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histological study of crop and proventriculus in pre and post hatching period in Blondinette pigeons

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Abstract

This study investigates the histological and histochemical changes in the crop and proventriculus of blondinette pigeons (Columba livia domestica) during the pre-hatching and post-hatching periods. Histological sections revealed distinct structural modifications associated with functional demands. In the crop, pre-hatching samples exhibited a stratified squamous epithelium with well-vascularized lamina propria and minimal desquamation. Post-hatching samples showed significant epithelial thickening, desquamation, and lipid droplet accumulation, indicating active crop milk production influenced by prolactin. In the proventriculus, mucosal folds lined by simple columnar epithelium and active glandular cells (chief and parietal) were observed. Post-hatching samples showed increased glandular activity, deeper gastric pits, and thicker muscular layers, suggesting enhanced digestive functionality. Histochemical PAS staining confirmed the presence of neutral mucopolysaccharides in mucosal glands

I. Introduction

Like in mammals, the avian digestive tract is an open, double-ended tube that extends from the beak to the vent. The mouth, esophagus, crop, proventriculus, ventriculus (gizzard), intestine, ceca, rectum, and cloaca make up this structure in that sequence. During a species' evolutionary process, some of these structures could become vestigial or even disappear. Food moves through the digestive system in a certain order, which includes premoistening and softening, acidifying, grinding, hydrolyzing, emulsifying, and propelling the final products. Klasing (1999)

The crop is an inflated structure located in the middle and lower portion of the bird esophagus Ibrahiem(2013) found that during nonbreeding periods, the pigeon crop displayed a thin epithelial lining with keratinized, stratified, squamous cell types. In contrast, in breeding pigeons, the crop consists of mucosa, lamina propria and submucosa,(Gillespie et al., 2011) the lamina propria has loose connective tissue, and there are many large ductal glands in approximately 2/3 of the proximal esophageal region.The glands are a mixture of mucous glands and serous glands with a cystic appearance (Bharathi, Shenoy, & Hegde, 1997) . Specifically, the muscularis mucosa is a thick layer of smooth muscle fibers; while the submucosa contains loose blood vessels and nervous connective tissue . Muscularies externa was inner circular and outer longitudinal smooth muscles fibers (Saleem, 2013)

The glandular stomach (proventriculus) varies in size between species, being relatively small in graminivores, but often quite large and distensible in carnivores that ingest large food items and in ostriches which use it for water storage, In most species, however, food passes rapidly through the glandular stomach and is held in the muscular stomach where in the actions of the gastric secretions (pepsin, HCL, and mucus) occur (Duke, 1997)

II. Materials and methods

Eighteen healthy, mature Blondinette pigeons were used in this study. The pigeons were collected during the winter months (October to December) from Al-Muthanna Province. Their body weights ranged from 370 to 400 grams, and their ages were between 4 and 6 months. All birds were housed in cages under standardized environmental conditions and were provided with food and water

After the observation period, the pigeons were divided into two groups. Anesthesia was administered using chloroform via inhalation. A longitudinal dissection was then performed through the thoracic cavity, and the following digestive organs were carefully extracted in order: crop and proventriculus

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III. Results and Discussion

4.1 Histological and Histochemical study

4.1.1 Crop:

The crop sac wall consists of four tunicae; mucosa, submucosa, musculosa and serosa figure (1,3) **(Deka, Sarma, Rajkhowa, Sarma, & Ahmed, 2017)**

4.1.1.1 Mucosa:

The lining epithelium of the crop, a of blondinette pigeons is composed of stratified squamous non-keratinized epithelium (2,4) similar to that reported in pigeons, quails, and cattle egrets, **(El-naseery, Mohammed, Abuel-Atta, & Ghonimi, 2021) (Sa\ugsöz & L\iman, 2009)**. In contrast, stratified squamous keratinized epi thelium is mainly found in the common rock and wood pigeons, collared doves, domestic fowl, house sparrows, kestrels, and linnets **(Ali, Ali, & Taha, 2023) (Al-Juboory, Daoud, & Al-Arajy, 2015) (Mobini, 2014)(Rajabi & Nabipour, 2009).**

Esophageal mucosa were folded and composed of stratified squamous epithelial tissue, and numerous ducts from mucous glands were opened on the sides of the mucosal folds, and these observations are similar to some birds that reported by many researchers **(Mohammed, Abuel-Atta, Ghonimi, & El-Naseery, 2021)(Nagy et al., 2005)**. Like in other avian species **(Srisai, Juntaravimol, Pongkete, Koonjaenak, & Suprasert, 2002)**. In guinea fowl, the mucous glands of the esophagus were located in the lamina propria and the shape of its secretory units was branched tubular alveolar type. There are reports that the shape of the secretory units of these glands can be different in animals of the same species **(Srisai et al., 2002)**.

Histological changes in the crop wall of pigeons post hatching was observed in the epithelial lining only. The thickness of the stratified squamous epithelium increased significantly during the brooding period.This is due to heightened mitotic activity in the basal layer this result agree with **(Reshag, 2009)**.

the epithelial layer suffers from Cell Desquamation of superficial epithelial cells starts to occur but is minimal on the first day figure (4). This process intensifies over the next few days as the crop milk production fully develops We assumed that these cellular processes facilitate the desquamation process of the superficial cells to share in the crop milk production,this result agree with **(Gillespie et al., 2011)**.

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Crop milk is highly nutritious; it is rich in protein and fat, but poor in carbohydrates. It provides an easily digestible food that is rich in immunoglobulin A, and contains glyco proteins, transferrin, and pigeon milk growth factor **(Wang, Zhu, Xie, & Gong, 2023)** . Crop milk consists of desquamated mucosal epithelial cells lining the crop, and is mainly produced in the lat eral diverticula **(Mahdy & Mohammed, 2024)**. During the productive period, the walls of the diverticula thicken under the influence of prolactin hormone. Crop milk is strongly similar to mammalian milk, but is deficient in carbohydrates and calcium **(Gussekloo, 2006)**. In hoatzin, the crop is considered the site of food digestion by microbes **(Evans, 2016)**. Furthermore, crops play an essential role in innate immunity as the first major defense against foodborne pathogens **(van Dijk et al., 2007)**

On the first day, epithelial cells begin accumulating lipid droplets within the cytoplasm this result with **(Gillespie et al., 2011)**

but the degree of lipid storage is moderate compared to later stages of crop milk production

The process is under the control of prolactin, which begins to exert its effects strongly during the late incubation period and reaches peak influence during hatching and feeding

lamina propria:

Located beneath the epithelium is composed of loose connective tissue which may contain mucous glands that containing blood vessels, nerve endings, immune cells, and lymphatic tissue, contributing to vascular supply and immune defense figure (1,2,3) **(Rajabi & Nabipour, 2009)** that's appare in the period of pre hatching till day of hatching . while post hatching the lamina propria are Contains dense connective tissue but vascularization decreases compared to the peak of crop milk production with increased fibroblasts and collagen fibers to support epithelial proliferation **(Mukherjee, Das, & Banerjee, 2023)**.

Muscularis mucosa:

The muscularis mucosa in all groups were well developed and consisted of longitudinal bundles of smooth muscle this result similar to that in the Guinea fowl observation by**(Hameed & ; Jihad A. Ahmed, 2019)**.

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4.1.1.2 Submucosa:

The submucosa is poorly developed, made up of loose connective tissue with submucosal nerve plexuses along its structure **(Herrero, 2023)** in pre hatching.

While post hatching the submucosa Highly vascularized, with an increased number of capillaries and blood vessels, that may be due to ensuring an adequate supply of nutrients for crop milk formation The submucosa remains glandless, as pigeon crops lack secretory glands.

4.1.1.3 Muscularis Externa:

The tunica muscularis in all groups consists of two distinct layers of smooth muscle: the inner layer is thick and circular, while the outer layer is thin and longitudinal. These findings are consistent with the results reported by Nagay et al.,**(Nagy et al., 2005)**, who studied the histology of chicken esophagus. that tunica muscularis of the chicken esophagus was composed of two layers, an inner thick circular and thin outer longitudinal layer. It was similar to our results in blondinette pigeon. while disagreement with Rossi et al.,**(Rossi et al., 2006)** which has reported, tunica muscularis of the esophagus in Partigad Raynchotus consists of three layers of smooth muscle, two inner and outer longitudinal layers and a circular middle layer **(Hameed & ; Jihad A. Ahmed, 2019)**

4.1.1.4 Tunica serosa:

consists of connective tissue with vessels and nerves, and endothelial cells there is no difference between this layer and the tunica serosa in all groups **(Rajabi & Nabipour, 2009).**

Histochemical of the results of staining of crop by PAS showed a positive to reaction mucosal glands of crop as in figure (5) While mucosal glands revealed a positive reaction to PAS stain as an indication to the presence of mucopolysaccharides.These results agreed **with (Hameed & ; Jihad A. Ahmed, 2019)** who pointed that in Guinea Fowl

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Histomorphometry measurements of crop epithelium measerment was in pre hatching period 506.15±23.

While in post hatching period the thickness of the epithelium was 247.68±23.96

TaЬIe (1). Histomorphometry measurements of crop showed significant differences in epithelium thickness between groups

Period	Mean±SD
pre hatching	506.15±23.07
Post hatching	247.68±23.96

4.1.2 Proventriculus:

The proventriculus The result of histological sections of the wall of proventriculus in blondinette pigeon consist from four layers: tunica mucosa, tunica submucosa, tunica muscularis and serosa outer figure (6). This result was consistent with **(Kareem & Al-Zubaidi, 2021)** who noticed in Guinea fowl while this result matches with **(Marshall & Folley, 1956)**, who found in Asiatic swiftest only three layers only in it: mucosa, muscularis and serosa were detected.

4.1.2.1 Tunica Mucosa:

A mucous membrane of the all groups tunica mucosa was folds which differed in heights figure(6) that correspond with **(Abdul Ridha, Salih, Bargooth, & Wali, 2019)**, who record in quail, some fold confluent to each other this folds lined by simple columnar epithelium

lamina propria

Pre hatching The lamina propria distributed to the mucosal folds as a core of loose connective tissue, include blood, lymphatic vessels and numerous superficial glands, these glands was simple tubular founded in the foundation of the villi figure(6) in agreement with **(Abdul-Ridha, Salih, Bargooth, & Wali, 2019)** record in Domestic pigeon (Columba liviadomestica), these glands were lined by simple cuboidal epithelium and scattered at the top portion in the lamina propria, the propria was discarded from the underlying submucosa by

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fibers of smooth muscle known muscularis mucosa. This result agreement with **(Selvan, Ushakumary, & Ramesh, 2008)** in guinea fowl and **ostrich (Tadjalli, Parto, & Shahraki, 2011)**.

The laminae properia is extended toward folds of mucosa which is included simple gastric tubular gland, which is mucus secretion. These glands are drained (opened) inside the lumen on the epithelial surface, a result same with to those of the fowl **(Hodges, 1974)**.

The glandular region becomes more active, with Chief cells (zymogenic cells) and parietal cells .

The chief cells were found to be the main type of cells that lined the glandular tubules. These cells appeared as high columner in shape with round or elongated nuclei producing digestive enzymes figure(7) **(Khan & James, 1998)** .

Parietal cells These are specialized cells found in the stomach lining, specifically within the gastric glands. These cells are generally **pyramidal or triangular**, with their broad base anchored to the basal lamina and their apex pointing towards the lumen of the gastric gland figure(7) **(Hunt, Harrington, & Robinson, 2014)**.

Gastric gland isolated by septa between Loose connective tissue with missions plexus Adipose tissue figure (6) between gastric gland in septa—Mucous Cells Increase in number, possibly linked to a heightened immune response and protection against gastric acidity figure(8) .

Post hatching the Gastric pits extending deeper into the lamina propria suggest an increase in glandular depth . This could be an adaptive response to increased digestive secretion requirements or a reorganization of glandular structures . Overall Interpretation These changes indicate a functional and structural reorganization in the proventriculus. The loss of connective tissue between glands, mucus reduction, and disappearance of the muscularis mucosa suggest a shift toward increased glandular activity and secretion. This might be a response to higher metabolic demands or changes in digestive.

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4.1.2.2 tunica submucosa:

The tunica submucosa in pre and post hatching formed the biggest part of the wall thickness of the proventriculus; it was formed from loose connective tissue containing pear-looks like branched tubular proventriculus glands enclosed by fibrous capsule in deep gastric gland . This result disagree with **(Rocha, 1991)** in the burrowing owl whom described these glands as oval shape.

4.1.2.3 tunica mucularis:

The tunica muscularis was composed of two layer inner thick arranged circular and outer thin manner longitudinal . This result similar finding by **(Rocha, 1991)** who record in duck and pigeon, different with **(Rodrigues et al., 2012)**, who finding the tunica muscularis of macaw consist of one layer of muscle.

4.1.2.4 tunica serosa:

The serosa in all groups were loose connective tissue which rich bynerves, blood vessels, adipose cells were observed and coated by layer of mesothelium . This result were corresponding with **(Ahmed, Kamel, & Ahmad, 2011)**

Histochemical of the results of staining of proventriculus by PAS showed a positive to reaction because the found neutral mucin figure (9). This result agreed with (Hassan & Moussa, 2012) who record in duck

Histomorphometry measurements of proventriculus inner muscle measerment was in pre hatching period 666.67 ±121.1 and outer 1483.33±313.16

While in post hatching period the thickness of the muscle was 2150±796.87 and outer2833.33±314.11

TaBle (2). Histomorphometry measurements of crop showed significant differences in muscle thickness between groups

Proventriculus Inner

period	Mean ± SD
Pre hatching	666.67 ±121.1
Post hatching	2150±796.87

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Proventriculus outer

group	Mean±SD
pre hatching	1483.33±313.16
Post hatching	2833.33±314.11

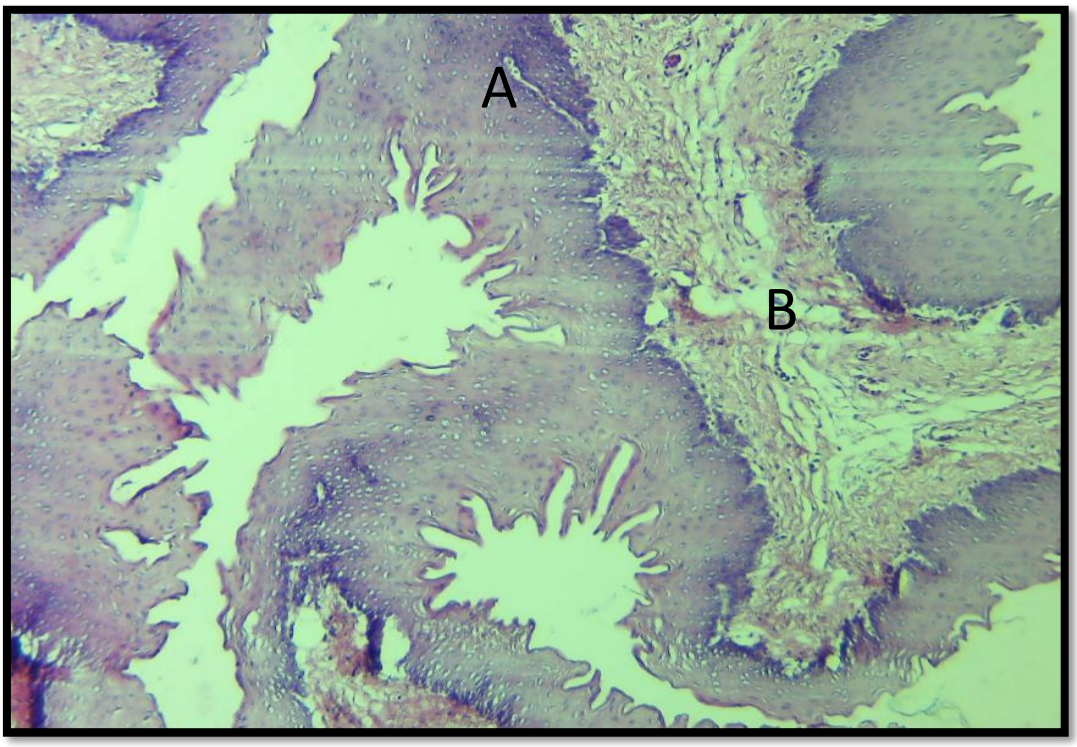

Figure (1): Longitudinal sections of the Crop in pre hatching show the: **A**. Stratified Squamous epithelium **B**. Lamina properia.(H&E,10X)

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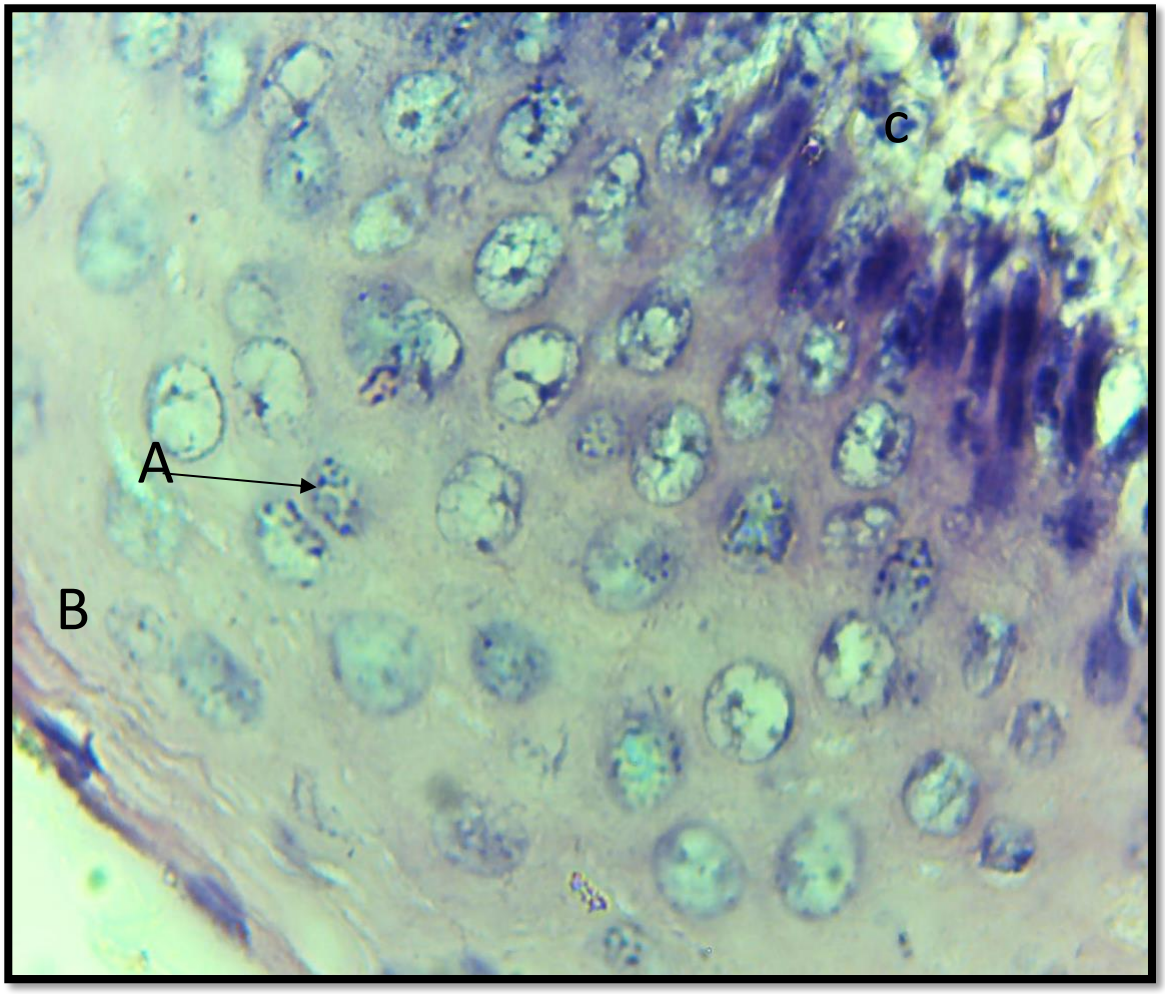

Figure (2): Longitudinal sections of the Crop of pre hatching show the: **A**. squamous cell **B**. stratified squamous epithelium c. Lamina properia.(H&E,100X)

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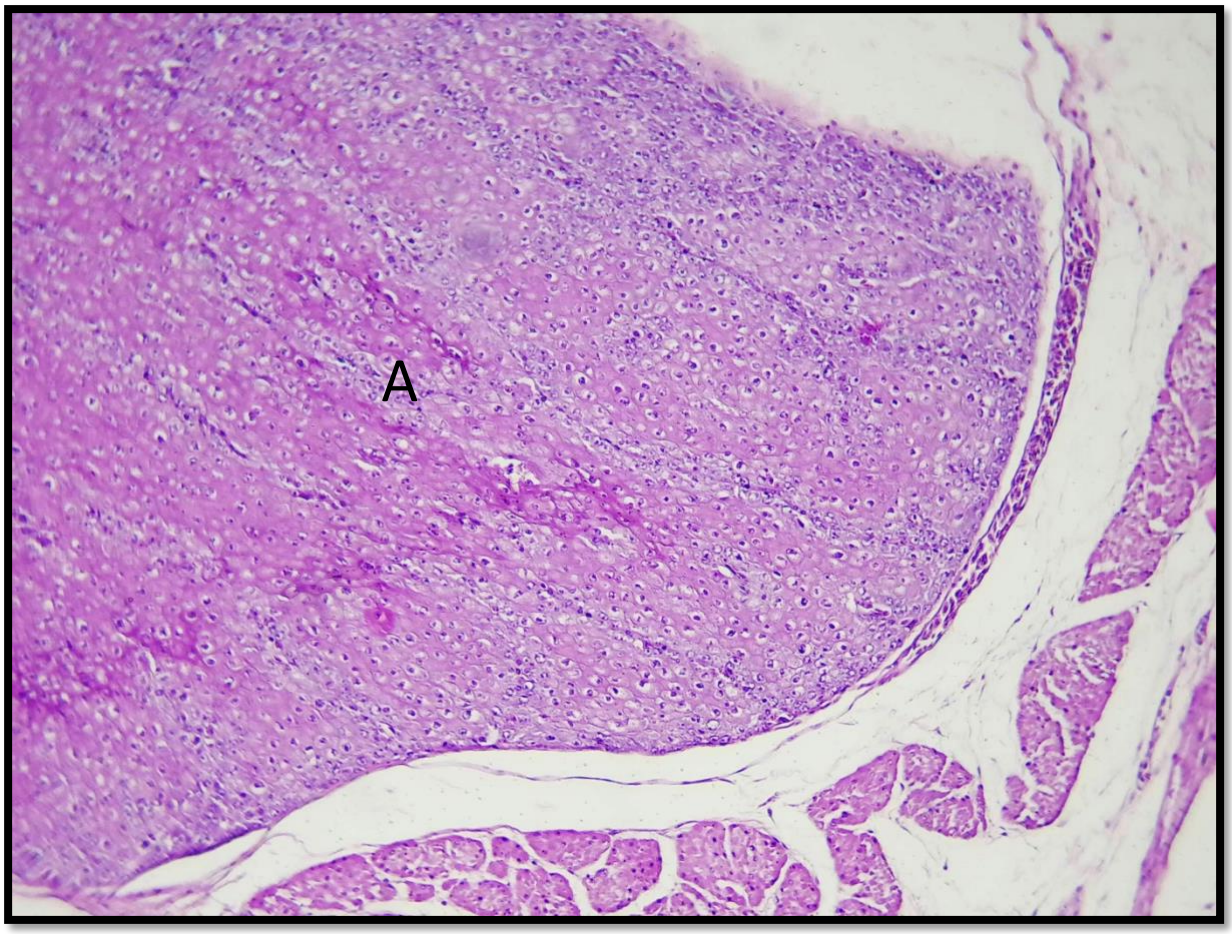

Figure (3): Longitudinal sections of the Crop of the post hatching show the: **A**. stratified squamous epithelium B. Lamina properia c. muscularis mucosa . (H&E,10X)

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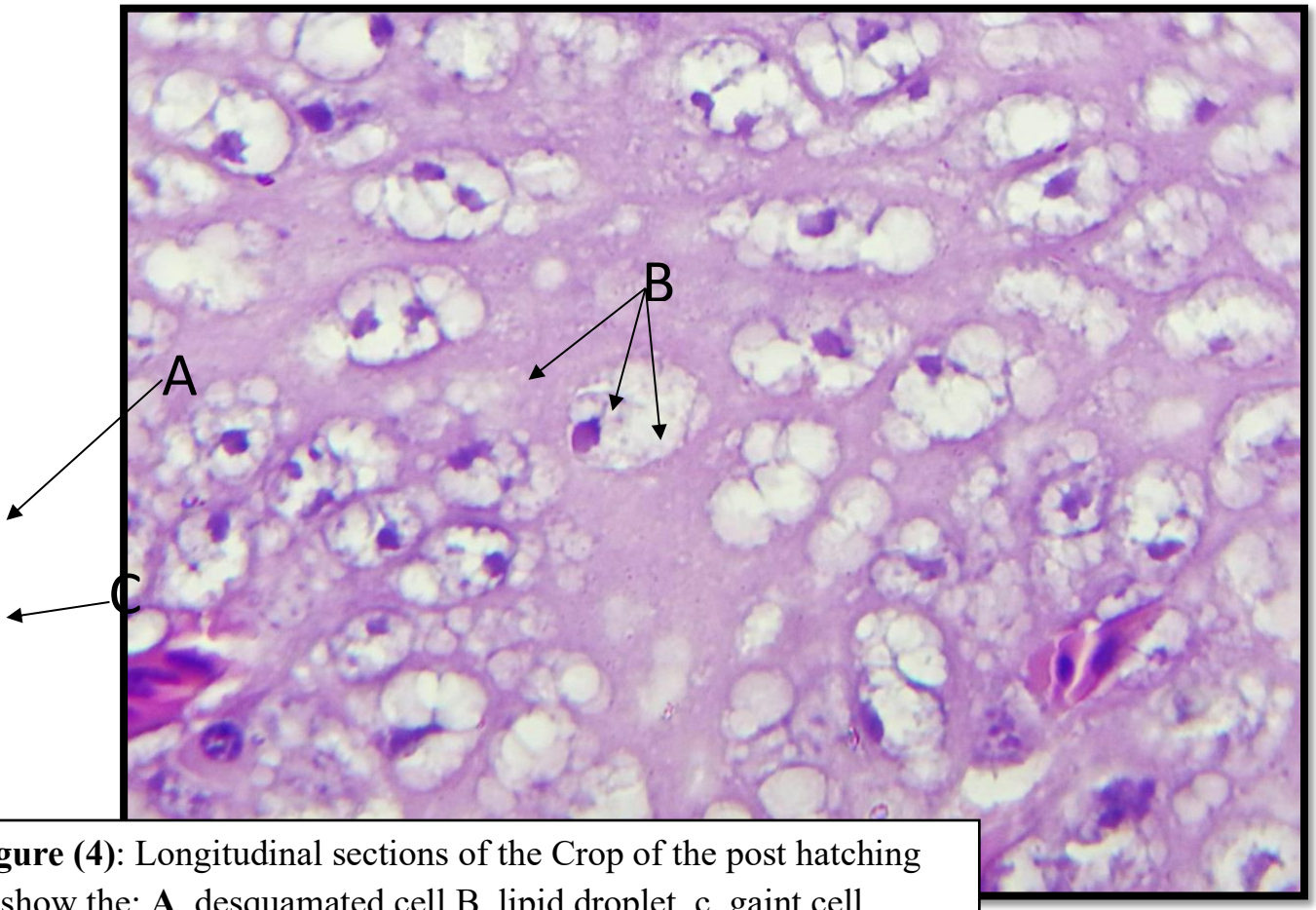

Figure (4): Longitudinal sections of the Crop of the post hatching show the: **A**. desquamated cell B. lipid droplet c. gaint cell . (H&E,100X)

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Figure (5): Longitudinal sections of the Crop of the show the: **A**.stratified squamous epithelium B. mucosal gland . (PAS,100X)

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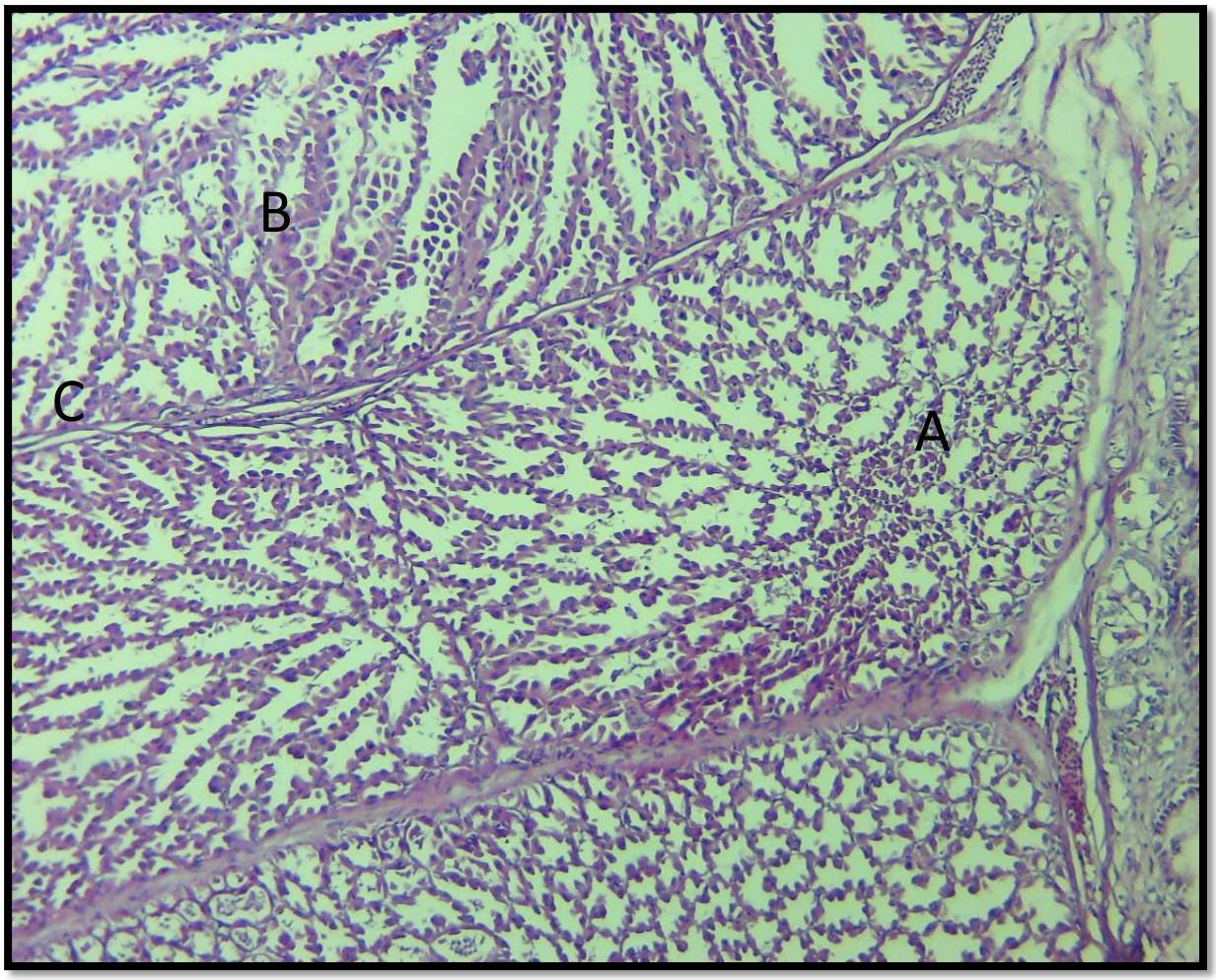

Figure (6): Longitudinal sections of the Proventriculus of the show the: **A**.simple columnar epithelium B. gastric gland .C. septa (H&E,10X)

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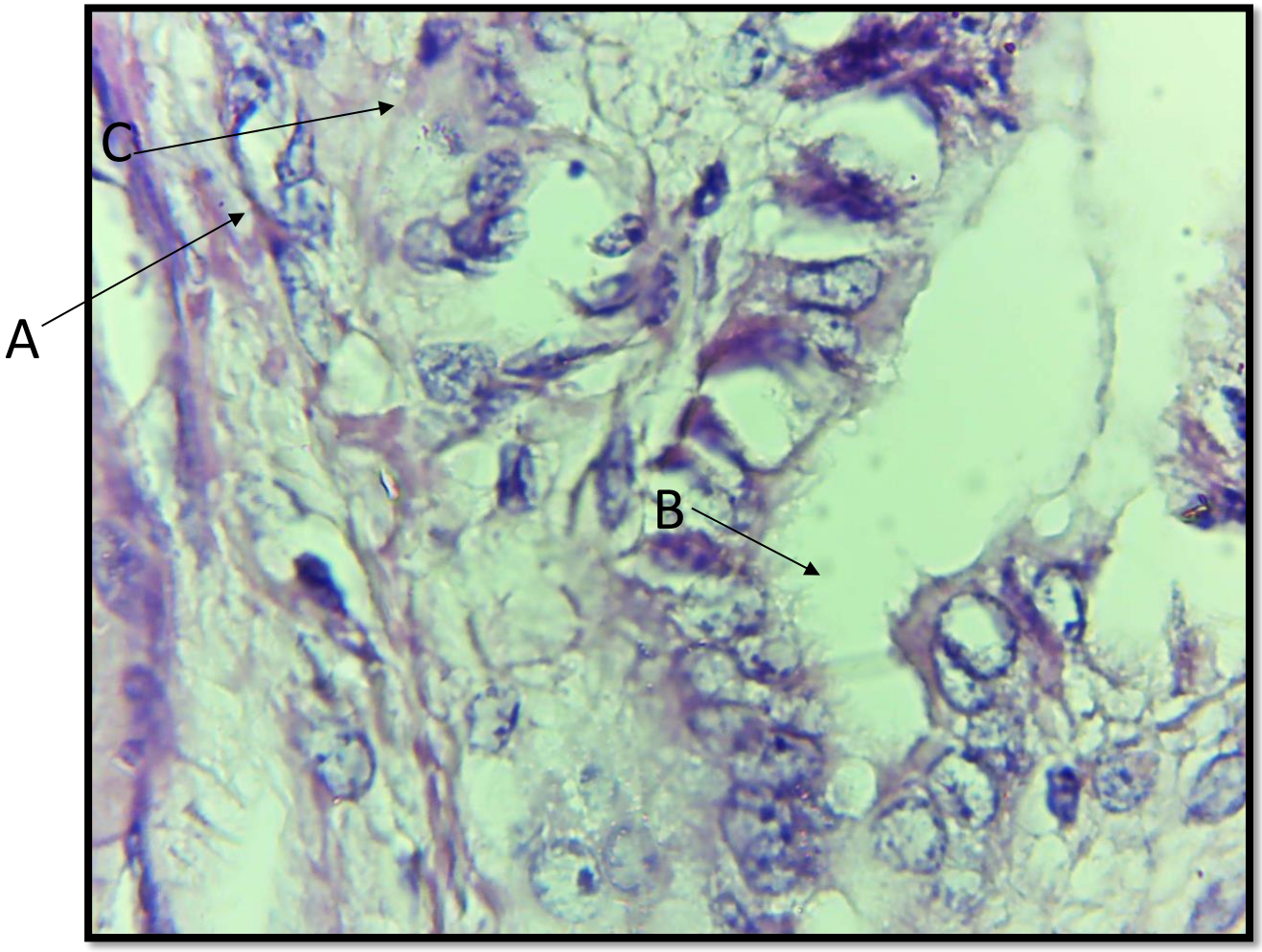

Figure (7): Longitudinal sections of the Proventriculus in the pre hatching period show the: **A**.cheif cell B. parietal cell .C. mucus cell (H&E,100X)

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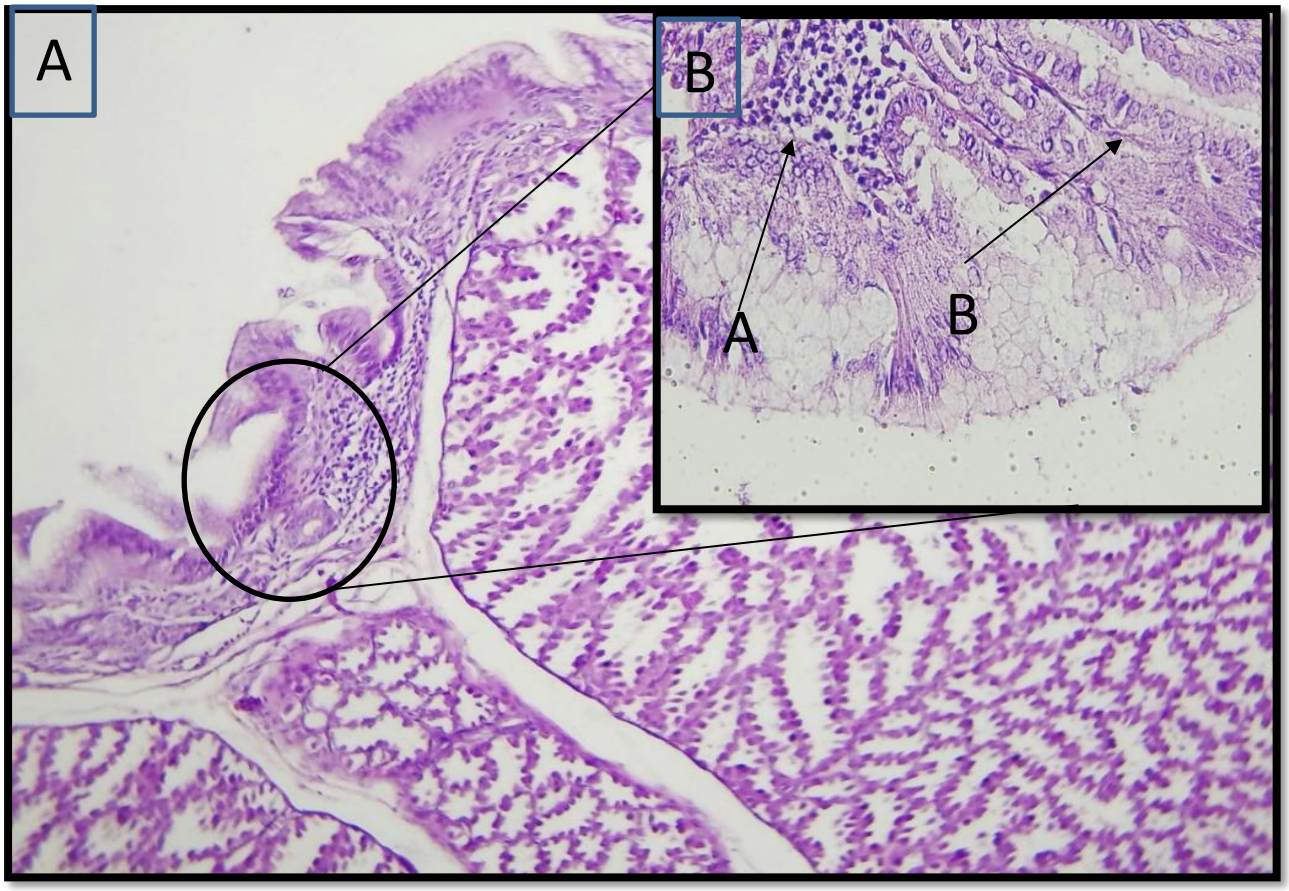

Figure (8): Longitudinal sections of the Proventriculus in thepost hatching period show the: **B.A**.simple columnar cell B.lumphoid tissue (H&E,A.10X, B.40X)

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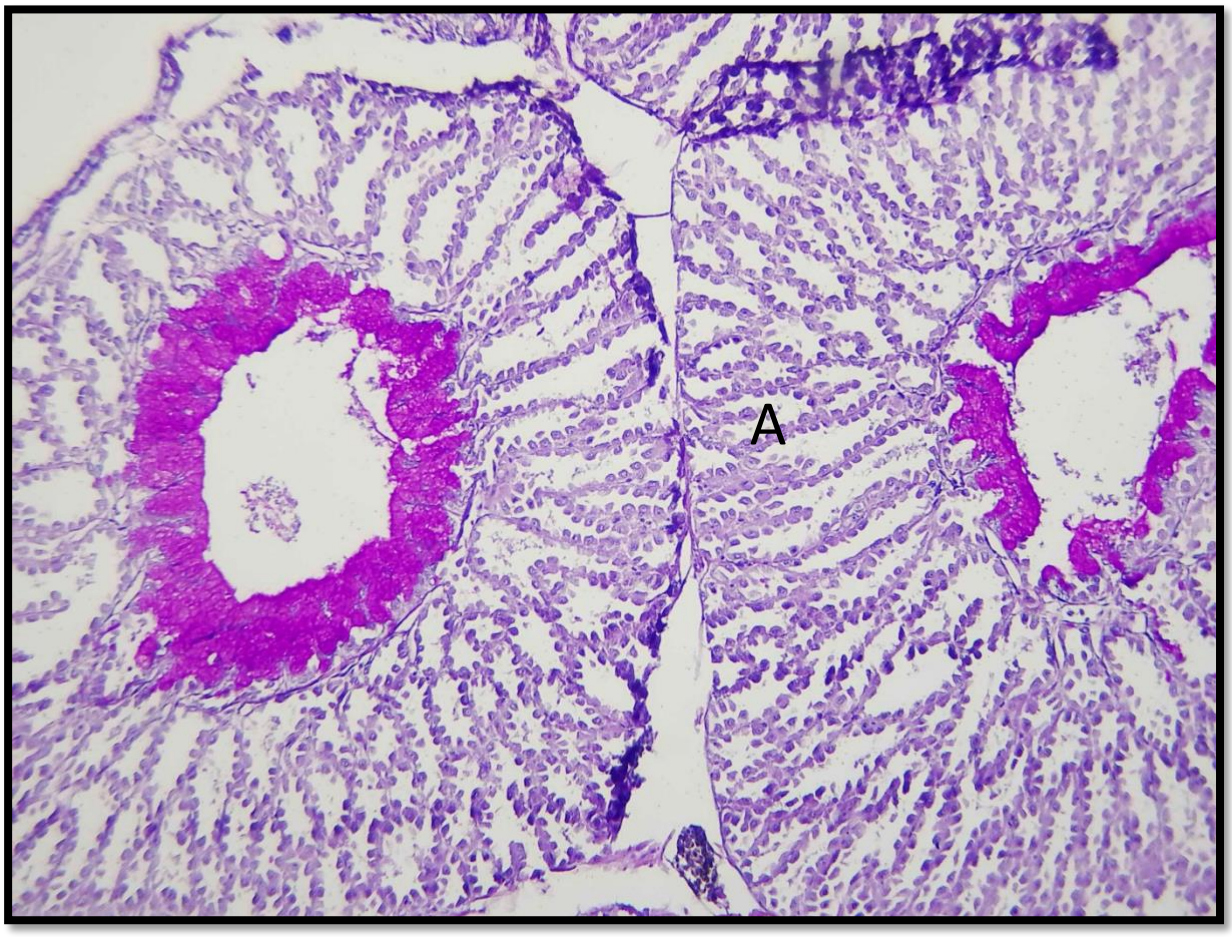

Figure (9): Longitudinal sections of the Proventriculus show the:A.mucus B.gastric gland (PAS ,10X)

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