0.001</td>
</tr>
</tbody>
</table>

Note: intergroup differences were assessed using the non-parametric Mann-Whitney test; p – probability of discrepancy of indices relative to control group; p1 – probability of discrepancy of indices relative to group 1; p2 – probability of discrepancy of indices relative to group 2; p3 – probability of discrepancy of indices relative to group 3; p4 – probability of discrepancy of indices relative to group 4; p5 – probability of discrepancy of indices relative to group 5; n – number of animals.

![Fig. 2. The pancreas of a rat with 11-day experimental diabetes mellitus. Islet of Langerhans: A – located in the central parts of the pancreas, B – located under the capsule of the pancreas. Islet cells are mostly in a state of necrosis (karyopyknosis and karyorrhexis). Staining of histological section with hematoxylin and eosin. Ob.20. Oc.10.](image-url)
In the case of 26-day alloxan-induced EDM, all previous trends were maintained. Thus, the size of islets of Langerhans and their cytology parameters on day 26 of the experiment were as small as in the intact animals and in the experimental animals with 11-day EDM as in the previous term of the experiment (Fig. 5A). In particular, the average number of cells in the islets of Langerhans composition profiled by histological section in 26-day EDM was 82.0% lower than the control level and 43.9% lower than that of rats with 11-day EDM. The average diameter of Langerhans islets decreased by 20.1 times compared to the level of intact animals and by 10.1 times compared to the 11-day EDM index. The specific volume of islets of Langerhans in the pancreatic tissue of experimental rats at this stage of observation remained 87.7% lower than the control level.

On day 26 of the experiment, the majority of Langerhans islet cells were in a necrotic state, but in addition to the karyopyknosis and karyorrhexis observed previously, signs of karyolysis were observed here, as well as on day 21 of the experiment, mainly in the center of the islets (Fig. 5A).
It should be emphasized that the cells of the exocrine pancreas showed no visible signs of alteration, indicating their stable recovery after 11 to 21 and 26 days of experimental alloxan diabetes and confirming the β-cell selectivity of the pancreatotoxic effect of alloxan.

On the 31st day after alloxan administration, all the main trends found on the 21st and 26th days of the experiment were preserved, with the exception of necrotic changes in the cells of the islets of Langerhans (Fig. 5B). Therefore, on the 31st day of the experiment, an average number of cells in the composition of islets of Langerhans, profiled by the histological section, was 4.9 times less than that of intact animals, an average diameter of islets of Langerhans was 17.7 times shorter, and the specific volume of islets of Langerhans in the pancreatic tissue of experimental rats with 31-day EDM – by 8 times, respectively. At the same time, no necrotic insulocytes were detected on the 31st day of the experiment (Fig. 5B). However, no β-cells were found in the pancreatic islets, similarly to the 21st and 26th day of the experiment. Therefore, despite the cessation of the progression of the changes in the islets of Langerhans on the 31st day of the experiment, there is no evidence of restoration of β-cells. It should also be noted that the cells of the exocrine pancreas showed no visible signs of alteration on the 31st day of experimental alloxan diabetes, which confirms the stable repair of these cells after the damage caused during the first 10 days of the experiment.

On the 41st day of the experiment, all the main trends determined on the 31st day after alloxan administration were maintained (Fig. 5C). The above refers to both endocrine and exocrine pancreas of experimental animals. Morphometric analysis showed that in response to alloxan administration after 41 days in rats there was a statistically significant decrease in the number of pancreatic islets, a significant reduction in the cytarity of the preserved islets, and necrosis of their cells. Thus, the average number of cells in the composition of islets of Langerhans, profiled by histological section, in case of 41-day EDM was 83.1 % less than that of intact animals, the average diameter of islets of Langerhans was 21.2 times shorter, and the specific volume of islets of Langerhans in the pancreatic tissue of experimental rats was reduced by 8.2 times, respectively.

At the end of the experiment, the specific volume of islets in rat pancreatic tissue was reduced by an average of 8.4-fold compared to intact animals (Figure 6A). Since the diameter of the islets was also significantly reduced, on average by 22.1-fold, the area of all islets per unit area of the gland is reduced even more. The decrease in cytarity of the preserved islets was maximal for the entire duration of the experiment – the average number of cells in the composition of Langerhans islets, profiled by histological section, was 84.1 % less than the control index on the 46th day after alloxan administration.

It is important to emphasize that in the case of 46 days of alloxan diabetes, as well as on the 11th, 21st, 26th, 31st, and 41st days of the experiment, the applied histochemical staining technique with aldehyde-fuchsin did not reveal even a single β-cell in the islets of Langerhans of the pancreas (Fig. 6B). Selective damage to islet β-cells by cytotoxic alloxan resulted in their death. This fact reliably confirms the adequacy of the simulated model of alloxan diabetes in terms of irreversible limitation of the functional capabilities of the endocrine pancreas and induction of absolute insulin deficiency in experimental rats.
Conclusions
1. A single intraperitoneal administration of an alloxan solution at a dose of 160 mg/kg of body weight to experimental animals induced pathomorphologic changes in the islet part of the pancreas, which had a significant degenerative-destructive character already on the 11th day of the experiment, which increased and persisted at all subsequent stages.

2. The diabetogenic cytotoxin alloxan caused severe dystrophic changes in the pancreatic islets of varying intensity at all stages of the experiment – from karyopyknosis/karyorrhexis already from the 11th day of observation to their burden with karyolysis from the 21st day of observation. The necrotic processes led to a reliable decrease in the endocrine parenchyma and a decrease in the specific volume of the islets of Langerhans in the pancreatic tissue, a decrease in the average size of the islets of Langerhans with a significant reduction in their cellular composition.

3. β-cells of the islets of Langerhans exceptionally served as a morphofunctional substrate for the pancreatotoxic effect of alloxan, whereas the cells of the exocrine pancreas did not show any noticeable signs of alteration already after the 11th day of the experiment. The absence of even single β-cells in the pancreatic islets at all times of the experiment (when staining histological sections with aldehyde-fuchsin) proves the irreversibility of their damage and the absence of reparative regeneration.

4. Alloxan-induced destruction of β-cells with subsequent loss of their secretory activity proves the validity of the simulated experimental model for the development of decompensated diabetes in animals due to insulin deficiency.

Prospects for further research can be considered in the assessment of the insulin-producing function of the β-cells of the Langerhans islets in correlation with the results of histomorphometry in the dynamics of the development of alloxan-induced experimental diabetes.

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ГІСТОМОРФОЛОГІЧНІ ОСОБЛИВОСТІ ПАРЕНХИМИ ПІДШЛЮНКОВОЇ ЗАЛОЗИ ЩУРИВ ПРИ АЛОКСАН-ІНДУКОВАНИМ ДІАБЕТІ РІЗНОЇ ТРІВАЛЬНОСТІ

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Резюме.
Вивчення патогістологічних, морфофункціональних змін в ендокрінному апараті підшлюнкової залози (ПШЗ) є обов’язковою частиною експериментальної діабетології, яка дозволяє отримати значний об’єм інформації стосовно різноманітних аспектів етології та патогенезу цукрового діабету та його ускладнень через використання експериментальних моделей на тваринах.

Мета дослідження – проведення комплексної оцінки особливостей гістоструктури ПШЗ та репараційного потенціалу уражених панкреатичних остривців Лангерганса у щурів з алоксан-індукуваним експериментальним підшлюнковим діабетом (ЕПД) різної тривалості.

Матеріали та методи. Дослідження проведено на 63 статевозрілих нелінійних самців білых щурів, у яких 53-х яких моделювали алоксановий експериментальний підшлюнковий діабет (ЕПД) (10 інтенсивних тварин слугували контрольом). Через 10, 20, 25, 30, 40 та 45 діб після уведення діабетогенної речовини експериментальних тварин вводили з експерименту, вищухали підшлюнкову залозу (ПШЗ), виготовляли серії зрізів, котрі забарвлювали гематоксилином та сеоком за стандартною методикою, а також альдегід-фуксином – для ідентифікації β-клітин остривців Лангерганса. Для кількісної оцінки ендокрінної частини ПШЗ на серійних гістологічних зрізах вивчали середні діаметри остривців Лангерганса (мкм), середню кількість клітин остривців Лангерганса у профілі гістологічного зрізу, а також питомий об’єм остривців Лангерганса в тканині підшлюнкової залози (%).

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