

Experimental Evaluation of the Effectiveness of Submucosal Administration of Hemoben Gel During Laser Mucosectomy from the Stomach in Terms of the Potential for Endoscopic Treatment of Polypoid Formations

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Abstract The article presents an experimental study to evaluate the effectiveness of submucosal administration of Hemoben gel followed by laser mucosectomy of the gastric mucosa. The study is aimed at the potential possibility of developing a new method of endoscopic treatment of polypoid neoplasms of the gastrointestinal mucosa. Experimental excision of the gastric mucosa using laser radiation allows precision resection of the mucosa without bleeding from the vessels of the submucosal layer, while the gel pad prevents the penetration of exposure beyond the submucosal layer, and the defect heals within 7-10 days. In the control group of animals, where only 0.1% methylene blue solution was used, the pillow did not allow to clearly delineate the area of surgical intervention, quickly resolved and did not give a noticeable hemostatic effect, while the created lifting did not make it possible to completely protect against the penetration of laser radiation into the deep layers of the stomach wall, which can lead to necrosis of the serous membrane and the development of postoperative purulent-septic complications.

Keywords Submucosal administration of hemoben gel, Laser mucosectomy, Stomach, Endoscopic treatment, Polypoid formations

1. Introduction

The development of endoscopic technologies has made it possible to significantly expand the range of interventions performed, including operations for various superficial polypoid formations of the mucous membrane of the gastrointestinal tract [1,2,3,4,5]. This study is based on the possibility of developing a new method for endoscopic removal of superficial polypoid formations, which is aimed at reducing the risk of developing specific complications and recurrence of their formation. The experimental study is devoted to the study of the possibilities of using a high-energy diode laser in conjunction with a submucosal injection of a gel substance, including antiseptic and hemostatic agents, when performing the removal of superficial tumors of the mucous membrane. The objectives of the study were: assessment of the provision of dosed destruction of the mucous membrane of the gastrointestinal tract for further

use in excision of superficial tumors of the mucous membrane in humans; analysis of the characteristics of regeneration in the affected area; assessment of hemostatic and anti-inflammatory activity in the affected area.

Morphological changes occurring during the healing of postoperative wounds of the gastrointestinal tract indicate the complexity and multi-stage nature of this process. Understanding the mechanisms of healing, as well as the development and application of new methods and technologies to improve tissue repair, play a key role in modern medicine. The importance of an integrated approach, including the use of innovative treatment methods, cannot be overestimated in the process of achieving optimal healing results and restoring the functions of the gastrointestinal tract after surgery.

The stomach of some laboratory animals (mice, rats, guinea pigs, hamsters) has two morphologically and functionally different parts. After the esophagus flows into the stomach, the gelatinous part begins, lined with a multilayer squamous epithelium. It is followed by a glandular part lined with a single-layer prismatic glandular epithelium and containing

gastric glands. These two zones are clearly separated by a bounding ridge. In rats, mice and hamsters, the gelatinous part of the stomach occupies about 2/3 of the total volume [6,7].

The stomach wall of rats has a typical structure and is represented by a mucous membrane, a submucosal base, muscle and serous membranes. The mucous membrane of the esophageal region (the non-gelatinous part) is formed by a multilayer keratinizing epithelium, in which 3 to 6 layers of flat epithelial cells without signs of keratinization, their own connective tissue plate and the muscular plate of the mucous membrane (lam. Muscularis mucosae) are visualized. The transition of the multilayer epithelium into the glandular part is well visualized. The mucous membrane of the glandular (fundal, cardiac and pyloric) part is represented by a single-layer single-row cylindrical epithelium lining the gastric pits, at the base of which the gastric glands open. Diffusely located leukocytes are detected in places in the rat's own plate of the gastric mucosa, among which lymphocytes and polymorphonuclear neutrophils predominate. The muscular plate and the muscular lining of the stomach are formed by smooth muscle cells. The cells of the muscle plate are arranged in a single layer. The submucosal base, represented by connective tissue, does not contain glands. It contains the vascular plexuses and the nervous submucosal plexus of Meissner. The muscular membrane is well developed and consists of three layers. From the outside, the stomach is covered with a serous membrane consisting of a single-layer squamous epithelium - mesothelium - and a layer of connective tissue [8].

2. Materials and Methods

Experimental studies were conducted at the State Institution "Republican Specialized Scientific and Practical Medical Center for Surgery named after academician V.Vakhidov" in 2022-24. Laboratory animals (rats) were kept in the vivarium of the center, equipped with supply and exhaust ventilation, lighting and constant temperature (22-25°C). The animals were kept in separate rooms (according to the type of animals) and cages of 2 individuals. Meals in the form of balanced dry mixes and vegetables, vitamins were provided 2 times a day. Water was constantly present in special drinking bowls. After the manipulations performed by him, the animals were dynamically monitored and anesthetized (if necessary) by adding ipobrufen tablets to drinking water.

Surgical operations and manipulations were performed in the laboratory of experimental surgery, equipped with the necessary surgical instruments and equipment, sterilization and medical supplies. Surgical interventions were performed after placing the animals under a bell with evaporation of an inhalation anesthetic from a moistened gauze ball, followed by anesthesia using a special mask with a gauze pad moistened with an inhalation anesthetic. The number of animals is shown in Table 1.

Table 1. The number and timing of studies on laboratory animals

Rats	1 day	3 days	5 days	7 days	14 days	Total
Control	4	4	2	2	4	16
Experience	4	4	2	2	4	16
Total	8	8	4	4	8	32

Hemobene is a powder with particle sizes from 50 to 300 microns in sterile vials of 1.0 g. This preparation, made from cellulose derivatives, is hygroscopic, dissolves well in water, actively absorbs moisture and turns into a gel at a concentration of more than 1%. To prepare the gel to be used as an injectable solution, Hemoben was previously dissolved in 0.1% methylene blue (MB) solution at concentrations of 1, 2, 3, 5 and 10%.

The results of the research showed that when the gel is formed at a concentration of up to 5%, the permeability through the needle channel is maintained for 5-15 minutes, and it also remains quite viscous, since it retains its shape on the slide. The gel concentration of less than 4% retains a liquid consistency for 20 minutes, however, the viscosity of the drug is quite low and spreads easily over the surface of the glass. Also, to form a pillow, you have to inject a larger amount of gel, as it spreads over the submucosal layer. A Hemobene concentration of more than 10% leads to a rapid increase in the viscosity of the drug, which makes it difficult to inject it through the needle channel with the formation of a blockage with a dense clot of gel.

The concentration of the drug in the form of a 4-5% solution met the requirements of the experiment to the greatest extent. In this case, the gel was injected into the submucosal layer with a sufficient force of 10-20 kPa and formed a pillow in the submucosal layer, which lasted for quite a long time – up to 30 minutes. The time for preparation and administration of the drug was limited to 5-10 minutes. After the introduction of the gel, the needle channel should be rinsed with enough water so that a clot does not form, which can lead to obstruction of the lumen of the needle.

In the experiment, a part of the gastric mucosa was resected after the formation of a submucosal cushion using 5% Hemobene gel with 0.1% MB (experiment), and in another group of rats 0.1% MB (control). In rats, hair was removed from the anterior abdominal wall under general anesthesia. Upper median laparotomy up to 3 cm long. The stomach was removed into the wound. Dissection of the anterior wall of the stomach up to 1 cm long. After visualization of the posterior wall of the antrum of the stomach, submucosal administration of 5% Hemoben gel in 0.1% MB solution in an amount of 0.3 ml was performed (Fig. 1) (0.1% MB in an amount of 0.3 ml was administered in the control group). 2 minutes after the formation of the submucosal cushion with a diameter of up to 1 cm, excision of the mucosa with a diameter of 0.5 cm was performed using a Gbox (GIGAA) laser with a wavelength of 1470 nm, a power of up to 7W in a pulsed mode with a frequency of 2-5 Hz and a spot area of up to 2 mm (Fig. 2). The wound of the anterior wall of the stomach was sutured with a

continuous seam of Prolene 6/0. The laparotomy wound was sutured with double-row stitches. 1 hour after the operation, the rats of the experimental and control groups woke up from anesthesia and moved around the cage. However, food and water were not touched. When trying to pick it up, they sluggishly resisted. In the following periods (up to 3 days), 500 mg of Ipobrufen was added to 100 ml of drinking water for anesthesia.

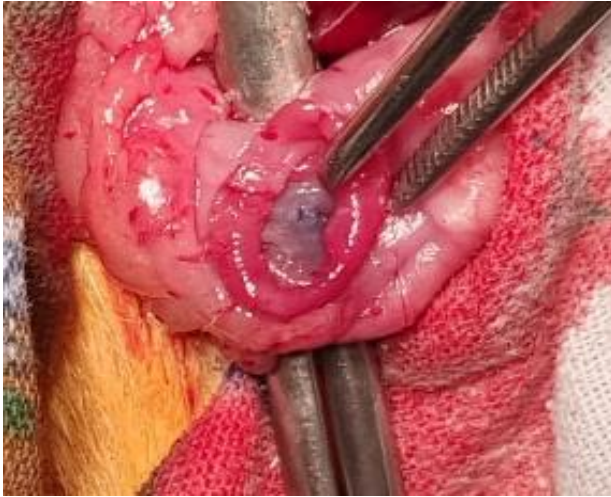


Figure 1. Experience. Formation of a cushion of Hemoben gel in the submucosal layer of the stomach



Figure 2. Experience. Destruction of the gastric mucosa using a laser

3. The Results of the Study

1 day after the operation, the rats of the experimental group actively moved around the cage. They began to take food and water. There were no signs of active bleeding in animal droppings. The postoperative wound is clean, moderately painful. On palpation, the abdomen is soft. The mucous membranes are clean and moist.

3 days after the operation – the animals are quite active. They take food and water. Healthy bladder and bowel habits. When trying to pick up, they actively resist. The abdomen is soft and painless. The wound is clean.

After removing 2 rats from the experiment by overdosing on a narcotic drug, the abdominal cavity was opened. There was no pathological effusion or changes in the abdominal cavity. There is a moderate amount of food in the stomach. There were no signs of damage to the serous lining of the stomach. When opening the lumen of the stomach at the site of excision of the mucosa, there is a small stellate wound in the likeness of an ulcer measuring 2 mm in diameter (Fig. 3). The edges of the mucosa are without necrotic plaque. There is no hematoma around the wound. The bottom of the defect is covered with a thin layer of fibrin. No damage to the serous cover was detected. There were also no traces of the injected Hemoben gel.

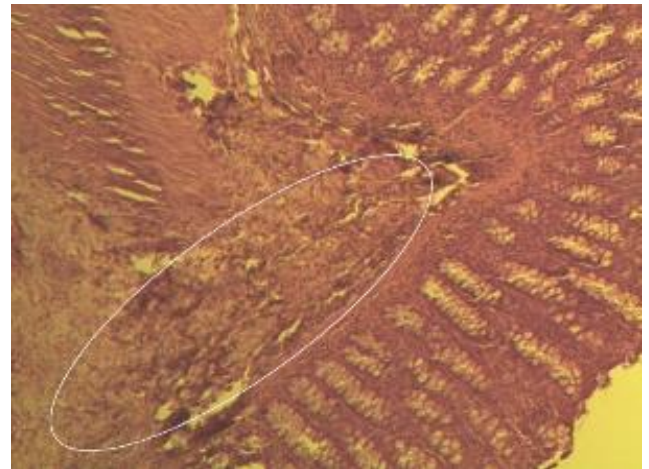


Figure 3. Gel in the submucosal layer, collapsed (soldered) blood vessels of medium caliber, edema and dystrophy of muscle fibers. Micrograph of the stomach wall in the experimental group, 3 days. Hematoxylin and Eosin $\times 40$

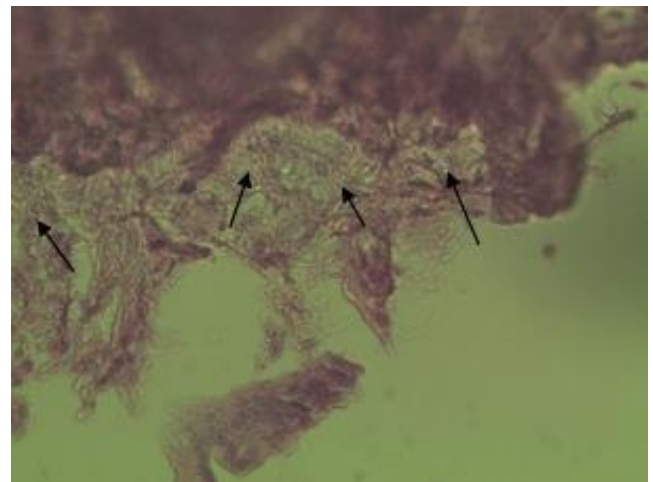


Figure 4. Gel in contact with endothelial and epithelial cells (indicated by arrows). Micrograph of the stomach wall. Experience, 3 days. Hematoxylin and Eosin $\times 400$

During morphological studies of drugs of the experimental group (3 days), with the use of Hemoben gel in the submucosal layer of the stomach, coagulation thermal necrosis of the mucous membrane, submucosal and muscular layers connecting the tissues of all anatomical layers of the

stomach wall is noted. This factor can prevent the gel from penetrating into all the free spaces of the submucosal layer of the stomach wall. In the wall of the stomach, the blood vessels are moderately hyperemic, the walls are thickened.

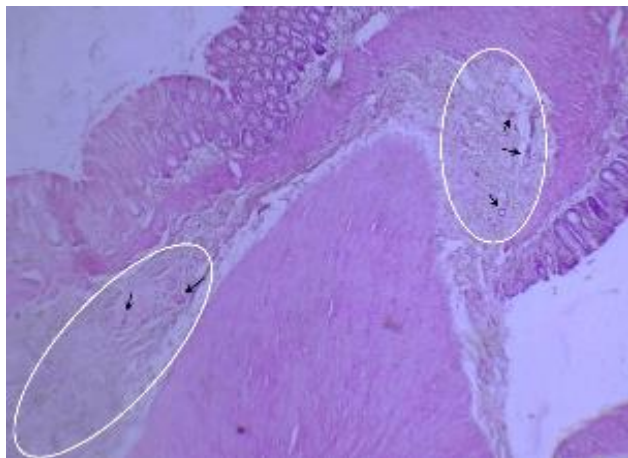


Figure 5. The blood vessels in the stomach wall are moderately hyperemic and the walls are thickened (indicated by arrows). Residual swelling of the submucosal layer, with gel residues (highlighted in white). Experimental group, 5th day. Hematoxylin and Eosin $\times 40$

In some places, there are mild dystrophic changes in muscle fibers. There are separate erythrocytes between the muscle fibers and under the mucous membrane (Fig. 3). The creeping of the regenerating epithelium onto the granulation tissue, edema, loosening of connective tissue, absence of neutrophil infiltration is determined in the submucosal layer (Fig. 4). In the gastric mucosa of the experimental group, on the 5th day, there was a positive dynamics of morphofunctional indicators characterizing a decrease in acute inflammation in the stomach wall (degree of vascular blood filling, desquamation index) against the background of persistent moderate polymorphocellular infiltration of the mucous membrane and submucosa. There are no hemodiscirculation phenomena in the stomach wall. There are almost no hemorrhages in the submucosal and subserous parts of the intestine and stomach, parenchymal and fuchsinophilic dystrophy and necrobiosis of smooth muscles do not appear. The mucous membrane with signs of moderate atrophy (Fig. 5).

7 days after mucosectomy in an experimental group of animals. Rats differ little from healthy individuals in their physical and physiological parameters. They actively take food and water, There are no deviations from the norm By the nature of the litter and urine excreted. After euthanasia, the abdominal cavity was opened. Abdominal organs without visible organic changes. The stomach is of the usual shape and consistency. There are no signs of a defect or necrosis of the stomach wall. When opening the lumen, the remains of food were evacuated. The mucous membrane is pink and clean, a small scar is determined in the area of mucosectomy without infiltration. There were no signs of inflammation or necrosis. The drug was taken for histological examination.

Morphologically, at these terms of the experiment,

complete gel resorption is noted, the number of polymorphic leukocytes and macrophages decreases in the field of inflammation. Epithelialization of previously damaged sections is observed in the mucous membrane. The epithelial layer is in a row with single goblet cells. A large number of mesothelial cells have a dystrophic condition and desquamation. Irregularities of the relief surface of the connective tissue base of the basement membrane are observed. A clear change in mesothelial cells, indicating the appearance of vacuoles, granules in their cytoplasm. A pronounced vascular reaction occurs in the microvascular network: stagnation of blood, increased permeability of capillary vessels, perivascular edema, migration of leukocytes and monocytes into surrounding tissue structures, loss of fibrin.

On the 14th day after the operation, the operated animals are active and move freely around the cage. After euthanasia and opening of the abdominal cavity, no pathological formations and signs of inflammation were revealed. There are remnants of food in the lumen of the stomach. Mucosa - a barely noticeable scar is determined at the site of the performed mucosectomy. There were no signs of inflammation or necrosis. The picture of the preparations of the experimental group on day 14 is represented by the processes of resolving inflammation with the formation of granulation tissue followed by its fibrous transformation, epithelialization and complete restoration of the histological structure of the mucous membrane (Fig. 6). There is an increase in the proportion of fibrillar structures in the connective tissue of the own plate of the mucous membrane, which indicates an increase in synthetic cell activity.

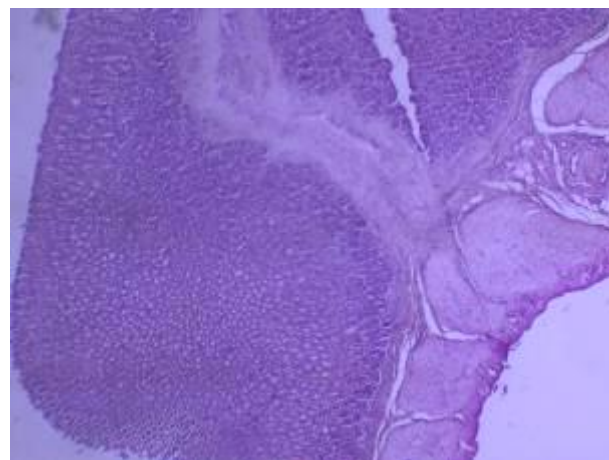


Figure 6. Experience. Gel injection zone, day 14. Restoration of the histological structure of the mucous membrane of the stomach wall. The mucous membrane and epithelial cells of the villi are without any pathological changes, there is no scarring. Hematoxylin and Eosin $\times 40$

The results of studies in the control group of animals. 1 day after the performed mucosectomy using a laser (similar to the experimental group), the animals of the control group were more in the corner of the cage, sedentary. They took water and food in limited quantities. The coat is dull. When trying to pick it up, they weakly resisted. The abdomen is moderately painful during palpation. Mucous membranes

with a tendency to dryness. After euthanasia, the abdominal cavity of 2 rats was opened. There is a small amount of clear, odorless liquid in the abdominal cavity. The stomach is in loose adhesions with the surrounding organs, which are separated in a blunt way. The stomach is reduced in volume, there are leftovers. In the area of the performed mucosectomy, a hematoma site and a change in the color of the serous cover are determined. After opening the lumen of the stomach at the site of mucosectomy, a mucosal defect of 8mm in diameter covered with fibrin with signs of inflammation is determined. The hair is fixed to the bottom of the defect. The serous cover is worn out with signs of impaired blood supply. There are no defects in the stomach wall.



Figure 7. Control. 3 days after excision of the gastric mucosa. There is a mucosal defect measuring 7 mm in diameter

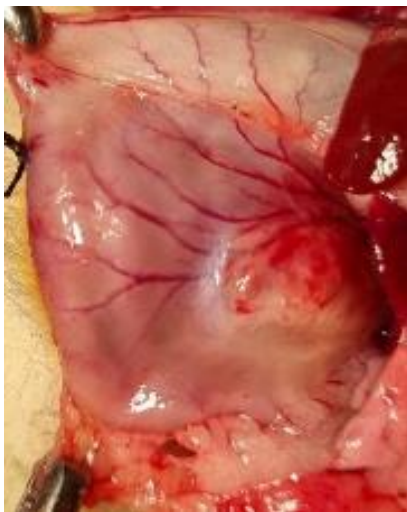


Figure 8. Control for 3 days after mucosectomy. Infiltration of the stomach wall with signs of inflammation

On 2-3 days after the operation, 1 rat of the control group was fatal. Upon opening the abdominal cavity, the presence of a cloudy, odorless liquid is determined. The loops of the intestine are swollen, the phenomena of paresis. The stomach is in loose adhesions with the surrounding organs. When examining the stomach, there is a defect of serous covering with leakage of stomach contents. In another case, the defect is covered with an omentum with the formation of an abscess,

which subsequently broke into the abdominal cavity. In other rats of the control group, the condition was assessed as satisfactory during these study periods. They moved around the cage, took food and water. No effusion was found on opening the abdominal cavity. The stomach was in adhesions with the surrounding organs. After the adhesions were separated, the infiltrate at the site of the performed mucosectomy was determined. Upon opening the lumen of the stomach at the site of mucosectomy, a mucosal defect resembling a chronic ulcer covered with fibrin up to 7 mm in diameter was determined. The edges are edematous, with signs of inflammation (Fig. 7-8).

Histomorphological analysis of preparations of the gastric mucosa of rats of the control group (3 days) of the experiment showed signs of inflammatory and degenerative processes, the dynamics of which had their own characteristics. Morphologically, both arterial and venous blood vessels in the gastric mucosa are sharply dilated, hyperemic, and in a state of stasis. There is a widespread hemorrhage between the muscle fibers and under the mucous membrane. Muscle fibers in a state of parenchymal dystrophy and necrobiosis, edema, inflammatory infiltration of the submucosa (Fig. 9). On the 5th day of the experiment, a slightly different morphological picture was observed in the gastric mucosa of the control group. Destructive foci are more clearly expressed, hemodynamic, alternatively necrotic and edematous-inflammatory changes. The number of inflammatory cells in its own connective tissue base increases. During this period of experience, perivascular accumulation of mast cells is associated with hemodynamic disorders, which may be involved in the implementation of dyscirculatory changes, diffuse lymphocytic infiltration and focal necrosis were determined. The mucous membrane of the stomach, significantly swollen due to edematous phenomena of the interstitial tissue, dystrophy of glandular cells. On the surface of the mucous membrane, there is an expansion and deepening of the gastric pits, an elongation of the villi (Fig. 10).

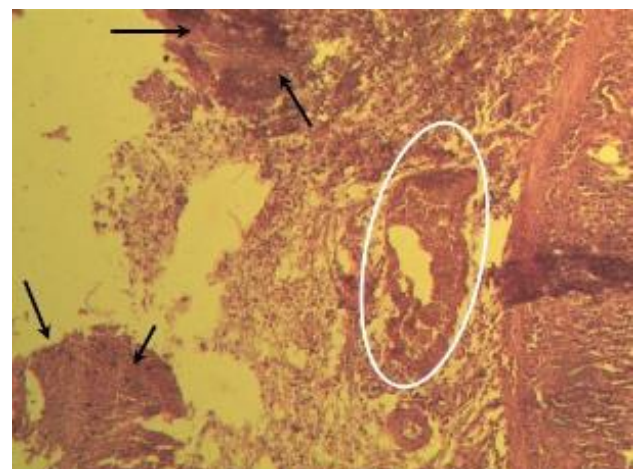


Figure 9. Hemorrhage between muscle fibers and under the mucous membrane (indicated by arrows), stasis in large vessels (highlighted in white), edema. Micrography of the stomach wall in control, 3 days. Hematoxylin and Eosin × 40

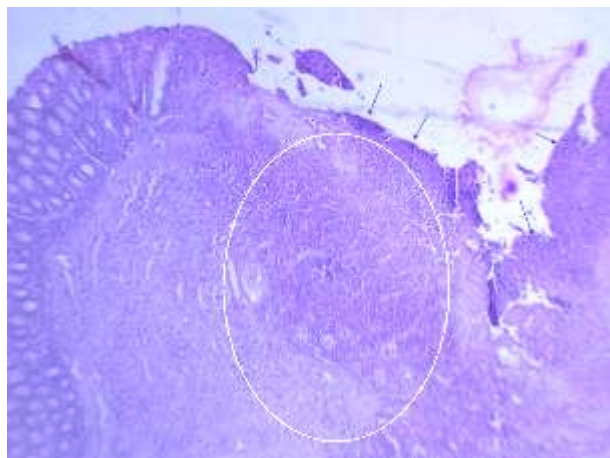


Figure 10. Erosive and ulcerative lesions (highlighted with arrows), diffuse lymphocytic infiltration and focal necrosis (highlighted in white), swelling of the submucosal layer. Gastric mucosa, after excision of the polyp with a coagulator, control. Hematoxylin and Eosin $\times 40$

7 days after the operation. The animals were actively moving around the cage. They took water and food in limited quantities. They resisted moderately when trying to get a grip. The abdomen is moderately painful. When opening the abdominal cavity after euthanasia, no pathological fluid was detected. The stomach in the area of the performed mucosectomy is in a junction with the surrounding organs. There is food in the lumen of the stomach. Upon opening the lumen of the stomach, a mucosal defect of 2-3 mm in diameter is determined. The bottom is covered with fibrin. The mucous membrane is moderately edematous. There were no signs of perforation and necrosis of the serous wall. During histological examination of the drugs of the control group, on the 7th day of the experiment, it was found that lymphoplasmocytic infiltration was noted in the gastric mucosa to varying degrees, lymphoid follicles formed, dilation of blood vessels with the phenomenon of hyperemia and stasis, i.e. stagnation persists in them. The glands shorten (atrophy), their density decreases. Reduction of specialized gland cells, their replacement with mucoid ones.

14 days after mucosectomy in the control group of animals. Rats differ little from healthy individuals in their physical and physiological parameters. They actively take food and water, There are no deviations from the norm. By the nature of the litter and urine excreted. After euthanasia, the abdominal cavity was opened. Abdominal organs without visible organic changes. The stomach is of the usual shape and consistency. There are no signs of defect or necrosis. When opening the lumen, the remains of food were evacuated. The mucous membrane is pink and clean, a small scar is determined in the area of mucosectomy without infiltration (Fig. 11). There were no signs of inflammation or necrosis. Histologically, during these periods, the damage zone was replaced by a layer of fibroblasts, plasma and mast cells. Accumulation of leukocytes and macrophages was noted in the submucosal base. The predominance of polymorphonuclear leukocytes, macrophages, purulent bodies and lymphocytes, the presence of plasma cells, and single fibroblasts is observed.

There is an uneven distribution of cellular composition, localization of infiltration into the area of multiple thin-walled vessels (Fig. 12).



Figure 11. Control. 14 days after mucosectomy. Mucosal healing. The seams of the gastrotomy incision are visible

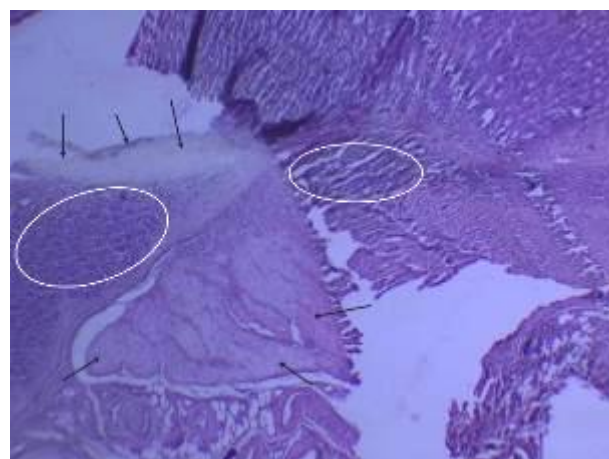


Figure 12. Control. 14th day. Fibroblasts in significant numbers, intensive process of synthesis of interstitial tissue. Only occasionally there are isolated fragments of tissue, purulent corpuscles and a decrease in interstitial edema. Hematoxylin and Eosin $\times 40$

4. Conclusions

The results of the conducted studies allowed us to establish that the introduction of 4-5% Hemoben gel in 0.1% methylene blue solution is optimal for the formation of a pillow with subsequent resection of the gastric mucosa. The injected gel takes a distinct shape, does not blur and allows you to clearly delineate the limits of performing surgical manipulation. Excision of the gastric mucosa using laser radiation in the range of 1470 nm, with a power of 7 W and focusing of radiation allows precision resection of the mucosa without bleeding from the vessels of the submucosal layer. The gel pad prevents the penetration of laser radiation beyond the submucosal layer. The healing of the defect sites occurs within 7 days without pronounced inflammation and postoperative complications. In the control group of animals, where a 0.1% solution of MB was used, the pillow did not

allow to clearly delineate the area of surgical intervention. It quickly resolved and did not give a noticeable hemostatic effect. When exposed to laser, the water cushion did not make it possible to completely protect against the penetration of laser radiation into the deep layers of the stomach wall, which led to complications in the form of necrosis of the serous wall and postoperative purulent-septic complications.

Thus, the conducted experimental studies have shown the high efficiency of the proposed method of excision of the gastric mucosa, which can later be used in clinical practice for endoscopic removal of polypoid neoplasms of the gastrointestinal mucosa.

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