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THE PROTECTIVE ROLE OF PEDICLED OMENTAL GRAFT ON UNSAFE SMALL INTESTINAL ANASTOMOSIS: AN EXPERIMENTAL STUDY WITH HISTOLOGICAL EVALUATION IN DOGS

By
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Background: *The pedicled omental graft has wide uses in surgery. Some authors have described its clinical use for protection of gastrointestinal anastomoses, particularly after rectal and oesophageal anastomoses. It was found that the rate of anastomotic leakage after small bowel anastomoses in generalized peritonitis is high. After resection of a necrotic intestinal segment, there will be a low blood flow at the anastomosis due to collateral circulation and the healing may therefore be impaired. In these conditions the anastomosis of the bowel is unsafe and a defective anastomosis usually results.*
Aim: *This work is an experimental study on dogs to test the ability of the pedicled omental graft to protect the mechanically impaired (interrupted) small intestinal anastomosis with partially ischaemic edges i.e. unsafe. The possibility for induction of anastomotic healing was also investigated.*
Material and Methods: *Fifteen male dogs were randomly divided into three groups. Group (I) composed of three dogs were used as control (sham operated control group); group (II) composed of 3 dogs with mechanically impaired unsafe anastomosis without omental wrap, group (III) composed of 9 dogs with mechanically impaired unsafe anastomosis covered with omental wrap. The animals were housed in Medical Research Center, Faculty of Medicine, Ain Shams University. All animals of group (I) and only 7 dogs of group (III) were sacrificed after 6 weeks and the anastomoses together with the omental wrappings were histologically examined. One of the remaining two dogs of group (III) was sacrificed after 3 days and the other dog was scarified after 7 days to examine the gross appearance of the sites of the anastomoses to determine the progress of healing..*
Results: *All the animals of group (II) died within 2-5 days after the operation. Autopsy revealed frank peritonitis with intestinal leakage. All the experimental animals of group (I) and (III) survived till the end of the experiment. All the anastomoses of group III dogs appeared to be in good condition by naked eye and no leakage was observed. The blood supply of the omentum in all animals was good. The most significant histological results were the restoration of the mucosal continuity and the increase collagen content at the gaps in-between the sutures of all anatomoses of animals of group (III). Heavy mononuclear cellular infiltration was also observed in- between the muscle fibers of musculris externa as well as in the omental wrap of the anastomoses.*
Conclusion: *Covering of an impaired unsafe intestinal anastomosis with a pedicled omental graft can secure the anastomosis and give it some strength. Subsequently the incidence of leakage and peritonitis is reduced thereby, ensuring the survival of the experimental animals.*

Key words ***:Pedicled omental graft, Unsafe small intestinal anatomosis, Omental wrap.***

INTRODUCTION

The pedicled omental graft has wide uses in surgery. Since Bennett first described its use in 1896 to plug a perforated gastric ulcer[1], it has been described for clinical use in the repair of vesico-vaginal fistulae[2], liver injuries[3], body wall defects[4], urinary bladder reconstruction[5], and the protection of vascular grafts[6] and great vessels[7].

Some authors have described its clinical use for protection of gastrointestinal anastomoses, particularly after rectal[8] and oesophageal anastomoses[9,10]. However,

others claimed that omental pedicled graft does not provide any real advantages to protect anastomoses after intestinal resection[11].

Omentum can be used both as a free graft and as a vascularised omental flap to protect intestinal anastomoses. Pettet and his colleagues discovered that free omental transplants protect against leakage from end-to-end anastomoses of the large intestine. They believed that free omental transplant within a few hours could adhere firmly to the intestinal surface on which they had been placed[12]. However, intraperitoneal use of protective free omental grafts is extremely limited, due to the risk of development of adhesions and possibility of intestinal obstruction[13]. Goldsmith and his coworkers have demonstrated the advantages of pedicled omental grafts and their beneficial action on intestinal blood supply. They found that, pedicled segments of the omentum deceased the likelihood of perforation of intestinal loops whose vascular supply had been interrupted from the mesentery[14].

It was found that the rate of anastomotic leakage after small bowel anastomoses in generalized peritonitis is high. After resection of a necrotic intestinal segment, there will be a low blood flow at the anastomosis due to collateral circulation and the healing may therefore be impaired. In these conditions the anastomosis of the bowel is unsafe and a defective anastomosis usually results. Leakage of a bowel anastomosis remains the most serious postoperative complication in gastrointestinal surgery [15,16,17].

This work is an experimental study on dogs to evaluate the ability of the pedicled omental graft to protect the mechanically impaired small intestinal anastomosis with partially ischaemic edges i.e. unsafe anastomosis. The possibility for induction of anastomotic healing was also investigated.

PATIENTS AND METHODS

Fifteen male dogs were randomly divided into three groups. Group (I) composed of three dogs were used as control (sham operated control group); group (II) composed of 3 dogs with mechanically impaired and unsafe intestinal anastomosis without omental wrap, group (III) composed of 9 dogs with mechanically impaired and unsafe anastomosis covered with omental wrap. The animals were housed in Medical Research Center, Faculty of Medicine, Ain Shams University.

Surgical procedure:

The animals were anesthetized with intravenous thiopental (15 mg/Kg of body weight), intubated and connected to a Manley respirator. Povidone-iodine and 70% ethyl alchohol were used to clean and disinfect the skin. A midsupra-umbilical incision was made under strict aseptic conditions. Animals of group (I) were subjected to sham operation, where abdominal incision was performed with subsequent manipulation of the jejunal loops. This was followed by careful closure of the abdomen.

In group (II) and (III) a small intestinal (jejunum) loop was selected and was completely severed transversely and intestinal continuity was restored in all cases by means of an end-to-end anastomosis in single layer and by 5-7 individual spaced 000 vicryle sutures (mechanically-impaired anastomosis) according to the circumference of the intestinal loop. The edges of the anastomosis were made partially ischaemic i.e. unsafe; by ligation of the mesenteric vessels 2 cm on either side of the anastomosis but leaving the marginal vessels intact (Fig. 1). In group (III) the anastomosis was wrapped with pedicled (vascularised) omentum for a distance 6-8 cm. This band passed through the gap developed between the two sides of the mesentery surrounded the anastomosis at its entire periphery and covered the intestinal anastomosis carefully. After wrapping, the graft was immobilized by sutures on the remaining omentum (Figs. 2,3). Hemostasis was achieved with electro-cautary as the vessels were clearly identified. The procedure was completed by closure of the abdominal wall in layers without drainage after careful peritoneal lavage.

In group (II) all dogs died within 2-5 days, while, all the experimental animals of group (I) and (III) survived. All animals of group (I) and only 7 dogs of group (III) were sacrificed after 6 weeks where the sites of the jejunal anastomoses and the attached omentum were excised (Fig.4) and fixed in 10% formlin to be subjected to histological study.

One of the remaining two dogs of group (III), was sacrificed after 3 days and the other one was sacrificed after 7 days to examine the gross appearance of the sites of the anastomoses to determine the progress of healing.

Histological study (tissue preparation and techniques)

The dissected jejunal loops (of the dogs of group I and group III) were immediately fixed in 10% formalin, then paraffin sections (4-6 micrometer thick) were prepared. They were stained with H&E., PAS reaction[18], Prichard 's technique for mitochondria[19] ,and Mallory triple stain for collagen [20].

Morphometric study

The height of randomly chosen 50 villi and the depth of randomly chosen 50 crypts were measured in each animal of group (I) and in 7 animals of group (III). Moreover, the mean number of goblet cells per field (x 20) was also calculated. These were performed by the use of computerized image analyzer Leica Q 500 in Histology Department, Faculty of Medicine, Ain Shams University.

All the data where statistically analyzed by the use of student's t-test.

RESULTS

All the animals of group (II) died within 2-5 days after the operation. Autopsy revealed frank peritonitis with intestinal leakage.

All the experimental animals of group (III) survived and 7 dogs were sacrificed after 6 weeks.

1- Gross (naked eye) appearance:

By naked eye all the anastomoses appeared to be in good condition and no leakage was observed. The blood supply of the omentum in all animals was good. The condition of the intestinal wall appeared more or less healthy.

The remaining two dogs of group (III) were sacrificed after three and seven days respectively to examine the site of the anastomosis and to determine the progression of healing . The omental wrap was found to form an effective bridge over anastomotic defects in the first 72 hours, which will provide the bulk of the granulation tissue in their subsequent healing.

2- Histological Results :

Group I: Control group (sham operated group)

By light microscopic examination, the dog jejunum was found to be consisted of four concentric layers: mucosa, submucosa, muscularis externa and serosa. The mucosa was formed of villi, crypts, lamina propria and muscularis mucosa (Fig.5). The surface of the villus, was covered by tall columnar cells with basal oval nuclei (Fig.6), and apical PAS positive brush borders (Fig.7). The apical part of the columnar cell showed a rich mitochondrial content (Fig.8). Goblet cells were observed scattered in between these columnar cells (Fig.6), with strong PAS positive reaction (Fig.7). The lamina propria represented a loose connective tissue filling the core of the villi and extending in-between the crypts. The crypts of Lieberkühn appeared as simple tubular glands opening at the base of the villi. They were lined by simple columnar cells as well as goblet cells. Numerous mitotic figures could be observed (Fig.9). The muscularis mucosa consisted of inner circular and outer longitudinal smooth muscle fibers (Fig.5). The submucosa was formed of loose connective tissue. The muscularis externa was arranged as inner circular and outer longitudinal smooth muscle fibers (Fig.10). Serosa was seen covering the jejunum from outside and it was formed of loose connective tissue covered by single layer of mesothelial cell (Fig.10). Few collagen fibers were seen scattered through all the layers of the jejunum (Figs.11,12).

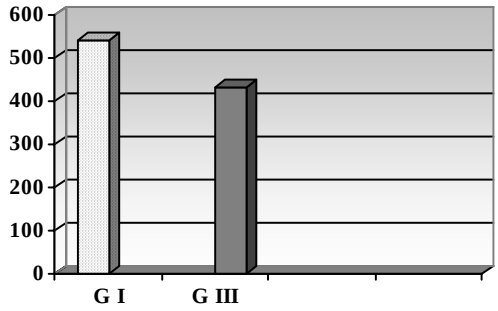
Group (III): Experimental group

Significant reduction in the height of the villi and the depth of the crypts was detected as compared to the control (Figs.13&14), (Table 1 and Histogram 1 & 2). The enterocytes were more or less comparable to that of the control except for the focal decrease in the intensity of apical PAS positive brush borders (Fig.15), with relative decease in the mitochondrial content in some of them (Fig.16). The number of the goblet cells per high power field was significantly less than that of the control (Fig.15), (Table 1 and Histogram 3). The muscularis externa appeared disorganized and interrupted with heavy mononuclear cellular infiltration in-between the muscle fibers (Figs.17&18). The collagen fibers of the submucosa and in-between the muscle fibers of muscularis externa were increased. The gaps in between the muscle fibers were filled with collagen fibers (Figs.19&20). The majority of the outer circumference of the jejunum at the site of the anastomosis was covered by highly vascular fatty omentum with outer methothelial cells covering (Figs. 13&17). At some areas the omentum revealed heavy mononuclear cellular infiltration (Fig.18). The collagen fibers appeared condensed at the omental covering (Fig.20).

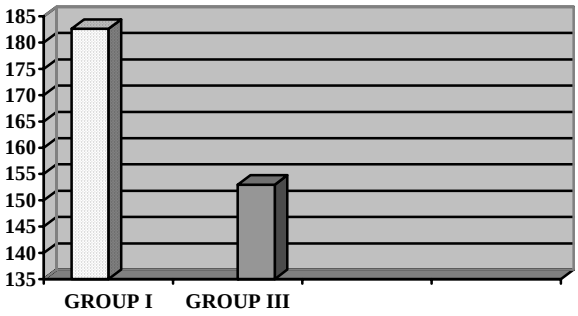
Table (1): ***Comparison between groups III & I as regards height of villi, depth of crypts and number of goblet cells***

	GROUP I	***GROUP III***
Mean height of the villi ±SD	537.29±38.61	431.42±52.26
t		3.55
p		<0.05*
Mean depth of the crypts ±SD	182.60±12.61	152.96±16.40
t		3.1
p		<0.05*
Mean number of the goblet cells per field ± SD	63.1±1.9	42.1±1.6
t		16.8
p		<0.001**

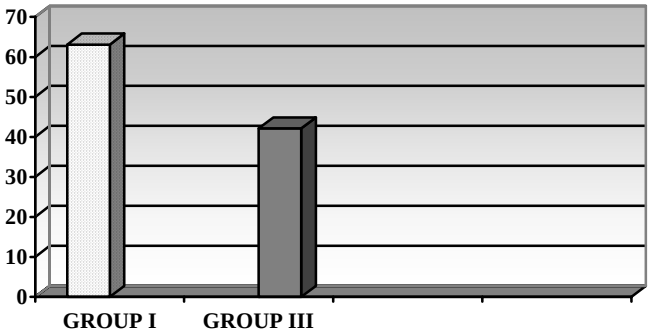
SD= Standard deviation T= Student t-test P= Probability
** Significant ** Highly significant*



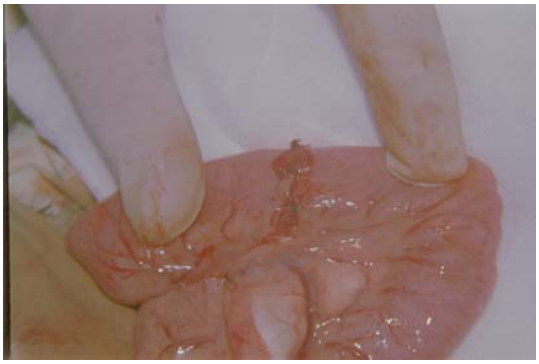
Histogram (1) : showing the mean height of the villi.



Histogram (2): showing the mean depth of the crypts.



Histogram (3) : showing the mean numbers of the goblet cells per field.



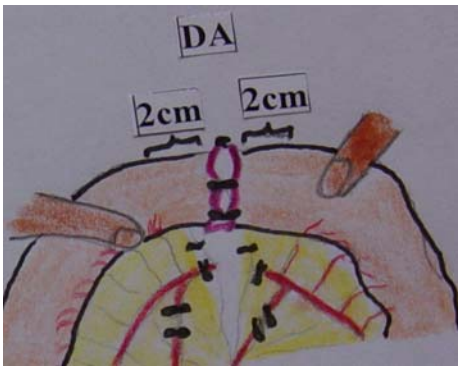
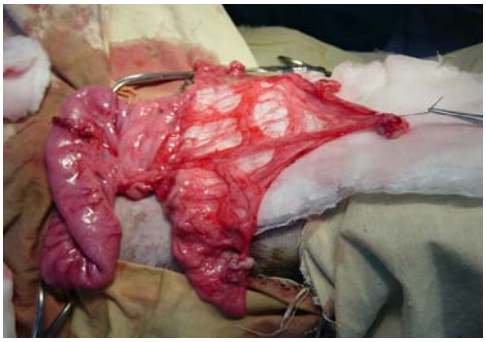


Fig (1): Spaced mechanically impaired anastomosis of the small intestine, with partial ischemia for 2 cm on either side.



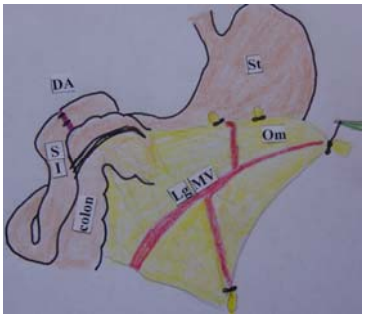
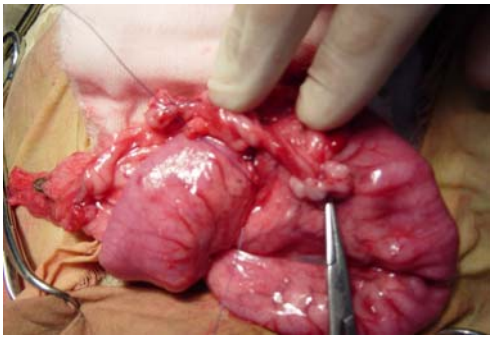
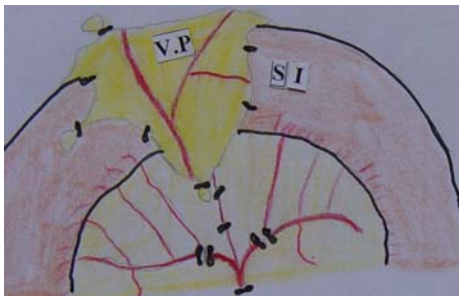


Fig (2): DA= defective anastomosis St= stomach SI= small intestine O= omentum Lg MV= large feeding vessel VP= vascularised omental pedicle





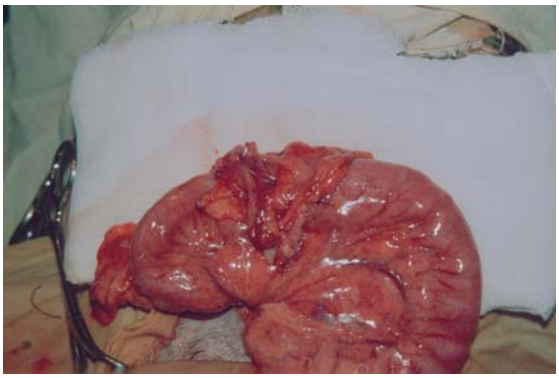


Fig (3): Complete covering of the unsafe anastomosis by the omental graft, which was immobilized by sutures.

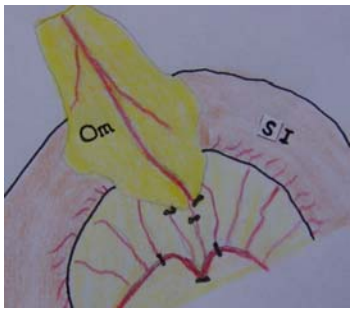


Fig (4): Part of the intestine excised after 6 weeks with the fixed omental graft.

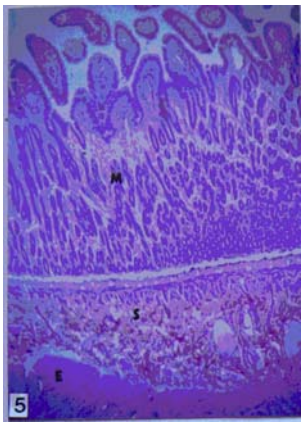


Fig. (5): Photomicrograph of section of jejunum of a dog showing that the wall is formed of mucosa (M), submucosa (S) and muscularis externa (E). (Group I, H&Ex 50).

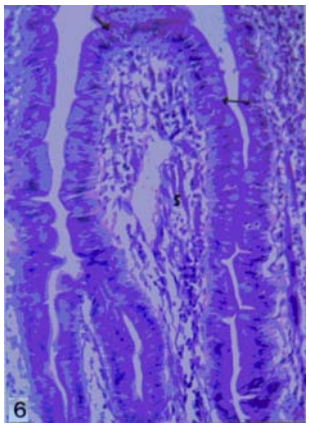


Fig. (6): A higher magnification showing the tall columnar cells (enterocytes) covering the villi with interspersed goblet cells (↑). The core of the villus contains smooth muscle fibers (S). (Group I, H&E x 250).

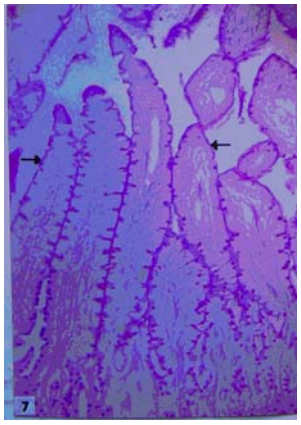


Fig . (7): Photomicrograph showing strong PAS positive reaction of the brush border of the enterocytes (↑) and the goblet cells. (Group I, PAS x 100).

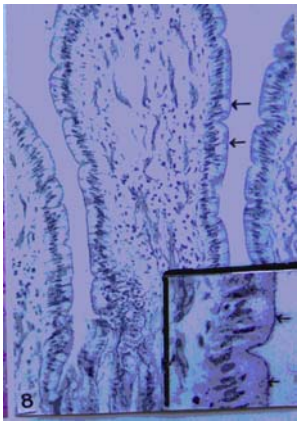


Fig. (8): Photomicrograph showing rich mitochondrial content at the apical part of the enterocytes. (Group I, Prichard technique x 250, inset x 640).

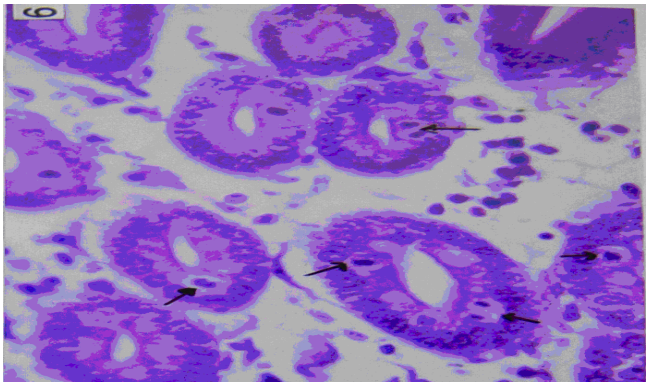


Fig . (9): Photomicrograph showing numerous crypts with many mitotic figures. (Group I, H&E x 250).

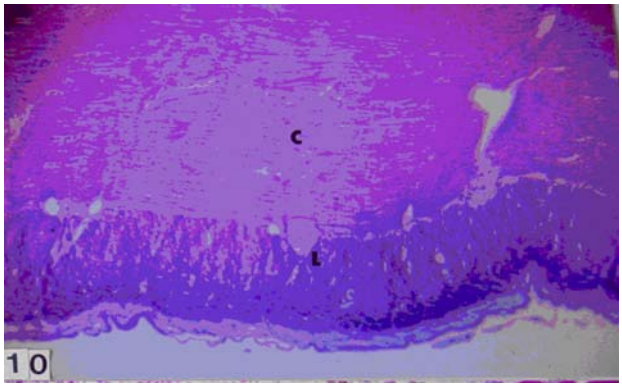


Fig . (10): Photomicrograph showing inner circular (C) and outer longitudinal (L) layer of muscularis externa that is invested by loose connective tissue covered by flattened methothelial cells. (Group I, H&E x 250).

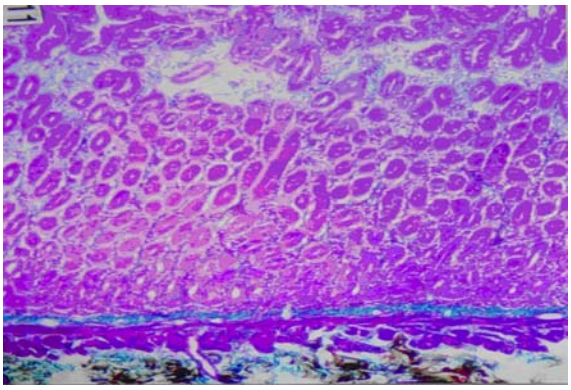


Fig. (11): Photomicrograph showing the normal distribution of the collagen fibers in the mucosa and submucosa. (Group I, Mallory triple stain x 100).

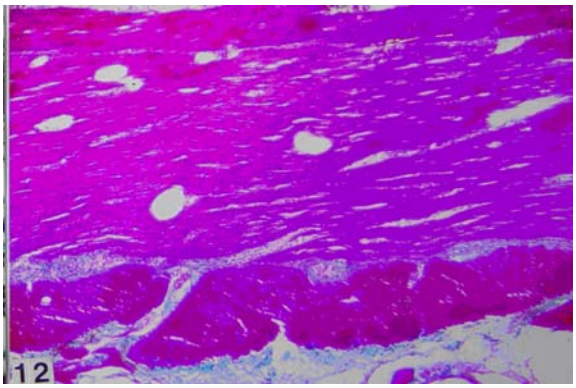


Fig (12): Photomicrograph showing the normal distribution of the collagen fibers in the muscularis externa. (Group I, Mallory triple stain x 100).

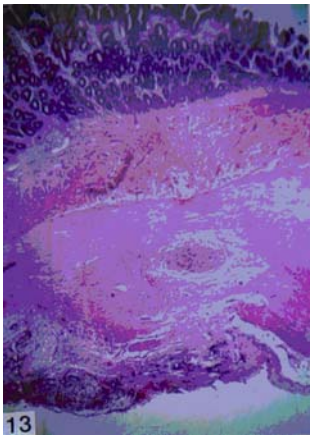


Fig. (13): Photomicrograph showing the different layers of the jejunum. The muscularis externa is interrupted and surrounded with fatty omentum. (Group III, H&E x 50).

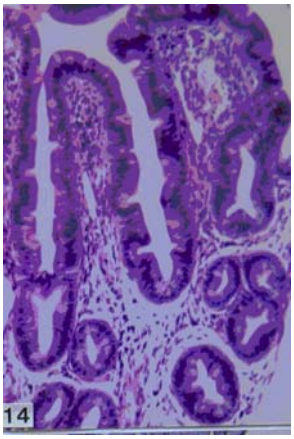


Fig. (14): A higher magnification showing normal structure of the villi that is comparable to that of the control. Notice apparent shortening of the villi and the crypts. (Group III, H&E x 250).

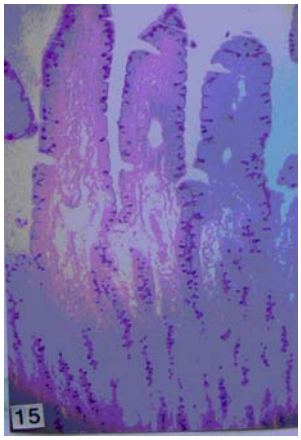


Fig. (15): Photomicrograph showing focal reduction in the intensity of the PAS positive brush borders with apparent decrease in the number of goblet cells. (Group III, PAS x100).

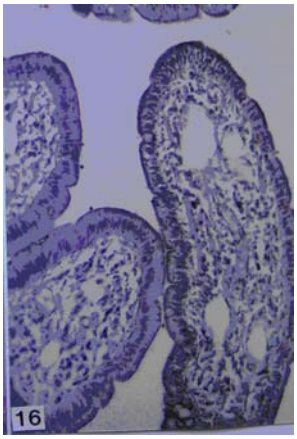


Fig.(16): Photomicrograph showing decease in mitochondrial content of some enterocytes (↑) as compared to control. (Group III, Prichard s technique x 250).

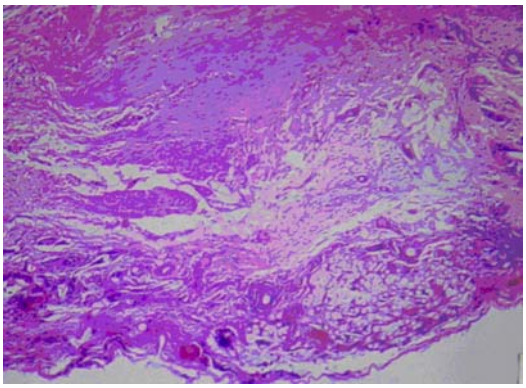


Fig. (17): Photomicrograph showing disorganized muscularis externna with mononuclear cell infeltration. The muscle is covered with highly vascular fatty omentum. (Group III, H&E X 100).

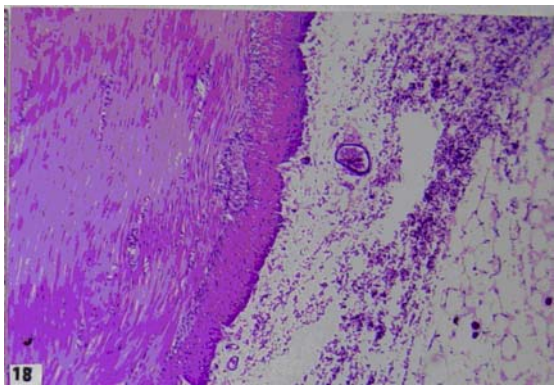


Fig. (18): Photomicrograph showing mononuclear cellular infeltration in between the muscle fibers and in the adjacent omentum. (Group III, H&E x 100).

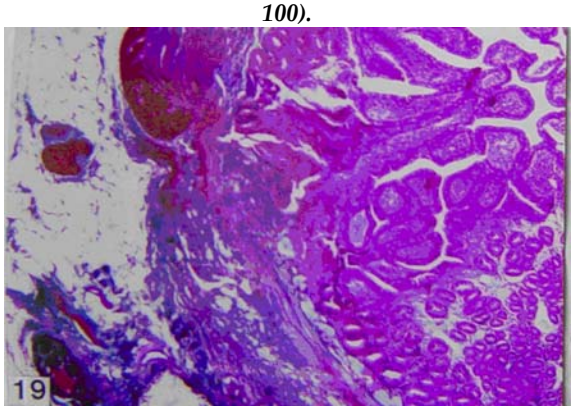


Fig. (19): Photomicrograph showing increased collagen fibers content in the submucosa, in between the muscle fibers and in the adjacent omentum. (Group III, Mallory triple stain x 75).

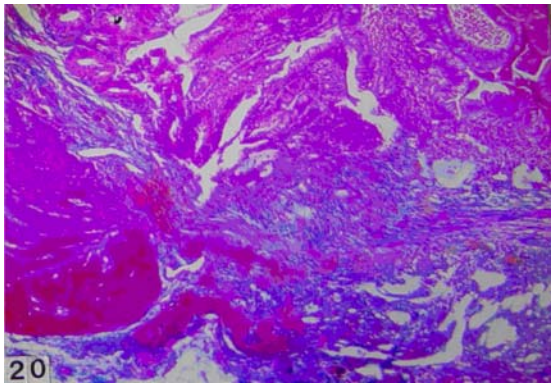


Fig. (20): A higher magnification showing condensed collagen fibers: in the submucosa, filling the gaps between the muscle fibers and in the surrounding omentum. (Group III, Mallory triple stain x 100).

DISCUSSION

The greater omentum has been found to be an ideal structure for a protective role. It has been shown to absorb fluid and particulate matter and transport them to tissue macrophages and immunocompetent cells. It has been found to readily envelope-injured tissues and to control hemorrhage. It appears to be intrinsically better than adhesions from other organs for assisting injured or ischemic tissues[13].

The safety of small intestinal anastomosis depends upon the good blood supply and watertight approximation of the edges. Using a lethal (mechanically interrupted) anastomotic technique, the omental pedicle wrap has provided a dramatic improvement in experimental animals survival by maintaining intestinal continuity and integrity over a distance between two separated ends of bowel[1].

The biologic viability of the omental wrap appears to be critical for its function. Devascularised omentum (free omental graft) has been previously studied for its potential to protect anastomosis. It has been found that; the free omental graft is not of benefit and actually leads to a worse anastomotic outcome than no wrapping at all. This is because it draws the blood supply away from the underlying bowel and causes infection secondary to necrosis of the pedicle graft in addition to late adhesive intestinal obstruction secondary to extensive adhesions[8].

The value of a viable omental wrap has clinical implications for those gastrointestinal anastomoses with a

known propensity to leak, particularly extraperitoneal esophageal and low rectal anastomosis. In both these settings successful clinical experience with an omental wrap has been reported[1,9,10].

In the current study, protection of the small bowel anastomosis was achieved by the use of an intact vascularized omentum, thus maintaining all of its mechanical and biologic properties. All dogs of group (III) whose anastomoses were covered by omental graft survived while all dogs of group (II) with uncovered anastomoses died. Wrapping of an unsafe intestinal anastomosis with a pedicled omental graft secured the anastomosis and ensured survival of the experimental animals.

When two dogs of group (III) were sacrificed after three and seven days respectively, the omental wrap appeared forming an effective bridge over the anastomotic defects in the first 72 hours to provide the bulk of the granulation tissue in their subsequent healing. This could be due to development of an anastomosis between the omental and bowel wall vessels as early as the third postoperative day to aid in anastomotic healing[1].

In the present work, restoration of the mucosal continuity was observed all around the anastomotic sites. This might be due to the fact that, the intestinal mucosa has the fastest rate of turnover, of any tissue of the body[21]. However, there was a significant reduction in the height of the villi and the depth of the crypts at the anastomotic sites of group III animals. This might be accompanied by malabsorption due to the decrease in the surface area of such portion[22].

The function of the goblet cells is the production of acid mucus, which lubricates the intestinal epithelium[23] and prevents adherence and invasion of microorganism to the mucosa[24]. Thereby, the significant reduction in the number of goblet cells per field observed in group III animals of the present study, might lead to increased susceptibility to infective agent with subsequent mononuclear cellular infiltration. The PAS positive brush borders of the enterocytes is due to the thick mucous and cell coat which protect against auto digestion of the enterocytes[21]. In the current study, focal loss of the PAS positive brush borders of some enterocytes covering the villi of jejunum of group III animals could be observed. This might be accompanied by focal increased susceptibility of the epithelium to auto digestion, resulting in mucosal abrasions with increased tendency for invasion by micro-organisms at the anastomotic site[22].

As the rich mitochondrial content of the enterocytes is involved to provide energy, needed for absorption[25], hence the mild depletion of mitochondrial content of the enterocytes of group III might lead to malabsorption. In group III animals, the collagen fibers in the submuosa, in-between the muscle fibers of muscularis externa and in the omental covering were notably increased as compared to the control. This actually protected against the inventible leak from the interrupted suture. In the present work, the majority of the circumference of the jejunum of group III animals; was covered by an outer layer of mesothelial cells at the anastomotic sites. This mesothelial covering is essential to guard against the postoperative peri-anastomotic adhesive disorders of the gut[21].

Despite the presence of some microscopic structural difference at the anastomotic sites of group III dogs, as compared to the control, however, the restoration of the mucosal continuity and the increased collagen content at the gaps in-between the sutures would protect against the inventible leakage and peritonitis. This demonstrated the omental wrap protective ability.

The omentum has a plastic property, that makes it capable of lowering the rate of anastomotic failures[8]. The fact that all animals of group III with a wrapped compromised anastomosis actually had an untroubled convalescence, with an intact anastomoses after six weeks demonstrated the wrap's protective ability.

In the study done by Tocchi and his colleagues,[8]on the protective role of the pedicled omentum graft in protection of the colorectal anastomosis, they found that, when the colorectal anastomosis was performed with intact omental pedicle, it provided a circumferential soft-tissue contact to the anastomosis that would plug early anastomotic leakages during the dangerous period before the occurrence of revascularization. Omental pedicle also provided a source of granulation tissue and a neovasculature for dehiscence repair, which would possibly contribute to lowering the rate of subsequent anastomotic strictures. However, the omental wrap served as a stimulus to induce new vessels formation[26].

In conclusion, covering of an unsafe intestinal anastomosis with a pedicled omental wrap can secure the anastomosis and give it some strength and subsequently reduce the incidence of leakage and ensure the survival of the experimental animals. Hence, viable pedicled omental wrap is highly recommended for gastrointestinal anastomosis with a known tendency to leak.

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