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EARLY AND LATE ULTRASTRUCTURAL CHANGES IN PANCREATIC CELLS FOLLOWING LOW TEMPERATURE EXPOSURE: AN EXPERIMENTAL STUDY

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Purpose. A number of theoretical and experimental studies, both in vitro and in vivo, have been performed to explain the action of low temperatures on tissue. It is now evident that the thermal parameters used in the past for freezing during cryosurgery were not precise, which may have resulted in the failure of treatment. **Methodology.** Experimental cryogenic destruction was performed as follows. After anesthesia, an upper medial laparotomy was performed and the abdominal cavity was opened in layers. The pancreas was brought as close to the opening as possible. The anterior surface of the organ was exposed and the cryogenic instrument of the Cryoelectronic device was firmly applied to the organ.The operation control unit was adjusted to the temperature required to produce the cryogenic effect of –80°C and of –180°C, because cryozone formation is dependent on different parameters, particularly on these temperatures. An in vitro experimental study used this method. Cryogenic destruction, which involves only freezing, was performed for three minutes. The time parameter was also investigated in this study. The freeze–thaw cycle was monitored using intraoperative ultrasound before, during and after cryosurgery. The cryogenic applicator was then automatically warmed up and removed from the tissue. Automatic thawing of the destruction area was continued up to four minutes. In order to study ultrastructural changes in the pancreatic parenchyma after exposure to low temperatures, biopsy samples were taken immediately, one hour and 24 hours after warming up. For transmission electron microscopy, the specimens were taken immediately and one hour after the finishing of the freeze–thaw cycles intraoperatively, after which the abdominal cavity was closed. The next specimens were taken after 24 hours, this time also intraoperatively. **Results.** The present study examined the effects of exposure to different low temperatures on pancreatic cells. The results clearly suggest that damage to pancreatic cells after freezing and thawing at various intensities is acute, and the pancreatic cell architecture cannot be returned more or less to normal. The disruption of pancreatic cells occurred after freezing and thawing at different low temperatures, in particular at temperatures of –80°C and –180°C. Pancreatic cells are entities with a highly specific intracellular morphological content, separated from the non-specific extracellular solution by the cell membrane. **Originality**. The pathophysiologic consequences of freezing a significant portion of the primate pancreas have been investigated using a liquid nitrogen cryosurgical probe. Histologically, typical cryonecrosis occurred in the treated area, while the untreated pancreas and surrounding tissue remained normal. This study demonstrates that cryonecrosis of a significant portion of pancreatic tissue can be produced in a primate without the development of diffuse pancreatitis or other ill effects. **Conclusions.** The present observations on the early ultrastructural changes in the pancreatic tissue clearly enable a better understanding of the mechanisms of damage and the pathogenesis of frostbite during cryosurgery. The properties of the pancreatic parenchyma response after low temperature exposure provide important insights into the mechanisms of damage and cryogenic lesion immediately after thawing in cryosurgery. Progressively damaged pancreatic cells in the post-cryosurgical zone lead to cryoaponecrosis and cryoapoptosis. Vascular changes and circulatory stagnation indicate the anti-angiogenesis mechanism of biological tissue injury after low temperature exposure at –180°C and –80°C. Thus, cryoaponecrosis, cryoapoptosis and anti-angiogenesis are some of the most important mechanisms by which living tissue suffers damage as a response to cryosurgery, finally leading to complete local cryodevitalization of the pancreatic parenchyma. **References** 16.

Keywords: cryosurgery, low temperature, pancreatic cell changes, anti-angiogenesis, cryoaponecrosis, cryoapoptosis, oncology, animal study

РАННІ ТА ПІЗНІ УЛЬТРАСТРУКТУРНІ ЗМІНИ В КЛІТИНАХ ПІДШЛУНКОВОЇ ЗАЛОЗИ ПІСЛЯ ДІЇ НИЗЬКИХ ТЕМПЕРАТУР: ЕКСПЕРИМЕНТАЛЬНІ ДОСЛІДЖЕННЯ

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В роботі наведено ряд теоретичних і експериментальних досліджень, які проводились *in vitro* та *in vivo*, для того, щоб пояснити дію низьких температур на тканини. Вони дозволяють стверджувати, що температурні параметри кріохірургії, які використовувались раніше не були достатньо контрольовані, що в ряді випадків приводило до провалу лікування. У цьому дослідженні вперше описуються ранні ультраструктурні особливості паренхіми підшлункової залози після низького температурного впливу, тобто кріохірургії, в природних умовах. Показано вплив заморожування-розморожування з використанням температури різної інтенсивності. Була досліджена кріохірургічна реакція паренхіми підшлункової залози, в тому числі ультраструктурні клітинні зміни в тканинах підшлункової залози. Аналіз за допомогою електронного мікроскопа, показав, що аналогічні процеси відбувалися в тканинах підшлункової залози після локальної кріодеструкції при температурі -80°С і -180°С. Вони були близькими в період від безпосередньо кріохірургічної фази до 24 годин після того, як тканина піддається впливу низької температури.

Ключові слова: кріохірургія, температура, панкреатичні зміни клітин, анти-ангіогенез, кріоапонекроз, кріоапоптоз, онкологія, дослідження.

PROBLEM STATEMENT. The exocrine pancreatic cells in the center of the cryozone changed upon thawing. Ultrastructural changes in the exocrine pancreatic cells, where the first signs of dystrophic processes had been noticed, were increased. These ultrastructural changes in the pancreatic cells provide a means of better understanding the mechanisms of damage and the pathogenesis of frostbite after cryosurgery. The properties of the pancreatic parenchyma response to low temperature exposure provide important insights into the mechanisms of damage and the cryogenic lesion immediately after thawing in cryosurgery. Our new insights prove on the cell level that suddenly and progressively damaged pancreatic cells in the post-cryosurgical zone lead to aseptic cryonecrosis and then to aseptic cryoapoptosis of vital normal tissue. The vascular capillary changes and circulatory stagnation demonstrate the anti-angiogenesis mechanism, which, together with cryoaponecrosis and cryoapoptosis, are some of the main mechanisms of biological tissue injury following low temperature exposure.

The use of deep freezing as a method of destroying biological tissue has been known for a long time and has been described in detail [1].

Cryosurgery is based on the application of low temperatures to destroy abnormal tissue. The use of cryogenic techniques for the resection of parenchymal organs [2–4] and *in situ* ablation of tumors using subzero temperatures for surface malignancies [5] has been described. Advances in this technology have aroused interest in the use of cryosurgery for the treatment of parenchymal malignancies [6].

Tumors are not cut out but shock-frozen. Recent scientific research on the application of freezing in the fields of biology and medicine, and numerous theoretical and experimental studies *in vitro* and *in vivo*, have demonstrated the effect of low temperatures on tissue, as well as the impairment and destruction of cells under cryoinfluence [7].

Interest is increasing in using low temperatures to treat diseases of the pancreas, notably pancreatic malignancies [8]. It has been suggested that cryoablation should be used where pancreatic tumors are deemed unresectable [9].

The pathophysiologic consequences of freezing a significant portion of the primate pancreas have been investigated using a liquid nitrogen cryosurgical probe [10]. Histologically, typical cryonecrosis occurred in the treated area, while the untreated pancreas and surrounding tissue remained normal. This study demonstrates that cryonecrosis of a significant portion of pancreatic tissue can be produced in a primate without the development of diffuse pancreatitis or other ill effects.

Another study was designed to evaluate the effects of direct pancreatic surface cooling on the exocrine pancreas [11]. The changes in serum amylase levels, pancreatic water, amylase and cathepsin B as a lysosomal enzyme, content, histological changes of acinar cells, and the subcellular distribution of cathepsin B after one to two and three hours of direct pancreatic cooling in rats have been measured. In addition, the *in vivo* amylase and cathepsin B output stimulated by caerulein, *in vitro* lysosomal and mitochondrial fragility, as well as the pancreatic adenylate energy metabolism, were evaluated.

Electron microscopy of acinar cells of the pancreas cooled to –30°C with subsequent thawing has already been reported [12].

Quick-freezing and freeze-drying were used in preparation for high-quality morphology and immunocytochemistry at the ultrastructural level to obtain ultrastructural localization of insulin in the pancreatic beta cell by the unlabeled antibody-enzyme technique [13].

Also, fetal rat pancreases, cultured for eight days in PRMI 1640, were successively frozen to –196°C [14]. The effects of culture, various cooling times, warming rates, thawing procedures, dimethyl sulfoxide concentration, and ultrastructural changes in cellular elements were analysed.

Currently, no data are available on ultrastructural cellular changes of cryosurgical response in normal and pathological pancreatic tissue when exposed to low temperatures. Neither the *in vivo* sensitivities of the pancreatic parenchyma to different cryosurgical exposures, nor the minimum temperature required to cause adequate cryodestruction and prevent tumor recurrence, have as yet been defined or described in the medical literature. The effect of different temperature applications on the freeze–thawing process and the cryosurgical response of normal pancreatic tissue in animals have not been investigated by electron microscopy. Such studies would permit an assessment of cryosurgical techniques as a treatment option for diseases of the pancreas, especially for pancreatic tumors.

To our knowledge, this is the first study investigating ultrastructural findings in the acinar cells of animal pancreatic parenchyma after exposure to different low temperatures. An experimental study in pancreatic cryosurgery, revealing hitherto unreported ultrastructural changes in the exocrine cells of the dog's pancreas after the freeze–thawing process, is presented here.

EXPERIMENTAL PART AND RESULTS OBTAINED. *Animals.* The aim was to investigate the processes that occur in pancreatic parenchyma subjected to destruction by the application of different low temperatures and the degree of their early ultrastructural manifestations.

Experimental cryogenic destruction was performed as follows. After anesthesia, an upper medial laparotomy was performed and the abdominal cavity was opened in layers. The pancreas was brought as close to the opening as possible. The anterior surface of the organ was exposed and the cryogenic instrument of the Cryoelectronic (Scientific-Production Company 'Pulse', Kyiv, Ukraine) device was firmly applied to the organ [15].

The operation control unit was adjusted to the temperature required to produce the cryogenic effect of –80°C and of –180°C, because cryozone formation is dependent on different parameters, particularly on these temperatures. An *in vitro* experimental study used this method [16]. Cryogenic destruction, which involves only freezing, was performed for three minutes. The time parameter was also investigated in this study [16]. The freeze–thaw cycle was monitored using intraoperative ultrasound before, during and after cryosurgery. The cryogenic applicator was then automatically warmed up and removed from the tissue. Automatic thawing of the destruction area was continued up to four minutes. In order to study ultrastructural changes in the pancreatic parenchyma after exposure to low temperatures, biopsy samples were taken immediately, one hour and 24 hours after warming up. For transmission electron microscopy, the specimens were taken *immediately* and *one hour* after the finishing of the freeze–thaw cycles intraoperatively, after which the abdominal cavity was closed. The next specimens were taken after *24 hours*, this time also intraoperatively.

A total of 48 healthy adult mongrel dogs of both sexes, each weighing 12.1 to 14.6 kg, were used for the experiment. All animals were quarantined at the University Animal Care Facility for one week, and the dogs were kept strictly in accordance with the guidelines for the care and use of research animals established by the animal ethics committee of the National Medical University and the Ukrainian National Veterinary University in Kyiv. All animals were provided with standard pellet food and water, but were not fed for 12 hours prior to the experiment. All dogs were kept in their cages and observed for the next 24 hours postoperatively.

Experimental Design. Thirty-two animals were used for the experiment. The dogs were divided into two groups of 16 each. In group A, the pancreatic parenchyma was frozen at –80°C and in group B at –180°C in each of 11 animals. The cryoprobes were in contact with the animal pancreas for three minutes at each of these temperatures. The remaining 10 animals were used as controls and again divided into two groups each of 5 animals. In the first control group (n=5 animals), the cryoprobe was inserted into the pancreas but no freeze–thaw cycle was conducted. The second control group of 5 animals underwent sham (simulated) laparotomy (opening of the abdominal cavity).

Operative Procedure. After a 12-hour fast, the animals were anesthetized by intravenous injection of 50 mg/ml ketalar (0.2 mg/kg body weight) and xyla (xylazine base, 0.2 mg/kg body weight) in a ratio of 1:1 (InterChemie, Netherlands). After endotracheal intubation, the animals were ventilated with a respirator. The pancreas was exposed for laparotomy using an oblique abdominal section and a vertical or hockey-stick-shaped incision towards the left costal arch. The pancreas was examined bimanually and the peritoneal cavity was explored to rule out extrapancreatic disease. The pancreas was then isolated by disconnecting all peritoneal attachments. One hour after cryosurgery, the abdominal wall was closed in two layers. After 24 hours, this operative procedure was repeated. When the dogs regained

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consciousness, they were returned to their cages.

The Cryosurgical Approach. A disc probe with a diameter of 20 mm was placed on the pancreas. Temperatures of –80°C and –180°C were selected for cryosurgical exposure, at a temperature stabilization of ± 1%, for contact with the pancreatic parenchyma. A three-minute freeze followed by a complete thaw of the pancreas was used for each freeze–thaw cycle. Every cryolesion was observed for 60 minutes after thawing. The two freeze–thaw cycles were monitored by intraoperative ultrasound before, during and after cryosurgery. Liquid nitrogen was applied by Cryoelectronic, a cryosurgical device of our own, produced by the Scientific Company, Kyiv, Ukraine, suitable for universal application. Liquid nitrogen was circulated through the probe at –196°C (15).

Freezing with a Cryoprobe. Specific lesions in the pancreas could be identified, targeted and cryoablated in the animal experiment. A cryoinstrument with a disk-shaped cryoprobe measuring approximately 20 mm in diameter was placed on the pancreas tissue to induce necrosis (tissue destruction). Guided by continuous intraoperative ultrasonic monitoring, the pancreas tissue was frozen to –80°C and –180°C in order to achieve complete tissue destruction. The ice-ball (cryogenic zone) was generated without difficulty. The line of demarcation between the area that was cryosurgically destroyed, followed by cryogenic necrosis, and the healthy pancreatic parenchyma was clearly outlined by the rim of the cryozone. The cryozone included a 7-mm margin, which surrounded the normal-looking pancreas parenchyma. When the pancreas tissue had frozen to an ice-ball at a temperature of –80° and –180°C, liquid nitrogen refrigeration was stopped. Immediately after a single freeze–thaw cycle, the cryozone was measured using a cryoprobe with a diameter of 20 mm, and found to measure 34 mm. During the freezing process, the diameter of the cryogenic zone was 7 to 15 mm larger than that of the cryoprobe. No intraoperative or postoperative complications were encountered. All dogs tolerated pancreas cryosurgery well and none died.

Thawing the Tissue. Thawing each freeze–thaw cycle took approximately four minutes in the automatic cryosurgical unit. The cryoprobe was warmed up and removed from the tissue. The two freeze–thaw cycles that were performed for each cryolesion in the course of experimental cryosurgery revealed the same cryozone with an ice crater in the middle and an ice margin with a demarcation line immediately after cryosurgery.

Electron Microscopic Study of the Pancreas. For transmission electron microscopy, the specimens of the pancreas tissue were divided into fragments and then secured in a 1% solution of osmium tetraoxide for a period of 2 hours at a temperature below +4°C. The objects were dehydrated for 15 minutes in each of the following solutions: a) 70° and 80° ethanol at +4°C, b) room temperature (+18°C) of 96° ethanol, c) 3 portions of 100° ethanol, d) in a mixture of ethanol and acetone, and e) in 2 portions of acetone. The objects were immersed in 3:1, 1:1, 1:3 mixtures of acetone and epoxy resin. They were left in each of the mixtures for one hour, in pure resin for 12–24 hours and then embedded in epoxy resin. Sections of 0.5-μ thickness were cut and then stained with 2% solution of uranyl acetate on 70°

ethanol for 15 minutes, and left in a lead citrate(s) concentration for a further 15 minutes.

For light microscopic studies, specimens from the pancreatic parenchyma were collected from the cryozone immediately, one hour and 24 hours after cryosurgery. The biopsy was taken from the margin between the frozen and normal pancreatic parenchyma, i.e. from the center of the cryonecrosis, and investigated under a light microscope. The histological results have been published separately (2, 8).

RESULTS. *Before the Freeze–Thaw Cycle.* The electronic micrographs show the structure of the dog pancreas cells before cryosurgery (Figures 1A-B).
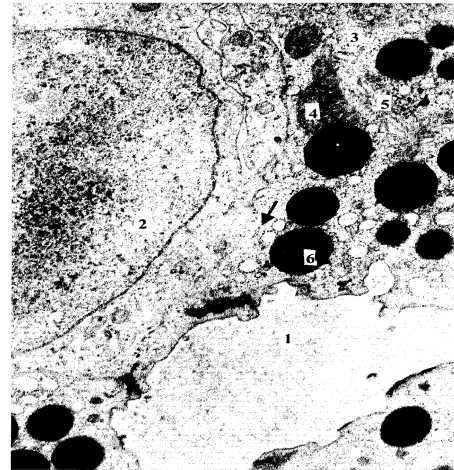
Figures 1A-B – Dog pancreas cells before cryosurgery, followed by gradual freezing using liquid nitrogen (i.e. refrigeration at –196°C). Electron micrograph (Figure 1A), showing the pancreatic cell with the space of pancreatic acini (1), a centro-acinar cell with nucleus (2), exocrine pancreatic cells (3) with mitochondria (4), Golgi body (5), canaliculi of the rough endoplasmic reticulum (↑), and zymogen granules (6)
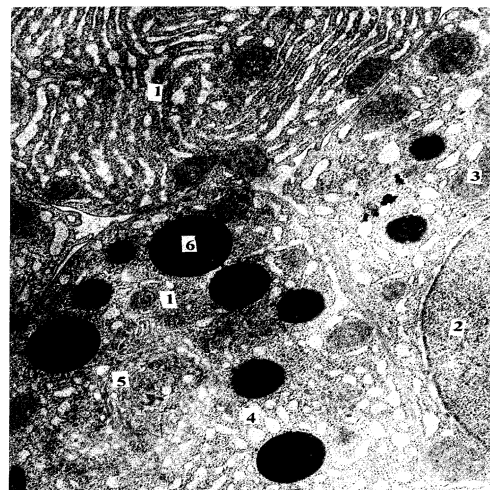

Figure 1B – shows the exocrine pancreatic cells (1) with the basement side. Nucleus with an evenly distributed chromatin (2), mitochondria with a dense electronic matrix and a small number of cristae (3), canaliculi of the rough endoplasmic reticulum (4), Golgi body (5), and zymogen granules (6)

The Freeze–Thaw Cycle at –180°C. For the first time, tissue was taken directly from the central portion of the cryozone in the dog's pancreas immediately after thawing to observe the ultrastructural changes in pancreatic parenchyma (Figure 2A).
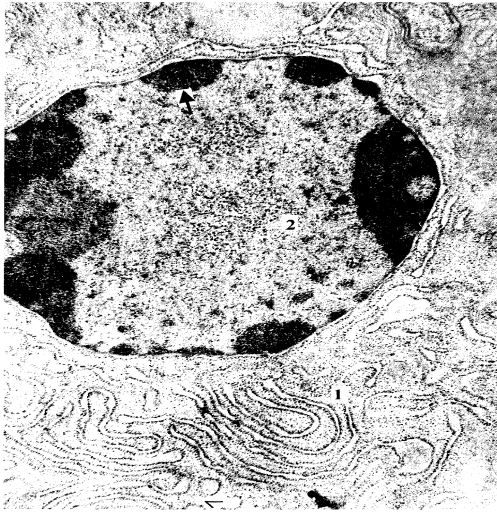

Figure 2A – Freeze–thaw cycle at –180°C: exocrine pancreatic cell (1), nucleus (2), margination of the chromatin into large aggregates (↑), canaliculi of the rough endoplasmic reticulum (∠)

An exocrine pancreatic cell (1) and the nucleus (2) with a well-preserved nuclear envelope (membrane) are seen. The perinuclear space is insignificantly dilated all over the cell. The chromatin is divided into large aggregates (↑). The canaliculi of the rough endoplasmic reticulum are slightly dilated and partly fragmented (∠). Zymogen granules are absent.
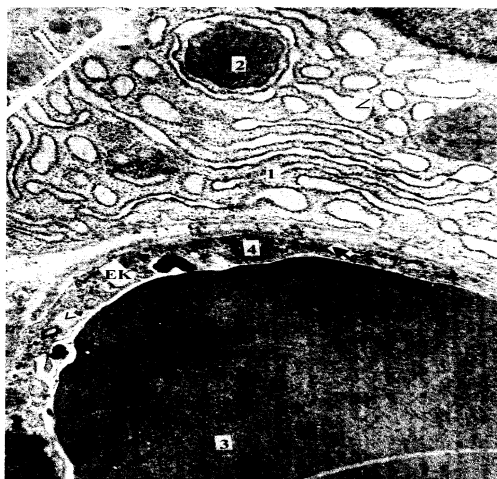

Figure 2B – Pancreatic exocrine cell (1), mitochondria and a small number of cristae (2), canaliculi (∠), erythrocytes (3), endothelium cell (4), canaliculi of the endoplasmic reticulum (∧), and basement membrane (↑)

In Figure 2B, the bottom part of the pancreatic exocrine cell (1), mitochondria with a dense electronic matrix, and a small number of cristae (2) are seen. Canaliculi of the rough endoplasmic reticulum are slightly dilated and partly fragmented (∠). Erythrocytes

(3) are found in the vascular capillary space. An endothelium cell (EK) with electron-dense mitochondria (4), canaliculi of the endoplasmic reticulum (∧) and ribosomes are visible. The basement membrane (↑) of the endothelium cell is reduced to powder.

Figure 3A – Pancreatic exocrine cell (1), nucleus (2), mitochondria (3), dilated canaliculi of endoplasmic reticulum (∠), and myelin-like structures (↑)

Figure 3A shows the central portion of the cryozone in the dog's pancreas one hour after thawing. An exocrine pancreatic cell (1) with a nucleus (2) possessing a dense chromatin and mitochondria with an electron-dense matrix, and a small number of cristae, are seen. Dilated canaliculi of the endoplasmic reticulum full of an amorphous flake-like substance (∠) are also observed. No ribosomes are found on the membranes. The number of ribosomes in the cytoplasm is suddenly reduced. The myelin-like structures (↑) in the cytoplasm testify to the increase in free radicals.

Figure 3B – Pancreatic exocrine cell (1), plasmatic membrane (↑a), canaliculi (∠), erythrocyte (2), myelin-like structure (3), endothelial structure (↑b), myelin-like structure (4), and cell organelle (6)

Also, Figure 3B illustrates the central portion of the cryozone in the dog's pancreas one hour after thawing. An exocrine pancreatic cell is seen (1). The plasmatic

membrane on the bottom (↑a) surface is not clearly structured. Canaliculi of the endoplasmic reticulum (∠) fragmentize upon intensification of the edema and loosen the ribosomes. An erythrocyte (2) and a myelin-like structure (3) are present in the vascular capillary space where the endothelial structure is locally damaged (↑b). The endothelial cell reveals a myelin-like structure (4). An erythrocyte (5) and the remains of the cell organelles (6) in the interstitial space are also seen.
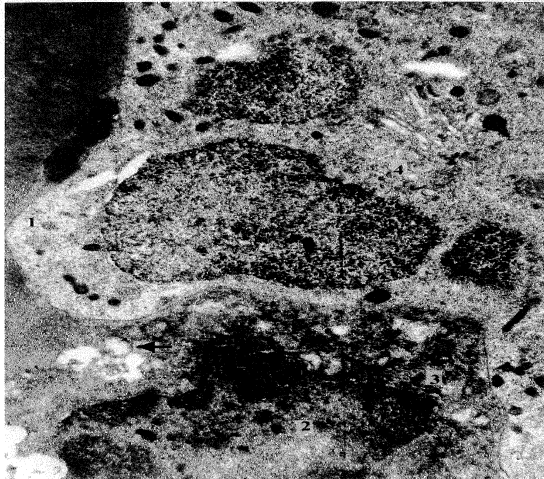

Figure 4A – Pancreatic cell (1), pyknotic nucleus (2), cytoplasm (3), endoplasmic reticulum (↑), and neutrophil (4)

Figure 4A illuminates the central portion of the cryozone in the dog's pancreas 24 hours after thawing. An exocrine pancreatic cell (1) with a pyknotic nucleus is seen. The cytoplasm is filled with an electron-dense homogeneous content (3) and with the canaliculi of the endoplasmic reticulum (↑). A segmented neutrophil is seen in the center of the inflammation (4).
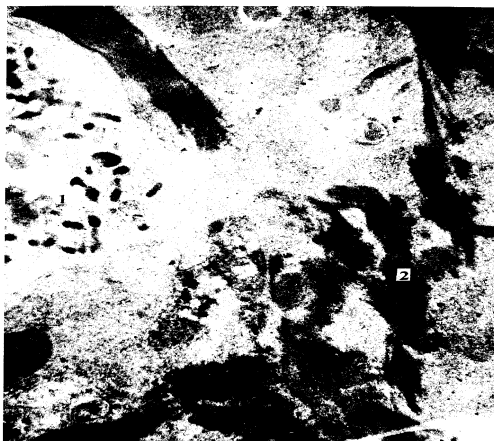

Figure 4B – Cell detritus (1) and fibrin fibers (2)

Twenty-four hours after thawing, the central portion of the cryozone in the dog's pancreas (Figure 4B), cell detritus (1) and fibrin fibers (2) are observed, which are signs of cryonecrosis and cryoapoptosis following the exposure of the pancreatic parenchyma to deep low temperatures.

Discussion. The present study examined the effects of exposure to different low temperatures on pancreatic cells. The results clearly suggest that damage to pancreatic cells after freezing and thawing at various intensities is acute, and the pancreatic cell architecture cannot be returned more or less to normal. The disruption of pancreatic cells occurred after freezing and thawing at different low temperatures, in particular at temperatures of –80°C and –180°C. Pancreatic cells are entities with a highly specific intracellular morphological content, separated from the non-specific extracellular solution by the cell membrane.

An inadequately studied aspect of cryosurgery is the mechanism by which damage occurs during the freezing process. This is important because, although cryosurgery is clinically used to treat parenchymal tumors, especially liver tumors, neither the freezing process nor the mechanism of damage involved in this kind of treatment is fully understood. Therefore, research into the process of freezing in liver tissue is of great significance for pancreatic surgery.

An experiment-based investigation of pancreas cryosurgery must consider the following aspects: 1) the mechanism of tissue destruction with respect to pancreas freezing; 2) the viability and safety of cryosurgical applications in the pancreas; 3) biological perspectives of freezing pancreas tumors; and 4) experimental studies focused on refining the technology of the equipment used for pancreatic cryosurgery.

The data presented here constitute the results we obtained from the experimental investigations in the field of pancreatic cryosurgery. Healthy pancreatic parenchyma was investigated grossly and morphologically by electron microscopy. The study was focused on the mechanism of the freeze–thawing process with applications of –80°C and of –180°C to pancreatic tissue in terms of early pancreatic cell changes. Endothermic transformation of the cryogenic lesion in the pancreatic parenchyma immediately, one hour and 24 hours after freeze–thawing does correlate with various low temperatures.

In this study, the following early ultrastructural changes in the post-cryosurgical zone occurred in the pancreas at a temperature of –180°C more than at a temperature of –80°C: the division of chromatin into large aggregates, dilation and partial fragmentation of the canaliculi of the rough endoplasmic reticulum, reduction of the basement membrane of the endothelium cell, reduction in the number of ribosomes and the presence of myelin-like structures in the vascular capillary space. Further, the cell detritus and the fibrin fibers, resulting from cryonecrosis and cryoapoptosis, were more clearly visible at the temperature of –180°C than at –80°C. The cryonecrosis and cryoapoptosis indicated the cryodevitalization of the living matter, i.e. pancreatic tissue.

Cell changes in the pancreatic tissue augment with time in animals, both after exposure to –180°C and after exposure to –80°C. Immediately after the freeze–thaw cycle at –180°C, no myelin-like structures in the cytoplasm can be found. They occur only after one hour after thawing, indicating an increase in free radicals. On the other hand, a segmented neutrophil as well as cell detritus and fibrin fibers are not observed immediately or one hour after the freeze–thaw cycle, but only after 24 hours.

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Similarly, in this study, the cell changes in the pancreas tissue depend on time after the freeze–thaw cycle at –80°C. Thus, cell detritus in the interstitial space can first be seen one hour after thawing in the cryozone, and damaged exocrine pancreatic cells are visible 24 hours after thawing in the dog's pancreas.

Further, our investigation demonstrated that the damaged endothelial structure and the damaged blood cells in the vascular capillary space including segmented neutrophils are the initial signs of aseptic inflammation and circulatory stagnation after the freeze–thaw cycle.

The results of our study show that the early pancreatic cell changes in the post-cryosurgical zone observed in the first 24 hours after the freeze–thawing cycles are the beginning phase in the whole post-cryosurgical process. They lead to the next phase of aseptic cryoaponecrosis and then to aseptic cryoapoptosis, which, in many weeks, will clinically finish with the formation of a post-cryosurgical crust and a post-cryosurgical scar. The cryodevitalization of the pancreatic parenchyma will be complete at that time too.

Further, fundamental investigation of the cryodestruction mechanisms in pancreatic tissue would allow the researcher to list the main requirements for cryosurgical equipment. The main technical parameter for effective cryodestruction is the provision of a sufficiently low, sub-zero temperature in the biological tissue, followed by deliberate thawing. Our findings, i.e. early ultrastructural changes in the pancreas parenchyma, show that research into cryosurgical techniques will yield data on the main medical requirements for the application of cryomethods. However, studies that will reflect the late ultrastructural changes in the pancreas after low temperature exposure, e.g. one week, two weeks, four weeks, two months, three months, six months, are yet to be conducted. Such data would enhance the efficiency of cryosurgical methods in the different fields of medicine, primarily for the treatment of malignant tumors in the pancreas and other parenchymal organs. Thus, our findings and additional further studies should lead to a fuller understanding of the ultrastructural features of parenchymal tissue after cryosurgery, giving rise to a new concept concerning the technical requirements of cryosurgical equipment to perform modern cryosurgical operations, especially in oncology. The optimal technical data for the modern universal cryosurgical systems should be of a high cooling capacity and of great accuracy, thus guaranteeing the cryodestruction of the specified volume (16).

Prospective randomized clinical trials concerning cryogenic surgery and conventional surgical techniques in patients with pancreatic carcinomas have not been published hitherto. It is, however, necessary to work out a clinical concept of cryosurgical and conventional surgery focused on intraoperative tumor reduction in pancreatic cancer, as this would improve patients' survival rate and quality of life.

CONCLUSIONS. The present observations on the early ultrastructural changes in the pancreatic tissue clearly enable a better understanding of the mechanisms of damage and the pathogenesis of frostbite during cryosurgery. The properties of the pancreatic parenchyma response after low temperature exposure provide important insights into the mechanisms of damage and cryogenic lesion immediately after thawing in cryosurgery. Progressively damaged pancreatic cells in the post-cryosurgical zone lead to cryoaponecrosis and cryoapoptosis. Vascular changes and circulatory stagnation indicate the anti-angiogenesis mechanism of biological tissue injury after low temperature exposure at –180°C and –80°C. Thus, cryoaponecrosis, cryoapoptosis and anti-angiogenesis are some of the most important mechanisms by which living tissue suffers damage as a response to cryosurgery, finally leading to complete local cryodevitalization of the pancreatic parenchyma.

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РАННИЕ И ПОЗДНИЕ УЛЬТРАСТРУКТУРНЫЕ ИЗМЕНЕНИЯ В КЛЕТКАХ ПОДЖЕЛУДОЧНОЙ ЖЕЛЕЗЫ ПОСЛЕ ДЕЙСТВИЯ НИЗКИХ ТЕМПЕРАТУР: ЭКСПЕРИМЕНТАЛЬНЫЕ ИССЛЕДОВАНИЯ

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В работе представлен ряд теоретических и экспериментальных исследований, которые проводились *in vitro* и *in vivo*, были выполнены, чтобы объяснить действие низких температур на ткани. Они позволяют утверждать, что температурные параметры криохирургии, которые использовались ранее, не были достаточно контролируемые, что в ряде случаев приводило к провалу лечения. В этом исследовании впервые описываются ранние ультраструктурные особенности паренхимы поджелудочной железы после низкого температурного воздействия, то есть криохирургии, в естественных условиях. Показано влияние замораживания-размораживания с использованием температуры различной интенсивности. Была исследована криохирургическая реакция паренхимы поджелудочной железы, в том числе ультраструктурные клеточные изменения в тканях поджелудочной железы. Анализ с помощью электронного микроскопа, показал, что аналогичные процессы происходили в тканях поджелудочной железы после локальной криодеструкции при температуре -80° С и -180° С. Они были близки в период от непосредственно криохирургической фазы до 24 часов после того, как ткань подвергается воздействию низкой температуры.

Ключевые слова: криохирургия, температура, панкреатические изменения клеток, анти-ангиогенез, криоапонекроз, криоапоптоз, онкология, исследования.