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PANCREATIC MORPHOMETRY UNDER STRESS

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Abstract: This study investigated the impact of maternal stress on pancreatic development in rat offspring. Morphological analyses of the pancreas at various postnatal stages revealed a developmental delay and altered acinar cell development in the experimental group compared to controls. Notably, changes in both exocrine and endocrine cells were observed from the third day of the experiment in all stressed animals, including destructive changes in the mitochondria of acinocytes. Specifically, morphometric analysis showed a decrease of up to 4% in pancreatic acini diameter and an 18% reduction in acini height in the experimental group relative to controls. These hemomicrocirculatory changes, characterized by interstitial stroma swelling and cellular dystrophic changes, were most pronounced on day 14 and correlated with destabilization of acinocyte membrane structures. These findings suggest that maternal stress induces significant morphological changes in offspring pancreatic cells, potentially leading to chronic pancreatitis. The experimental design involved subjecting pregnant rats to a stress-inducing maze environment, with offspring pancreases examined histologically at 3, 7, 14, 21, and 30 days postnatal.

Keywords: stress, pancreas, pancreatic lobes, acinus, morphological changes, experimental stress, postnatal ontogenesis, exocrine cells, endocrine cells, rat model, maternal health, chronic pancreatitis, histological preparation, morphometry, laboratory rats, saline solution, experimental conditions, abdominal cavity.

Introduction. Stress is a universal nonspecific neurohormonal response of the body in the form of an increase in the body's resistance to damage or as a signal that threatens the life or well-being of the organism. Today, stress disorders are one of the main problems of public health concern. In addition, the COVID-19 pandemic and the consequences of social isolation have had a profound impact on the mental health of society. These factors constitute a heterogeneous cluster of mental disorders that can manifest as general physical symptoms (for example, tachycardia, shortness of breath, or gastrointestinal disorders) accompanied by anxiety. Other clinical symptoms associated with stress include excessive or disproportionate anxiety and fear, persistent issues associated with impairment in social, occupational, or other important areas of functioning.

Relevance of the work. Gastrointestinal problems are commonly reported in studies of occupational stress. Unfortunately, it is difficult to assess the physiological systems underlying gastrointestinal symptoms in the workplace. Acute psychological stress has a variable effect on pancreatic secretion, stimulating large increases in some individuals and reducing production in others. Gastrointestinal problems are particularly high in many workers, manifested by disruptions in the circadian rhythms of the central nervous system in controlling pancreatic secretion. A growing body of research suggests that prenatal stress can have significant effects on pregnancy, maternal health, and human development

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throughout an individual's life. These effects may occur directly through the impact of prenatal stress-related physiological changes on the developing fetus or indirectly through the effects of prenatal stress on maternal health and pregnancy outcomes, which in turn affect infant health and development (The University of Colorado, Colorado Springs, and The Colorado School of Public Health, 1420 Austin Bluffs Parkway, Colorado Springs, CO 80918, USA). The prevalence of stress in the United States has been found to have increased by approximately 6.7% among individuals aged 18 to 25 from 2008 to 2018. The increasing prevalence of anxiety and stress in society is considered to be the beginning of several mental health problems, cardiovascular diseases, digestive diseases, and metabolic syndrome. With changes in the environment and lifestyle, the incidence of pancreatic diseases has been increasing year by year in recent years, and pancreatic diseases have become a common ailment of the digestive system. The etiology of pancreatic diseases is diverse; the condition is complex, treatment is relatively difficult, and the mortality rate is high. The harm of pancreatic diseases to human health has attracted great attention from researchers. Reducing the mortality rate and improving the prognosis of patients with pancreatic diseases is becoming a major problem in medicine today.

Purpose: To determine the nature of morphological and morphometric changes in the pancreas under experimental stress.

Materials and methods of research. To achieve the goal, 60 white laboratory rats were subjected to experimental stress. The rats were divided into 2 groups. Group 1 is a control group of healthy rats. Mother rats in the control group were given 1.0 ml of saline solution every morning in the stomach. A subclavian catheter was used as a probe. Rat pups were anesthetized under ether anesthesia on days 3, 7, 14, 21, and 30 after birth. Group 2 is an experimental group, in which 50 female white laboratory rats were kept in specially prepared maze cages to induce experimental stress. This stress model continued after the rats became pregnant and gave birth. After opening the abdominal cavity of the animals, the pancreas was removed, fixed in a 12% formalin solution, paraffin blocks were prepared, and histological preparations were made from them. Experiments and slaughter of animals were carried out in accordance with the "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" (Strasbourg, 1985). At a thickness of 8–10 μm, histological sections prepared on a rotary microtome were stained with hematoxylin-eosin according to the standard method. Micropreparations were photographed using an MBI-6 microscope. The obtained material was processed using the methods of variation statistics (Boyarsky A.Ya. 1965; Kaminsky L.S. 1953; Sepetliev.D. 1968; Venchikov A.I.; Venchikov V.A.1974, G.G. Avtandilov, 1990) using the tables of R.B. Strelkova (1986).

Results and Discussion. The pancreas accounts for 2.45% of the total body weight of rats. According to our studies, the pancreas of rats can be divided into three parts: 1. Duodenum - located in the mesentery of the duodenum. 2. Biliary - located along the common bile duct. 3. Stomach - located to the right of the spleen. The right lobe of the pancreas of rats is caudally wrapped and is bordered by the duodenum, while the middle lobe surrounds the abdominal wall and the cranial part of the mesenteric artery, and is limited by the transverse colon. The left lobe, which has the shape of an elongated triangle, communicates with the spleen. Externally, the pancreas is covered by a serous membrane and a connective tissue capsule of the small intestine. In rats, the pancreas is located intraperitoneally. The exocrine (exocrine) and intrasecretory (endocrine) parts are distinguished in the pancreas of rats. The delicate stroma of the organ is represented by connective tissue, which divides the gland into lobes. When viewed macroscopically, a connective tissue capsule surrounding it from all sides is

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revealed. From the capsule, a membrane penetrates deep into the organ, resulting in lobes appearing in the parenchyma of the organ, giving the gland a lobed structure. The exocrine part of the pancreas of rats forms a structural and functional unit - the acinus, the cross-sectional area of which is (943.22±47.1) μm². The acinus consists of 8-12 exocrine pancreatocytes or acinocytes. The average area of an exocrine pancreatocyte is (133.2 ± 2.5) μm².

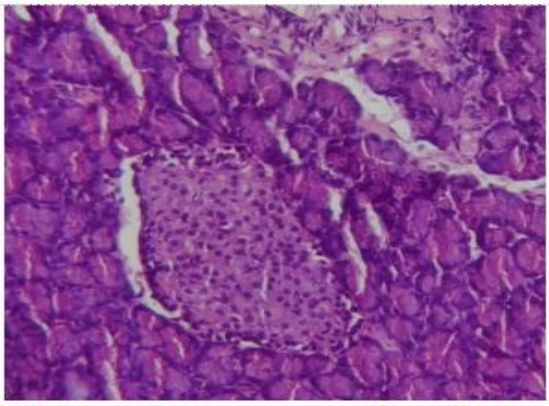

Figure 1. Rat pancreas on the 7th day of the experiment. Dystrophic changes in acinar cells, edema in the islets of Langerhans. Color: hematoxylin-eosin.

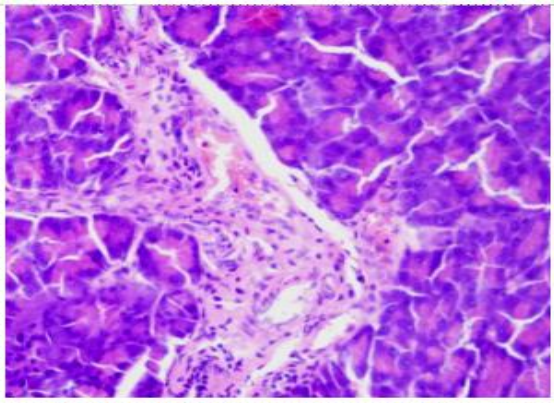

Figure 2. Rat pancreas on the 14th day of the experiment. Increased swelling in the pericapillary spaces. Color: hematoxylin-eosin.

Morphological examination of the pancreas by the 14th day of the experiment showed that changes in the vessels developed even more. An increase in edema was noted in the stroma, mainly in the perivenular and pericapillary spaces (Fig. 2).

Collagen fibers are swollen and loose; the capsule is unevenly located, and swelling of connective tissue along with thickening of trabeculae was clearly observed. The diameter of the capillaries was 2.15±0.2 μm (1.88±0.056 μm in the control group), the walls were dilated, and the stroma was filled with erythrocyte accumulation and tissue edema. It was found that the diameter of the capillary blood vessels increased 1.2 times compared to the previous group (Fig. 3).

Some of the acinocytes around the areas of hemorrhage were observed to be distant and compressed, and the architecture of the islets was disturbed. In the peripheral parts of the pancreas, inflammatory infiltration of the interacinar spaces between the acini and hyperplasia of ductal epithelial cells were observed (Fig. 4).

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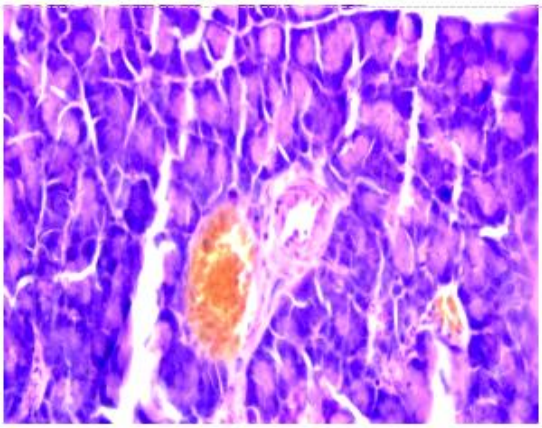

Figure 3. Rat pancreas on the 14th day of the experiment. Thickening of the diameter of capillary blood vessels. Color: hematoxylin eosin.

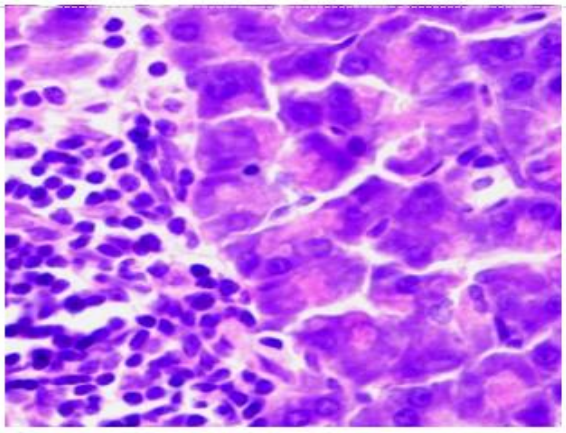

Figure 4. Rat pancreas on the 14th day of the experiment. Inflammatory infiltration of the interacinar spaces between the acini. Color: hematoxylin eosin.

Significant structural changes occurred in endocrine cells. The average area of the nuclei decreased, and karyopyknosis occurred. The area of α-cells decreased by 8.5% compared to the control group. The α-cell nucleus showed enlarged pores and reduced electron density. The area of secretory granules increased in α-cells, but the diameter of granules decreased from 22.7±0.9 nm to 18.4±0.5 nm.

As a result of morphometric studies, the diameter of pancreatic acini is 11.4±0.3 μm, and their height reaches 2.15±0.18 μm. Compared to the control group, acinar cell diameter and height were delayed by 3% and 12% in the experimental group. These changes were evident at 14 days.

On day 21, the segmental structure of the gland was preserved, but the volume of the segments was slightly reduced. An increase in the intensity of swelling in the stroma of the pancreas was observed, which spread throughout the gland and became diffuse. Swelling and fragmentation of collagen fibers were observed in some areas. Reparative processes were enhanced in the preserved parts of the stroma, with intensive proliferation of fibroblasts and the formation of fibrils detected. The capillary diameter of the exocrine parenchyma reached 2.24±0.26 μm (in the control group, it was 1.91±0.07 μm). The capillary diameter increased by 1.3 times. In the exocrine cell acini, the location and structure of the nucleus and nucleoli changed; cytoplasmic vacuolization, decreased zymogen granules, and disturbed structure of the endoplasmic reticulum were noted. Loss of degranulation was observed in the cytoplasm of endocrine cells. Interlobular venous vessels were enlarged, leukocyte accumulation was detected, along with edema and myxomatous changes on the vessel wall.

Plasmorrhagia was observed in the fibrous structures of the walls of small lobular vessels. An increase in the number of degranulated eosinophils was found in the organ stroma.

As a result of morphometric studies, we found that the diameter of pancreatic acini is 11.7±0.2 μm, which is up to 4% compared to the control group, and their height is 2.2±0.15 μm, which is up to 18%.

The results of morphometric studies confirm that in hypothyroidism, the height of the pancreatic gland acinar cells and their diameter gradually decrease compared to the control group, indicating the development of an atrophic process in the gland parenchyma (See Table 1).

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Table 1. **Age-related changes in pancreatic acinar cell morphometric parameters.**

Rat children age (in days) of	Atsinus cell diameter, µm	Acinus cell height, µm
3	10,7±0,2	1,9±0,1
7	11±0,25	2,05±0,15
14	11,4±0,3	2,15±0,18
21	11,7±0,2	2,2±0,15
30	11,9±0,2*	2,3±0,2*

Note:* - p<0.05 is reliable compared to the control group.

Conclusion. Thus, changes are observed in the pancreatic acinocytes of offspring born to mother rats under stress. These changes are hemomicrocirculatory in nature, manifested as swelling of the interstitial stroma and destructive and dystrophic changes in the cells. These changes are more pronounced on the 14th day of the experiment and are characterized by destabilization of membrane structures in acinocytes. As a result, destructive changes are detected in the mitochondria of acinocytes. Disorganization of acinocytes and their structures indicates a violation of the contractile function of these cells. The data obtained prove that changes in the pancreas at the cellular and subcellular levels lead to chronic pancreatitis. As a result of morphometric studies, we observe that the diameter of the pancreatic acini shows a decrease of up to 4%, and their height lags behind growth by up to 18% compared to the control group. Disruption of the architecture of the acinus was seen in the sections located on the periphery of the pancreas. It can be observed that some acinocytes around the blood-stained branches are separated and compressed.

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