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## Tendinous part of the diaphragm under the influence of carboxyperitoneum and simulated jaundice: an experimental study

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*Abstract: understanding the increase in intra-abdominal pressure during pneumoperitoneum due to carbon dioxide insufflation as part of laparoscopic surgery is important as systemic changes due to carbon dioxide have become important. Despite the benefits, laparoscopic surgical procedures and insufflation affect many systems and organs, such as the brain, lungs, and liver. The aim of the study was to investigate the histological structure of the tendon parts of the diaphragms of rats with obstructive jaundice under the influence of standard pneumoperitoneum pressure formed by carbon dioxide, which is used in laparoscopic surgery, for a variety of time periods. The experimental study was performed on 70 mature rats weighing (235.0±20.0) grams. Obstructive jaundice was modelled by ligation of the common bile duct through a previously made laparotomy access. Pneumoperitoneum was created after puncture of the abdominal wall with a Veresch needle connected to an insufflator that injected carbon dioxide and maintained a given intra-abdominal pressure for a certain time. Obstructive jaundice led to thickening of the tendon due to edema. Collagen fibres acquired a tortuous direction. The cellular infiltration was distinct, with macrophages appearing in the structure. In 1 hour after pneumoperitoneum, there was an undulating direction of collagen fibres separated by intercellular substance, in which fibroblasts, lymphocytes and multiple macrophages were visualised. After 2 hours, there was a thickening of collagen fibres, homogeneity with multiple foci of decay and delimitation by the main substance containing fibroblasts and macrophages. After 3 hours, in addition to the disintegration, loss of unidirectional arrangement, in some cases, foci of chaotic accumulation of cell-fibre mass and haemorrhages were detected. In cellular infiltrates, plasmacytes appeared in small numbers among lymphocytes. The results obtained indicate that obstructive jaundice leads to structural changes in the tendon part of the diaphragm. Pneumoperitoneum with carbon dioxide exacerbates the changes in the morphological picture, which depend on the duration of the latter.*

**Keywords:** Laparoscopy; Pressure; Pneumoperitoneum; Diaphragm; Obstructive Jaundice; Rats; Histology.

### Introduction

Obstructive jaundice is a common clinical phenomenon caused by various benign or malignant diseases. The most common benign biliary

pathology worldwide is choledocholithiasis, defined as the presence of stones in the common bile duct [1]. Previous studies have shown that 4–15 % of patients with cholecystolithiasis

also have concomitant choledocholithiasis [2]. According to a recent systematic review, when cholecystolithiasis and choledocholithiasis are diagnosed at one time, laparoscopic cholecystectomy with simultaneous intraoperative cholangiography and revision of the common bile duct is recommended. In this strategy high technical success rate and short-term hospital stay is predicted [3].

Understanding the effect of intra-abdominal pressure increase during pneumoperitoneum (PP) due to carbon dioxide (CO<sub>2</sub>) insufflation as part of laparoscopic surgery is underestimated yet relevant. This is because systemic changes due to CO<sub>2</sub> insufflation have been reported as significant [4]. Despite the benefits, laparoscopic surgical procedures and CO<sub>2</sub> PP affect many systems and organs, such as the brain, lungs, and liver [5]. Also, the kidneys are among the critical organs where such effects can be observed. Moreover, changes in the small intestine have also been noted [4]. Animal studies have shown that elevated intra-abdominal pressure established during PP, a necessary procedure for performing laparoscopic surgery, leads to ischemia-reperfusion injury of the intestine, which disrupts the intestinal barrier [6].

Our experimental study is conducted to determine the impact of CO<sub>2</sub> in PP on the structure of the diaphragm parts in the presence of obstructive jaundice (OJ). This is an important topic for research, since these pathological conditions occur at laparoscopic surgery for cholelithiasis with choledocholithiasis or other diseases accompanied by impaired bile outflow, and the CO<sub>2</sub> PP effect on organs and systems of the body has not yet been studied.

## Aim

The aim of this study is to investigate the histological structure of the tendon parts of the diaphragm of rats with obstructive jaundice under the influence of standard pressure pneumoperitoneum formed by carbon dioxide, which is used in laparoscopic surgery, for various periods of time.

## Materials and methods

Research on laboratory animals was performed in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), Directive 2010/63/EU of the European Parliament and of the Council, and the Law of Ukraine No. 3447 – IV «On the Protection of Animals from Cruelty». The experimental study was carried out on mature reproductive age rats (5-6 months), with body weight (235.0±20.0) g. The animals were kept in an accredited vivarium of I. Horbachevsky Ternopil National Medical University (Table 1). The experiments were conducted in the morning indoors at a temperature of (18-20) °C, relative humidity of (60-80) % and illumination of 200 lx. In the end of the modelling all rats were sacrificed by a single overdose of thiopental sodium solution injected intra-peritoneally (75 mg/kg of body weight).

Animals of all groups, except the intact group, underwent OJ simulation. Anesthesia was performed with ketamine hydrochloride (90 mg/kg) and xylazine (10 mg/kg) intramuscularly. Before surgery, the anterior abdominal wall was shaved, and the surgical field was treated three times with a disinfectant solution and covered with sterile drapes. This was followed by a median laparotomy approach

**Table 1.** Distribution and characteristics of animals in groups

Animal group	Group characteristics
Intact group (n = 10)	Diaphragm harvesting in animals without simulating any pathology
Control group (n = 15)	Diaphragm sampling after laparocentesis using a Veress needle without creating PP
First experimental group (n = 15)	Diaphragm sampling after creating PP at 10 mmHg for 1 hour
Second experimental group (n = 15)	Diaphragm sampling after creating PP at 10 mmHg for 2 hours
Third experimental group (n = 15)	Diaphragm sampling after creating PP at 10 mmHg for 3 hours
Total – 70 animals	

of 3 cm in length. The stomach and duodenum were carefully removed through the laparotomy wound, the common bile duct and pancreatic duct were identified, after which only the bile duct was ligated. After 7 days, the animals had yellow ears, yellow urine, and a yellow tint of the sclera. When the abdominal cavity was opened, jaundice of the fatty tissue, icteric muscle tissue, and dilatation of the common bile duct were noted. According to the common literature data, 5 to 7 days is sufficient to study the effects of bilirubin intoxication in experimental animals and the occurrence of changes in the organs and systems of the body.

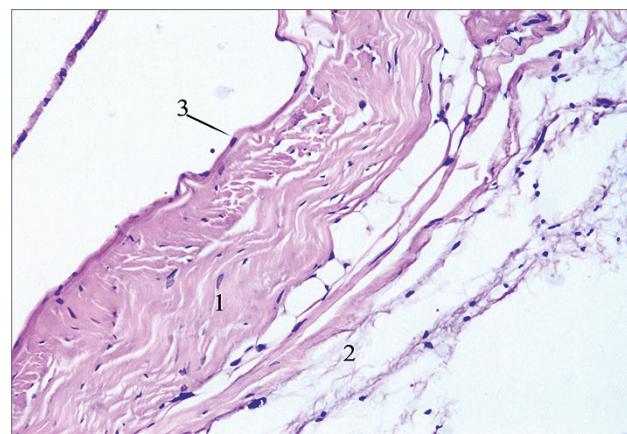
In the control group, the abdominal wall was punctured with a Veress needle and the animal was in this state for 2 hours in order to study whether anaesthesia and laparocentesis affect the morphology of the diaphragm. In the experimental groups, carboxyperitoneum was created, according to the author's certificate for the work No. 126409 [7], with a KARL STORZ electronic laparoflator 264300 20 at a level of 10 mmHg and a carbon dioxide supply rate of 0.1 l/min, for 1, 2 and 3 hours. According to the studies, this pressure level in rats corresponds to a pressure of 15 mmHg in an adult. During the experiment, the animal breathed independently and did not require external lung ventilation. After the experiment, the rat diaphragm was harvested in accordance with the author's certificate for the work No. 126059 [8].

The tendon part was isolated from the macro specimen, which was fixed in a 10 % neutral formalin solution. Afterwards, the material was dehydrated in ethyl alcohols of increasing concentration and embedded in paraffin. Paraffin sections 5–7  $\mu$ m thick were made from each paraffin block on a microtome. After deparaffinization, they were stained with hematoxylin and eosin and Alceal blue.

## Results

The tendon part of the diaphragm of intact group rats is represented by an aponeurotic sheet, which is formed by dense bundles of collagen fibres. In different sections of the micro specimen its thickness varies slightly. On the cranial side of the tendon, it is covered with diaphragmatic fascia, on the caudal side it is covered with

parietal peritoneum. Under the microscope, it has a loose consistency with inclusions of fatty tissue. Collagen fibres are homogeneously eosinophilic, the intensity of staining slightly differs, very rarely they are loosened without damaging their integrity. The cellular component is poorly represented, somewhere consisting of fibroblasts, fibrocytes and histiocytes (Fig. 1).

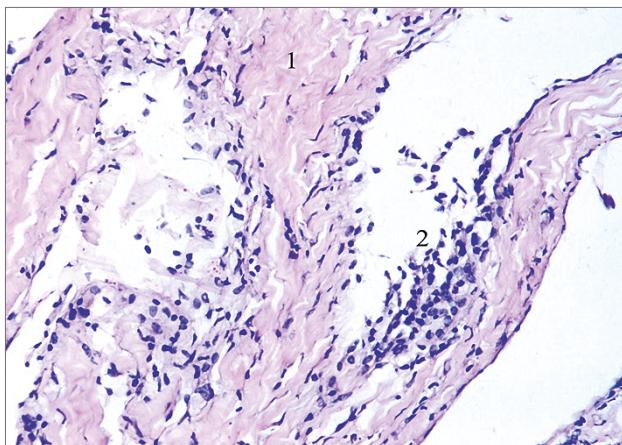


**Figure 1.** Histological structure of the tendon part of the diaphragm of the intact group rats: 1 – dense bundles of collagen fibres; 2 – loose tissue of the thoracic surface; 3 – parietal peritoneum. Staining with haematoxylin and eosin.  $\times 200$

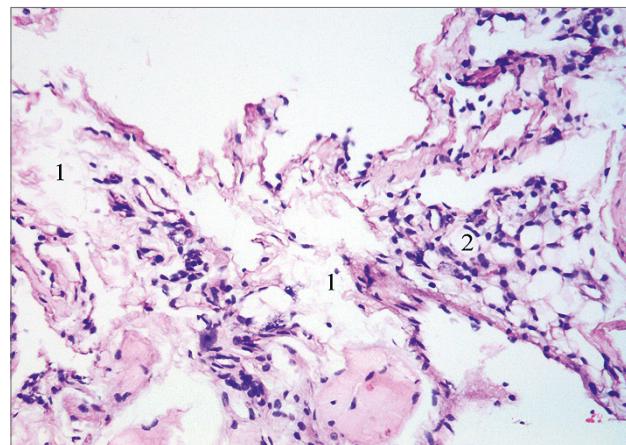
In the micro specimens of the control group, the tendon part of the diaphragm has an identical tissue composition, as in the previous group, but differs in the ratio of structural components. Its thickness is mainly increased. This can be explained by tissue loosening due to oedema. Collagen fibres acquired a winding direction. In this group, uneven staining was sharply noted. Cellular infiltration was pronounced, both diffuse and with the formation of focal infiltrates with macrophages (Fig. 2).

The PP effect in the presence of OJ in the main experimental groups caused other morphostructural changes along with the changes observed in the control group with OJ. The equidirectional arrangement of collagen fibres of the tendons has changed to undular. The fibres were also strongly delimited by the intercellular substance, in which fibroblasts, lymphocytes and multiple macrophages were visualized (Fig. 3).

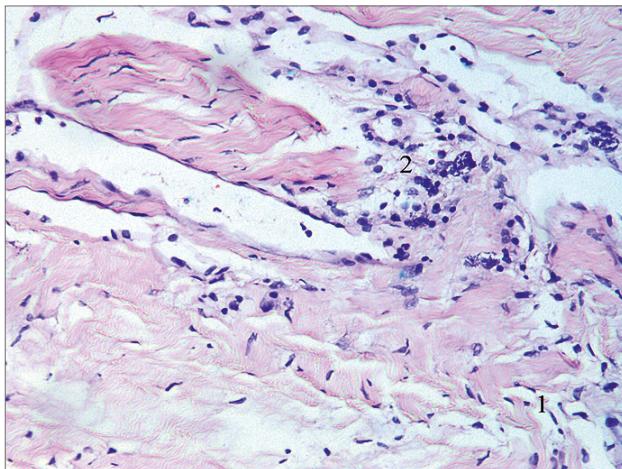
The microscopic picture after 2 hours of PP was characterized by sickening of the collagen fibres, becoming homogeneous, with multiple foci



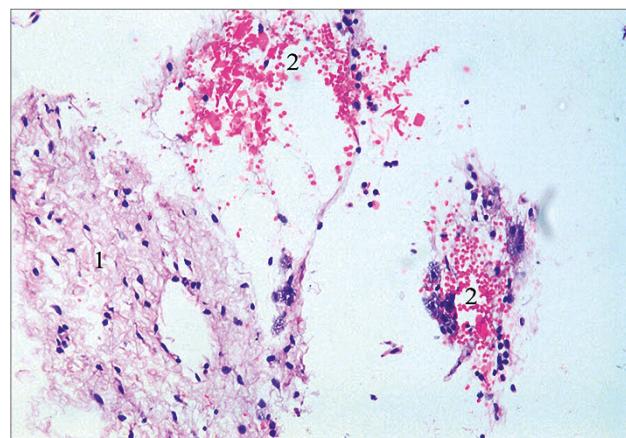
**Figure 2.** Histological structure of the tendon part of the diaphragm of the control group rat: 1 – tortuous and unevenly stained collagen fibres; 2 – cellular infiltrate. Staining with haematoxylin and eosin.  $\times 200$ .



**Figure 4.** Histological structure of the tendon part of the diaphragm of the II experimental group rat: 1 – oedema; 2 – disorganization and destruction of collagen fibres with diffuse polymorphocellular infiltration. Staining with haematoxylin and eosin.  $\times 200$ .



**Figure 3.** Histological structure of the tendon part of the diaphragm of the I experimental group rat: 1 – tortuous collagen fibres, disintegrated by oedema; 2 – polymorphocellular infiltrate. Staining with haematoxylin and eosin.  $\times 200$ .



**Figure 5.** Histological structure of the tendon part of the diaphragm of the III experimental group rat: 1 – disorganization and destruction of collagen fibres; 2 – haemorrhages and surrounding cellular infiltrates. Staining with haematoxylin and eosin.  $\times 200$ .

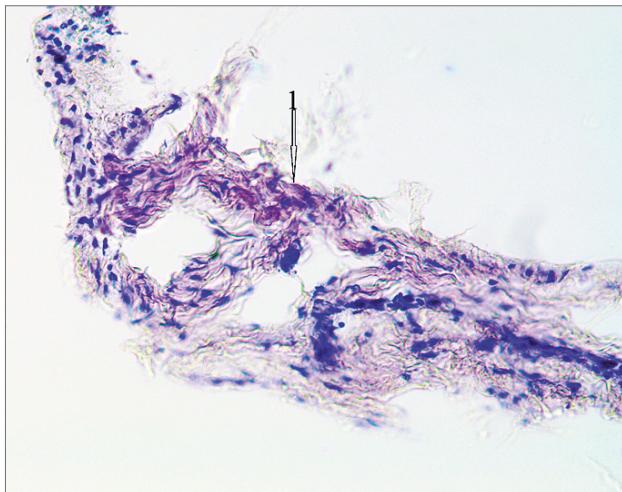
of decay and delimited by the ground substance, which contained fibroblasts and macrophages. The destroyed fibres were replaced by cellular infiltrates, which were dominated by macrophages and lymphocytes (Fig. 4). Alcian blue staining did not reveal signs of mucoid degeneration.

3 hours after the created PP, the tendons underwent significant structural changes. In addition to their defibreization, loss of equidirectional arrangement, in some cases, foci of chaotic accumulation of cell-fibre mass and haemorrhages were detected (Fig. 5). In the cellular infiltrates, plasma cells appear in

small numbers among lymphocytes. Alcian blue staining has shown slight metachromasia (Fig. 6).

### Discussion

When searching for scientific works that would study the morphology of the diaphragm under the influence of OJ and PP, we can state that we are the first in this direction of research. We have not found other similar data from other authors. Most of the works that study OJ concern changes in the liver and other organs of the abdominal cavity. When including the words



**Figure 6.** Rat tendon with jaundice after 3 hours of pneumoperitoneum. Metachromasia phenomenon in the ground substance. Staining with Alcian blue.  $\times 100$ .

«laparoscopy», «pneumoperitoneum», «intra-abdominal pressure» in the search field, most of the works again concentrate their scientific attention to the other organs of the abdominal cavity. The significance of this study is that laparoscopic methods of treating surgical pathologies of the abdominal cavity are constantly expanding. And the influence of PP on organs and systems of the body, especially with different intra-abdominal pressure formed by carbon dioxide, is beginning to be actively studied.

A further perspective of the study is to analyse the changes in different parts of the diaphragm at different intra-abdominal CO<sub>2</sub> pressures. Recent studies are conducted in the direction of reducing the standard pressure in laparoscopic surgery to lower limits than 12 mmHg, explaining this by the fact that lower pressure does not contribute to a decrease in the volume of instrument manipulations, and the postoperative period is more favorable, especially in patients with concomitant pathology [9].

In the one of our other studies, where the modelling of the OJ was not conducted, we found that the PP created by carbon dioxide leads to a change in the morphological structure of the tendon part of the diaphragm. It has caused a loss of compactness of collagen fibres, collagen fibrilization was observed, areas of lysis were occasionally determined, and cellular infiltration with a predominance of erythrocytes was

detected. The severity of these changes depends on the duration of created pneumoperitoneum. It was found, that two weeks after the simulated pathology, the diaphragm fully restores its structure.

Although rats are readily available and economically viable for any experimental study, the question arises whether the results are comparable to other species. There are several studies examining the effects of PP on the body of various animals, including humans, which indicate that a pressure of 8-10 mmHg in the abdominal cavity of a rat is similar to a pressure of 12-15 mmHg in the human body [10].

The number of laparoscopic surgical interventions on the biliary tract has increased significantly in recent years. These interventions range from conventional cholecystectomies for chronic calculous cholecystitis to operations on the biliary tract, such as those on the right and left hepatic ducts and the choledochus. The initial step in the process is to create the PP using CO<sub>2</sub>. As the volume of operations increases, the duration of the PP also increases. This method is frequently utilised in elderly and senile patients with concomitant cardiovascular and pulmonary diseases, for whom additional ‘aggression’ in the form of PP becomes clinically significant. The created intra-abdominal pressure of the PP causes hypercapnia and acidosis, which are avoided by hyperventilation [11]. The diaphragm is displaced cranially, reducing the volume of the pleural cavity and the lungs’ pliability, and creating high pressure in the airways. This leads to a decrease in the diaphragm’s functional capacity. [12].

In our professional opinion, the practical significance of this work for clinical medicine is that, in addition to the effects of PP on the body mentioned above, intra-abdominal hypertension during laparoscopy affects the microstructure of the diaphragm. It should be noted that these changes occur when standard pressure is applied in the abdominal cavity. Research conducted over the last decade has been restricted to the utilisation of low-pressure PP at 7 mmHg, which equates to a pressure of 5 mmHg in rats. When the results were analysed, it was found that pain (a phrenic symptom) was 36.7% more common

in patients treated with standard pressure than in those treated with lower pressure. There were also fewer postoperative complications when lower pressure was used [13, 14, 15]. Our research is focused on studying the structure of the diaphragm at low pressure within the abdominal cavity and comparing the results with those at standard pressure. The outcomes of this research will be detailed in upcoming scientific papers.

The observed changes in the control group of animals can be attributed to the direct toxic effect of bilirubin, which leads to oedema, resulting in the loosening and tortuous fibres with cellular infiltration. The mechanism of the detected changes in the main groups is associated with intra-abdominal hypertension created by PP, which led to stretching of the fibrous structures of the tendon due to the displacement of the diaphragm in the cranial direction and the duration of pressure exposure. Increased pressure resulted in fibre breakdown in cases of oedema and cellular infiltration. Duration led to the appearance of larger areas of chaotic accumulation of cell-fibre mass with haemorrhage, also in cases of bilirubin intoxication.

### Conclusions

The obtained results indicate that obstructive jaundice leads to structural rearrangement of the tendon part of the diaphragm in the form of edema, loosening and the presence of cellular infiltration. Pneumoperitoneum with carbon dioxide deepens the changes in the morphological picture due to

the created intra-abdominal pressure, tension and displacement of the diaphragm to the thoracic cavity. These changes in direction depend on the durations. Stretching of the fibrous structures of the tendon part of the diaphragm leads to rupture of the fibers and a change in their direction. After 2 and 3 hours, areas of destroyed fibers appeared, which contained macrophages and fibroblasts. The greatest changes are observed after 3 hours and are characterized by the appearance of areas with a chaotic location of cellular-fibrous mass in which single plasma cells and lymphocytes appeared.

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### Consent for Publication

The authors provide consent for the publication of the manuscript detailed above.

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## Сухожильна частина діафрагми під впливом карбоксиперитонеуму та змодельованої жовтяниці: експериментальне дослідження

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**Анотація:** розуміння збільшення внутрішньочеревного тиску під час пневмоперитонеуму внаслідок інсуфляції вуглекислого газу, як частини лапароскопічної хірургії є важливим, оскільки системні зміни через вуглекислий газ стали важливими. Незважаючи на переваги, лапароскопічні хірургічні процедури та інсуфляція впливають на багато систем і органів, таких як мозок, легені та печінка. Метою роботи було дослідити гістологічну структуру сухожильних

частин діафрагм щурів із механічною жовтяницею під впливом стандартного тиску пневмoperitoneума утвореного вугекислим газом, який використовується в лапароскопічній хірургії, на протязі різноманітного проміжку часу. Експериментальне дослідження виконано на 70 статевозрілих щурах масою ( $235,0 \pm 20,0$ ) грам. Механічну жовтяницю моделювали методом перев'язки загальної жовчної протоки через попередньо зроблений лапаротомний доступ. Пневмoperitoneum створювали після проколу черевної стінки голкою Вереша з'єднаної із інсуфлятором, який нагнітав вугекислий газ і підтримував заданий внутрішньочеревний тиск на протязі певного часу. Механічна жовтяниця призводила до потовщення сухожилка за рахунок набряку. Колагенові волокна набувають звивистого напрямку. Клітинна інфільтрація була виразною, в структурі якої з'являлися макрофаги. Через 1 годину після пневмoperitoneуму сопстерігали ундулярне направлення колагенових волокон розмежованих міжклітинною речовиною, в якій візуалізуються фібробласти, лімфоцити і множинні макрофаги. Через 2 години відмічалось потовщення колагенових волокон, гомогенність з множинними осередками розпаду і розмежованість основною речовиною, в якій містяться фібробласти і макрофаги. Через 3 години крім розволокнення, втрати рівнонаправленого розташування, в ряді випадків виявлялися осередки хаотичного скупчення клітинно-волоконної маси та крововиливи. В клітинних інфільтратах серед лімфоцитів з'являються плазмоцити в невеликій кількості. Отримані результати свідчать, що механічна жовтяниця призводить до структурної перебудови сухожильної частини діафрагми. Пневмoperitoneum вугекислим газом поглиблює зміни морфологічної картини, які залежать від тривалості останнього.

**Ключові слова:** лапароскопія, внутрішньочеревний тиск, пневмoperitoneum, діафрагма, механічна жовтяниця, щурі, гістологія.



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