Original Article

A Histological Study of the Rat Duodenal Mucosa During Pre- and Post-weaning Periods

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ABSTRACT

Background: Changes in food constituent during suckling, weaning and post-weaning periods documented histological and functional adaptation in rat small intestine.

Objectives: Identify histological changing events, which represent such adaptation in the rat duodenal mucosa.

Material and Methods: Twenty four male rat pups are divided into equal 4 groups including day one, 7 &15 (pre-weaning) and day 21 (weaning). In addition, two other groups including 12 male adult rats equally divided, aged 45 days (post-weaning) and 90 days, were used. The animals are euthanized at the fore mentioned days and the duodenum is processed for light and scanning electron microscopic examination. Paraffin sections are stained with haematoxylin & eosin and combined alcian blue/ PAS. Computer image analysis and statistical study are done for the height of the villi, depth of the crypts and villous / crypt ratio.

Results: The villi are broadening with the advance of age. The height of the villi and the depth of the crypts show a statistical non-significant increase in the pre-weaning groups. At weaning, there is a significant decrease in the height of the villi with a decrease of the villous/crypt ratio. The villi are covered by brushed columnar enterocytes and some vacuolated enterocytes are seen only in pre-weaning period. Acidic and neutral mucin positive stained goblet cells are distributed on villi and within the crypts of pre-weaning pups. At weaning, goblet cells are positive for acidic mucin only. Crypts are lined with pyramidal enterocytes and paneth cells are dominating in day 21. Both villi and crypts at postnatal day 21(weaning) acquire structural appearance similar to that of adult.

Conclusion: This study shows the structural changes that occur at weaning in the mucosa of the duodenum in the rat, coinciding with the transition of food from milk to solid food. This illustrates the importance of enteral nutrition during the weaning process in animals and humans, and the expected dangers of intravenous feeding in the early stage of infancy.

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Key Words: Intestine; postnatal development; rat; weaning.

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INTRODUCTION

The mucosa of the small intestine of the rat passes through several morphological and physiological adaptive changes during postnatal development[1]. In addition to diet, age, hormones (glucocorticoids) and growth factors (epidermal growth factor) are important factors affecting postnatal gastrointestinal tract development[2]. Significant postpartum intestinal tissue changes occurs in response to initial starting of feeding[3]. The intestinal epithelium is stimulated via specific enteral nutrients and secrets trophic hormones that accelerate its proliferation and decrease apoptosis[4]. The circadian rhythm of cell proliferation that occurs in rat small intestine during the first three weeks after birth is concurrent with diet[5].

In humans, breast feeding and weaning influence the small intestine growth and morphology also[6]. During the third week or weaning, the milk-based diet is replaced by solid food and is accompanied by growth and

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maturation acceleration of small intestine in rat[7]. Rat's intestinal mucosa is altered by early weaning, which affects food absorption[8].

Morphological and functional alteration of intestinal mucosa occurred in response to types and constitution of different food molecules and reaches constant state in adulthood[9].

Reviewing the literature, some studies were carried on the rat jejunum[5] and on human small intestine[6] regarding the effect of weaning on epithelial growth, but few studies on rat duodenal mucosa are met with concerning weaning[1]. Accordingly, the current study aims to demonstrate the histological changes in rat duodenal mucosal layer in the pre-and post-weaning periods.

MATERIALS AND METHODS

Animals

Pregnant albino rats (Wister strain) were obtained from the animal house, Faculty of Medicine, Ain -Shams University. Animals were left to deliver spontaneously. The pups with their mothers from birth up to 21 days of age were housed in standard conditions. After 21 days, pups were weaned and caged separately. In addition, 12 male adult rats aged 45 days (n= 6) and 90 days (n=6) were used. All animals received standard rat solid pellets and water ad libitum with suitable environmental conditions and good ventilation. The animals were maintained according to guidelines and principals of the Committee of Animal Research Ethics (CARE). Male pups were sorted into 4 groups (N= 6). All animals were euthanized under deep ether anaesthesia by cervical dislocation at the following postnatal days:

Pre -weaning period; Group I: day one aged pups (just 3-6 hours after birth, started suckling), group II: day 7 and group III: day 15.

Weaning; Group IV: day 21

Post-weaning period; Group V: day 45 (young adult).

Group V I: day 90 (adult).

Light microscopic study

The duodenum of all groups was extracted, and specimens from the proximal part were fixed in 10% neutral formalin. Paraffin embedded transverse sections (5 µ thick) were prepared, stained with haematoxylin and eosin (H&E) for general structure[10]. For visualization of mucins of mucosal cells, the sections were stained with combined alcian blue-PAS for acid and neutral mucins, respectively[11]. Acid mucins were stained blue color, neutral mucin stained magenta color while mixed neutral and acidic mucins were stained dark blue or purple. Light microscope (Olympus 268 M) coupled via digital camera to a computer was used to examine and photograph all sections.

Scanning electron microscopic examination

Specimens from duodenal mucosa were pinned flat to prevent curling and fixed in 4% paraformaldehyde and 3 % glutaraldehyde in 0.1 M cacodylate buffer then were processed for scanning electron microscopic study[12]. Specimens were examined by scanning electron microscope (Philips-XL30, 8 kV) at the Electron Microscope Unit, Anatomy Department, Ain Shams University.

Morphometric study (Computer image analysis)

The height of duodenal villi and depth of crypts were measured in um from five non-overlapping fields in five different haematoxylin and eosin stained sections, (using the microscope objective lens of X10) and from five different animals in each group, using an image analyzer Leica (Q 500 MC program). Villus height was taken starting from villus apex to its junction with intestinal crypt. Crypt depth was also measured from the base till the junction of villi.

Statistical analysis

Statistical analysis was done by using the Statistical Package for Social studies (SPSS) program version 17. Comparison of means was done using one-way analysis of variance (ANOVA) test with Bonferroni Post Hoc comparison between groups. *P-values* equal or less than 0.05 were considered significant and those equal or less than 0.001 were highly significant. Data were represented in tables and histograms.

RESULTS

1. Histological results

Pre-weaning period (postnatal days one, 7 and 15)

Examination of haematoxylin and eosin (Hx & E) stained sections revealed the different layers of the duodenum; mucosa, submucosa, musculosa and serosa. The wall thickness and the perimeter showed apparent increase in day 7and 15 more than day one. The mucosa was formed

of multiple villi between the bases of which were crypts of Lieberkühn. The density of the villi and crypts, heights of villi and depths of crypts were found to be increased by day 7 and 15 (Figures 1 a,b,c). At postnatal day one, the villi were short, few and have broad apices and shallow crypts with relatively wide lumina (Figure 2 a). At day 7 and 15, the villi increased in height and width and the crypts increased in depth, too. (Figures 2 b,c). At day 7, the mucosa became differentiated from the underlying submucosa by the presence of very thin layer of muscularis mucosae, which consisted of 1-2 smooth muscle layers at the base of the crypts (Figure 2 b). At day 15, long villi were seen alternating with short ones. The muscularis mucosae (2-3 muscle thick) was seen separating the mucosa from the submucosa which became more differentiated with prominent blood vessels (Figure 2 c). In the preweaning period, the epithelial cells covering the villi and lining the crypts included the enterocytes (surface absorptive cells), goblet cells and paneth cells. In day one &7, the villi were covered mainly by low columnar brushed border enterocytes. Numerous enterocytes showed vacuoles of different sizes (Figures 3 a,b). At day 15, most of the enterocytes were not vacuolated (Figure 3 c). The lamina propria of the mucosa, at day one appeared undifferentiated. The core of the villi showed thin walled endothelial lined spaces, some of them with blood cells and others appeared empty and dilated (Figure 3a). In days 7 and 15, the core of the villi became more cellular with many capillaries. The endothelial lined spaces were narrowed (Figures 3 b,c).The duodenal crypts in postnatal day one, 7 and 15 were lined by pyramidal enterocytes with basophilic cytoplasm and rounded basal nuclei (Figures 4 a,b,c). Paneth cells could be identified at day 7 as single or twin cells located in the basal parts of the crypts. They were recognized by their apical acidophilic granules (Figure 4 b) and were seen more frequently in day 15 (Figure 4 c).

In combined alcian blue-PAS stained sections, postnatal day one and 7 showed few positively stained goblet cells with acidic and neutral mucin present away from the vacuolated cells along the sides of the villi and within the crypts (Figures 5 a,b). In postnatal day 15, many positively stained goblet cells with acidic mucin were present along the villi and within the crypts. Few goblet cells were positively stained with neutral mucin (Figure 5 c).

In scanning electron micrographs, at postnatal day one, the villi appeared as finger like projections. The apical brush borders of the polyhedral enterocytes were recognized with their velvety appearance. The vacuolated cells could be seen as ballooned ones in-between the enterocytes while the mucus secreting goblet cells were identified as depressed spots (Figure 6 a). In day 7, the enterocytes, the vacuolated cells and the goblet cells were also recognized with same picture as in day one (Figure 6 b). In day 15, the ballooned (vacuolated) cells couldn't be recognized while the goblet cells were numerous (Figure 6 c).

Weaning period (postnatal day 21)

On examining Hx & E stained sections, the wall thickness and the perimeter of the duodenum showed an apparent increase. The density of the villi and crypts and the depth of the crypts increased while the height of the villi showed apparent decrease (Figure 7 a). The villi tended to vary in width and shape and were closely packed with narrow inter-villous space. Frequent joined villi were seen (Figure 7 b). The enterocytes became tall columnar cells and goblet cells were more dominating, well developed and scattered along the whole length of villi. Multiple transverse side furrows were seen. The villi core showed increased cellularity (Figure 8 a). The muscularis mucosae was seen separating the mucosa from the submucosa. In the duodenal crypts, group of paneth cells were clearly seen and goblet cells were more prominent, dominating and larger. Mitotic figures were frequently encountered as well (Figure 8 b).

Combined alcian blue-PAS stained sections revealed the acidic nature of the mucin secreting goblet cells between enterocytes in the villi and the neutral PAS positive brush border of enterocytes (Figure 9 a). Larger positively stained goblet cells were prominent and dominating in the crypts (Figure 9 b).

Scanning electron micrographs showed broad villi with multiple transverse side furrows and some goblet cells between enterocytes (Figures 10 a,b).

Post-weaning period (postnatal day 45) and adult (90 days old rat)

In Hx & E stained sections, both days 45 and 90 showed marked apparent increase in the wall thickness and the perimeter of the duodenum. The density of the villi and crypts and the depth

of the crypts increased as well (Figures 11 a,b). In day 45, the villi were numerous and elongated while the crypts were relatively shorter with high villus/crypt ratio (Figure 11 a). At day 90, the villi decreased in height and still joined villi could be seen (Figure 11 b). The villi were covered by tall columnar enterocytes in day 45 that exhibited well defined brush border and many goblet cells. Transverse side furrows were scarcely seen. The villus core was highly cellular with many lymphocytes (Figure 11 c). Similar picture was observed in day 90 with many large cup shaped goblet cells (Figure 11 d). The crypts in day 45 and 90 were similar with many large goblet cells and some paneth cells (Figure 12 a,b).

In combined alcian blue-PAS stained sections, both postnatal day 45 and 90 showed numerous goblet cells with typical globular appearance. The cells showed positive alcian blue staining of acidic mucin (blue color) along the villi and within the crypts. Also, positive PAS staining of neutral mucin along the brush border of the enterocytes and the apical border of acini forming the Brunner's glands (magenta color) (Figures 13 a,b,c,d).

Scanning electron micrographs of day 45 showed group of enterocytes with well-developed brush border and some goblet cells in-between (Figure 14 a). Day 90 showed similar picture but more goblet cells appeared as irregular white buttons and interruption of cell continuity and presence of cell remnants were observed (Figure 14 b).

Morphometric results

The mean height of the villi (Table 1), the mean depth of the crypts (Table 2) and the villus / crypt ratio (Table 3) of the duodenum were measured in the different postnatal ages and were represented in (Histogram I).

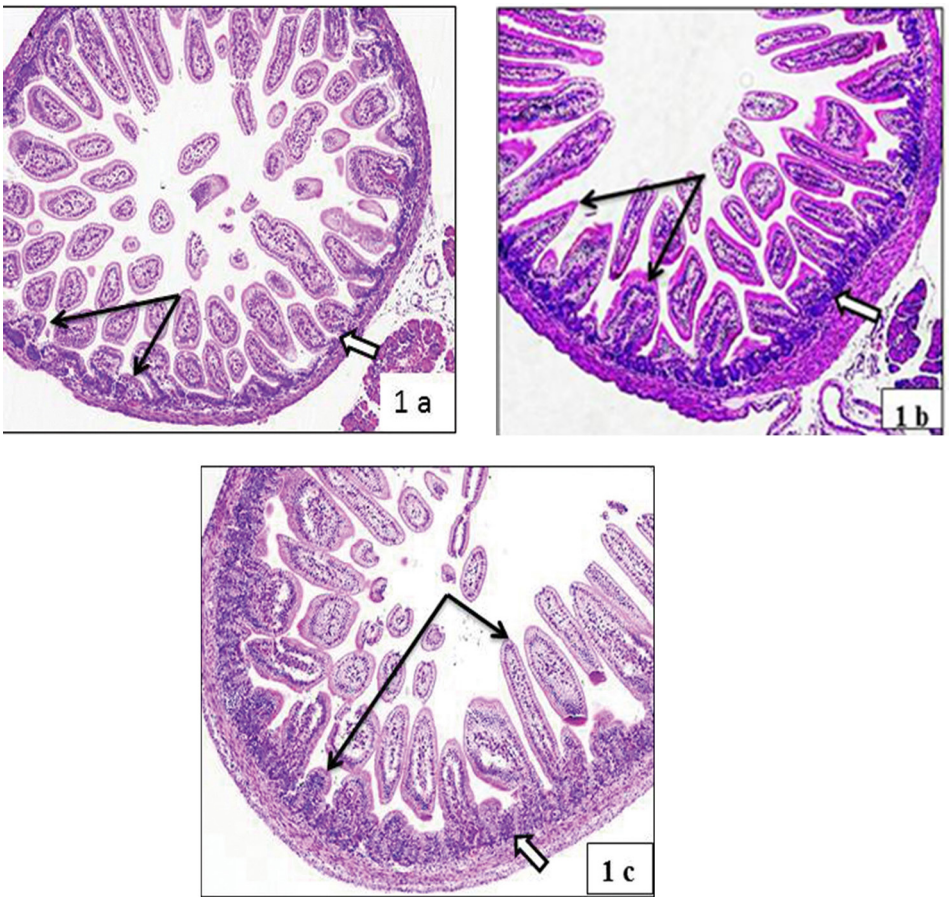


Fig. 1: Photomicrographs of transverse sections of rat duodenum during the pre-weaning period (a. day one, b. day 7 & c. day 15) showing **a:** the different layers . Note that the mucosa is formed of villi (thin black arrows) alternating with crypts (white arrow). **b:** an increase in the density of villi (thin black arrows) & crypts (white arrow). **c:** an increase of its perimeter. Note the apparent increase in the density of long villi alternating with short ones (thin black arrows) and the crypts (white arrow). (H. & E. a, b & c X 100)

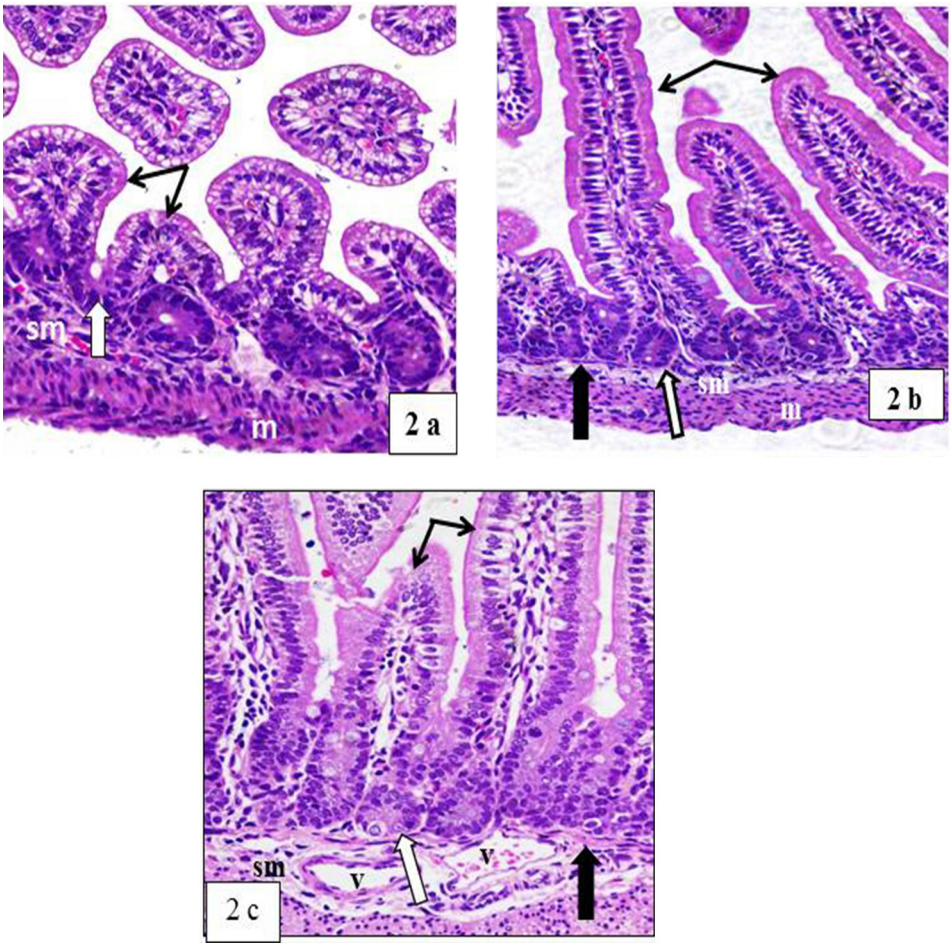


Fig. 2: Photomicrographs of transverse sections of rat duodenum during the pre-weaning period (a. day one, b. day 7 & c. day 15) showing **a:** short broad shaped villi (thin black arrows), shallow crypts (white arrow), submucosa (sm) and musculosa (m). **b:** mild increase in height &width of the villi (thin black arrows) &in depth of crypts (white arrow). Note the presence of one layer of muscularis mucosae (thick black arrow), ill-defined submucosa (sm) and musculosa (m). **c:** showing long villi alternating with short ones (thin black arrows) & crypts (white arrow). Notice the well-developed muscularis mucosae (thick black arrow) & submucosa (sm) with prominent blood vessels (v). (H. & E. a, b & c x 200)

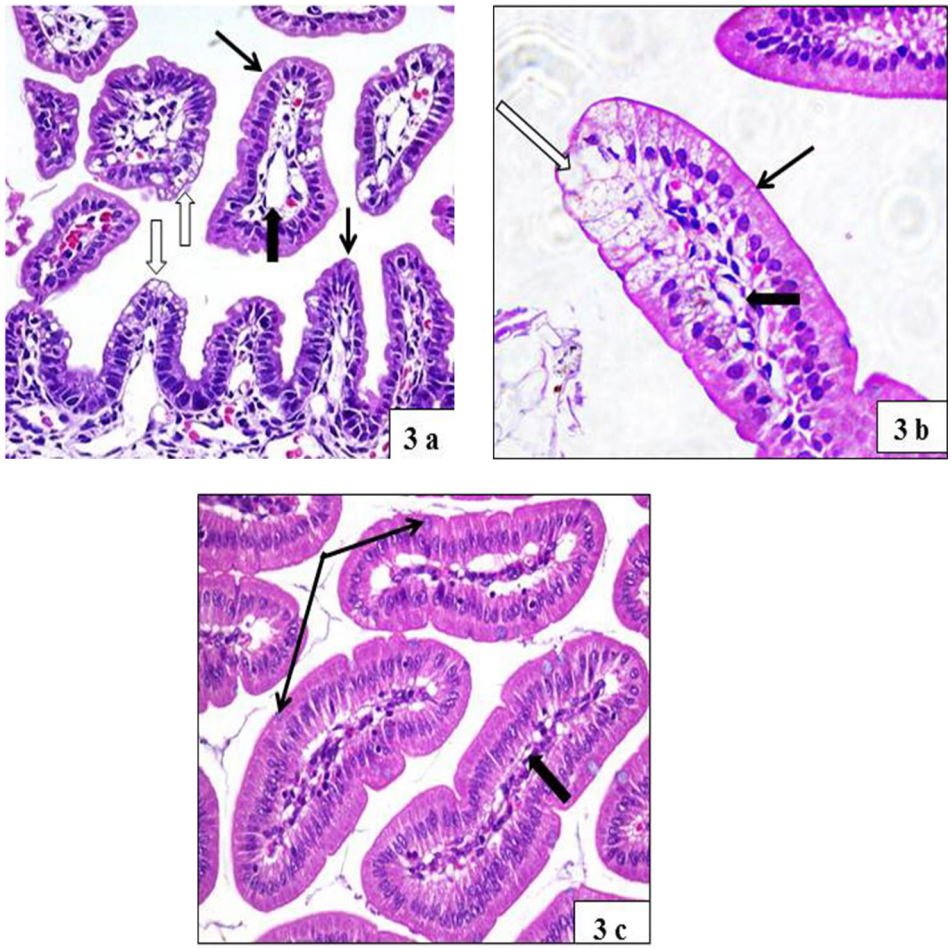


Fig. 3: Photomicrographs of transverse sections of rat duodenum during the pre-weaning period (a. day one, b. day 7 & c. day 15) showing **a:** villi lined with low columnar enterocytes with well-defined brush borders (thin black arrows). Notice numerous cells with vacuolated cytoplasm (white arrows). The villous core shows thin walled endothelial lined spaces, some of them show blood cells and others appear dilated and empty (thick black arrow). **b:** the presence of vacuolated cells (white arrow) and enterocytes with well-defined brush borders (thin black arrow). Note the cellular villous core with narrow thin walled endothelial lined spaces (thick black arrow). **c:** most of the enterocytes are non-vacuolated with many goblet cells (thin black arrows). The villous core shows cellular density and many capillaries (thick black arrow). (H. & E. a, b & c X 400)

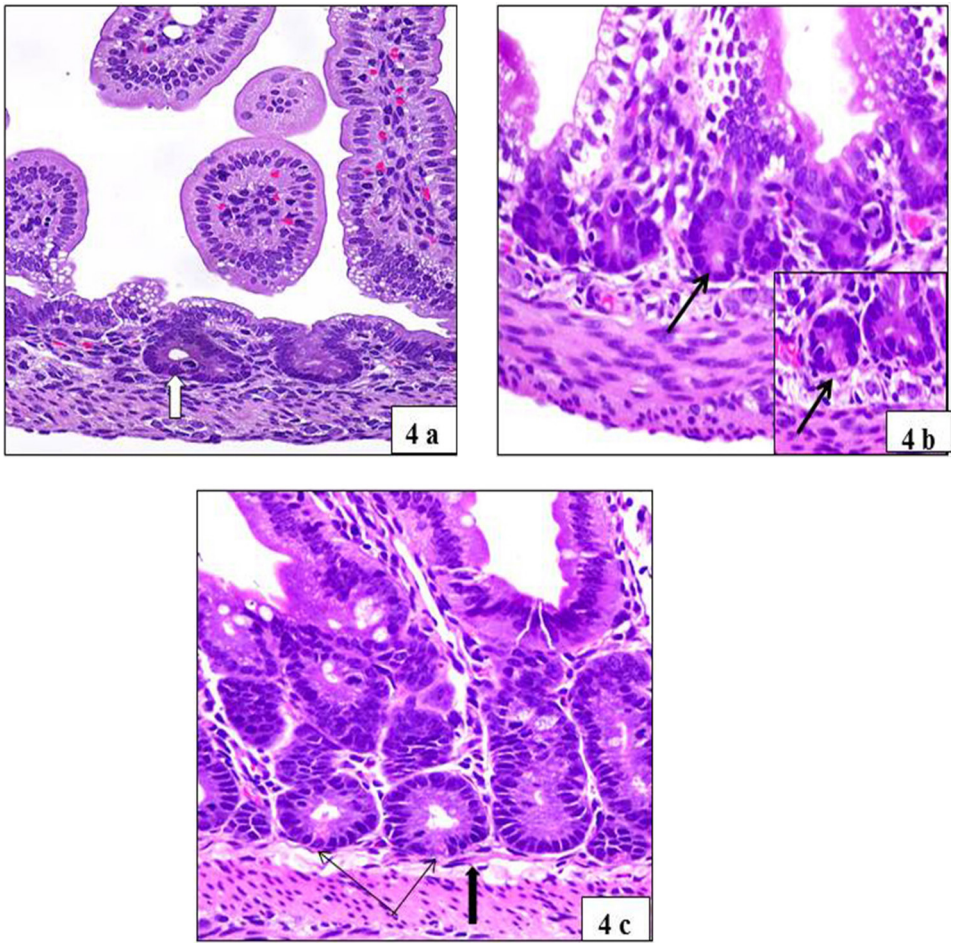


Fig. 4: Photomicrographs of transverse sections of rat duodenum during the pre-weaning period (a. day one, b. day 7 & c. day 15) showing **a:** the shallow short crypts (white arrow) are lined by pyramidal cells with basophilic cytoplasm. **b:** enterocytes and paneth cells with granular acidophilic cytoplasm at the base of the crypts (black arrow). Inset: black arrow points to paneth cells. **c:** enterocytes and paneth cells with granular acidophilic cytoplasm at the base of the crypts (thin black arrows). The thick black arrow points to muscularis mucosae. (H. & E. a, b, c & Inset in b X400)

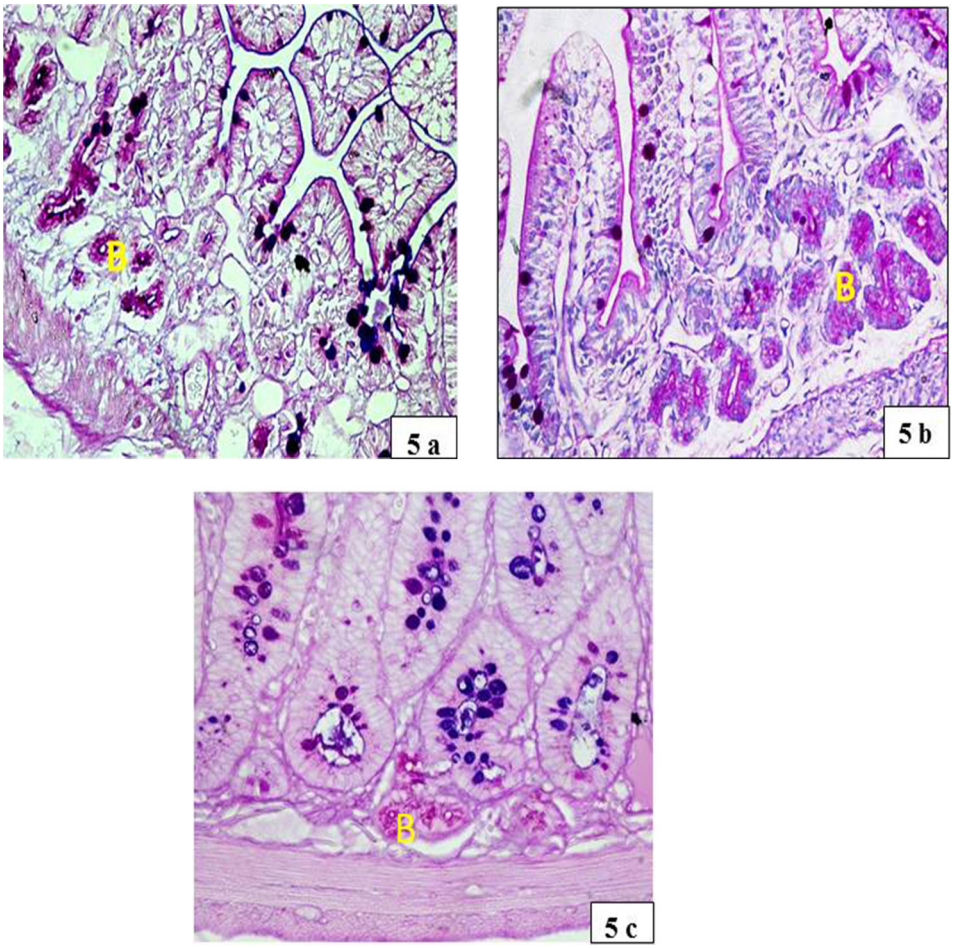


Fig. 5: Photomicrographs of transverse sections of rat duodenum during the pre-weaning period (a. day one, b. day 7 & c. day 15) showing **a:** the positive staining of few goblet cells with acidic and neutral mucin (purple colour) especially away from vacuolated cells along the side of the villi and within the crypts. The Brunner's glands (B) show neutral mucin secretion (magenta colour). **b:** few mucin secreting goblet cells (purple color) on the villi especially away from vacuolated cells and within the crypts. The Brunner's glands (B) show mucin secretion (magenta colour). **c:** the basal parts of the villi and the crypts rich in +ve mucin secreting goblet cells (purple colour) with few ones with magenta colour. The Brunner's glands (B) have neutral mucin secretion (magenta colour). (Combined alcian blue-PAS. a, b & c X 400)

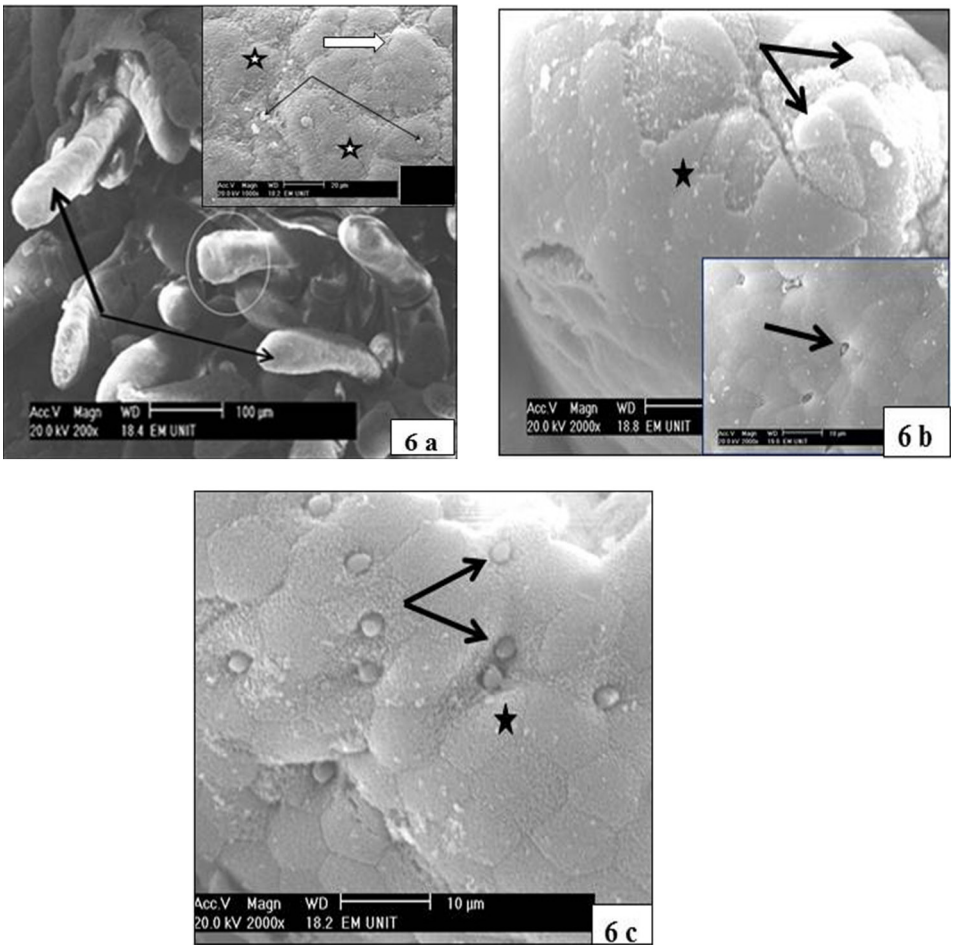


Fig. 6: Scanning electron micrographs of rat duodenum during the pre-weaning period (a. day one, b. day 7 & c. day 15) showing **a:** the finger like projecting villi of the mucosa (black arrows). Inset: the apical border of polyhedral enterocytes have velvety appearance (*) with few ballooned cells in between (white arrow). Note also the mucous secretion of goblet cells (black arrows). (a X 200 & inset X 1000) **b:** the apical border of enterocytes with a velvety appearance (*) and few ballooned cells in-between (arrows). Inset: Note that the goblet cells appear as depressed spots (arrow) among the polyhedral shaped enterocytes. (b & inset X 2000) **c:** the apical border of enterocytes with a velvety appearance (star) with many mucous secreting goblet cells in between (arrows). (x 2000)

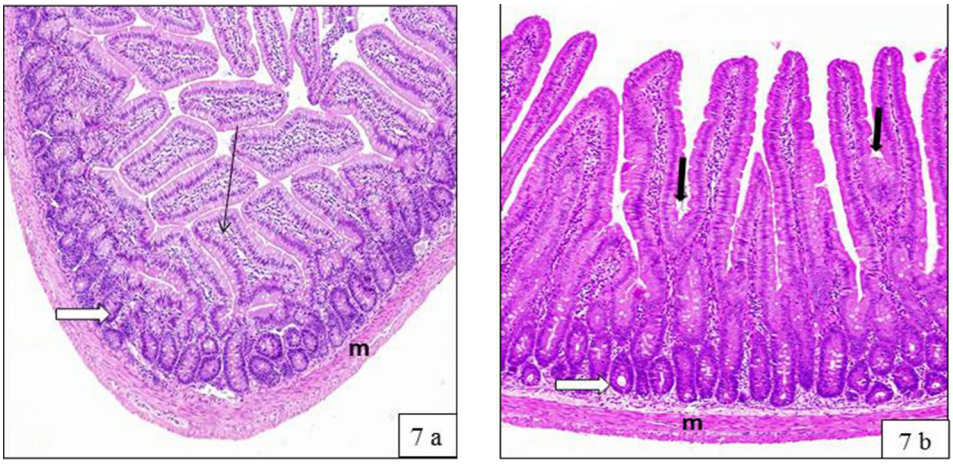


Fig. 7: Photomicrographs of transverse sections of rat duodenum at weaning (day 21) showing **a:** the apparent increase in density of the villi (black arrow) and increased density and depth of the crypts (white arrow). Notice narrow submucosa & developed musculosa (m). **b:** the villi vary in width and shape and are closely packed. Note frequent joined villi (black arrows). (H. & E. aX40 & bX100)

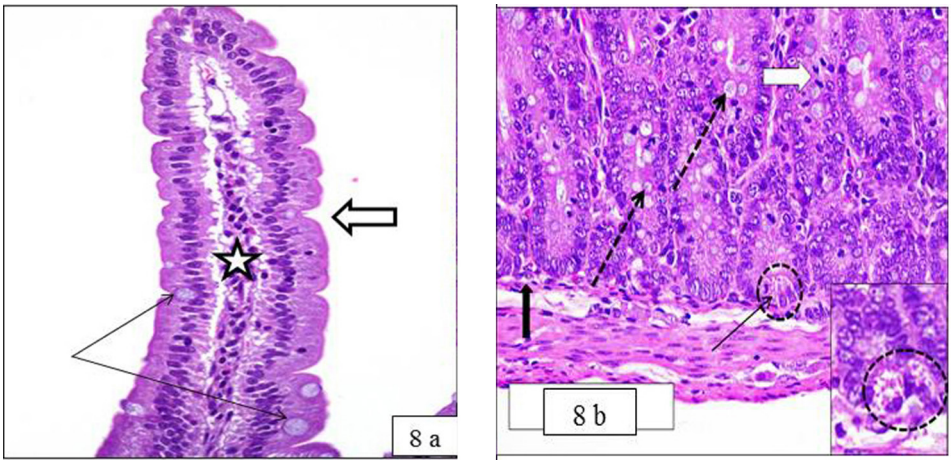


Fig. 8: Photomicrographs of transverse sections of rat duodenum at weaning (day 21), showing **a:** multiple transverse furrows (white arrow) of one villous that is covered by tall enterocytes and many goblet cells (thin arrows). Notice also high cellularity of the villous core (star). **b:** the crypts are lined with pyramidal enterocytes. Goblet cells (dotted arrows) &paneth cells (circle) are clearly seen. Numerous mitotic figures (white arrows) are encountered. The thick black arrow points to muscularis mucosae. Inset: shows a group of paneth cells with granular apical acidophilic cytoplasm at the base of the crypt (circle). (H. & E. aX400, bX200 & insetX400)

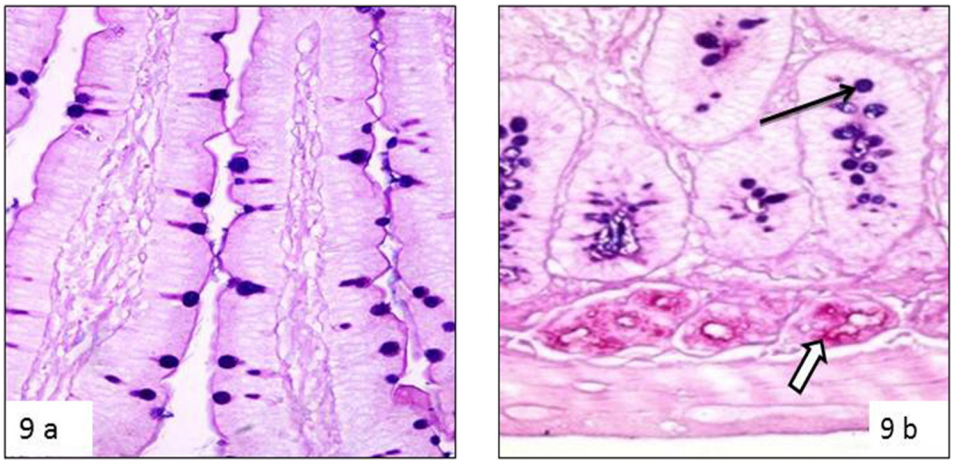


Fig. 9: Photomicrographs of transverse sections of rat duodenum at weaning (day 21) showing **a.** the acidic nature (blue colour) of the +ve mucin secreting goblet cells between enterocytes in the villi and the neutral +ve brush border of enterocytes (magenta colour). **b.** the acidic nature (blue colour) of the +ve mucin secreting goblet cells of the crypts (black arrow). The Brunner glands show neutral mucin secretion (magenta colour) (white arrow). (Combined alcian blue- PAS. a ,b X 400)

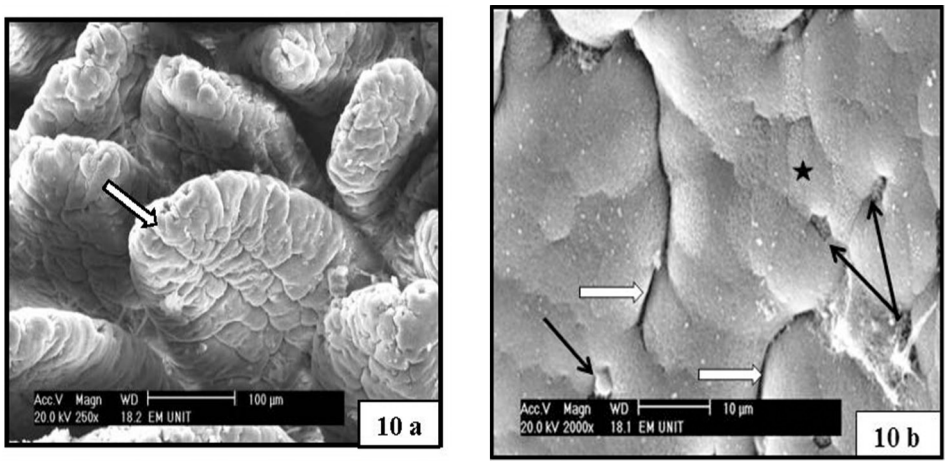


Fig. 10: Scanning electron micrographs of rat duodenum at weaning (day 21) showing **a.** broad villi with multiple transverse side furrows (arrow). **b.** multiple furrows of the villi (white arrows) with goblet cells (black arrows) between the enterocytes(star). (a X 250 & b X 2000)

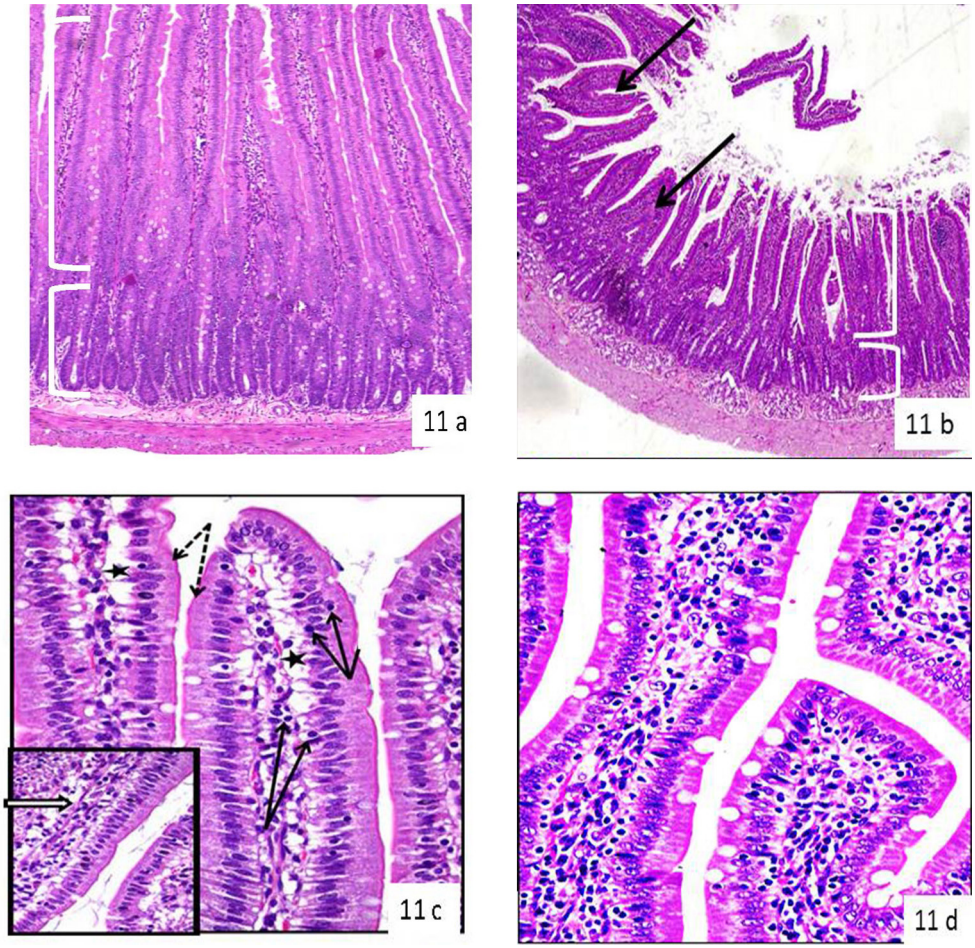


Fig. 11: Photomicrographs of transverse sections of rat duodenum in postweaning period (a & c day 45 and b &d day 90) showing **a.** numerous elongated villi &relative shorter crypts with high villus/crypt ratio (white brackets). **b.** apparent decrease in the height of the villi with short villus/crypt ratio (white brackets). Note the presence of the joined villi (black arrows). **c.** the upper part of villi lined by tall columnar enterocytes and well defined brush border (dotted arrows). Notice highly cellular villous core (black star). The black arrows point to lymphocytes. Inset: The white arrow points to smooth muscles in the villous core. **d.** numerous large cup shaped goblet cells in between the tall columnar enterocytes. (H. & E. a&b X100, c& inset X400, d X 600)

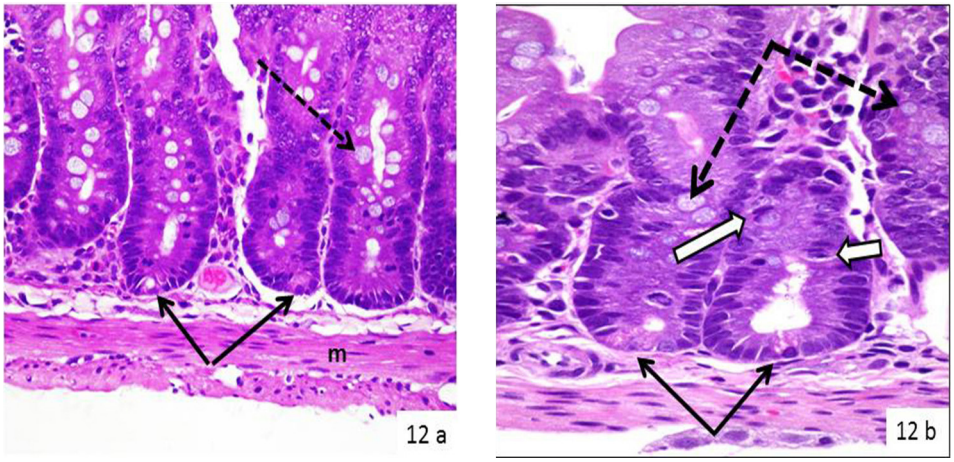


Fig. 12: Photomicrographs of transverse sections of rat duodenum in postweaning period (a. day 45 and b. day 90) showing **a.** the lining epithelium of the crypts; mainly enterocytes, goblet cells (dotted arrow) & paneth cells (black arrow). Notice the well-developed musculosa (m). **b.** the enterocytes lining the crypts together with large goblet cells (dotted arrows) and paneth cells (black arrows). Notice the frequent mitotic figures within the crypts (white arrow). (H. & E. a X400 & b X 600)

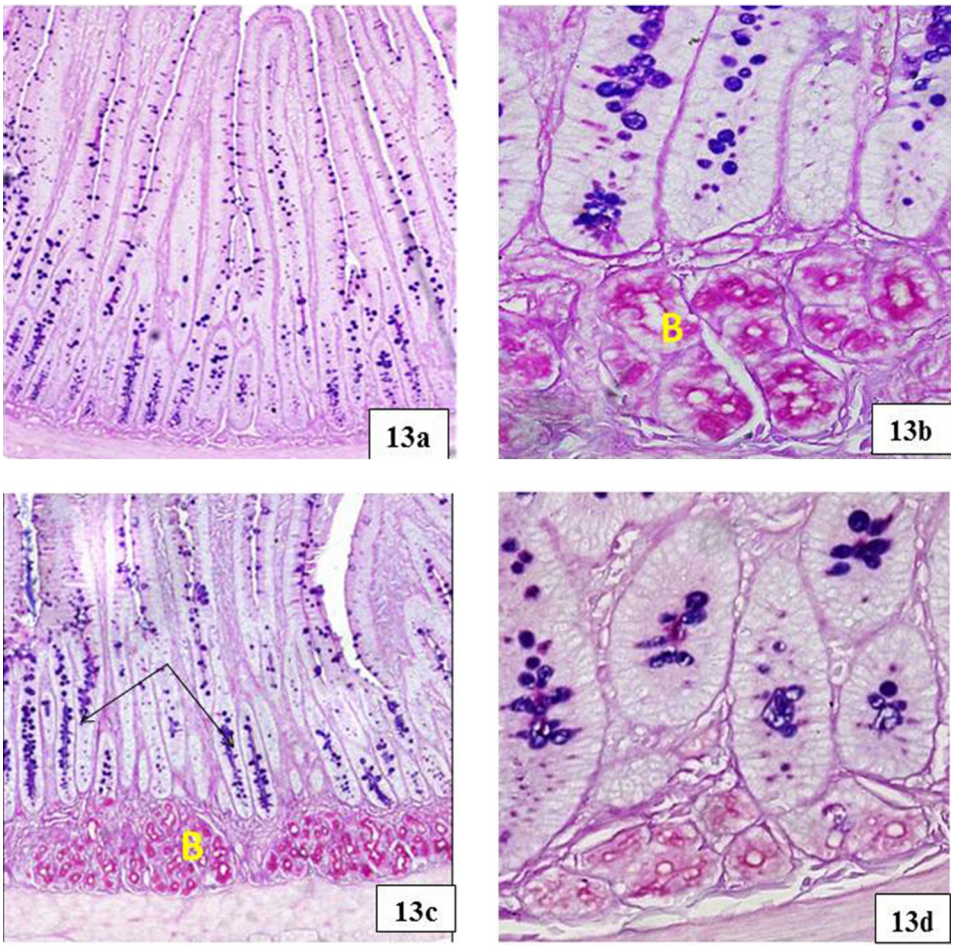


Fig. 13: Photomicrographs of transverse sections of rat duodenum in post-weaning period (a & b day 45 and c & d day 90) showing **a.** Neutral mucin (magenta colour) along the brush border of the enterocytes and acid mucin (blue colour)in the goblet cells along the villi & among the crypts. **b.** the globular appearance of goblet cells with acidic mucin secreting (blue colour) between other enterocytes. Notice the positive PAS neutral mucin staining (magenta colour) of the apical border of acini forming the Brunner's glands (B). **c.** numerous alcian blue +ve goblet cells in crypts. B =Brunner's gland. **d.** typical globular appearance of alcian blue +ve goblet cells. (Combined alcian blue-PAS. a,c X 100 & b,d X400)

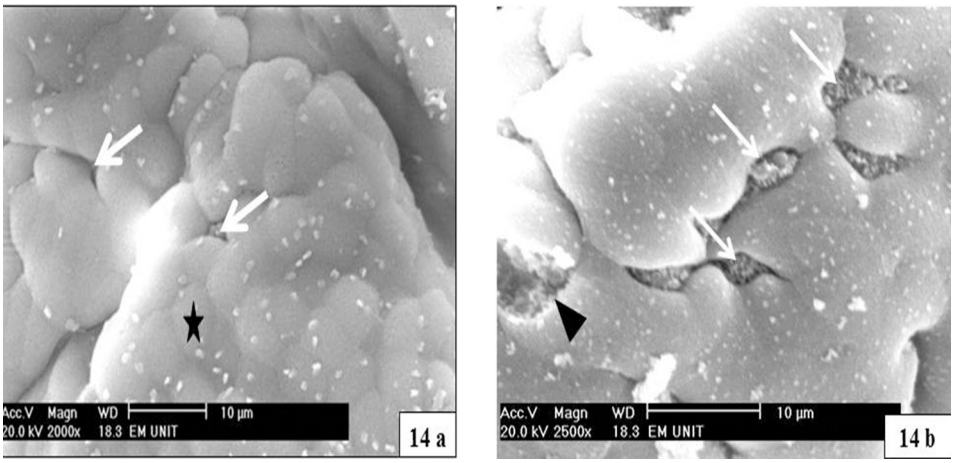


Fig. 14: Scanning electron micrographs of rat duodenum in post-weaning period (a. day 45 and b. day 90) showing **a.** the lateral border of a duodenal villus revealing a group of apical border of enterocytes with well-developed brush border (star) and goblet cells (white arrows). **b.** the upper border of a group of enterocytes and several irregular white buttons (white arrows) between enterocytes containing secretion of goblet cell. Notice interruption of cell continuity and presence of cell remnants (head arrow). (a X2000 & b X 2500)

Table 1: The mean height and standard deviation (SD) of villi in um in the duodenum in different postnatal ages and their significance

Postnatal ages	Height of Villi (mean±SD)	*P-value*
Day 1	245.12 ± 106.57	
Day 7	299.20 ± 113.49	0.205
Day 15	376.35 ±156.36	0.147
Day 21	273.71 ± 92.17	0.044*
Day 45	713.60 ± 269.93	0.000004**
Day 90	440.13 ± 83	0.00124***

* Significant decrease in day 21 as compared to day 15
** Highly significant increase in day 45 as compared to day 21
***Significant decrease in day 90 as compared to day 45

Table 2: The mean depth and standard deviation (SD) of crypts in um in the duodenum in different postnatal ages and their significance

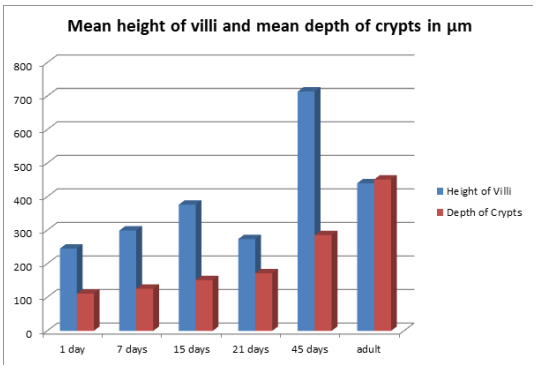
Postnatal ages	Depth of Crypts (mean±SD)	*P-value*
Day 1	110.8 ± 21.2	
Day 7	125.4 ± 19.81	0.13
Day 15	151.4 ± 34.9	0.055
Day 21	171.8 ± 84.6	0.49
Day 45	285.4 ± 67.2	0.004*
Day 90	451.4 ± 123.2	0.001**

* Significant increase in day 45day as compared to day 21

** Significant increase in day 90 as compared to day 45

Table 3: The villus / crypt ratio in the duodenum in different postnatal ages

Age	One day	7 Days	15 days	21 Days	45 days	Adult
Mean Villi Heights	245.1	299.2	376.4	273.7	713.6	440.1
Mean Crypts Depths	110.8	125.4	151.38	171.8	285.4	451.4
Ratio of Villi to Crypts	2.21	2.38	2.49	1.59	2.50	0.98



Histogram I: The mean height of villi and the mean depth of crypts of the duodenum in um in different postnatal ages

DISCUSSION

In the present study, rat pups in the pre-weaning period (days one, 7 & 15) suckle milk and were weaned by postnatal day 21. However, in a previous work, the rat pups at postnatal day 16 (when eyes become opened) started to take small bits of solid matter present around them like feces or rodent diet and at postnatal day 21, they became normally weaned[2].

In the present study, the mucosa of the rat duodenum showed short finger shaped villi in pre-weaning day one and their width increased in days 7 and 15. Similar results were observed in the proximal intestine of suckling rats[1,13] and in the small intestine of human neonates[6]. In the present work, the villi became more broad and frequently joined with multiple transverse side furrows at day 21 (weaning) and day 45. In small intestinal rat mucosa, the increase in the width of the villi at day 35 was explained by fusion of adjacent villi[2]. The density of the villi increased in the present work with advance of age and this coincide with findings of previous work[1]. This increase expand the surface area of the mucosa to get ready for the large amount of diet introduced at weaning[6]. In the present work, the height of the villi showed statistical non-significant increase in the pre-weaning period which coincided with the results observed in rat small intestine[14]. The height of the villi at weaning then showed statistical significant decrease with low villus/ crypt ratio followed by highly significant increase in day 45. This decrease might be due to lowered cell proliferation and increased apoptosis[15]. In jejunum of piglets, this might result from the decrease in energy intake (nutrient intake) during weaning leading to decrease energy metabolism[16]. In the present study, the crypts were shallow at day one, and then increased in density and depth with advance of age until it reached the adult period. This increase in depth was insignificant before and after weaning. Similar results were observed in rat duodenum in previous work[1] and in rat jejunum and ileum[8]. During postnatal development, crypts replicate via a process of crypt fission and crypt hyperplasia. Crypt fission

was the main mechanism of epithelial growth during milk feeding in neonatal rats and human while crypt hyperplasia was then the main mechanism after weaning[6]. Factors present in breast milk and growth factors acting on intestinal receptors, influence such growth[17]. In rats and in humans, the cells of the crypts proliferated and differentiated to carry on their function after drifting up to the villi[9]. In that respect, mitotic figures were present at weaning in the present study, which agreed with the observation of previous work in which the intestinal crypts contain active cycling stem cells at their bases and precursor cells along the sides[18].

In the present work, the epithelium of the duodenal villi and crypts was formed mainly of enterocytes (columnar cells with well-defined brush border). The enterocytes of the villi showed morphological changes during postnatal development that correlate with its functional role in nutrient transport. In the first postnatal days (one & 7), some of them exhibited apical vacuolation with variable sizes .These vacuoles were seldom to be found at day 15. The vacuolated enterocytes were described in fetuses (vacuolated fetal enterocytes =VFE) and preceded the occurrence of adult-type in mammals (including humans), and showed a high level of similarity of structural appearance across the species[19]. In rats, VFE were demonstrated at day 7 & 14 mainly in the distal small intestine[13]. In piglets, the VFE in the duodenum and the proximal jejunum remained for 2-3 days after birth and in the mid and distal jejunum till day 14. At day 21, the adult type enterocytes were present instead of fetal type vacuolated ones. The disappearance of VFE is a characteristic feature of mucosal remodeling and may be used as a marker of maturation of the small intestine[20]. During early postnatal life, a tubulo-vesicular network or apical canalicular system (ACS) existed and closely associated with invagination of the luminal plasma membrane of enterocytes. ACS vesicles fuse together in small vacuoles, cluster in larger vacuoles, often filling the majority of enterocyte[21]. Macromolecules and IgG present in maternal milk is absorbed through the epithelium forming these vacuoles during the suckling period. Consequently, the number of vacuoles and neonatal-Fc-receptor increased but later decreased after weaning[22]. Vacuoles within enterocytes may represent absorbed colostral macromolecules and other milk proteins from intestinal lumen to reach the circulation without losing their biological activity (VFE containing transport vacuoles)[20]. In addition, VFE produce digestive vacuoles to support the digestion of milk inside the cell (VFE containing digestive vacuoles)[19]. The large vacuoles seen in the VFE of pig duodenum and proximal jejunum are considered transport vacuoles, whereas in the lower parts of the gut - digestive vacuoles[20]. During the suckling period in rats, binding and transfer of IgG across the small intestine needs FcRn expression with highest intensity which serve also for its protection from degradation[23]. After weaning FcRn was markedly decreased and in adult it was found in few cells at tips of intestinal villi and is involved in transporting the IgG- antigen complexes to the immune cells of the lamina propria[13].

In the present study, few goblet cells were observed in days 1 and 7 old rats away from the vacuolated cells on the villi of the duodenum and within the crypts and increased gradually in day 15. They appeared well developed in days 21 and 45 and adults. Similarly, fewer goblet cells with poor mucus layer were described in young rat pups susceptible to physical or infectious injury compared to its matching structures in older resistant animals[24]. Mucin, a glycoprotein secreted by goblet cells moistures the epithelium and protects it from different injurious factors resulting from diet or pathogens[25]. Mucins attach initially to the invading infectious agent at the cell surface thus preventing it to bind to other glycoproteins and counteract it[26].

In the present study in pre-weaning period (days 1, 7 & 15), goblet cells showed positive staining for acidic and neutral mucin along the sides of the villi and within the crypts. At day 21 (weaning) the cells were positive mainly to acidic mucin. This may be due to change of the nature of diet from maternal milk to solid food which may be contaminated with different pathogens. In that respect, the neutral mucin revealed a physical barrier function while acidic mucin (sulphomucin)appear to be less decomposable by bacterial glycosidases and host protease thus protected against bacterial translocation[27].

In this work, paneth cells could be identified at day 7 as single or twin cells located in the basal parts of the crypts. They were recognized by their apical acidophilic granules and were seen more frequently in day 15 and in groups at weaning (day 21). In that respect, the paneth cells enhanced the defensive capacity of intestinal mucosa via the antimicrobial peptides rich granules[28].

This study may throw light about the effect of food element consistency on structural and functional alteration of intestinal wall and raised a question of mandatory clinical awareness. So prolonged parenteral feeding used in treatment of infants suffering from debilitating diseases might induce reduction of paneth and goblet cells leading to disturbed function of epithelial barrier and make the mucosa vulnerable to luminal aggression and increased incidence of duodenal ulcers[29]. So giving small amounts of enteral nutrition enhances the development of gastrointestinal tract of premature newborns thus improving milk tolerance, decreasing septicemia and early exiting from hospital[30].

CONCLUSION

The results of this study demonstrate the different structural changes occurring during the postnatal development of the mucosa of the duodenum in the rat. These adaptive structural changes occur when diet changes from milk to solid food. This may further add to our understanding the importance of enteral feeding during the weaning process in animals and humans and the expected hazards of parenteral nutrition in early infancy.

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CONFLICT OF INTERESTS

There are no conflicts of interest.

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دراسة نسيجية للغشاء المخاطى للاثنى عشر فى الجرذ خلال فترة ما قبل وما بعد الفطام

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ملخص البحث

الخلفية : التغيرات فى مكونات الاكل خلال مراحل الرضاعة والفطام وما بعد الفطام توثق التكيفات الهستولوجية والوظيفية فى الأمعاء الدقيقة للجرذ

الاهداف: التعرف علي التغيرات الهستولوجية التى تمثل تلك التكيفات فى الغشاء المخاطى للاثنى عشر فى الجرذ

المواد والطرق: تم تقسيم اربعة وعشرون جرو من ذكور الجرذان الى اربع مجموعات متساوية تشتمل على الاعمار الاتية: اليوم الاول والسابع والخامس عشر (قبل الفطام) واليوم 21 (يوم الفطام) .كذلك تم استخدام اثنى عشرجرذ مناصفة لمجموعتين من الذكور البالغين وكانت اعمارهم 45 يوم (بعد الفطام) و 90 يوما . تم القتل الرحيم لجميع الحيوانات السابق ذكرهم. تم معالجة الاثنى عشر للفحص بالمجهر الضوئى والمجهر الالكترونى الماسح . تم صبغ قطاعات البارافين بالهيماتوكسيلين والايوسين وصبغة الالشيان الازرق والبريوديك اسيد شيف مجتمعة. وتم استخدام محلل الصور لقياس ارتفاع الزغب وعمق الخبايا ونسبة الزغب / الخبايا وعمل تحليل إحصائى .

النتائج: يتسع الزغب مع تقدم العمر. يظهر أرتفاع الزغب وعمق الخبايا زيادة إحصائية غير ذات أهمية فى مجموعات ما قبل الفطام. تظهر مجموعة الفطام (21 يوم) إنخفاض فى ارتفاع الزغب وكان ذات دلالة إحصائية وانخفاض فى نسبة الزغب/ الخبايا . تغطي الزغب بخلايا معوية عمودية مع فرشاة و توجد بعض الخلايا المعوية مفرغة فقط فى فترة ما قبل الفطام. يتم توزيع القدح الحمضية والمصبوغة ايجابيا للالشين الازرق والبريوديك اسيد شيف على الزغب وداخل الخبايا فى ايام ما قبل الفطام وكانت موجبة للبريوديك اسيد شيف فقط عند الفطام. تصطف الخبايا بواسطة الخلايا المعوية الهرمية وخلايا بانيث والتى كانت واضحة في اليوم 21.تكتسب كل من الزغب والخبايا فى يوم 21 ما بعد الولادة (الفطام) المظهر الهيكلى مماثلة لتلك الخاصة بالبالغين.

الخلاصة: تظهر هذه الدراسة التغيرات الهيكلية التى تحدث عند الفطام فى الغشاء المخاطى للأنثى عشر فى الجرذ والمتزامنة مع تحول الغذاء من الحليب الى غذاء صلب. وهذا يوضح أهمية التغذية المعوية اثناء عملية الفطام فى الحيوانات والأنسان ومدى الأخطار المتوقعة للتغذية عن طريق طريق الوريد فى المرحلة الأولى للطفولة.