Light microscopic study on the absorptive cells and goblet cells in the intestine of adult common carp *Cyprinus carpio*

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Summary

The purpose of this study was to describe of some histological structures and histochemical features of the absorptive cells and goblet cells in intestine of common carp. In this study, Fifteen adult male common carp were catching alive from the AL-Forat river, with age about (7 - 12) months and mean of their weight was (2100 ± 81 g) and mean standard length was (50.4 ± 3.1 cm), immediately after death.Incision was made through the midventral line of the fish just from cranial to the anus to expose the intestine, specimens of intestine were taken and washed with (0.9 %) normal saline solution. Ten samples were obtained from different regions of each portion of the intestine (anterior, middle and posterior), fixed by either 10% neutral buffered formalin or Bouin's solution approximately 24 hours at room temperature and then treated by routine histological processing. The stains were used, Hematoxylin and Eosin, periodic acid Schiff, Alcian blue pH 2.5, and Combinated Alcian blue PH 2.5 plus periodic acid Schiff. The Mean number, height and width of mucosal folds were measured and counts of goblet cells in the mucosal folds of each portion of the intestine. The results showed that the intestinal epithelium is simple columnar, the enterocytes were tall columnar cells with brush border; goblet cells were ovoid, located between the enterocytes. The folds composed of the entire thickness of the mucosa, mean number, height and width of the mucosal folds were few in posterior portion of intestine compare with that in other portions. The anterior portion of the intestine has largest number of mucosal folds, while has less number of goblet cells compare to middle and posterior portions of the intestine. The epithelial cells stained red-purple by periodic acid Schiff stain with Alcian blue, the goblet cells were take positive reaction with periodic acid Schiff stain, and with Alcian blue plus periodic acid Schiff, while negative with only Alcian blue stain. Glycoprotein staining intensity varied from very weak to moderate, whereby the presence of neutral, acidic and sulphatedglycoconjugates. In conclusion, numbers of goblet cells were observed to be increased towards posterior portion of intestine required as lubricant for fecal expulsion, and reversed relation between number of the folds and goblet cells.

Keywords: Common carp, Intestine, Enterocytes, Goblet cells.

Introduction

The family *Cyprinidae*, commonly known as cyprinids, is the largest of all fish families found throughout the world; members of the family have a wide geographical distribution and occur in Europe, Asia, Africa, and North America.There are about 2900 species of cyprinids worldwide (1). The common carp is a widespread freshwater fish in lakes and large rivers in Europe and Asia, its mostly omnivores, feeding on small crustaceans, insects and some minute plants but some specialize in eating large plants, certain species are used in experimental studies (2). In fish, as in other vertebrates, the digestive tract consists of the alimentary canal, a hollow tube of varying diameter which is divided into the esophagus, stomach, intestines and rectum, also some associated glands, salivary glands, liver, gall bladder, and pancreas (3). The digestive tract of fishes shows a remarkable diversity of morphological and functional characteristics. This is related to different feeding habits and to taxonomy, as well as to shape, size and weight of body and sex (4). Intestinal tissue is exposed to a permanent challenge of bacteria, parasites, viruses and toxins from the luminal content. The epithelial cells are protected against pathogen by a mucus layer, which covers the intestinal

epithelium (5 and 6). The intestinal barrier can be divided into the extrinsic, intrinsic, and immunological barriers. The extrinsic barrier is mainly formed by mucus which counters pathogens and toxins. The intrinsic barrier consists of epithelial cells and cell junctions. Immunological barriers are formed by the innate immune system and the adaptive immune system that are present in the intestinal wall (7and 8). The intestinal epithelium is made up of cells named enterocytes, goblet cells, lymphocytes, stem cells and enteroendocrine cells are scattered through the epithelium and rodlet cells are also found in some species of teleost fishes (9-11). Enterocytes, they are numerous, tall columnar cells, with oval nuclei, lie in the epithelium of the folds. The columnar epithelial cells that dominate the intestinal epithelium and possessing microvilli, the enterocytes in the hindgut of fish have an antigen-transporting capability and also many macrophages and lymphoid cells are distributed among the epithelial cells and in the lamina propria. Goblet cells derive their name from challis-like shape. The nucleus is located in the tapered stem, which widens and then constricts to from an apical pore through which mucus is discharged, goblet cells are common components of the intestinal mucosa in fish (12-14).

Materials and Methods

The study was performed using fifteen of healthy male adult common carp Cyprinuscarpio during January and February 2019. Were caught alive from the AL Forat river, with age about (7- 12) months, mean their weight was (2100 ±81 g) and mean standard length was (50.4 ± 3.1) cm (1), immediately after death, incision was made through the midventral line of the fish from just cranial to the anus to expose the intestine. The ventral abdominal wall of each fish was removed, the intestinal tract was separated, each intestinal specimen was divided into three portions (anterior , middle and posterior) immediately after death was washed with normal saline solution (0.9%).Small pieces from different regions of anterior, middle and posterior portions of intestine were taken and fixed by either 10% neutral buffered formalin or Bouin's solution approximately 24 hours at room temperature and then treat by routine histological processing. Embedding with paraffin wax (58-60 C^0) and sectioning to 5-7µm. The stains were used, Hematoxylin and Eosin for the general histological components, periodic acid Schiff for mucopolysaccharides, Alcian blue pH 2.5 for acidic polysaccharides, and Combinated Alcian blue PH 2.5 with periodic acid Schif for neutral polysaccharides (15). The slides were then dipped in xylene and mounted with cover slip using DPX mounting medium. Mean height, width and number of mucosal folds per microscopic fields (X 100) and counts of goblet cells in the mucosal folds of each portion of the intestine. The mean and the standard error were calculated for 5 slides for portion of intestine (16).

Results and Discussion

The histological structure of common carp intestine formed by four tunicae, mucosa, sub mucosa, muscularis and serosa (Fig.1and 2). The intestinal mucosa composed of a three structures contained epithelium, lamina propria and muscularis mucosa (Fig.3), were similar with other teleost fishes like Catlacatla (17), Anguilla anguilla (18), Clariusbatrachus (19), Salmosalar (20). The mucosal epithelium is simple columnar epithelium comprised of the enterocytes and interspersed with goblet cells (Fig.3 and 4). This result agrees with that of Gargiulo et al. (21) in Tilapia spp. and (22) in Liza aurata. Enterocytes, they were principal cells type of epithelium, the nucleus of enterocytes was pale oval vesicular situated nearly in the base of the cell, with numerous apical microvilli, forming the brush border (Fig.3and 4), they are generally similar to those in other vertebrates (23).Authors (24) Suggested that the junctional complexes of the enterocytes may prevent the paracellular leak of ions and macromolecules. In addition, (25) reported that the intestinal brush border membranes of some marine teleosts are linke to enzymes, such as peptidases and disaccharidases, which lead to the total digestion of the diet components and allowing their absorption by the enterocytes. These cells are both absorptive and secretory in function and important for nutrient uptake and ion regulation(7).

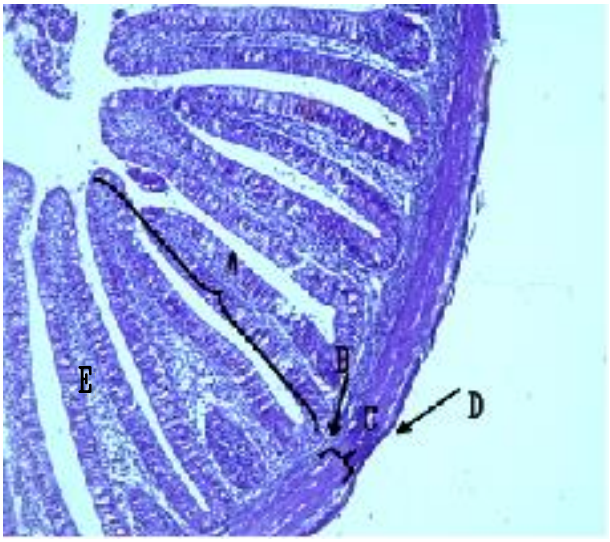
They were transverse folds composed of the entire thickness of the mucosa and appear deep finger entering the lumen; the folds have a loose connective tissue core (Fig.1 and 2). Mean height and width of mucosal folds in anterior, middle and posterior portions of intestine were (575.5 ± 13.2; 538.1 ± 10.3; 410.5 ± 12.1 µm) (91.12 ± 0.25; 75.91 ± 0.45; 64.55 ± 0.50 µm) respectively, and mean their number in per microscopic field in anterior, middle and posterior portions of intestine were (11.2 ± 0.22; 8.6 ± 0.29; 6.6 ± 0.21) respectively (Table, 1). In middle and posterior portions of intestine, the mucosal folds were fewer and less height as compared to the anterior portion of intestine (Fig.5 - 8). The arrangement of folds (have no lymphatic vessels so that are considered as not villi), that slow the speed of food transport and allow longer digestive period within the intestine and consequently better utilization of the nutrients (19 and 22) reported the morphology of the mucosal folds of the Liza aurata intestine, bear a relationship with environmental pollution.

The goblet cells appear like swollen, ovoid and flask shaped scattered among the absorptive cells (Fig.3). Their nucleus were close to the base, the cells appeared white vacuolated view with H&E (Fig.3), The distribution of goblet cells was observed to have increased in the posterior portion of intestine showing high mucous secretion, as compared to anterior and middle portions of intestine. Mean their number in each fold of anterior portion was (18.7 ± 0.41) was lesser than that in other portions (Table, 1) and increased toward the end of the intestine. Mean their numbers in each fold in the middle and posterior portions of intestine were (24.4 ± 0.34) (29.8 ± 0.38) respectively (Table, 1). The increased number of goblet cells toward the posterior portion of intestine that observed in this investigation, these results is in agreement with those found in Clariusbatrachus (19), Monopterusalbus (13), Schilbemystus (24), in common carp (25). Alestesbaremoze (26). Whereas in other species of fishes like Oncorhynchusmykiss (23). The goblet cells are decreased towards posterior intestine. The mucus produced by these cells, plays a key role in protecting the intestinal mucosa, besides lubricating the food bolus, buffering of intestinal fluid, facilitate the absorption of nutrients, as well as prevention of proteolytic damage of the epithelium and defense against bacteria and other pathogens, may serve to lubricate the feces in posterior intestine and rectum (27).

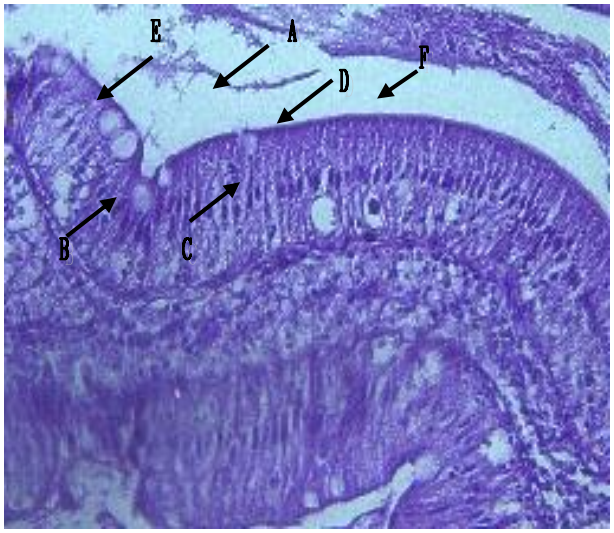
Histochemically the epithelial cells stained red-purple by periodic acid Schiff stain with Alcian blue, indicating that content of a mixture of both acidic and neutral mucus, and the epithelial cells stained red, indicating the presence of neutral mucus (Fig.9). The entire intestine is characterized by mucous secreting goblet cells that reacted strongly positive to periodic acid Schiff stain (Fig. 5 and 7). However, the goblet cells from all anterior, middle and posterior portions of intestine appeared to be negative with alcian blue stain (Fig. 9 and 10). The goblet cells reacted positively to companied the periodic acid Schiff with Alcian blue pH 2.5 in each portions of intestine (Fig. 11 and 12). All these results indicating found the neutral glycoprotein and acid glycoconjugatesulphates and carboxylated. The mucin granules that fill the greater part of the goblet cells have variable sizes and densities and occupy almost the whole cytoplasm of the cells (5). The intestinal goblet cells of many fish species, synthesize neutral and sulphatemucins, and sialomucins containing sialic acid, reported that neutral mucous compounds of the intestine participate in enzymatic food digestion, formation of food which in turn may directly affect the function of the alimentary tract. The presence of mucosubstances, especially those sulfated in the intestine; possibly regulate the transfer of proteins, as well as of ions and fluids. The possible role of mucosubstances in intestinal absorption processes is supported by the findings of (26) who observed that starvation induced an increase in the number of intestinal gobletcells.

Table,1: The mean height and width of mucosal folds (µm), their number in per microscopic field (X100) and number of goblet cells in each fold, in intestine of common carp (Mean ± S.E).

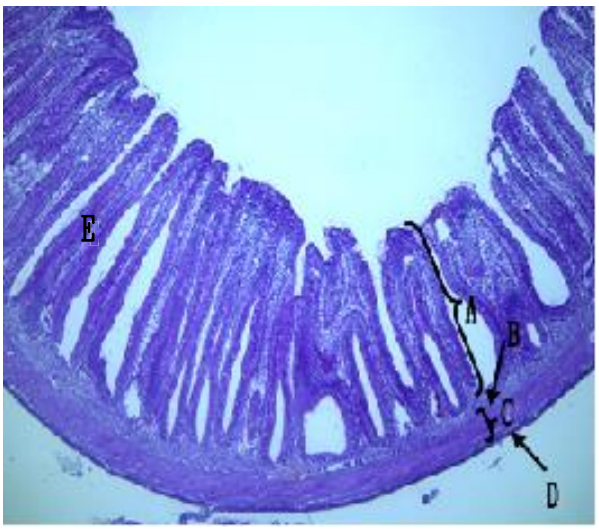
Part	Mucosal folds			Number of goblet cells
	height (µm)	Width (µm)	Number	
Anterior portion	575.5 ± 13.2	91.12 ± 0.25	11.2 ± 0.22	**18.7 ± 0.41**
Middle portion	538.1 ± 10.3	75.91 ± 0.45	8.6 ± 0.29	**24.4 ± 0.34**
Posterior portion	410.5 ± 12.1	64.55 ± 0.50	6.6 ± 0.21	**29.8 ± 0.38**



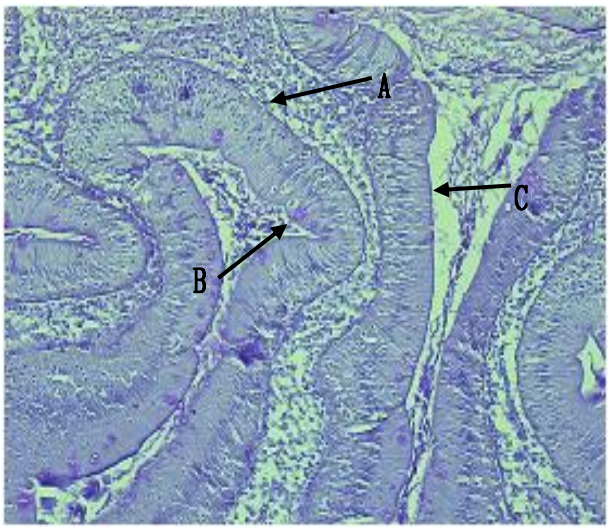
Figure, 1: Cross microscopic section of anterior portion of intestine of common carp, (A).mucosa (B).submucosa, (C). muscularis, (D). serosa, (E).fold, H & E stain (X40).



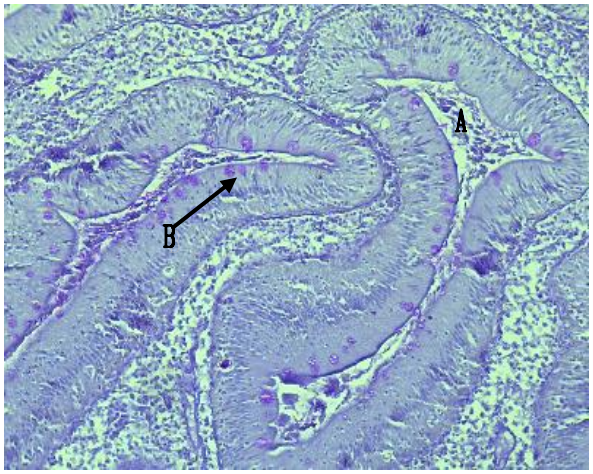
Figure, 3: Cross microscopic section of middle portion of intestine of common carp, (A) epithelium, (B) lamina propria, (C) muscularis mucosa,(D) columnar cell, (E) goblet cell,(F) microvilli, H & E stain (X400).



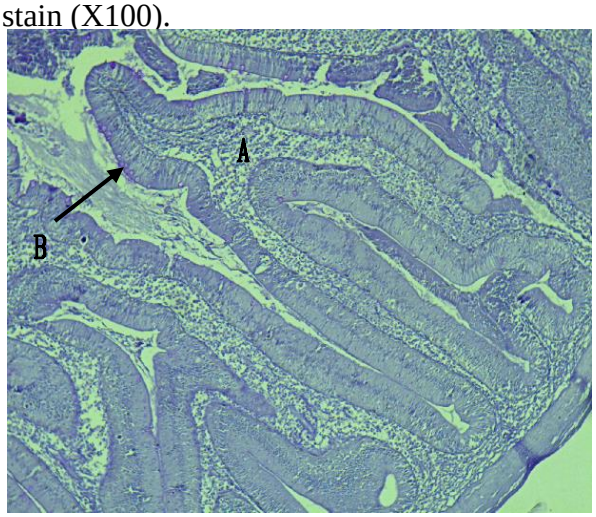
Figure, 2: Cross microscopic section of posterior portion of intestine of common carp, (A)mucosa, (B)submucosa, (C)muscularis, (D)serosa, (E)fold, H & E stain (X40).



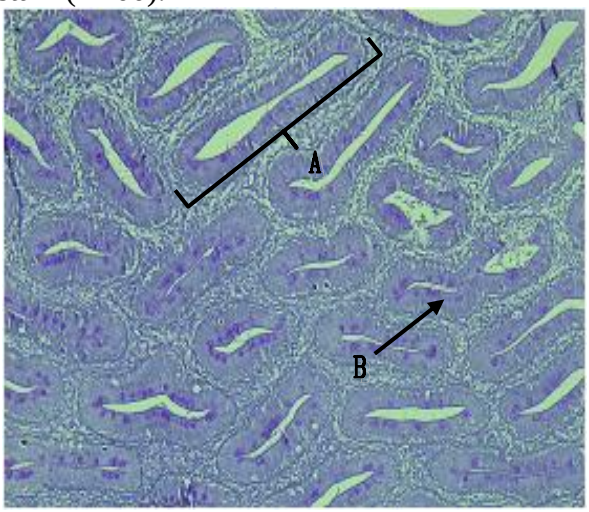
Figure, 4: Cross microscopic section of posterior portion of intestine of common carp, (A) mucosal fold, (B) goblet cell (arrow), (C) microvilli, PAS stain (X100).



Figure, 5: Cross microscopic section of anterior portion of intestine of common carp, (A)mucosal fold, (B)goblet cell (arrow), PAS stain (X100).

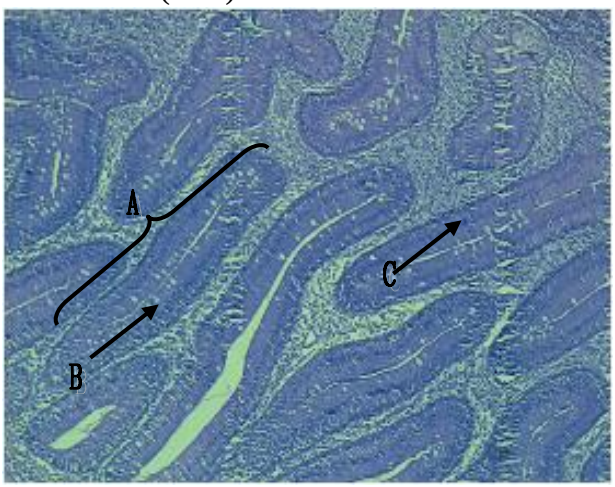


Figure, 6: Cross microscopic section of middle portion of intestine of common carp, (A)mucosal fold, (B)goblet cell (arrow),PAS stain (X100).

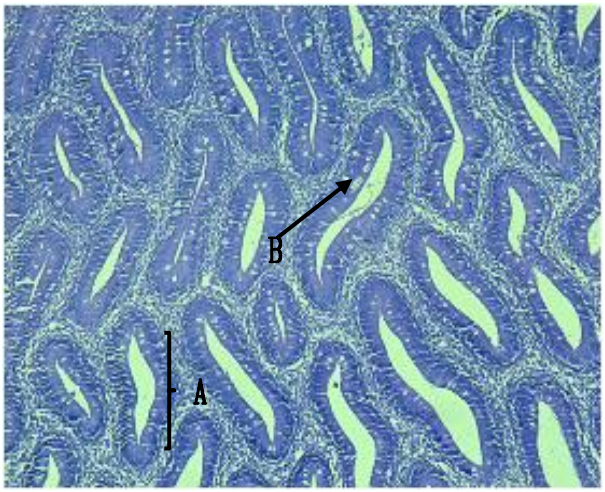


Figure, *7:* Longitudinal microscopic section of anterior portion of intestine of common carp, (A)fold, (B)goblet cell (arrow), PAS stain (X400).

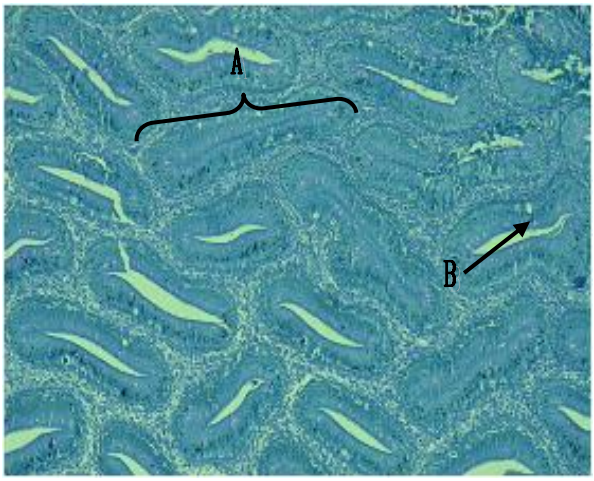
Figure, 8: Cross microscopic section of posterior portion of intestine of common carp, (A)fold, (B)submucosa, (C)Tunica muscularis, H & E stain (X40).



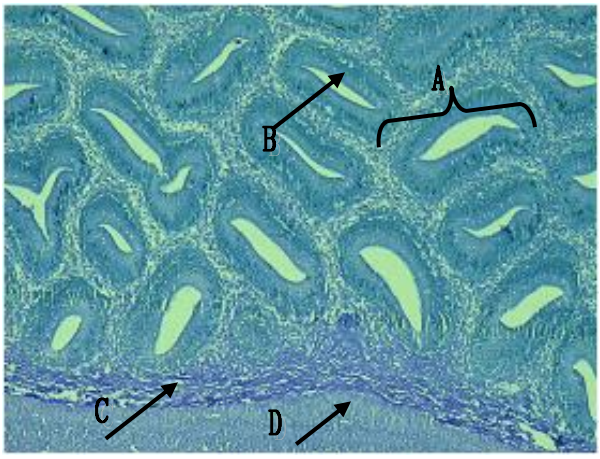
Figure, 9: Longitudinal microscopic section of anterior portion of intestine of common carp, (A) mucosal fold , (B) goblet cell (arrow),(C) Columnar cells, AB stain (X100).



Figure, 10: Longitudinal microscopic section of middle portion of intestine of common carp, (A)mucosal fold, (B) goblet cells(arrow), AB stain (X 100).



Figure, 11: Longitudinal microscopic section of anterior intestine of common carp, (A) fold (B)goblet cell (arrow), PAS with AB ph2.5 stain (X100).



Figure, 12: Longitudinal microscopic section of posterior portion of intestine of common carp, (A) Fold,(B) goblet cell, (C) submucosa, (D) tunica muscularis, PAS with AB ph2.5 stain (X100).

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دراسة مجهريه على الخلايا الامتصاصية والخلايا الكاسية في أمعاء سمك الكارب البالغ (*Cyprinus carpio*)

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الخلاصة

تهدف الدراسة الحالية لوصف بعض التراكيب النسجية والخصائص الكيميائية النسجية للخلايا العمودية (الامتصاصيه) والخلايا الكاسية في أمعاء سمك الكارب . أجريت الدراسة على خمسة عشرعينة من سمك الكارب البالغ تم اصطيادها من نهر الفرات ، معدل وزنها (2100 ± 81) غرام وطولها القياسي (50.4 ± 3.1) سم بعد القتل مباشرة . تم إجراء شق خلال الخط ألبطني الوسطي لكل سمكه تماما أماميا لفتحة المخرج لإخراج الأمعاء، أخرجت عينات الأمعاء وغسلت في المحلول الملحي الفسيولوجي الطبيعي (0.9 %)، تم اخذ عشر عينات أخذت من مناطق مختلفة من كل جزء من الأمعاء (الأمامي والوسطي والخلفي) وثبتت إما في محلول الفورمالين المعادل (10%) أومحلول بوين ولمدة 24 ساعة بدرجة حرارة الغرفة بعد ذلك مررت العينات بالتقنية النسجية الروتينية ، وضعت الشرائح النسجية بملون الهيماتوكسيلين والايوسين وكاشف شف الدوري وملون الشيان الأزرق (اس هيدروجيني 2.5) واتحاد ملون الشيان الأزرق مع كاشف شف الدوري (اس هيدروجيني 2.5) تم قياس متوسط كل من عدد وارتفاع وعرض الطيات المخاطية وعدد الخلايا الكاسية في الطيات المخاطية لكل جزء من الأمعاء .أظهرت النتائج الحالية النسيج الظهاري للأمعاء عمودي بسيط والخلايا العمودية خلايا طويلة مع حافة الفرشاة، الخلايا الكاسية ظهرت بشكل بيضوي تقع بين الخلايا العمودية ، هنالك طيات مستعرضه في كل سمك الطبقة المخاطية مع قلب الطبقة تحت المخاطية. متوسط عدد وارتفاع وعرض الطيات المخاطية في الجزء الخلفي للأمعاء اقل مما هو عليه في بقية الأجزاء، والجزء الأمامي للأمعاء يمتلك اكبر عدد من الطيات المخاطية لكن اقل عدد من الخلايا الكاسية مقارنة مع الجزء الوسطي والخلفي للأمعاء. الدراسة الحالية أظهرت ازدياد عدد الخلايا الكاسية باتجاه الجزء الخلفي للأمعاء للتزييت وطرح الفضلات ووجود علاقة عكسية بين عدد الطيات المخاطية والخلايا الكاسية.

الكلمات المفتاحية: : سمك الكارب ،أمعاء، خلايا عمودية، خلايا كاسيه