The Histological Effect of Hunger Stress on the Stomach in Male Albino Rats: A Study of Light Microscope

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Abstract: Emotional changes can influence feeding behavior. Feed intake is the cornerstone of animal productivity. The consequences of inadequate intake include inhibited growth, delayed puberty, infertility, reduced milk production and lowered resistance to parasites and disease. In this study, the histological changes observed in male albino rats' stomach mucosa which was exposed to hunger were examined. Pieces from stomach were taken in the 1st-5th days following last feeding. These tissue specimens were fixed by using 10% neutral formalin which was compressed. After routine tissue checks, the tissue pieces were sunk into paraffin wax and some blocks were prepared. With the aim to examine histological structures of the pieces taken, they were stained with Hematoxylin and Eosin (H and E). An increase in the number of blood cells taken part in stomach tissue was observed in the first 24 h. At the end of the 3rd day of hunger, necrosis of fundic glands, especially the base of fundus and mild congestion of mucosal blood vessels were observed. A widening in glandular epithelium lumen was seen after 2 days. A tendency to slim was observed in mucosa layer of the surface and glandular lumen during hunger. In the stomach mucosa, structural changes were caused by hunger. It was found that these changes were in direct proportion with hunger duration.

Key words: Fasting, histology, stomach, hunger, rats, Jordan

INTRODUCTION

It is well known that food deprivation inhibits gastric and intestinal epithelial cell proliferation in adult rats (Alvares, 1987). The size of the crypts and villi (Altmann, 1972), the migration rate (Al-Dewachi et al.,1975) and the crypt cell production rate (Goodlad et al., 1988) are also decreased in food-deprived adult rats.

These starvation-induced hypoproliferative responses have been attributed to the increase in cell cycle time. Food deprivation has been found to stimulate cell proliferation in the gastric mucosa of suckling rats whereas the weanling period has been reported to be unresponsive in terms of proliferative activity (Palanch and Alvares, 1998).

Long-term hunger is a problem that all living species in the nature often have to cope with. It is a known fact that hunger has a considerable impact on many systems metabolically and structurally. In hungry living species, the speed of basal metabolism decreases rapidly and the amount of azoth in urine increases as an indicator of protein destruction. During hunger, first priority is given to the central nerve system and erythrocytes that can use pure glucose as energy source. At the same period, the level of serum insulin decreases but the amount of glucagons increases and thus gluconeogenesis, i.e., the process of producing glucose from some amino acids and glycerol speeds up in the liver. Since the level of insulin decreases, blood cells begin to absorb less glucose. Yet, fatty acids can enter into these cells and they are used as energy source.

All of these occur during the first 48-72 h of hunger. The process of gluconeogenesis reaches at maximum level during the 3rd day of hunger (Sonmez and Ozan, 2005). During hunger, the process of cell mitosis slows down and cell cycles prolong with some cells staying at the G1 stage. In parallel to this, epithelium cells rate of renewing decreases (Colakolu et al., 1999).

Blood pressure, glucose levels and body weight lower down depending upon the intensity level of hunger. The gastric pH of stomach decreases, i.e., the amount of gastric acid increases (Koc and Muslu, 2007). Emotional alterations, as well as pharmacological treatments can influence feeding behavior. For example, food intake is

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increased by acute administration of diazepam (Britton *et al.*, 1981; Cooper, 1983; Khattab, 2007). Exposure to stressors cause an array of biochemical, physiological and behavioral changes (Sakr, 2007) and it has been described that chronic exposure to stressors of a certain severity decreases food intake and body weight in the rat (Dess *et al.*, 1988; Marti *et al.*, 1994). In addition, increased intake of sucrose has been reported in rats submitted to an inescapable shock session (Dess, 1992). Other studies showed that painful tail stimulation produced food craving during the training session although, the intake was reduced to half in the post-training period (Rowland and Antelman, 1976). Chronically stressed animals show increased ingestion of sweet food (Ely *et al.*, 1997).

Studies on humans have provided further evidence of overeating induced by emotional+experiences (Yates, 1992; Silveira *et al.*, 2000). Peptic ulcers can occur anywhere in the digestive tract but are most common in the lower half of the stomach (gastric ulcer) or in the upper part of the duodenum, the first 30 cm of the small intestine directly below the stomach (duodenal ulcer). Despite their different locations, both types of ulcer are the result of excessive damage to the mucosal cells that line the stomach and duodenum (Peura and Czinn, 2004). To protect the stomach and small intestine from the corrosive gastric juices secreted to digest food (hydrochloric acid and pepsin), these cells produce a layer of mucin, a thick slippery mucus along with other factors that neutralize any acid that manages to come into contact with the stomach or intestinal lining. Mucosal cells lining the stomach and intestines are constantly renewed with normal turnover occurring every 72 h so any damaged cells are quickly replaced.

Problems only arise when normal mucosal cell function is disrupted by factors such as Helicobacter pylori infection, aspirin and other Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), alcohol, nutrient deficiencies, stress and other factors. Regardless of the general appearance of a gastric ulcer at the time of endoscope, histological evaluation is generally considered to be warranted to rule out the possibility of malignancy (Thirunavukkarasu *et al.*, 2010). When hunger begins in the first instance glycogen storages mobilize and they become emptied in 24 h. There are various studies focusing on the histological and histo-chemical changes occurred in stomach mucosa in different phases of hunger (Koc and Muslu, 2007). In this study, the effects of hunger stress on stomach mucosa was examined.

MATERIALS AND METHODS

This study was fulfilled in the Animal House of Department of Pharmacological Sciences, Faculty of Medicine, University of Jordan in August, 2011. All experiments in this study were accomplished according to the protocol recommended by Local Animal Care Ethical Committee. In this histological study, 15 male albino rats weighed 300 g±10 were used. During the experiment, each subject group was kept in separate cages and in night-day periods with 12 h.

Following last feeding, pieces were taken from stomach of the subjects after applying ether anesthesia at the end of the 24th h and 2nd-5th days following last feeding. These pieces were fixed by using 10% neutral formalin which was compressed. After washing and routine checking procedures, the pieces were sunk into paraffin wax and then, blocks were prepared. The sections being 5 μ in thickness and having been taken from paraffin blocs were stained with Hematoxylin and Eosin to examine general histological structure. These preparations were examined under the Zewiss light microscope and their photographs were taken.

RESULTS AND DISCUSSION

Stomach mucosa of the control groups were in normal histological appearance (Fig. 1). Tubular stomach glands under the surface epithelium were observed. Parietal cells were seen in gland epithelium. At the end of the first 24 h, following last feeding congestion of blood vessels were seen (Fig. 2 and 3). Some flattening and degeneration were observed in the surface epithelium and gland epithelium. An increase was seen in the congested blood veseles.
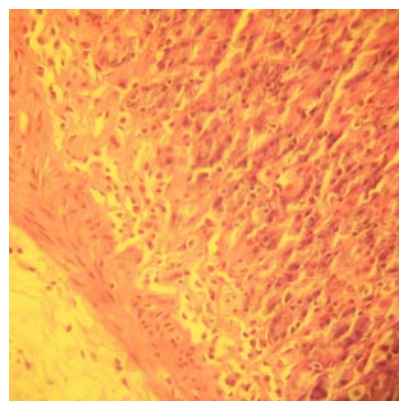

Fig. 1: Section of stomach (control), showing transverse section of fundic glands with chief cells at the base (H and E stain, ×400)
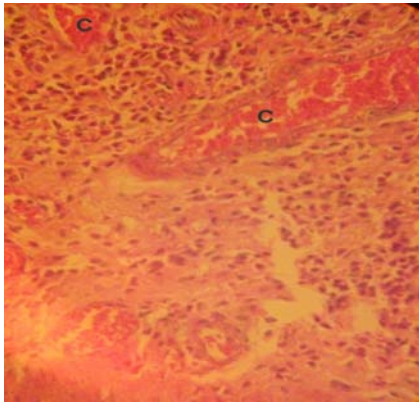
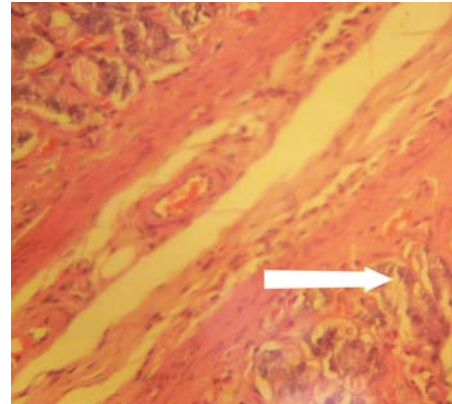Fig. 2: Section of stomach after 1st day of last feeding, showing congestion of blood vessels (H and E stain, ×400)
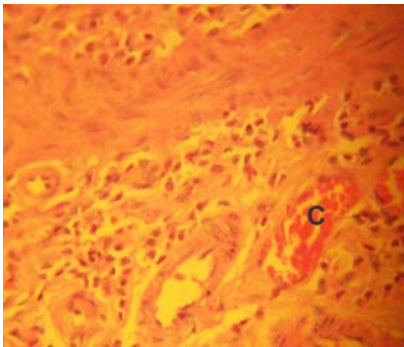

Fig. 3: Section of stomach after 1st day of last feeding, showing congestion of blood vessels of submucosal layer (H and E stain, ×400)
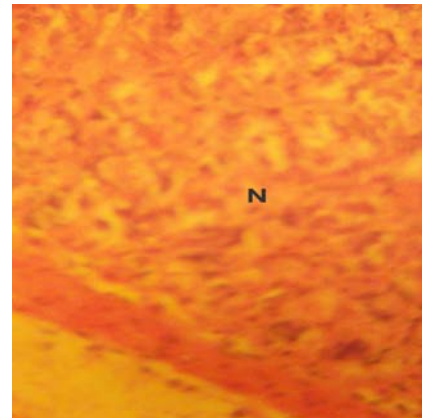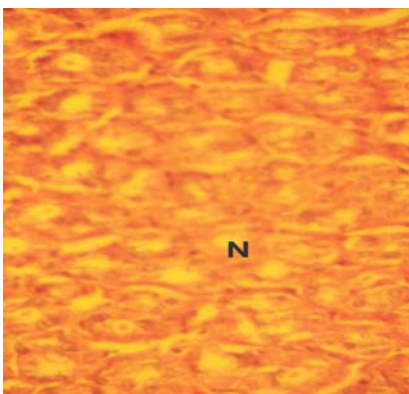

Fig. 4: Section of stomach after 2nd day of last feeding, showing necrosis of the base of fundic glands (H and E stain, ×400)

Muscular layer had normal histological appearance. In the 48th h, these changes became more obvious and degeneration and necrosis of fundic glands were

Fig. 5: Section of stomach after 2nd day of last feeding, showing degeneration and necrosis of fundic glands (arrow) (H and E stain, ×400)

Fig. 6: Section of stomach after 3rd day of last feeding, showing necrosis of fundic glands especially the base of fundus (H and E stain, ×400)

observed (Fig. 4 and 5). In addition to this, a partial destruction was observed in the surface epithelium. There was also degeneration in parietal cells morphologically. At the end of the 3rd day of hunger, necrosis of fundic glands, especially the base of fundus (Fig. 6) and mild Congestion of mucosal blood vessels (Fig. 7) were seen. It was observed that stomach glands of the rats had widened and there were disintegration in mucus cells and shrinkages in parietal cells. Necrosis of fundic glands were also seen at the end of the 4th day of hunger (Fig. 8) and congestion of blood vessels at the mucosa of the stomach (Fig. 9 and 10). At the end of the 5th day of hunger, necrosis of gastric glands and congestion of submucosal blood vessels were observed (Fig. 11 and 12). It was detected that the muscular structure was degenerated, parietal cells were suffered from structural destruction and there was a rise in the number of cells. Hunger is a situation affecting living organisms
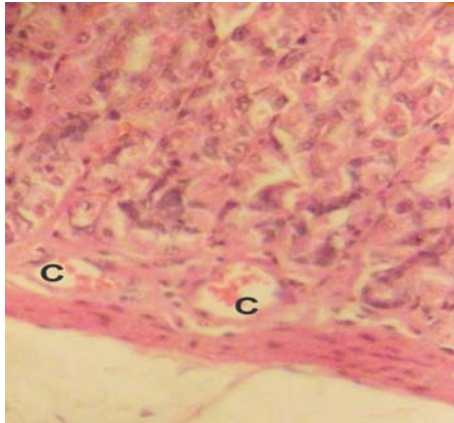
Fig. 7: Section of stomach after 3rd day of last feeding, showing mild Congestion of mucosal blood vessels (H and stain, ×400)
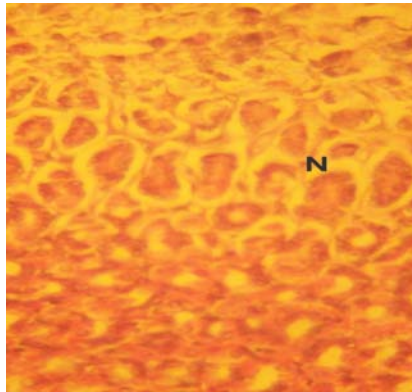

Fig. 8: Section of stomach after 4th day of last feeding, showing necrosis of fundic glands (H and E stain, ×400)
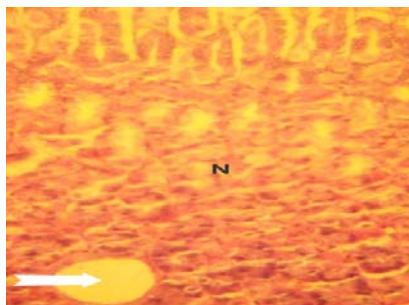

Fig. 9: Section of stomach after 4th day of last feeding, showing necrosis of mucosal area with unusual blood vessels (H and E stain, ×400)
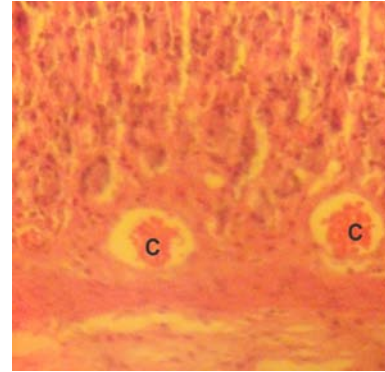

Fig. 10: Section of stomach after 4th day of last feeding, showing congestion of blood vessels at the mucosa of the stomach (H and E stain, ×400)
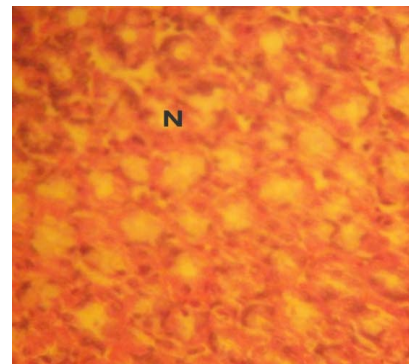

Fig. 11: Section of stomach after 5th day of last feeding, showing necrosis of gastric glands (H and E stain, ×400)
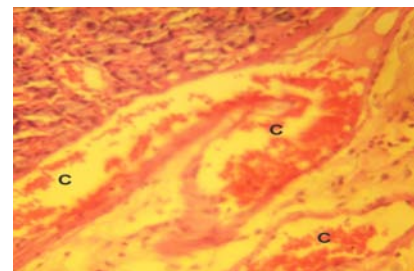
Fig. 12: Section of stomach after 5th day of last feeding, showing congestion of submucosal blood vessels (H and E stain, ×400)

metabolically and structurally. Secretions of the cells located in stomach glands enable the nutrients in the stomach to be decomposed and the digestion process to be started. There are studies which focus on the changes in various cells of the stomach mucosa due to short-term and long-term hunger and examine these changes under the light and electron microscopes. In these studies, histological findings varied upon the genus, age and hunger level of the selected animal were obtained (Koc and Muslu, 2007; Sonmez and Ozan, 2005; Colakolu *et al.*, 1999; Ucar *et al.*, 2004). Blood pressure

and gastrin levels and body weight lower down significantly depending upon the intensity level of hunger (Sonmez and Ozan, 2005; Colakolu *et al.*, 1999; Ucar *et al.*, 2004). In contrast to the fluid which is a response to cellular dehydration and hypovolemia (Toth and Gardiner, 2000), the knowledge of why animals feel hunger is still poor (Smith, 2009). The size of the last meal is probably more important than the length of time since that meal which suggest that calorie intake is the most important factor (Friedman and Stricker, 1976). A feeling of satiation is probably also important for animals as it is for human, this feeling is in turn influenced by a range of factors, not just the degree of distention of the stomach.

The loss of the feeling of satiation is probably more important for the re-initiation of feeling than a feeling of hunger. The lack of knowledge about the factors controlling feeding behavior makes it difficult to evaluate the effects of fasting which is complicated by the fact that even adult animals often exhibit marked variations in body weight and feed intake. For example, even genetically identical mice (B6C8F1) can vary in weight from 30-48 g (Allaben *et al.*, 1996). Feed intake and calorie demands can in addition vary between genetic lines, age groups environments, physiological status and the opportiunity to undertake physical exercise. Animals will also often overeat when large amounts of food are continually available. The body has better mechanisms for avoiding overhydration than it does for limiting overeating (Smith, 2009). In this study, the changes in stomach mucosa occurring during hunger were examined. It was assumed that hunger causes thinning in the mucus layer of stomach epithelium surface.

The first changes in the stomach mucosa resulting from hunger were detected at the end of the first 24 h following last feeding. In this period, it was observed that the lumen of stomach glands widened. The surface epithelium became flattened and degenerated. Colakolu *et al.* (1999) observed the widening in gland lumen after 3 days of hunger. Alvares (1992) detected no change in stomach mucosa of the mice after 18 h of hunger. Zaviacic *et al.* (1977) observed that there were some pouring in the surface mucus cells and shrinkages in the parietal and main cells after the 72 h of hunger. Matsumoto *et al.* (1989) observed that stomach ulcers occur in mice due to hunger.

Jacobs and Sturtevent (1982) found that there are more multi-vesicular elements in normally and limitedly feeding animals than the case in hungry animals. Furthermore, they detected that some irregularities take place in the granular endoplasmic reticulum of parietal cells during hunger. In that study, some degeneration was observed in stomach mucosa of the rats which were kept hungry in 5 days but no sign of ulcer was observed. In this study, it was seen that the surface mucus cells get poured and some cells became shrunk after 3 days of hunger. It was found that the microvillus structures of parietal cells in the stomach were affected from hunger. In the 4 and 5th days of hunger, the tendency of shortening and becoming sparse was observed. Another change occurred in the stomach mucosa due to hunger is the degeneration and pouring in the surface epithelium. It was seen that there was some pouring in the surface mucus cells during the 3rd day of hunger.

CONCLUSION

In this study, it was noticed that hunger causes an obvious reduction in the mucus mucosa of stomach epithelium surface. Considering this study and other related studies, researcher reach the conclusion that stomach mucosa is affected and some histo-pathological changes occur under hunger stress depending upon the duration of hunger.

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