

UDC: 616.37-002-036.11:616.37-018-078-08-092

Morphological and immunohistochemical characteristics of the pancreas and liver in experimental pancreatitis and its correction with cytochrome c

Ganiev A.K., Sabirova R.A., Israilov R.I., Tursunov D.Kh.

Tashkent Medical Academy, Republican Pathological Anatomical Center

SUMMARY

The results of the research showed that acute pancreatitis is characterized by a change morphological and immunohistochemical properties of the pancreas' and liver's sells, especially on the 10th day of the experiment. When correcting the revealed changes, it was found that the combined administration of cytochrome c with sandostatin has a more favorable corrective effect on the cytokine status than the individual administration of these drugs to experimental animals with acute pancreatitis.

Key words: acute pancreatitis, morphology, immunohistochemistry, liver, pancreas.

Recently, there has been a significant increase in the incidence of acute and chronic pancreatitis (1-3). Therefore, the study of the mechanisms of damage to the pancreatic tissue, the importance of the process of apoptosis, its enzymatic and cytokine mechanisms of development must be taken into account in the pathogenetic therapy of patients with acute pancreatitis (4). In the fight against acute and chronic pancreatitis, much attention is paid to the study of the role of apoptosis of pancreatic cells. Apoptosis occupies a leading place in maintaining homeostasis, maintaining cell renewal in both physiological and pathological conditions (5-6). The significance of apoptosis lies in the fact that in each organ the proliferation and maturation of functionally active cells occur simultaneously and the selection processes proceed in parallel. With alteration or inflammation, the process of

apoptosis is activated. The signals that induce apoptosis are diverse; they are activated during the inflammatory process.

Purpose of the study: to study the morphological and immunohistochemical characteristics of the pancreas and liver in experimental pancreatitis and its correction with cytochrome c.

Materials and research methods. The experiments were carried out on 40 mature outbred male rats with an initial body weight of 120-140 g, kept on a standard diet. The experiments were carried out in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1985). Acute experimental pancreatitis was induced in rats according to the method of P.S.Simovarian [7]: local freezing of the surface of the pancreas with ethyl chloride.

Morphological and immunohistochemical studies were performed on the 7th and 10th days after the operation in sections of the pancreas and liver. 3 groups of rats were identified - 10 animals in each group:

- 1st - intact group (norm);
- 2nd - animals with 7- and 10-day acute pancreatitis;
- 3rd - animals with acute pancreatitis daily (10 days) intramuscularly injected cytochrome with 0.15 mg/kg of body weight.

The animals were decapitated on the 7th and 10th days after the operation.

Histological sections were prepared on a microtome 5–8 μm thick and stained with hematoxylin and eosin. Sections were examined under a light microscope. For immunohistochemical studies, sections were stained for Ki-67 and Bcl-2.

Results and its discussion. The results of morphological studies of the pancreatic tissue on the 7th day after the modeling of acute pancreatitis showed the presence of foci of necrobiosis and necrosis of the parenchymal elements of the gland in the form of an unstructured mass. In this case, both exocrine acini and

endocrine islets were affected (Fig. 1). The study of pancreatic tissue on a large microscope objective showed that necrotic areas are represented by a non-structural mass consisting of a homogeneous eosinophilically stained protein substance, in the thickness of which single cell structures with signs of apoptosis are determined. In the circle of necrosis, there is interstitial connective tissue in which there are inflammatory cells from leukocytes, lymphocytes and macrophages. The interstitial vessels are sharply dilated, plethoric, their wall is thinned with diapedetic hemorrhages. Exocrine acini are loosened due to strong edema of the interstitial substance, acini cells have disturbed histotopography in the form of wrinkling of the nucleus in the form of apoptosis, vacuolization of the cytoplasm and deformation of the acinar arrangement of cells.

On the 10th day of the experiment, when studying under a large lens under a microscope, dystrophy, destruction of epithelial cells of the exocrine acinus is noted. Histotopography of acini is destroyed, glandular epithelial cells are arranged randomly. Their nuclei are of different shapes and sizes, most of them with signs of the process of apoptosis, nuclear chromatin is hyperchromized with a disorderly arrangement of heterochromatin clusters, uneven karyoplasm. Some nuclei are bare from the cytoplasm and fragmented. Other nuclei are broken up into separate parts, which are located in the intercellular space (Fig. 2). The listed morphological changes in the cytoplasm and nucleus are characteristic of apoptosis of the acini epithelium in acute pancreatitis.

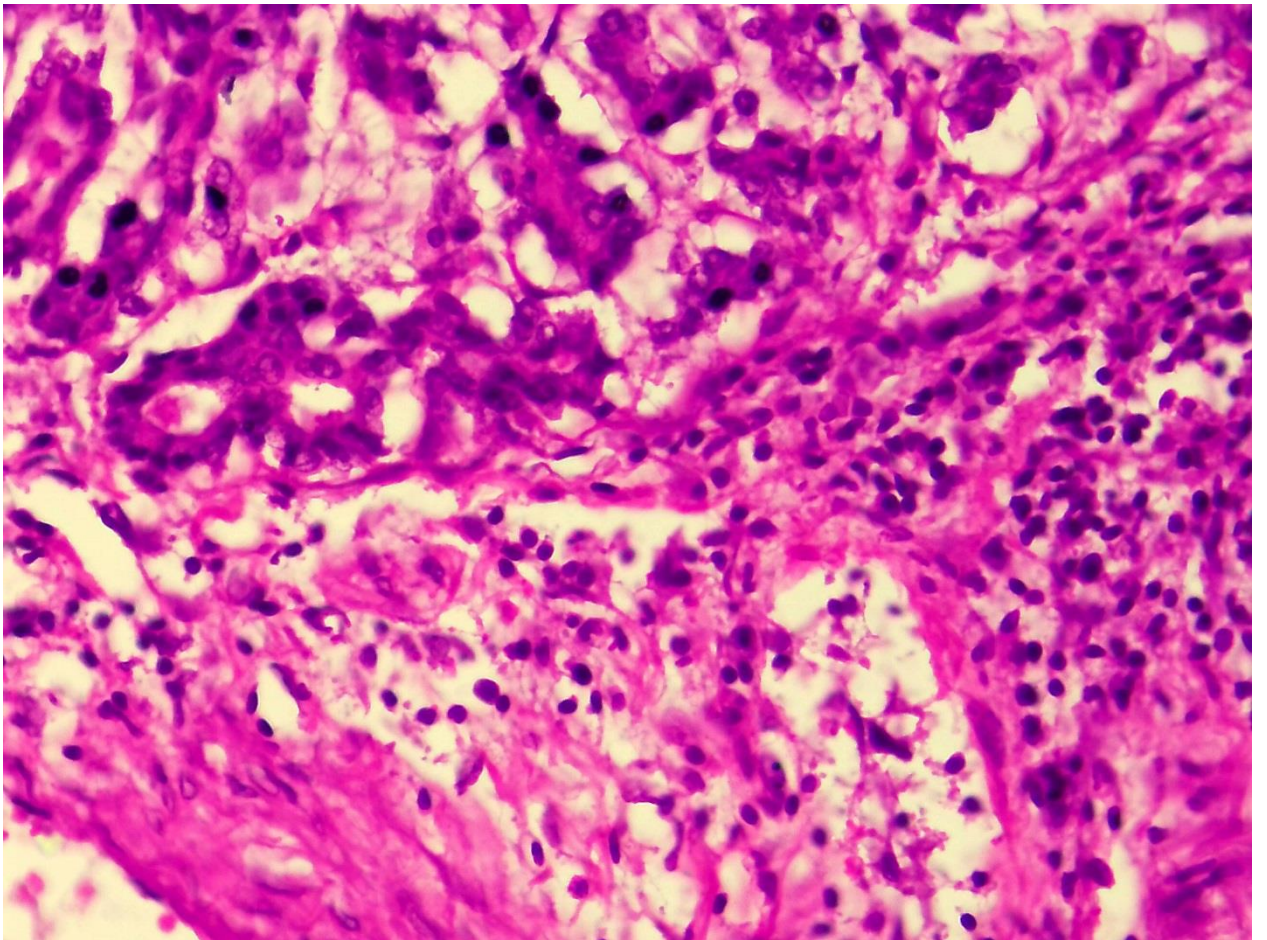


Figure 1. Experimental pancreatitis, day 7. Exocrine acini and endocrine islets are subjected to decay and necrosis. Coloring: G-E. Magnification: 10x40.

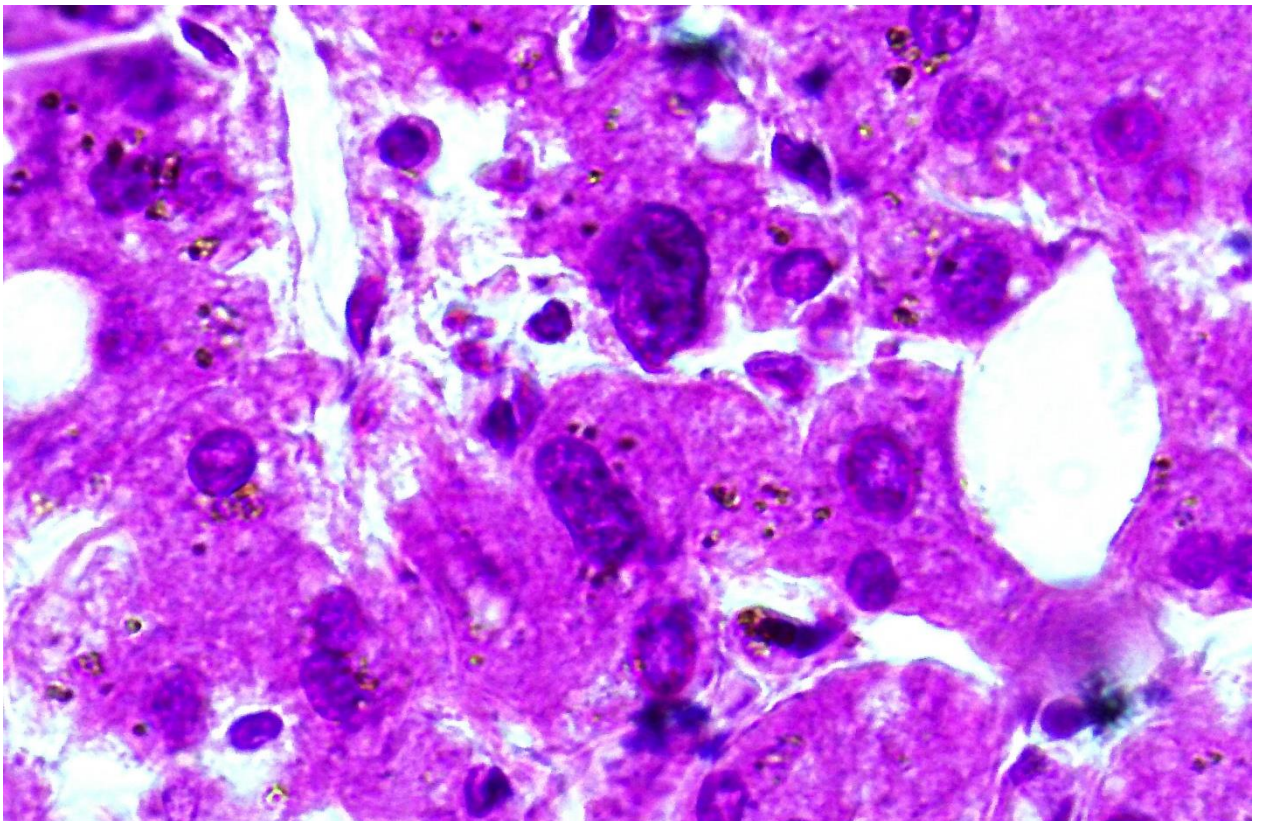


Figure 2. Experimental pancreatitis, day 10. The appearance of signs of apoptosis in the epithelium of the exocrine glands. Coloring: G-E. Magnification: 10x100.

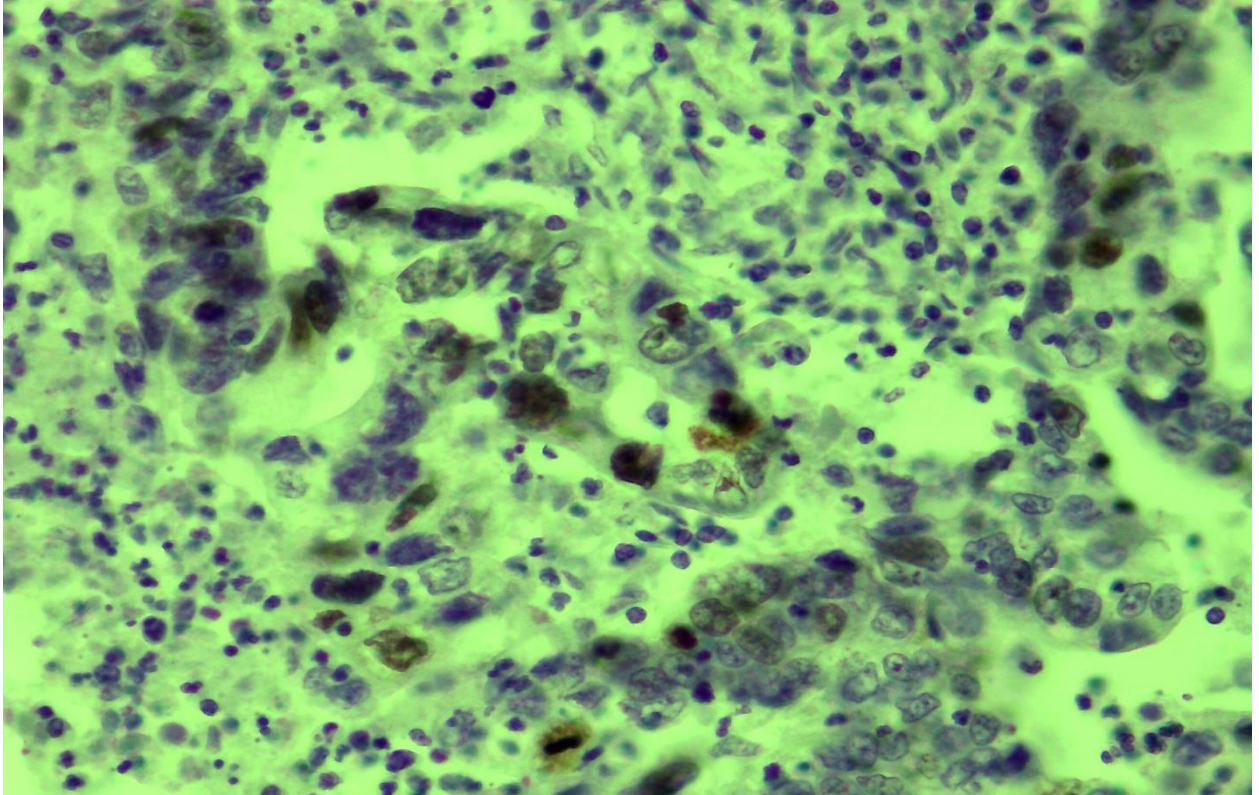


Figure 3. Experimental pancreatitis, day 7. Positive expression of the Ki-67 marker in the nuclei of individual epithelial cells of the exocrine part of the gland. Staining: Ki-67 immunohistochemistry. Magnification: 10x100.

On the 7th day after the modeling of pancreatitis, immunohistochemical studies on the Ki-67 protein marker, which shows the proliferative activity of cells, showed that in part of the acinar epithelium, this marker was positively expressed in the nuclear structures of the acinar epithelium (Fig. 3). At the same time, the complete destruction of the acini of the exocrine part of the gland is noted, the interacinar connective tissue is subjected to a diffuse inflammatory process. On day 10, exocrine epithelium with a positive expression of the Ki-67 marker is in a state of dystrophy and destruction, the nuclei of which are somewhat hypertrophied, the volume fraction of which averages 17.5% of the total volume of the epithelium of the exocrine part of the gland.

The study of the role of anti-apoptotic proteins Bcl-2 in acute and chronic pancreatitis can provide valuable information for understanding the mechanisms of damage to both the exocrine and endocrine parts of the pancreas. It is known that the mechanism of necrotic and reparative processes depends on the ratio of genetically programmed processes of apoptosis and anti-apoptotic protection. Bcl-2 proteins have an antiapoptotic effect, which regulate apoptosis by 2 mechanisms: the mitochondrial pathway and the process of caspase activation. In the first case, representatives of this family affect the permeability of the mitochondrial membrane, thereby controlling the release of cytochrome c from mitochondria. The process of activation of the caspase cascade is triggered by both the Bcl-2 family proteins and the presence of cytochrome c in the cell cytosol. Increased expression of Bcl-2 in any process with a low level of cell proliferation. Thus, the Bcl-2 protein is able to inhibit cell proliferation and affect the rate of cell differentiation and maturation.

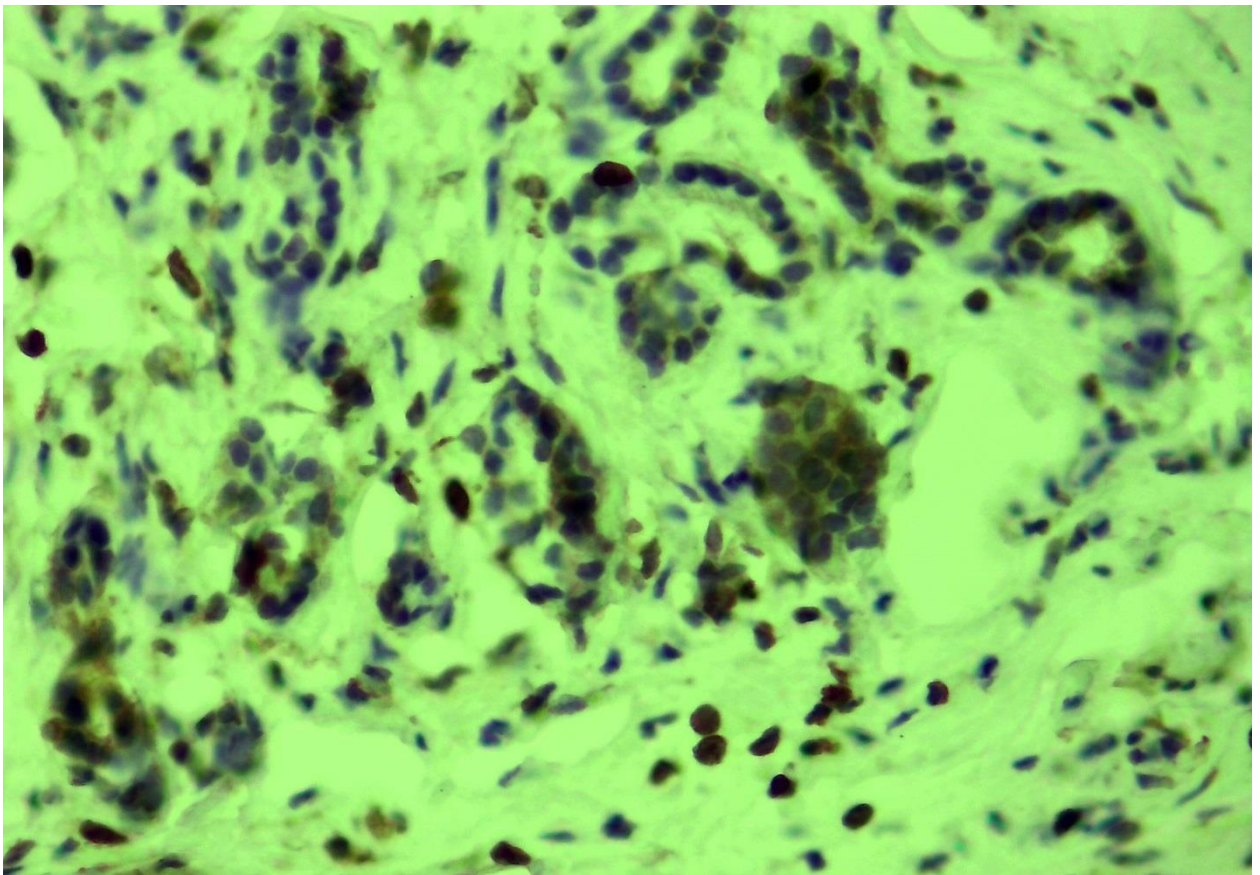


Figure 4. Experimental pancreatitis, day 7. Expression of Bcl-2 protein in both exocrine and endocrine parts of the pancreas. Staining: immunohistochemistry, Magnification: 10x100.

The results of our immunohistochemical study of the Bcl-2 protein on the 7th day of the experiment in experimental pancreatitis showed that in the composition of the epithelium of the exocrine part, the positive expression of the Bcl-2 protein was determined in a small part of the acinus cells in the form of brown staining of the epithelial cytoplasm (Fig. 4). This indicates the blocking of the apoptosis process in individual epithelial cells and the suppression of proliferative activity in almost all acinus cells. On the 10th day of the study, positive expression of Bcl-2 protein in stromal cells was noted. Apparently, suppression of the apoptosis process controls the process of proliferation and differentiation of stromal cells involved in the inflammation process. On the part of the endocrine islet, a moderate expression of Bcl-2 protein is noted in almost all endocrine cells, which shows, to one degree or another, damage to these cells by inflammatory factors.

The results of an immunohistochemical study of liver tissue on the 7th day of the experiment in experimental pancreatitis showed that the expression of the Ki-67 protein, which indicates the proliferative activity of cells in the liver tissue, has very low rates. In the liver tissue, the sinusoids and the Disse space are expanded, the expression of the Ki-67 protein is determined in single hepatocytes located in the nuclear structures and near the nuclear space, in an insignificant concentration (Fig. 5). On the 10th day of the study, the expression of the Ki-67 protein is determined as a dust-like distribution of a weakly colored light brown pigment, in some places it has a finely granular appearance. These pathomorphological and immunohistochemical manifestations of the expression of the Ki-67 protein indicate a slight proliferative activity of hepatocytes in response to experimental pancreatitis. The results of our immunohistochemical study of the Bcl-2 protein on the 7th day of the experiment in acute pancreatitis showed that in the liver tissue, positive expression of the Bcl-2 protein was determined in a small part of hepatocytes and

sinusoid wall cells. (Figure 6). This indicates the blocking of the process of apoptosis in individual hepatocytes and the suppression of proliferative activity, differentiation and maturation of hepatocytes. On the 10th day of the study, a positive expression of Bcl-2 protein was noted in endothelial and Kupffer cells of the wall of sinusoids. The suppression of the apoptosis process coincides in parallel with the processes of proliferation and differentiation of stromal cells involved in the inflammation process.

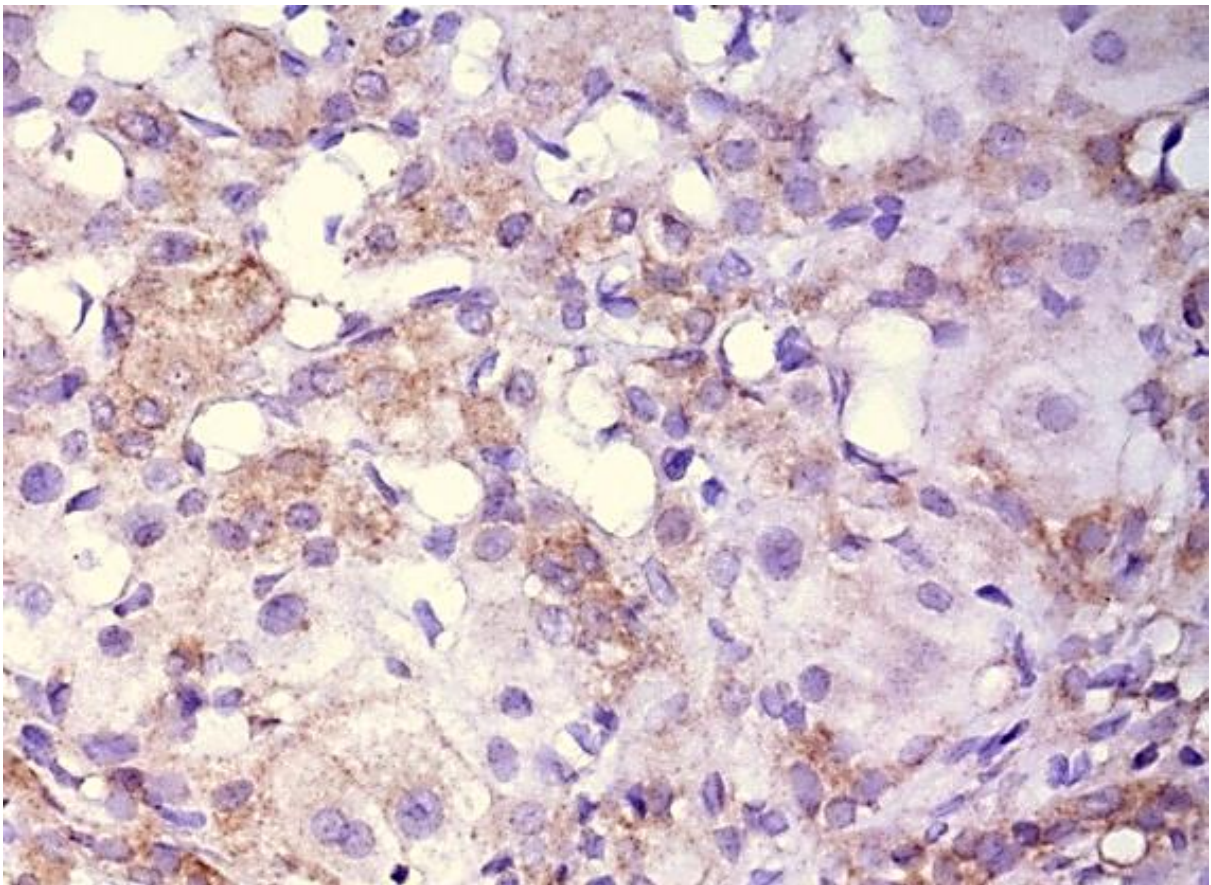


Figure 5. Experimental pancreatitis, day 7. Liver, Ki-67 protein expression in hepatocytes. Staining: immunohistochemistry. Magnification: 10x100.

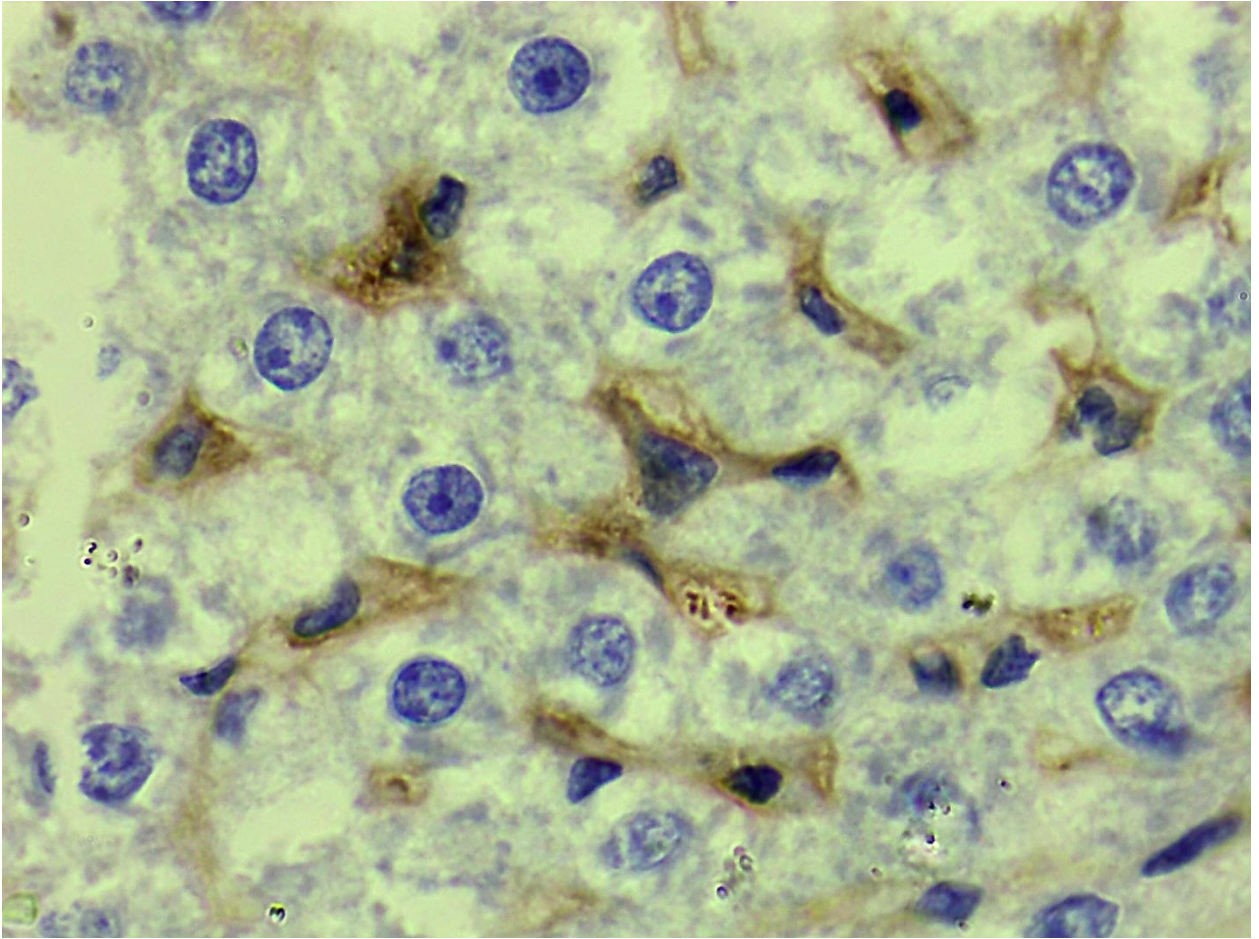


Figure 6. Experimental pancreatitis, day 7. Liver, Bcl-2 protein expression in both hepatocytes and sinusoid wall cells. Staining: immunohistochemistry. Magnification: 10x100.

After treatment of animals with 7 days of acute pancreatitis with cytochrome c, the results of a morphological study of pancreatic tissue showed some subsidence of general pathological processes. Necrobiotic and necrotic foci in the pancreatic tissue were resorbed, instead of them the growth of inflammatory granulation tissue was noted. A small number of inflammatory cells remained in the interstitial connective tissue. The acini of the exocrine part of the gland are located randomly with a violation of histotopography, and a strong swelling of the interstitium was determined between the acini. It is noted that the large objective of the microscope shows that the granulation tissue layers consist of separate bundles of fibrous-cellular structures, between which thin-walled blood vessels with diapedetic hemorrhages are determined. Inflammatory cells penetrate between the acini of the

exocrine part of the gland. The epithelium of the acini is subjected to dystrophic and edematous phenomena.

When studying the material of animals with 10 days of pancreatitis after treatment with cytochrome c on a large microscope objective, the presence of acinar epithelium with signs of apoptosis in the form of a concentration of nuclear chromatin, compaction of the cytoplasm matrix and uniform hematoxylin staining of the karyoplasm is noted (Fig. 7). As well as the absence of intercellular desmosoma, fusion of the cytoplasm of cells with each other, focal expansion of the intercellular space, which indicates the development of necrobiotic changes in the form of apoptosis.

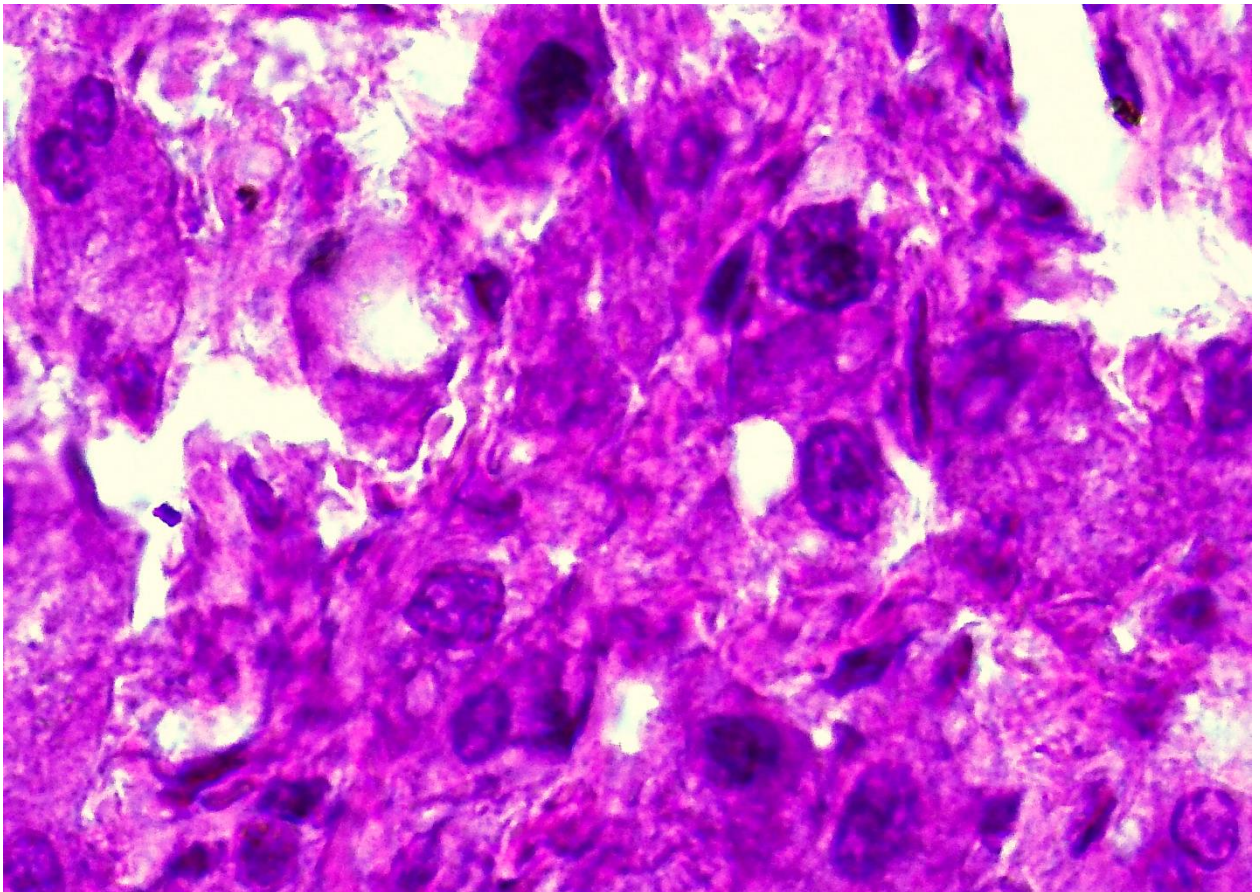


Figure 7. Experimental pancreatitis, day 7, treatment with cytochrome c. Necrobiotic and apoptotic changes in the exocrine part of the gland. Coloring: G-E. Magnification: 10x100.

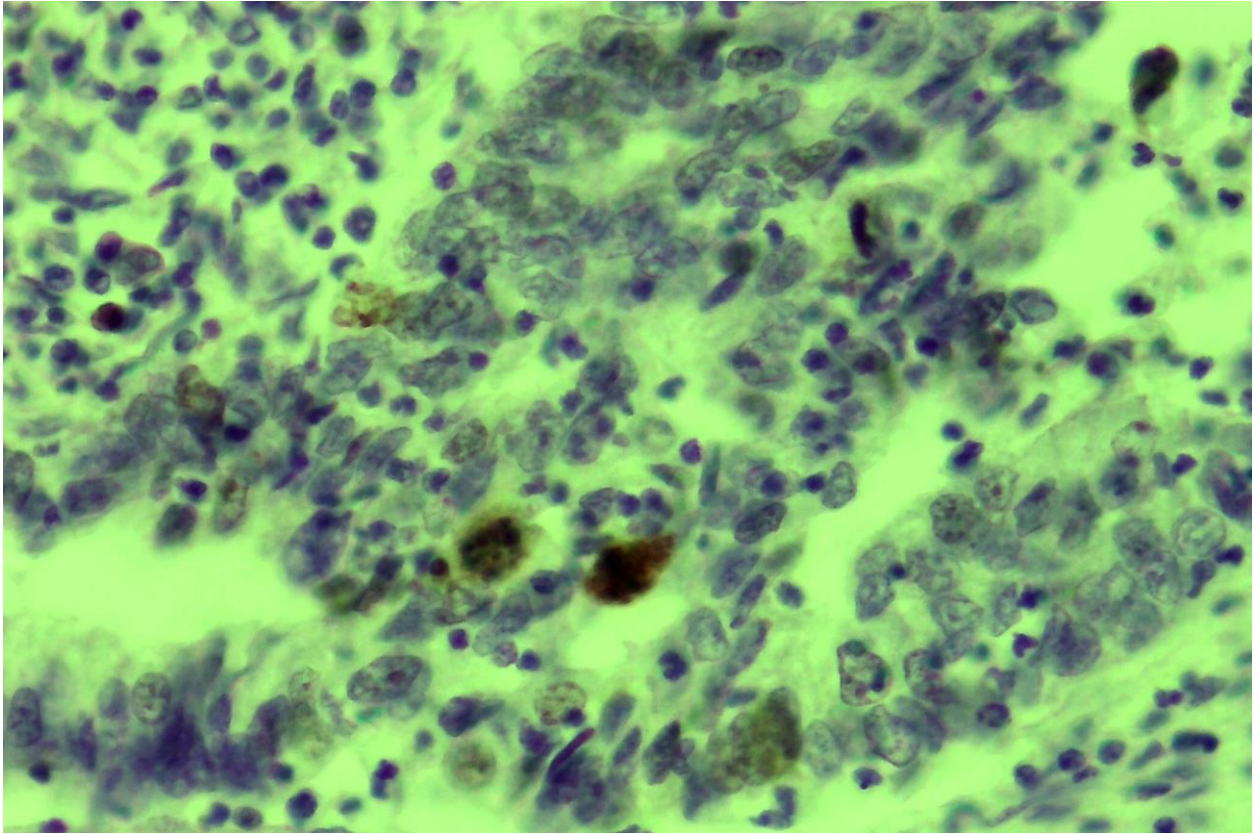


Figure 8. Experimental pancreatitis, day 10, treatment with cytochrome c. More intense positive expression of the Ki-67 protein in acinar epithelial cells. Staining: immunohistochemistry. UV: 10x100.

Immunohistochemical studies of pancreatic tissue in animals with 10 days of acute pancreatitis to determine the degree of activity of the Ki-67 apoptosis marker showed that, compared with the group without treatment, after treatment with cytochrome c, positive expression of this marker in acinar epithelial cells, it is more pronounced in nuclear structures. manifested by dark brown staining of nuclear structures (Fig. 8). At the same time, the complete destruction of the acini of the exocrine part of the gland is noted, the interacinar connective tissue is subjected to a diffuse inflammatory process. Exocrine epithelium with a positive expression of the apoptosis marker Ki-67 in a state of dystrophy and destruction, the nuclei of which are somewhat hypertrophied, the volume fraction of which averages 28.5% of the

total volume of the epithelium of the exocrine part of the gland.

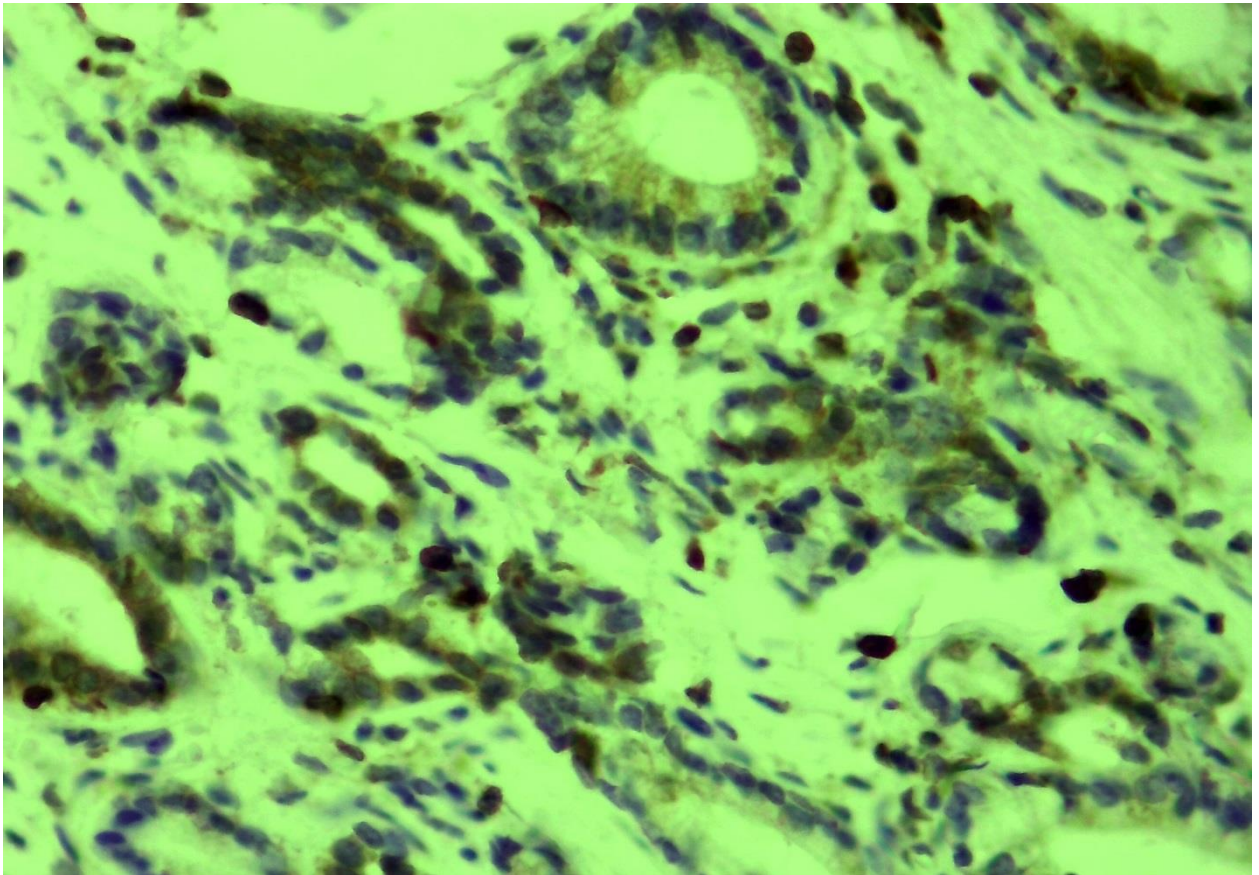


Figure 9. Experimental pancreatitis, day 7, treatment with cytochrome c. Expression of Bcl-2 protein in the cells of the exocrine and endocrine parts of the gland. Staining: immunohistochemistry. Magnification: 10x100.

The results of an immunohistochemical study of the anti-apoptotic Bcl-2 protein in animals with 7-day acute pancreatitis after treatment with cytochrome c showed that in the composition of the epithelium of the exocrine part with a positive expression of the Bcl-2 protein, the number of cells was manifested by a less intense staining of the marker to dark brown in both the cytoplasm and and nuclear structures (Fig. 9), which indicates a decrease in the degree of blocking of the apoptosis process in individual epithelial cells and an increase in the proliferative activity of acinus cells. It was noted that the degree of positive expression of Bcl-2 protein in stromal cells was also somewhat reduced. On the part of the endocrine part of the pancreas, a moderate expression of Bcl-2 protein is noted in almost all endocrine cells, which indicates damage to these cells by inflammatory factors to one degree or another.

The results of an immunohistochemical study of liver tissue in animals with 7 days of experimental pancreatitis after treatment with cytochrome c showed an increase in the expression of the Ki-67 protein, indicating the proliferative activity of hepatocytes. Positive expression of the Ki-67 protein is determined in hepatocytes as a significant concentration of dark brown pigment located in nuclear structures and near the nuclear space. These pathological and immunohistochemical manifestations of Ki-67 protein expression indicate an increase in the proliferative activity of hepatocytes in response to experimental pancreatitis.

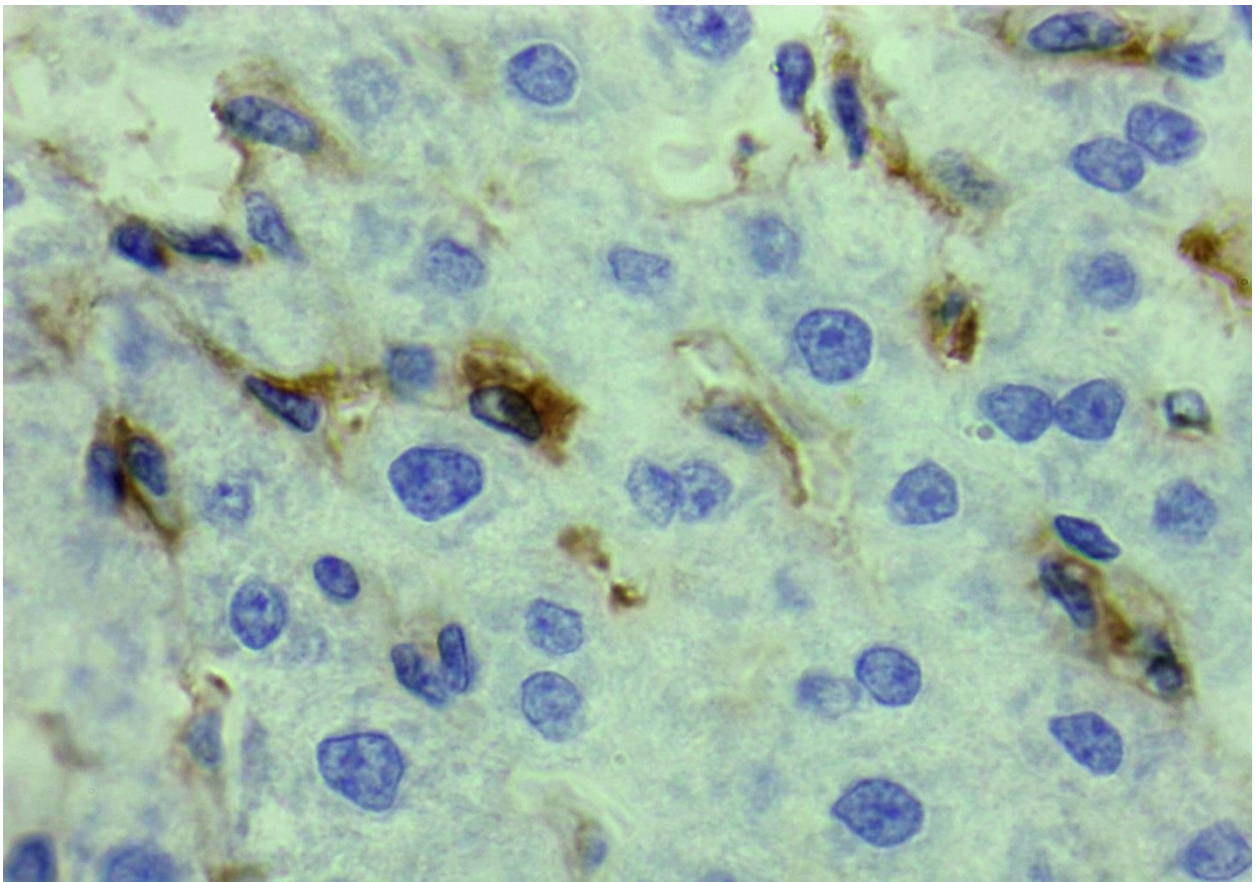


Figure 10. Experimental pancreatitis, day 7, treatment with cytochrome c. Expression of Bcl-2 protein in liver tissue. Staining: immunohistochemistry. Magnification: 10x100.

The results of an immunohistochemical study of the anti-apoptotic Bcl-2 protein in animals with 7 days of experimental pancreatitis after treatment with cytochrome c showed that positive expression of the Bcl-2 protein in liver tissue was detected both in individual hepatocytes and in cells of stromal structures (Fig. 10). This indicates

a decrease in the process of blocking apoptosis in individual hepatocytes and an increase in proliferative activity, differentiation and maturation of hepatocytes. A decrease in the degree of suppression of the apoptosis process coincides with an increase in the processes of proliferation and differentiation of stromal cells involved in the inflammation process.

Conclusions:

1. General morphological study of pancreatic tissue in experimental pancreatitis showed signs of apoptosis of cells of both the exocrine and endocrine parts of the gland.
2. Weak positive expression of the Ki-67 protein in the cells of the acinar part of the gland and endocrine cells showed a decrease in the proliferative activity of these cells in experimental pancreatitis.
3. A higher degree of Bcl-2 protein expression both in the epithelium of the exocrine part and endocrine cells indicates the suppression of the apoptosis process in experimental pancreatitis.
4. A more pronounced positive expression of the Ki-67 protein in the cells of the acinar part of the gland and endocrine cells indicates an increase in the proliferative activity of these cells after treatment with cytochrome c of experimental pancreatitis.
- five. A decrease in the degree of expression of Bcl-2 protein both in the epithelium of the exocrine part and in endocrine cells also showed an increase in the process of apoptosis after treatment with experimental pancreatitis with cytochrome c.

Literature:

1. Treatment of acute pancreatitis and its complications / S.V. Ivanov, O.I. Okhotnikov, G.A. Bondarev et al. - Kursk: KSMU, 2005. - 181 p.
2. Pancreatic necrosis: diagnosis, prognosis and treatment / I.I. Zatevakhin, M.Sh. Tsitsiashvili, M. D. Budurova [i dr.]. - M.: [b. and.], 2007. - 223 p.
3. Savelyev, V. S. Pancreatic necrosis / V. S. Savelyev, M. I. Filimonov, S. Z. Burnevich. – M.: Med.inform. agency, 2008. - 258 p.
4. Yuliya Viktorovna Evsyutina, Vladimir Trofimovich Ivashkin, EZ Abgadzava The role of mitochondrial and lysosome dysfunction in the pathogenesis of acute

- pancreatitis Russian Journal of Gastroenterology, Hepatology, Coloproctology. 2016;26(2):6-10.<https://doi.org/10.22416/1382-4376-2016-26-2-6-10>
5. M.A. Savitskaya, G.E. Onishchenko Mechanisms of apoptosis// BIOCHEMISTRY, 2015, volume 80, no. 11, p. 1613–1627
 6. Yu.A. Vladimirov, E.V. Proskurnina, A.V. Alekseev Molecular mechanisms of apoptosis. The structure of the complex of cytochrome c with cardiolipin// BIOCHEMISTRY, 2013, volume 78, no. 10, p. 1391–1404
 7. Simovaryan P.S., Timenina R.S. Indicators of fat-carbohydrate metabolism in experimental pancreatitis // Patol. physiol. And exp. ter.-M.: Medicine.-1973.-№2.- p.59-62.