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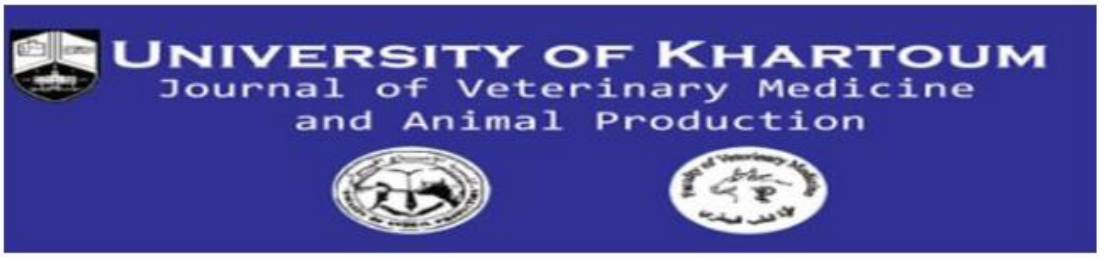

Morphology of the developing caecum and colon and histochemistry of the developing colon in male rabbits

BY
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Abstract: The objectives of the study were to investigate the histology and ultrastructure of the caecum and colon and histochemistry of the colon have been conducted on 50 Blanc de Bouscat male rabbits with ages varied between 0- 30 days. The investigation showed that the mucosa of the caecum and colon was composed of crypts with villar shape at 0-day of age and the length of the crypts decreased with advancing age. The crypts were lined by striated epithelial cells. Goblet cells were prominent. The absorptive columnar cells had apical microvilli and contained vacuoles of different size, ovoid to elongated nucleus, mitochondria, rough endoplasmic reticulum and lateral membranous interdigitations. Cells with polymorphic secretory granules were noticed. Varying degrees of positive reaction to PAS diastase resistant material and diastase- digested material (glycogen) was found in the colon.

Key words: Caecum &colon, postnatal development, rabbits, morphology

ملخص

أهداف هذه الدراسة التعرف على التركيب النسيجي والدقيق للأعور والقولون والتركيب الكيميائي النسيجي للقولون في ذكور أرانب البلانك دوبوسكات بعدد 50 أرنب. تراوحت أعمارهم بين 0- 30 يوم بعد الولادة. أوضحت الدراسة بأن الطبقة المخاطية للأعور والقولون تتكون من سراديب عميقة أشبه بالزغابات وذلك في عمر 0 يوم ويقل عمق هذه السراديب بتقدم العمر. يكسي السراديب ظهارة مخططة مع وجود خلايا كأسية واضحة. الظهارة المخططة عبارة عن خلايا عمودية إمتصاصية تختص بوجود زغابات دقيقة في سطحها الحر. كما تحتوي هذه الخلايا على فجوات بأحجام مختلفة، أنوية بيضاوية الى مستطالة، متقدرات، شبكة هيولى خشنة وتشابكات غشائية جانبية. كما لوحظ وجود نوع آخر من الخلايا يحتوي على حبيبات إفرازية بأشكال مختلفة. وأظهرت المخاطية للقولون تفاعلا مع صبغة ال PAS وإنزيم الدياستيز وذلك دليل وجود الجليكوجين.

Introduction

The histology, ultrastructure and histochemistry of the developing caecum and colon were investigated in few birds including, the chicks (Andrew, Kramer and Rawdon, 1982; Andrew, 1972a), the turkey (El-Zoghby, 2000) and quail (Ahmed, 2002).

In mammals, the colon was studied in piglets (Wooding, Smith and Craig, 1978; Krause, Cutts and Lesson, 1977), caecum in rat (Ono, 1980) and rabbits (Sabatakou, *et al*., 1999).

It was therefore decided that postnatal development of the large intestine should be investigated taking the rabbit as an experimental animal.

Material and methods

The gestation period of rabbit is remarkably short, ranging between 28- 35 days. Litters are usually weaned between 21-26 days old (Sandford, 1996). The litters began feeding at 18 days old and were weaned at 24 days old. The diet/ dry matter physical compositions per ton were yellow corn 30.15%, soybean meal 16.00%, hay 40.00%, wheat bran 10.35%, ground limestone 2.00%, dicalcium phosphate 1.00%, minerals mixture 0.10%, premix (vitamins for broiler) 0.10%, sodium chloride (common salt) 0.30% and anti coccidia.

Histology

Small pieces of tissue, about 1cm³ in size, were excised from the caecum and colon. The tissues were fixed by immersion in different types of fixatives, including Bouin's fluid, 10% formalin and 10% formal saline. The tissues were processed routinely and stained with heamatoxyline and eosin (H&E) (Culling, 1973)

Ultrastructure

Pieces of tissue for ultrastructural study were taken from animals of different ages. Small pieces of tissue (1x1mm in diameter) were taken immediately after the animal was anesthetized and fixed rapidly in 5% glutaraldehyde in phosphate buffer (PH 7. 4) for 2- 4 hours at 4°C. The tissues were post-fixed in 1% osmic acid for 2 hours, and then processed routinely for transmission electron microscope.

Semithin sections (0.5μ) were stained with toluidine blue and examined with the light microscope. The desired regions for electron microscopy were then selected and ultrathin sections were treated with 2% uranyl acetate and lead citrate for 15 minutes. They were then washed, dried and examined in a Zeiss EM 109 electron microscope (Bancroft and Stevens, 1996).

Histochemistry

Material for histochemical investigation was obtained from the suckling neonates (6 days), the feeding and suckling neonates (18days) and after weaning (30 days). Pieces of tissue were taken from the colon as quickly as possible after anesthesia.

Carbohydrates

Neutral mucopolysaccharids were investigated in tissues fixed in Bouin`s fluid and Gender`s fluid at 4ºC and processed for paraffin sections and stained with Periodic acid-Schiff (PAS) technique (Culling, 1973). The glycogen was investigated in tissue fixed in Gendre`s fluid and stained with PAS technique. Control sections for glycogen were treated either with saliva or 0. 1% malt diastase.

Results

Light microscopy

The caecum

At 0-day of age, the mucosa was composed of irregular intestinal crypts of villar-like shape and covered by simple striated columnar epithelial cells with homogenous acidophilic material in a vacuolated cytoplasm (Fig.1). The nuclei of the epithelial cells were oval and centrally situated. Goblet cells were conspicuous. The intestinal crypts were short and opened at the luminal surface. The lamina propria of loose connective tissue was found between the intestinal crypts. The muscularis mucosa was inconspicuous and this allowed the submucosa to merge with the lamina properia. At 6 days of age (Fig.2), the mucosa consisted of wide intestinal crypts. The epithelial cells contained elongated nuclei which were surrounded by pink homogenous vacuolated material. The intestinal crypts were straight and lined by short columnar epithelial cells. The muscularis mucosa consisted of a circular layer of smooth muscle fibres. At 12 days of age, the mucosa consisted of intestinal crypts in short villar–like shape (Fig.3). At the age of 18 days, suckling and feeding neonates, the mucosa consisted of irregular intestinal crypts. At 24 days of age (Fig.4), the mucosa consisted of short intestinal crypts lined by columnar cells

with oval nuclei and many vacuoles in cytoplasm and a few lightly stained cells were observed. The other layers were similar to those of younger groups. At the age of 30 days, weaned neonates, the mucosa consisted of short straight intestinal crypts lined by simple striated columnar epithelial cells with oval nuclei and the apical of the cytoplasm epithelial cells was lightly stained. The epithelial cells of the intestinal crypts contained prominent intracellular spaces. The other layers were similar to those of other age groups.

The colon

In neonates at 0-day of age, the mucosa was composed of crypts in form of villi, lined by simple or pseudostratified columnar epithelium with oval or round nuclei surrounded by pink homogenous material. There were few apical cytoplasmic vacuoles. Goblet cells were a prominent feature. The lamina propria of loose connective tissue was separated by a muscularis mucosa from the submucosa (Fig.5).

At 6 days of age, the layers were similar to those at 0- day of age. At 12 days of age, the mucosa consisted of regularly shaped intestinal crypts. The intestinal crypts were straight tubular glands and opened to the surface at the luminal margin. The other layers were similar to those of other age groups (Fig.6). At the age of 18 days, suckling and feeding neonates, the mucosa was lined by simple striated columnar epithelial cells with oval nuclei and most of them were surrounded by a small amount of pink and homogenous substance. The intestinal crypts were long and straight. Other layers were similar to those of other age groups (Fig.7). At 24 days of age, the epithelium of the mucosa contained oval and basally situated nuclei. The intestinal crypts were straight. There was no change in the other layers (Fig.8). At the age of 30 days, weaned neonates, folds with intestinal crypts (straight tubular glands) were embedded in the loose connective tissue of the lamina propria. Other layers were similar to those of other age groups.

Electron microscopy

The caecum

At 0-day of age, suckling neonates, the surface epithelium contained columnar cells with apical microvilli, mitochondria, rough endoplasmic reticulum, a few lateral membranous interdigitation and a large material of low electron density which displaced other cytoplasmic components apically and basally. Goblet cells were observed containing large number of premucin droplets (Fig.9). The intestinal crypts contained cells with polymorphic secretory granules which are situated mainly at the basal part of the cytoplasm (Fig.10). At 18 days of age, suckling neonates, the columnar cells contained apical microvilli, mitochondria, low electron dense material and oval nucleus. At 24 days of age, elongated nuclei with large amount of heterochromatin, and free ribosomes were seen in the columnar cells. In the intestinal crypts, the columnar cells contained rough endoplasmic reticulum, free ribosomes, mitochondria, oval nucleus with large heterochromatin and prominent nucleolus. Basally situated polymorphic secretory granules were found in the cytoplasm of a few cells that lined the intestinal crypts.

The colon

At 0-day of age, suckling neonates, apical microvilli, mitochondria, vacuoles, basal nuclei with heterochromatin and prominent nucleoli were seen in the columnar cells. Goblet cells with premucin droplets opened at the luminal surface (Fig.11). At 6 days of age, a large material of low electron density that displaced other cytoplasmic components apically and peripherally were observed in the columnar cells of the surface epithelium. There were lateral membranous modifications, seen as intracellular canaliculi. Columnar cells with elongated nuclei and clear intracellular spaces lined the crypts. A few cells with polymorphic secretory granules were scattered among the epithelial cells of the crypts (Fig.12). At 18 days of age, suckling and feeding neonates, large material of low electron density, which displaced other cytoplasmic components apically, basally situated nuclei and lateral membranous interdigitations were noticed in the columnar surface epithelial cells. The columnar cells of the crypts possessed large nuclei and contained many mitochondria.

Polymorphic secretory granules were found in a few cells which were dispersed between the lining epithelium of the crypts. At 24 days of age, small vacuoles, and oval nuclei with prominent nucleoli were observed in the columnar surface epithelium. Tight and intermediate junctions and desmosomes joined the columnar cells. A few cells with round and centrally situated nuclei with prominent nucleoli, mitochondria and polymorphic secretory granules were found in cells scattered between the cells of the intestinal crypts.

Histochemistry

Neutral mucopolysaccharides

At 6 days of age, the surface epithelium gave strong positive reaction for PAS stain and many fine glycogen particles were observed in the cell cytoplasm. A weak reaction was observed in the epithelium of the glands. At 30 days of age, the reaction was strong at the surface epithelium and less so in the glandular epithelium and a few fine glycogen particles were observed in the cytoplasm of the cells of the intestinal crypts (crypts of Lieberkuhn).

Figure 1: A photograph of caecum at 0-day of age
Note the presence of villus-like intestinal crypts (i), columnar cells (c), goblet cells (g), lamina propria (l), fibroblasts (f), smooth muscle fibers (m) and submucosa (s). **Toloiudine blue, X 25).**

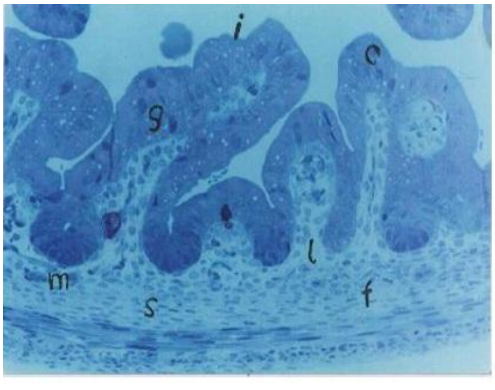


Figure 2: A photograph of caecum at 6 days of age
intestinal crypts (i) columnar cells (arrows), goblet cells (g), lamina propria (l), fibroblast (f), muscularis mucosa (m) and submucosa (S).

Toloiudine blue, X25

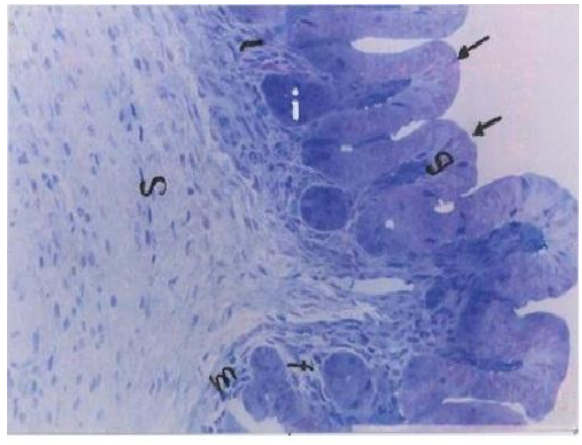


Figure 3: A photograph of caecum at 12 days of age
intestinal glands (i), columnar cells (arrows), goblet cells (g), lamina propria (l), fibroblasts (f), muscularis mucosa (m) and submucosa (S). **Toloiudine blue, X 25.**

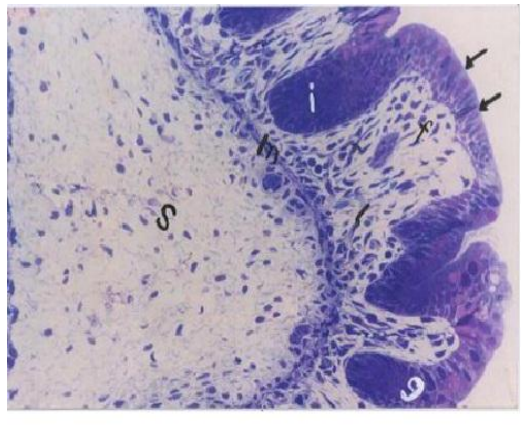


Figure 4: A photograph of caecum at 24 days of age
crypts (i), columnar cells (arrows), goblet cells (g), lamina propria (l), muscularis mucosae (m) and submucosa (s). Toloiudine blue, X 40.

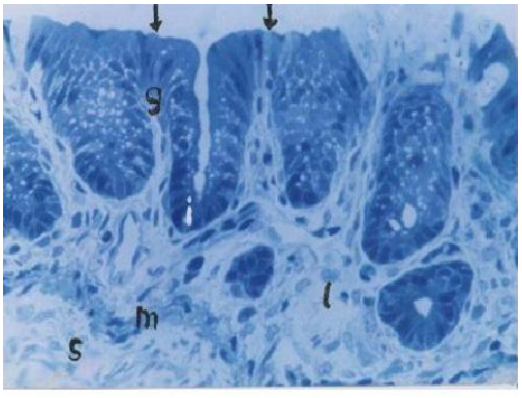


Figure 5: A photograph of colon at 0-day of age
Note the villar shape (arrow), the intestinal glands (i), goblet cells (g), lamina propria (l), fibroblasts (f), smooth muscle fibers (m) and submucosa (s). Toloiudine blue, X 25.

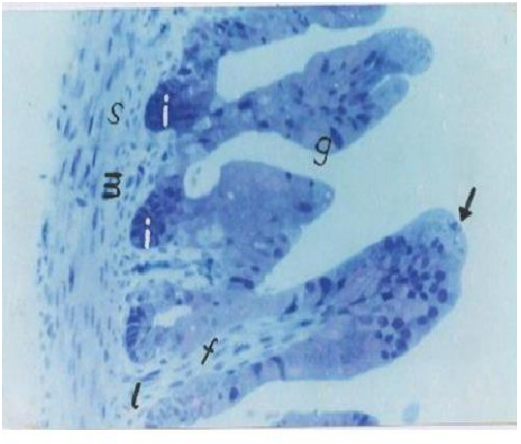


Figure 6: A photograph of **colon** at **12 days** of age
the crypts (i), columnar cells (arrows), goblet cells (g), lamina properia (l). Numerous blood vessels are present in the lamina propria and submucosa (v). Muscularis mucosae (m). Submucosa (s). **Toloiudine blue, X 40.**

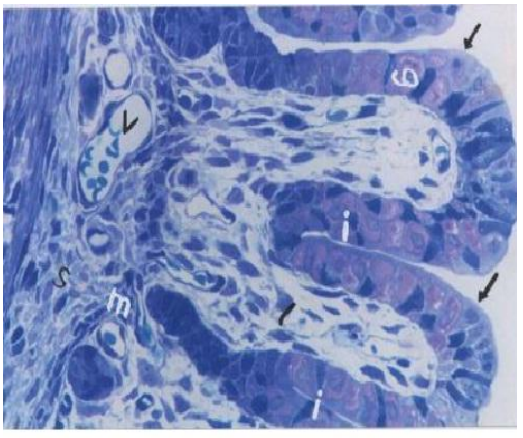


Figure 7: A photograph of **colon** at **18 days** of age
Note the presence of the intestinal crypts (i), columnar cells (arrows), goblet cells (g), lamina properia (l), muscularis mucosa (m) and submucosa (s). **Toloiudine blue, X 25.**

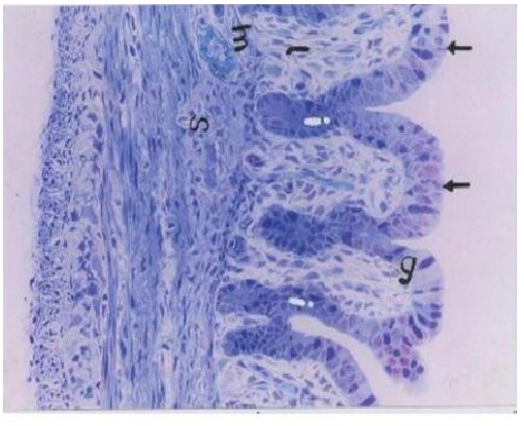


Figure 8: A photograph of **colon** at **24 days** of age
intestinal crypts (i), columnar cells (arrows), goblet cells (g), lamina propria (l), muscularis mucosae (m), blood vessel (v) and submucosa (s). Note the presence of many vacuoles in the crypts epithelial cells. **Toloiudine blue, X 16.**

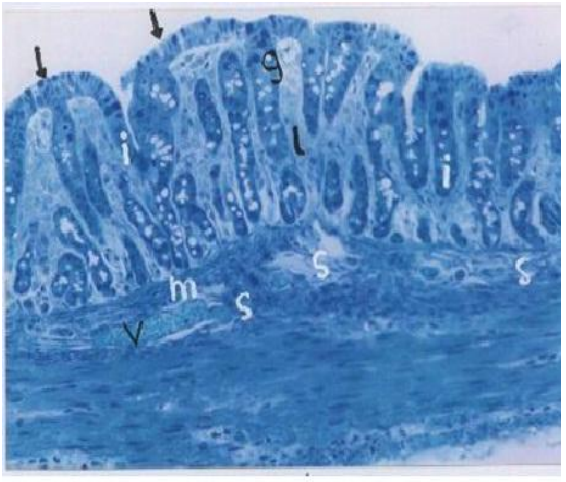


Figure 9: A micrograph of **caecum** at **0-day** of age
a goblet cell with premucin droplets (mg) and microvilli (arrow) on the surface of columnar cells. **Uranyl acetate and lead citrate, X 5000**

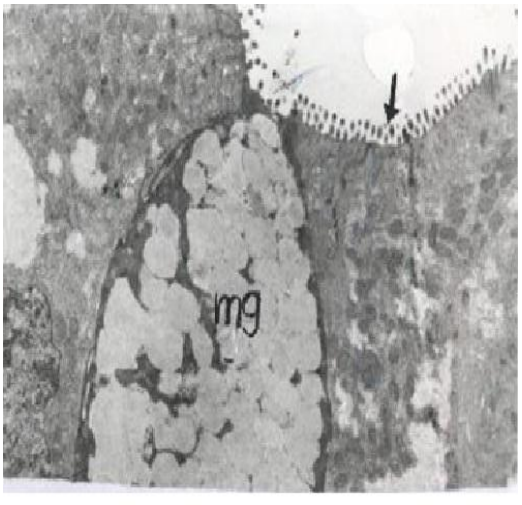


Figure 10: A micrograph of caecum at 0-day of age
a cell with polymorphic secretory granules (sg) in the crypt. **Uranyl acetate and lead citrate, X 5.000.**

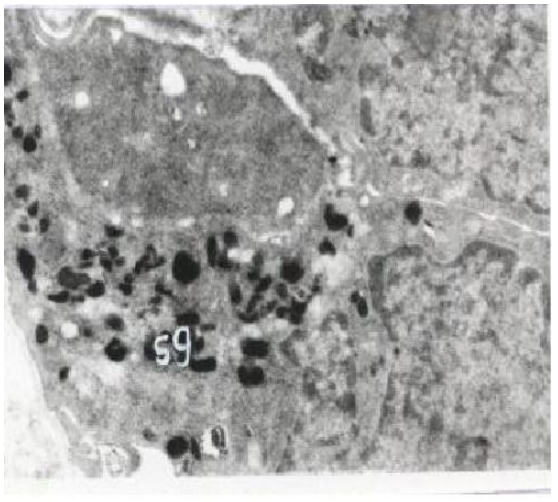


Figure 11: A micrograph of **colon** at **0-day** of age
a columnar cell with microvilli (arrow), vacoules (v), nucleus (n) and a goblet cell with premucin droplets (mg). **Uranyl acetate and lead citrate, X 5000.**

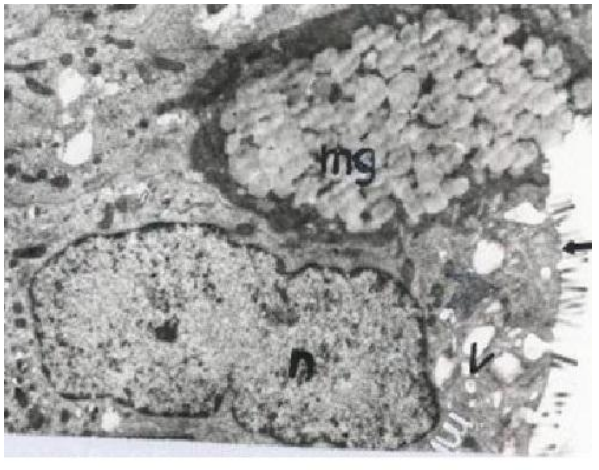
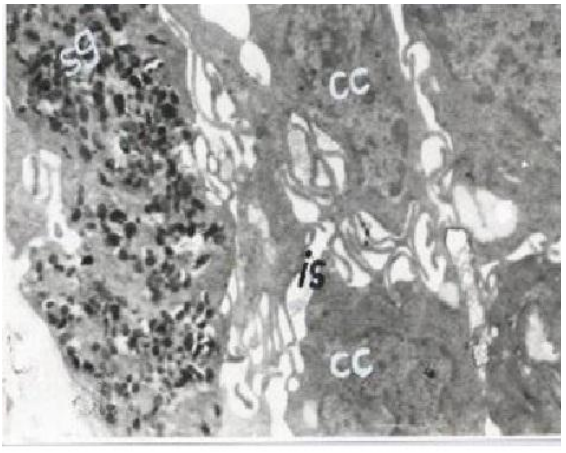


Figure.12. A micrograph of **colon** at **6 days** of age
crypt epithelial cells (cc) with intercellular spaces (is) and endocrine cell containing polymorphic secretory granules (sg). **Uranyl acetate and lead citrate, X 8000.**



Discussion

Ono (1980) in rat studied the caecum on day one presented villi which decreased rapidly in 5 days and disappeared in the 10th day of age. In the present investigation, the mucosa of the caecum at 0day of age was composed of irregular intestinal crypts of villus-like shape and covered by simple striated columnar epithelial cells with homogenous acidophilic material in the cytoplasm. Goblet cells were found. At 6 days of age the crypts became wide and at 12 days they became shorter and villus-like shape, while at 18 days of age the crypts became irregular, at 24 days they were short again and at 30 days of age they became short and straight and contained prominent intracellular spaces. This is in agree with Sabatakou *et al*., (1999) in rabbits the caecum was composed of villi in 1-day-old rabbit and by the 16th day of life these villi were replaced by ridges.

El-Zoghby (2000) is of the opinion that the caecum has the greatest number of goblet cells and the mucosa contains many crypts of Lieberkuhn in turkey at different ages. In the present investigation, the colon mucosa showed irregular crypts with villus shape at 0-day and 6 days of age, and regular crypts at 12 days of age. In the colon of piglets at birth have epithelial cells with villus-like protrusion and these protrusions are not seen in the 2-days old piglets (Wooding *et al*., 1978).

At 18, 24 and 30 days of age, the mucosa showed normal crypts as in the adult. The mucosa in this investigation was lined by simple or pseudostratified columnar epithelium at 0-day and 6 days of age and simple columnar epithelium in the other age groups. The authors could not be able to find any information in the literature regarding such development in any species.

In the present study the surface epithelium of the caecum and colon contained absorptive columnar cells with apical microvilli, mitochondria, rough endoplasmic reticulum, free ribosomes, elongated nuclei with large heterochromatin, and large material of less electron density. Goblet cells, contained premucin droplets and apical microvilli.

Andrew (1976a) reported that the chicks' intestinal endocrine cells, around the time of hatching, were very few in the caeca. A few of these cells were seen to reach the lumen where the narrow apices of these cells were flanked by junctional complexes and they may or may not bear microvilli. In the newborn opossum, the epithelium of the colon possesses an extensive apical endocytic complex, numerous dense irregular aggregates of material in the supranuclear region (Krause *et al*., 1977).

In the present study, the caecum contained cells with polymorphic secretory granules. These cells may be endocrine cells due to the location of their granules; these cells appeared at 0-day of age. This observation is in agreement with the report which is given by Ahmed (2002) in quail. In addition they are observed at 24 days in the caecum and at 6 days, 18 days and 24 days in the colon.

In the present Study at 6 days of age, the surface epithelium gave strong positive reaction for PAS stain and many fine glycogen particles were observed in the cell cytoplasm. A weak reaction was observed in the epithelium of the glands. At 30 days of age, the reaction was strong at the surface epithelium and less so in the glandular epithelium and a few fine glycogen particles were in the cytoplasm of the cells of the intestinal crypts (crypts of Lieberkuhn).

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