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## THE DELAYED HISTOLOGICAL CHANGES OF SEGMENTAL ISCHEMIA AND ISCHEMIA - REPERFUSION ON THE JEJUNAL VILLI OF RABBITS

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#### INTRODUCTION

Acute intestinal ischemia represents a broad spectrum of diseases with various clinical and pathological manifestations. The primary causes of insufficient blood flow to the intestine are diverse and include thromboembolism, neoplasms, vasculitis, abdominal inflammatory conditions, trauma, chemotherapy, radiation and corrosive injury (**Rha et al., 2000**). Despite of the advances in the pathophysiological, laboratory diagnosis and imaging techniques acute intestinal ischemia is still one of the major etiological causes of multiple organ failure (**Schlichting et al., 1995**). Ischemia induces continuous distrubance of hepatic microcirculation leading to liver dysfunction (**Nakamura et al., 2001**). Furthermore, it triggers the development of lung dysfunction (**Ito et al., 2003**). Ischemia is often associated with mortality rates more than 60% and thus it represents a major challenging clinical problem (**Lock, 2001**).

Reviewing the literature re reled that the intestinal mucosa in particular is one of the most sensitive tissues to ischemia and ischemia reperfusion injury in the human body (**Ikeda et al., 1998; Kalia et al., 2001**). Although the histopathological alterations in the intestinal mucosa after ischemic injury have been extensively described (**Puglisi et al., 1995; Takeyoshi et al., 1996; Ikeda et al., 1998**) yet most of the previous models of ischemia were achieved by complete occlusion of the superior mesenteric artery which often resulted in shock and early death of the experimental animals, hence rendering it rather difficult to examine the delayed morphological changes in the ischemic intestinal mucosa. Moreover, the intestinal segments distal to ischemia though clinically showed decreased contraction frequency, decreased relaxation responses and altered responses to norepinephrine (**Malone and Kanaan**  **2001)** yet the morphological changes in locations distal to ischemia were not properly investigated.

Recently, the role of modulators particularly nitric oxide has received special attention. It is hypothesized that nitric oxide might be involved in tissue protection and provide partial preservation of intestinal mucosa as the first line of defense following ischemia reperfusion (Kalia et al., 2001). Detection of nitric oxide could be achieved via the estimation of endothelial nitric oxide synthase (NOS) which is a member of a family of enzymes responsible for synthesis of nitric oxide from L arginine and molecular oxygen (Chan et al., 2002).

Accordingly, it was the aim of the current work to illustrate the delayed morphological and morphometric changes following segmental ischemia and ischemia reperfusion produced by selective occlusion of terminal branches of superior mesenteric artery. Moreover an attempt of detection of nitric oxide production was achieved via the estimation of nitric oxide synthase. The changes in both the ischemic segment as well as the segment distal to it were investigated. Such a study might be beneficial in post-ischemic resection anastomosis procedures, or in surgical revascularization.

## MATERIAL AND METHODS

Eighteen rabbits weighing 2000 - 2500 grams were obtained from the Research Unit and Bilharzial Research Center of Faculty of Medicine, Ain Shamus University. All animals were fasted 18 hours before commencing the experiment but had free access to water. At the beginning of the experiment rabbits were divided into two groups, nine rabbits each. Animals were anaesthesized by intravenous injection of sodium pentobarbitol (40 mg / kg). All surgical procedures were performed with the animals breathing spontaneously and under complete aseptic conditions. (Fig. 1 - a, b, c, d).

## **Group I : Ischemia Reperfusion**

Through a midline abdominal incision a single jejunal loop (10 cm) was delivered, all the terminal feeding branches of the superior mesenteric artery and their collaterals supplying the selected segment were occluded for 60 minutes with atraumatic vascular clamps, vessel occlusion was confirmed by loss of pulsations. The jejunal segment was returned to the peritoneal cavity and periodically irrigated with saline. At the end of the ischemic period the clamps were removed to allow reperfusion, the abdominal cavity was closed using monofilament polypropylene and a subcutical 2 / 0 vicryl. Three rabbits underwent the same procedures apart from occlusion of the terminal branches of superior mesenteric artery (Sham operated).

### Group II : Continuous Ischemia

Through a midline incision a single jejunal loop (10 cm length) was delivered and the terminal branches of the superior mesenteric artery and their collaterals supplying the selected segment were ligated in continuity with 4 / 0 polygalactin, vessel occlusion was confirmed by loss of pulsations. The jejunal segment was returned to the peritoneal cavity and irrigated with saline. The abdominal cavity was closed using monofilament polypropylene and a subcutical 2 / 0 vicryl. Three rabbits underwent the same procedure apart from ligation of the terminal branches of the superior mesenteric artery (Sham operated).

## **Tissue Preparation :**

On recovery from anesthesia, rabbits were allowed free access to water and soft diet to avoid abdominal over distention. Rabbits were sacrificed after 24 hours, the abdominal cavity was incised and the ischemic jejunal segment and the segment distal to it were resected. Specimens were immediately fixed in 10% formol saline and embedded in paraffin wax, Serial paraffin sections were cut at 5  $\mu$ m in thickness and stained with haematoxylin and eosin and Wilder Sliver stain for reticular fibers. Other specimens 1 mm3 in thickness were fixed in 2.5% glutraldehyde in phosphate buffer and proceeded for scanning electron microscopic studies. The specimens were examined and photographed with Philips scanning electron microscope (XL 30).

## **Image Morphometry :**

The villus height, thickness as well as, the height of the columnar epithelial cells (enterocytes) were measured using Leica Qwin 500 Image Analyzer. Morphometric analysis was carried out on routine haematoxylin and eosin stained slides. The height of the villus was measured by drawing a line from the tip of the villus to the base. The villus thickness was determined by measuring the width of the villus at several points throughout its length. The height of enterocytes was measured from surface of the cell to base passing through the nucleus. The height of villus and thickness were measured on magnification (X50) while the height of enterocytes on magnification (X 400). The measurements were repeated on several villi in serial sections. The data were represented as mean  $\pm$  standard deviation and were analyzed using student t-test.

#### Immunohistochemical procedure :

Paraffin sections 5 µm in thickness were mounted on slides coated with polysine, placed in 0.5% hydrogen peroxide methanol, washed with T.B.S buffer (PH. 7.5) then placed in diluted serum. Sections were incubated with the primary antibody. Nitric oxide synthase - 3 mouse monocolonal antibody (Nova Castra Laboratories), washed with T.B.S buffer for 5 minutes twice, incubated with biotinylated secondary antibody (Nova castra laboratories) after incubation sections were washed with T.B.S buffer for 5 minutes. Additional slides were treated with non immune serum instead of the primary antibody and were used as controls.

Endothelial cells with positive reactivity for nitric oxide synthase (NOS) showed brown colour in the cytoplasm and membranes.

### RESULTS

## The control groups (Sham Operated) :

Light microscopic examination of the jejunum of rabbits of the control groups (Sham operated) clarified that the jejunal villi were finger-like processes, the mean height of villi was  $321.070 \pm 132.475$  and the mean thickness was  $60.568 \pm 4.932$  (Tables 1, 2). Each villus was lined with tall columnar epithelial cells (enterocytes) with mean height 99.349  $\pm$  14.460 (Table 3). The enterocytes exhibited basal oval vesicular nuclei. At the surface, the epithelial cells were covered with homogeneous striated brush border (Fig. 2). Goblet cells were interposed at intervals between the columnar cells. They were rounded in shape with eccenteric nuclei (Fig. 3). Silverstained sections showed that the core of villi contained fine, elongated and continuous network of reticular fibers along the entire core (Fig. 4).

Scanning electron microscopic examination further revealed that the jejunum of rabbits of control groups was studded with villi; in some areas the villi were closely packed together. However, each villus had well-defined clear outlines and regular orientation (Fig. 5). The surface of the villi was formed by columnar epithelial cells. The tips of the closely packed microvilli of the columnar cells gave a slightly pebbled appearance to the surface. The pebbled surface was broken occasionally by small pits representing the goblet cell orifices (Fig. 6).

Immunohistochemical examination revealed strong nitric oxide synthase reaction in the endothelial cells of blood vessels within the core of the villi. The reaction was detected mainly in apical parts of the villi and to a lesser extent in the basal parts of villi (Fig. 7).

# **Group I : Ischemia Reperfusion**

## A) Ischemic Segment :

Light microscopic examination of the jejunum of rabbits of group I subjected to ischemia-reperfusion revealed that in the ischemic segment the general architecture of the villi was largely preserved. The villi were short and the mean height was significantly reduced ( $214.051 \pm 53.686 P < 0.01$ ) (Table 1). However, the mean thickness was significantly increased ( $84.971 \pm 36.86 P < 0.05$ ) (Table 2). Moreover, the typical striated brush border was indistinct and ill-defined. The enterocytes were significantly reduced in height ( $52.239 \pm 6.56 P < 0.001$ ) (Table 3). The pattern of organization of enterocytes was distorted and numerous cells were detached and lifted from the villus stroma. Goblet cells were infrequently encountered (Fig. 8). The core of the villi manifested mononuclear cellular infiltration, amorphous eosinophilic material and aggregation of red blood cells (Figs. 8, 9). Occasionally, some of the amorphous material and the red blood cells passed into the intestinal lumen through the dissociated enterocytes (Fig. 8). Less frequently vacuolated areas were noticed in the core of the villi (Fig. 9). Silver-stained sections revealed that the reticular fibers were partially disrupted and compressed along the sides of the villi (Fig. 10).

Scanning electron microscopic studies clarified a variety of surface alterations. The villi were frequently fused together with ill-defined boundaries and irregular orientation. The apical parts of the villi were either irregular and manifested small knobs or were partially detached (Fig. 11). The surface of villi showed marked sloughing of the epithelial cells and ill-defined microvilli with loss of the pebbled appearance. Goblet cells were rarely encountered (Fig. 12). The ischemic jejunal segment showed few flattened irregular shaped areas without surface villi (Fig. 11). Immunohistochemical examination clarified that nitric oxide synthase . eaction in the endothelial cells within the core was weak as compared with the control group and occurred at interrupted wide intervals (Fig. 13).

## **B) Distal Segment :**

Light microscopic examination of the jejunal segment distal to the ischemic segment revealed that the mean height of villi was  $285.578 \pm 90.343$  showing non

significant variation from the control group (P > 0.05) (Table 1). The mean thickness of villi was  $47.332 \pm 6.452$  showing significant variation than the control (P < 0.001) (Table 2). On comparing the distal segment to the ischemic segment, it was clear that the height of villi in the distal segment was significantly higher than in ischemic segment (P < 0.05) (Table 1). The mean thickness of villi was significantly reduced compared with the ischemic segment (P < 0.01) (Table 2). Though the villi generally exhibited intact brush border, numerous goblet cells and regular arrangement of enterocytes, yet the height of enterocytes was 65.145 ± 8.782 showing significant decrease from the control (P < 0.001) (Table 3). On comparing with the ischemic segment the height of enterocytes in the distal segment showed significant increase (P < 0.001) (Table 3). The core of the villi exhibited marked mononuclear cellular infiltration (Fig. 14). Moreover, silver-stained sections clarified the presence of extensive network of thickened branching reticular fibers particularly in the basal parts of the villi (Fig. 15).

Scanning electron microscopic examination revealed that jejunal segment distal to the ischemic segment was studded with villi with preserved outlines. However, numerous villi were irregularly oriented (Fig. 16). The surface was pebbled with numerous microvilli and prominent goblet cells which often formed multiple aggregates (Fig. 17). Immunohistochemical examination clarified that nitric oxide synthase reaction was intensified particularly in the endothelial cells in the apical parts of the villi (Fig. 18).

## Group II : Continuous Ischemia A) Ischemic Segment :

Light microscopic examination of jejunum of rabbits of group II subjected to continuous ischemia showed marked exacerbation of the mucosal injury. Most of the villi were atrophied, denuded and altered in shape with loss of their finger-like appearance (Fig. 19). The mean height of the villi was  $81.136 \pm 38.216$  showing significant decrease than the height of the villi of both the control (P < 0.001) and the group subjected to ischemia reperfusion (P < 0.001) (Table 1). Regarding the mean thickness of the villi it was  $40.66 \pm 17.92$  showing again significant decrease than the the control group (P < 0.001) and then the group subjected to ischemia reperfusion (P < 0.001) and than the group subjected to ischemia reperfusion (P < 0.001) (Table 2). The pattern of organization of enterocytes was completely lost (Fig. 19). The mean height of enterocytes was 54.16 + 8.32 showing significant decrease than the control (P < 0.001). However, on

comparing with the group subjected to ischemia reperfusion, the height of enterocytes did not show significant variation (P > 0.05) (Table 3).

Furthermore, numerous enterocytes were detached along the entire length of the villi. The detached cells had condensed or pyknotic nuclei (Figs. 19, 20). Large areas manifested ulceration and mononuclear cellular infiltration (Fig. 21). The villus stroma showed extensive hemorrhage and marked mononuclear cellular infiltration (Figs. 19, 21). Silver-stained sections clarified almost complete disruption of the reticular fibers as they appeared thin, short and fragmented (Fig. 22).

Scanning electron microscopic examination further demonstrated that the villi were atrophied, irregularly oriented and the apex of the villi was completely distorted. The jejunum showed wide areas of ulceration with complete loss of the surface epithelium and microvilli (Fig. 23). Immunohistochemical studies showed that the nitric oxide synthase reaction in the endothelial cells was extremely weak and localized (Fig. 24).

## **B) Distal Segment :**

Light microscopic examination of the jejunal segment distal to the segment subjected to continuous ischemia revealed that the villus structure was generally preserved apart from broadening and flattening of the apex of the villi (Fig. 25). The mean height of the villi was  $186.35 \pm 74.53$  manifesting significant decrease than the control (P < 0.01) (Table 1). The mean thickness of the villi was  $88.74 \pm 33.49$  showing significant increase than the control (P < 0.01) (Table 1). The mean thickness of the villi was  $88.74 \pm 33.49$  showing significant increase than the control (P < 0.01) (Table 2). On comparing with the ischemic segment, the height of the villi in the distal segment showed significant increase (P < 0.001) (Table 1). The mean thickness of the villi showed also significant increase (P < 0.001) (Table 2). Though the enterocytes were not detached but they exhibited few condensed nuclei (Fig. 25). Moreover, the height of enterocytes was  $68.60 \pm 8.35$  showing significant decrease than the control (P < 0.001) (Table 3). However, on comparing with the ischemic segment the height of enterocytes was significantly higher (P < 0.001) (Table 3). The core of villi showed severe, mononuclear cellular infiltration (Fig. 25). Silver-stained sections revealed that the reticular fibers were densely arranged in the basal parts of the villi (Fig. 26).

Scanning electron microscopic examination demonstrated that the jejunum was studded with closely packed villi and some villi showed detached apices. (Fig. 27). The characteristic finding in the distal segment was the presence of filamentous mucous threads encroaching on the villi (Fig. 28). Immunohistochemical studies

clarified that the nitric oxide synthase reaction was intense particularly in the endothelial cells in the apical parts of the villi (Fig. 29).

	Ischemic segment in	Distal segment in	Ischemic segment in	Distal segment in
	ischemia reperfusion	Ischemia reperfusion	continuous ischemia	continuous ischemia
	M : 214.051 -	M : 285.578 -	M : 81,136 -	M : 186.359 -
	SD : 53.686	SD : 90.343	SD : 38.216	SD : 74.531
Control	t : 2.581	t : 0.734	t : 6.252	t : 2.939
M: 321.070	P < 0.01	P > 0.05	P < 0.001	P < 0.01
SD: 132.475	HS	NS	HS	HS
Ischemic segment in ischemia reperfusion		t : - 2.322 P < 0.05 S		
Ischemic segment in continuous ischemia	t : 7.176 P < 0.001 HS			t :.9,889 P < 0.001 HS

Table 1 : The Height of Villi :

HS : Highly Significant.

S : Significant.

NS: Non-Significant.

t : Student's "t" test.

	Ischemic segment in	Distal segment in	Ischemic segment in	Distal segment in
	ischemia reperfusion	ischemia reperfusion	continuous ischemia	continuous ischemia
	M : 84.971 -	M : 47.332 -	M : 40.665 -	M : 88.743 -
	SD : 36.863	SD : 6.452	SD : 17.927	SD : 33.492
Control	t : - 2.276	t : 5.556	t : 3.717	t : 2.880
M: 60.568	P < 0.05	P < 0.001	P < 0.001	P < 0.01
SD: 4.932	S	HS	HS	HS
Ischemic segment in ischemia reperfusion		t : 3.335 P < 0.01 HS		
Ischemic segment in continuous ischemia	t : 3.956 P < 0.001 HS			t : - 4.6993 P < 0.001 HS

Table 2 : The Thickness of Villi :

Table 3 : The Height of Enterocytes :

	Ischemic segment in	Distal segment in	Ischemic segment in	Distal segment in
	ischemia reperfusion	ischemia reperfusion	continuous ischemia	continuous ischemia
	M : 52,239 -	M : 65.145 -	M : 54.164 -	M : 68.600 -
	SD : 6,560	SD : 8.782	SD : 8.326	SD : 8.358
Control	t : 9.491	t : 6.775	1 : 9.380	t : 6.377
M: 99,349	P < 0.001	P < 0.001	P < 0.001	P < 0.001
SD: 14.46	HS	HS	HS	HS
Ischemic segment in ischemia reperfusion		t : - 3.782 P < 0.001 HS		
Ischemic segment in continuous ischemia	t : - 5929 P > 0.05 NS			t : - 4.2381 P < 0.001 HS



Fig. (1): An illustration showing:
a) The selected jejunal segment.
b) The ligation of the terminal blood vessels.
c) The occlusion of the terminal blood vessels.
d) The jejunal segments following ischemia.





(Hx, & E., x 400)







Fig. (4) : A photomicrograph of a sec-tion of jejunum of rabbit of the con-trol group showing fine elongated, continuous reticular fibers (1). (Wilder Stain, x 200)



Fig. (5) : A scanning electron micrograph of jejunum of rabbit of the control group. Notice the closely packed, regularly oriented villi with well defined outlines.

(Scale bar 200 µm)



Fig. (6) : A scanning electron micrograph of jejunum of rabbit of the control group showing the pebbled surface of the villi (1). Notice the goblet cells (g).

(Scale bar 20  $\mu m$ )



Fig. (7) : A photomicrograph of a sec-tion of jejunum of rabbit of the con-trol group. Notice the nitric oxide syn-thase reaction in the endothelial cells along the entire core of the villus (\*) (NOS immuno-staining, x 400)



Fig. (8) : A photomicrograph of a section of the ischemic segment of jejunum of rabbit of group I showing distorted pattern of enterocytes. Notice the detached enterocytes (e), eosinophilic material (s) and the red blood cells  $(\uparrow)$ .

(Hx. & E., x 400)



Fig. (9) : A photomicrograph of a sec-tion of the ischemic segment of jeju-num of rabbit of group I showing eo-sinophilic material (s) vacuolation (v) and few red blood cells (T). (Hx. & E., x 400)





(Wilder stain, x 200)



Fig. (11) : A scanning electron micrograph of the ischemic segment of jejunum of rabbit of group (I) showing the villi with ill defined boundaries  $(\uparrow)$  and detached apex (a). Notice the plain area without surface villi  $(\uparrow\uparrow)$ .

(Scale bar 200 µm)



Fig. (12) : A scanning electron micrograph of the ischemic segment of jejunum of rabbit of group I showing sloughing of surface epithelium and microvilli  $(\uparrow)$ .

(Scale bar 500 µm)



Fig. (13) : A photomicrograph of a section of the ischemic segment of jejunum of rabbit of group I showing weak nitric oxide synthase reaction ( $\uparrow$ ). (NOS immuno-staining x 400)







Fig. (15): A photomicrograph of a section of the distal segment of jejunum of rabbit of group I showing thickened branching reticular fibers in basal parts of villi ( $\uparrow$ ). (Wilder stain; x 200)



Fig. (16): A scanning electron micrograph of distal segment of jejunum of rabbit of group (I). Notice the irregular orientation of villi. (Scale bar 200 µm)



Fig. (17): A scanning electron micro-graph of distal segment of jejunum of rab-bit of group I. Notice the aggregates of goblet cells (g) and the pebbled surface  $(\uparrow)$ .

(Scale bar 20 µm)



Fig. (18) : A photomicrograph of a section of the distal segment of jeju-num of rabbit of group I. Notice the intense nitric oxide synthase reaction in the apical parts of the villi (<sup>†</sup>). (NOS immuno-staining x 400)



Fig. (19): A photomicrograph of a section of the ischemic segment of jejunum of rabbit of group II. Notice the atrophied villi and the loss of finger like appearance.

(Hx. & E.; x 200)



Fig. (20): A photomicrograph of a section of the ischemic segment of jejunum of rabbit of group II showing villi with detached enterocytes (e). Notice the pyknotic nuclei (n) and the extensive hemorrhage  $(\uparrow\uparrow)$ .

(Hx. & E.; x 400)



Fig. (21) : A photomicrograph of a section of the ischemic segment of jejunum of rabbit of group II showing wide area of ulceration (a). Notice the mononuclear cellular infiltration ( $\uparrow$ ). (Hx. & E.; x 400)



Fig. (22) : A photomicrograph of a section of the ischemic segment of jejunum of rabbit of group II. Notice the thin, short and fragmented reticular fibers. (Wilder stain; x 200)

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Fig. (23) : A scanning electron micrograph of ischemic segment of jejunum of rabbit of group (II) showing atrophied villi with distorted apex  $(\uparrow)$ . Notice the areas of ulcration (u).

(Scale bar 200 µm)



Fig. (24) : A photomicrograph of a section of ischemic segment of rabbit of group II. Notice the weak, localized nitric oxide synthase reaction (1). (NOS immuno-staining x 400)

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Fig. (25) : A photomicrograph of a section of the distal segment of jeju-num of rabbit of group II showing the broad tip of the villi, mononuclear cel-lular infiltration (m) and condensed nuclei (n).

(Hx. & E., x 400)



**Fig. (26)**: A photomicrograph of a section of the distal segment of jejunum of rabbit of group II showing dense reticular fibers (r) in the basal parts of villi.

(Wilder stain; x 200)



 Fig. (27) : Scanning electron micrograph of the distal segment of jejunum of rabbit of group (II) showing villi with detached apex.
 (Scale bar 200 μm)



 Fig. (28) : Scanning electron micrograph of the distal segment of jejunum of rabbits of group II. Notice the filamentous mucus threads.
 (Scale bar 100 μm)





### DISCUSSION

The present study revealed that the intestine subjected to segmental ischemia reperfusion or continuous ischemia was dramatically affected. Furthermore, it provided evidences that delayed morphological changes were not limited to the ischemic segments but involved distal segments as well.

The present work revealed that following ischemia reperfusion the general architecture of the villi was largely preserved. However, the mean height of the villi was significantly reduced. In continuous ischemia, there was exacerbation of mucosal injury. Most of the villi were denuded, atrophied with loss of their finger like shapes. Significant reduction in the mean height of the villus was further noticed. It is hypothesized that the height of the villi is the most reliable and early detectable ischemic change. It could be considered as an excellent parameter for evaluating early ischemic changes (**Puglisi et al., 1995**). The present work showed that the changes in the height of the villi could be considered as one of the remarkable signs of the delayed morphological changes in both ischemia and ischemia-reperfusion. The significant shortening of the villi in continuous ischemia than in ischemia reperfusion would be expected to cause more reduction in the mucosal surface area (**Vatistas et al., 1998**).

On the other hand, in the ischemic segment the mean thickness of the villi in ischemia reperfusion was significantly increased, while during continuous ischemia the mean thickness of villi was significantly reduced when compared with the control or with the group subjected to ischemia reperfusion. The increased thickness of villi during ischemia reperfusion is most likely attributed to mucosal edema and acute accumulation of extracellular fluid rather than to the effects of diminished blood flow (Ostgaard and Reed, 1995; Rha et al., 2000). The reduction of thickness of villi encountered in the present study during continuous ischemia is probably due to the absence of edema. It is believed that mucosal edema develops only on reperfusion (Puglisi et al., 1995).

Although the villi preserved their finger-like shapes durin, ischemiareperfusion, yet the typical brush border was ill-defined. The enterocytes were significantly reduced in height and their pattern of organization was distorted. Numerous enterocytes were detached and lifted from the villus stroma. Similarly, during continuous ischemia the height of enterocytes was significantly reduced when compared to the control. However, it did not show significant variation from the height of enterocytes in ischemia-reperfusion. Unlike ischemia-reperfusion, the enterocytes in continuous ischemia were frequently detached along the entire length of the villi. Moreover, the detached cells had condensed or pyknotic nuclei.

Detachment of enterocytes from the tips of the villi was the early characteristic teature of ischemic damage to the small intestine (Ikeda et al., 1998). The present work showed that detachment of enterocytes was also a prominent delayed feature of ischemia-reperfusion. The reason for epithelial shedding is the appearance of membrane enclosed cytoplasmic blebs which arise at the base of the enterocytes and cause the detachment from the basement membrane (Ikeda et al., 1998). The condensation of nuclei and pyknosis are the characteristic morphological features of apoptosis (Puglisi et al., 1995). It has been proposed that the interaction between the extracellular matrix and detached cells plays a critical role in apoptosis (Frisch and Francis, 1994). Moreover, it was hypothesized that apoptosis might be due to increased plasma levels of histamine (Yoshida et al., 2000). Recently, oxidative stress was considered also as one of the causes of apoptosis (Jacob et al., 2003).

Scanning electron microscopic studies further revealed that following ischemia reperfusion the villi were frequently fused together with ill defined boundaries and irregular orientation. The morphological alterations were noticed mainly in the apical parts of the villi as they appeared irregular and manifested multiple small knobs or were partially detached. The surface of the villi showed sloughing of epithelial cells and ill-defined microvilli, Localized areas without surface villi were also encountered. On the other hand, during continuous ischemia, the apices of the villi were completely distorted and the jejunum exhibited wide areas of ulceration with complete loss of the surface epithelium and the microvilli. It was reported that superficial necrosis always started at the tips of villi in ischemia reperfusion (Chin et al., 2002). In the present work, it was evident that the degenerative changes were manifested in the apical parts of the villi in both models of ischemia performed. Schlichting et al. (1995) noticed shrinkage of the villi, mucosal ulceration, necrosis and complete loss of cpithelial cell renewal in ischemia-reperfusion achieved by complete occlusion of the superior mesenteric artery. Occlusion of arcades followed by reperfusion was assumed to cause reduction of epithelial converge up to 23% (Gangadharan et al., 1995).

The sloughing of epithelial cells noticed in the present work might greatly hinder the absorption of nutrients produced by the digestive process. Moreover, the absence of microvilli might abolish their physiological function and reduce contact between intestinal surface and the nutrients disturbing the absorption (Junqueira and Carneiro, 2003). The surface ulceration encountered in the present study following continuous ischemia might imply that the epithelial barrier integrity is impaired. The impairment is triggered by tissue hypoxia and the activation of the phagocytic system which might lead to macromolecular leakage and finally might lead to organ dysfunction (Sun et al., 2000).

In the present study, the core of the villi represented distinguishable changes in both models of ischemia. In ischemia reperfusion, the core manifested mononuclear cellular infiltration, amorphous eosinophilic material and agreggation of red blood cells. Less frequently, vacuolated areas were noticed. In addition, the reticular fibers were compressed and partially disrupted. In continuous ischemia, the core exhibited extensive hemorrhages and marked mononuclear cellular infiltration, while the reticular fibers were thin, short and fragmented.

The mononuclear cellular infiltration encountered in the present work might be a function of cytokine induced neutrophil chemoattractant which is elevated during ischemia or ischemia reperfusion playing an important role in neutrophil infiltration (Yagihashi et al., 1998). Leucocytic infiltration could be attributed also to increased vascular or mucosal permeability (Kanwar et al., 1998). The leucocytes were not only increased but were assumed to be adherent (Kalia et al., 2001). The present study clarified that the cellular infiltration during continuous ischemia was always accompanied by hemorrhage. It is believed that hemorrhagic mucosal lesions produced during ischemia are due to accumulation and activation of polymorphnuclear leucocytes in the tissues and might predispose to sepsis (Rollwagen et al., 2000). Inhibition of neutrophil accumulation could attenuate hemorrhagic mucosal lesions (Schoenberg et al., 1995).

Immunohistochemical studies revealed that endothelial cells of blood vessels within the core of the villi of the control groups exhibited strong nitric oxide synthase reaction mainly in the apical parts of the villi and to lesser extent in the basal parts of the villi. The present study revealed that nitric oxide synthase reaction was weak in ischemia reperfusion and occurred at interrupted wide intervals. In continuous ischemia the reaction was extremely weak and localized. The production of nitric oxide and its role during ischemia is still subjected to controvert ideas. The weak reduced nitric oxide synthase reactions encountered in the present work is suggestive of diminished nitric oxide production during ischemia. Microvascular impairments are generally noticed to be associated with reduction in nitric oxide production (Fruchterman et al., 1998). Inhibition of nitric oxide synthase might participate in intestinal apoptosis (Yoshida et al., 2000). Moreover, diminished

nitric oxie synthase could be the cause of structural and functional alterations in the mitochondria (Madesh et al., 2000).

Activation of nitric oxide synthesis and production to achieve a cytoprotective role was only noticed if ischemia was proceeded with ischemic preconditioning (Valsov et al., 2002). Contrary to the results of the present work, Takada et al. (1998) noticed accelerated nitric oxide production in ischemia reperfusion and suggested that it participated in breakdown of intestinal mucosa, exerting a cytotoxic effect.

The intestinal segments distal to ischemia are of special clinical importance. The morphological changes in such segments were not properly clarified. The present work illustrated that during ischemia reperfusion, the general architecture and the shape of villi in the distal segment were intact. The height of the villi did not exhibit significant variation from the control. On the other hand in continuous ischemia, the shape of the villi in the distal segments was also intact apart from slight broadening of the villus tips. The mean height of villi was significantly reduced. Such discrepancy in the height of villi might indicate that in ischemia-reperfusion the mucosal surface area is probably unaltered and preserved in distal segments. However, it might be disturbed in continuous ischemia (Vatistas et al., 1998). Morphometeric analysis comparing the height of villi in distal segments of both models of ischemia performed with height of villi in the ischemic segments showed that the height of villi in distal segments was always higher.

During ischemia-reperfusion the mean thickness of villi in distal segments was significantly decreased than both the control and ischemic segments, while during continuous ischemia the mean thickness of villi in distal segments was significantly increased than both the control and ischemic segment. The decrease in thickness of villi in ischemia reperfusion indicates absence of edema in distal segments and its restriction to ischemic segments only (**Puglisi et al., 1995**). The increase in thickness of villi in continuous ischemia is suggestive of occurrence of edema in distal segments. Clinically, incisions are preferred, to be done in distal segments to ischemic site in order to make it in less edematous area (Steen Voorde et al., 2003).

Scanning electron microscopic examination revealed that the villi in distal segments of both ischemia and ischemia-reperfusion had preserved outlines, intact surface epithelium and microvilli. However, during ischemia-reperfusion goblet cells formed multiple aggregates. Moreover, in continuous ischemia the surface of villi manifested filamentous mucus threads. The intense mucus secretion provides protection and lubrication to intestinal segments potentially exposed to inflammation (Jacob et al., 2003).

Furthermore, the present study clarified that the core of villi in distal segments exhibited marked mononuclear cellular infiltration. It is believed that during ischemia the inflammation is not restricted to the ischemic segment only or segments distal. It is postulated that ischemia favors the increase of microflora and the dissemination of different organisms even in the spleen, kidney and liver (Meddah et al., 2001). The increase in microorganisms triggered an increase in the resident inflammatory cells within the gut wall and raised the possibility that under conditions of ischemic stress, pro-inflammatory mediators might also be activated by toxic factors in gut lumen (Harari et al., 2000).

In addition to the marked mononuclear cellular infiltration the present work revealed that the core of villi had extensive branching thickened network of reticular fibers particularly in the basal parts of the villi. Generally, the small diameter of the reticular fibers and their loose deposition create a flexible network in organs subjected to motility or changes in volume (Junqueira and Carneiro, 2003). The thickening and branching of reticular fibers noticed in the core of the villi might explain the decreased motility, contraction frequency and relaxation frequency noticed after ischemia and might clarify the occurrence of clinical ileus or transit ingest in distal segments (Malone and Kanaan, 2001).

The present study further clarified that the core of villi in the distal segments exhibited intensified nitric oxide synthase reaction particularly in the apical parts of the villi. The intense reaction is suggestive of increased nitric oxide production. Nitric oxide formed in the endothelial cells diffuses in the blood to exert its effects (Kozlov et al., 2001). It is believed that nitric oxide could probably provide protection to the distal bowel (Chan et al., 2002). Administration of nitric oxide donner FK 409 in ischemia-reperfusion significantly reduced leucocytes adhesion, macromolecular leakage and maintained villous blood flow and was of great value in reducing tissue damage (Kalia et al., 2001). The intense nitric oxide synthase reaction noticed in the present study might indicate that nitric oxide seems to be involved in bowel protection after ischemia, and could be of value in maintenance of the distal segments.

In conclusion, the present work clarified that persistent morphological and morphometric changes were encountered in the jejunal villi following ischemiareperfusion. These changes were more evident following continuous ischemia. The distal segments manifested few alterations that should be considered during clinical management of ischemia or ischemia-reperfusion.

### SUMMARY

The aim of the present work was to illustrate the delayed morphological and morphometeric changes following segmental ischemia and ischemia-reperfusion in the jejunal villi of rabbits. Moreover, the present work aimed to investigate the changes that are likely to occur in intestinal segments distal to ischemia. Rabbits were divided into two groups. In the first group the terminal blood vessels of the selected jejunal segments were occluded for sixty minutes with a vascular clamp followed by reperfusion. In the second group the terminal blood vessels of the selected jejunal segments were continuously ligated with polygalactin. Both the ischemic and distal segments were excised after 24 hours.

Following ischemia-reperfusion, the ischemic segment exhibited significant decrease in height and significant increase in thickness of the villi. The enterocytes were disorganized and significantly reduced in height. The core of villi contained mononuclear cellular infiltration, cosinophilic material, aggregation of red blood cells and disrupted fragmented reticular fibers. Moreover, nitric oxide synthase reactions were weak. Scanning electron microscopic examination revealed distorted partially detached apices, sloughing of the epithelial cells of the villi and ill-defined microvilli. On the other hand, segments distal to ischemic ones, revealed non significant variation in height and significant reduction in thickness of the villi as compared to the control group. However, on comparing with the ischemic segment, the height of the villi was significantly higher and the thickness was significantly reduced. The villi had intact brush border and regular arrangement of enterocytes, but their height was significantly reduced. The core of the villi manifested severe mononuclear cellular infiltration, thickened dense reticular fibers and strong nitric oxide synthase reactions. Scanning electron microscopic studies revealed irregular orientation of some villi. However, the surface epithelium was intact and exhibited numerous microvilli. Aggregations of goblet cells were commonly noticed.

Following continuous ischemia, the ischemic segment presented atrophied villi. The mean height and thickness of the villi were significantly reduced as compared to the control or to the group subjected to ischemia-reperfusion. The pattern of organization of enterocytes was lost and the cells were significantly reduced in height. The enterocytes were detached along the entire length of the villi and the detached cells showed condensed and pyknotic nuclei. The core of the villi exhibited hemorrhage, mononuclear cellular infiltration, complete disruption of the reticular fibers and extremely weak localized nitric oxide synthase reactions. Scanning electron microscopic examination showed areas of ulceration with complete loss of surface epithelium and microvilli. Regarding the distal segment, the villi showed significant decrease in height and significant increase in thickness as compared to the control. On comparing with the ischemic segment, the height and thickness of the villi of distal segments were significantly increased. Though the enterocytes were not detached, they were significantly reduced in height and showed few condensed nuclei. The core exhibited marked mononuclear cellular infiltration, dense reticular fibers in the basal parts of the villi and strong nitric oxide synthase reactions. Scanning electron microscopic examination revealed characteristic filamentous mucus threads encroaching on the surface of the villi. It was concluded that persistent changes were noticed in jejunal villi following both models of ischemia. However, the changes were deleterious in continuous ischemia. Distal segments revealed slight alterations particularly in the core of the villi.

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# الملخص العربي

# التأثيرات المستولوجية المتاخرة لقطع الإمداد الدموى القسيمى وإعادة الإرواء على خملات المعى الصائم في الأرانب

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استهدفت هذه الدراسة توضيح التغيرات الشكلية والقياسية فى أمعاء الأرانب بعد قطع الإمداد الدموى القسيمى وقطع الإمداد الدموى القسيمى المصحوب بإعادة الإرواء . كما استهدفت الدراسة توضيح التغيرات المتوقع حدوثها فى القسيمية التى تلى القسيمية المصابة بقطع الإمداد الدموى . قسمت الأرانب إلى مجموعتين فى المجموعة الأولى تم إطباق الأوعية الدموية التى تغذى القسيم الصائمى لمدة ستين دقيقة بمشبك وعائى تم إعادة الإرواء وفى المجموعة الثانية تم ربط الأوعية الدموية ب ( بولى جلاكتين ) . بعد مرور أربع وعشرون ساعة تم استئصال القسيم الصائمى الذى تعرض لقطع الإمداد الدموى والقسيم التالى له .

قد تبين أن قطع الإمداد الدموى وإعادة الإرواء قد تسبب فى قصر نسبى فى طول الضملات ، بينما ازداد سمكها نسبيًا . أما الضلايا المعوية فقد أظهرت تباينًا فى تراصبها وقصر نسبى طولها . واحتوى قلب الضلات على تجمع خلايا أحادية النوايا وكرات دم حمراء وتحلل وتكسر فى الألياف الشبكية بالإضافة إلى نقص تفاعل أكسيد النيتريك المخلق وأظهر الفحص بالمجهر التفرسى الإلكترونى – تشوه وانفصال جزئى فى قمم الخملات – وتخشر فى الخلايا المعوية والزغبيات . أما فى القسيم الصائمى التالى للقسيم الذى تعرض لقطع الإمداد الدموى فلم تظهر الخملات تباين نسبى فى عولها ولكنها أظهرت زيادة نسبية فى سمكها بالمقارنة بالمجموعة الضابطة . ولكن عندما قورنت القسيمية الصائمية التالية بالقسيمية الصائمي تعرضت لقطع الإمداد الدموى تبين أن طول وسمك الخلات قد ازداد نسبيًا . حافظت الخملات على الحد الفرجونى وتراص الخلايا المعوية ولكن الخلايا المعوية أظهرت قصراً نسبياً فى طولها . أما قلب الخمائل فقد احتوى على خلايا أحادية النوايا كما ازدادت الألياف الشبكية سمكًا وإزداد أيضًا تفاعل أكسيد النيتريك المخلق وأظهر الفحص بالمجهر التفرسي الإلكتروني أن الخملات غير منتظمة الاتجاه لكن مع الحفاظ على الخلايا المعوية والزعيبات وظهرت تجمعات من الخلايا القدحية .

أما القسيمية المنائمية المعرضة لقطع الإمداد الدموى المستمر فقد اظهرت ضمور فى الخمائل ونقص نسبى فى طولها وسمكها بالمقارنة بالمجموعة الضابطة أو المجموعة التى تعرضت لقطع الإمداد الدموى المصحوب بإعادة الإرواء . كما أن نظام تراص الخلايا المعوية قد اختلط تمامًا وأظهرت نقص نسبى فى طولها وانفصال عن الغملات . احتوت الخلايا المعوية المنفصلة على نواييا متغلظة . وأوضح قلب الخملات وجود نزيف وخلايا أحادية النوايا وتفسخ فى الألياف الشبكية وتفاعل ضعيف لأكسيد الترح بلا خلايا معوية أو زغيبات . وأوضحت هذه التراسى وجود مساحات من القرح بلا خلايا معوية أو زغيبات . وأوضحت هذه الدراسة إن القسيم المنائمى التالى الترح بلا خلايا معوية أو زغيبات . وأوضحت هذه الدراسة إن القسيم المنائمى التالى وزيادة نسبية فى سمك الخملات بالمقارنة للمجموعة الضابطة ولكن عندما قورن وزيادة نسبية فى سمك الخملات بالمقارنة للمجموعة الضابطة ولكن عندما قورن بالقسيم الذى تعرض لقطع الإمداد الدموى قد أظهر قصر نسبى فى طول الخملات وزيادة نسبية فى سمك الخملات بالمقارنة للمجموعة الضابطة ولكن عندما قورن وزيادة نسبية فى سمك الخملات بالمقارنة المجموعة الضابطة ولكن عندما قورن بالقسيم الدى تعرض لقطع الإمداد الدموى المحموعة الضابطة ولكن عندما فورن وزيادة نسبية فى سمك الخملات بالمقارنة للمجموعة الضابطة ولكن عندما قورن بالقسيم المائمى الذى تعرض لقطع الإمداد الدموى المحموعة الضابطة ولكن عندما فورن بالقسيم المائمى الذى تعرض لقطع الإمداد الدموى المتور ولكنها أظهرت قصر نسبى فى وألياف شبكية متكائفة ، وازداد تفاعل أكسيد النيتريك المخلق . أوضح الفحص بالمجهر وألياف شبكية متكائفة ، وازداد تفاعل أكسيد النيتريك الملق . أوضح الفحص بالمجهر المؤسى الإلكترونى شرائط مخاطية على سطح الخملات .

وقد استنتج أن قطع الإمداد الدموى المصحوب بإعادة الإرواء أو المستمر قد سبب تغيرات فى الأمعاء وهذه التغيرات كانت أشد حدة فى القطع الدموى المستمر . كما أثر قطع الإمداد الدموى على أجزاء الصائمى التالية له .

مجلة التشريح المصرية ، ٢٦ (٢) ، يوليو ٢٠٠٣