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Comparison of Histological and Embryonic Development for Respiratory System in Two Types of Birds (Chicken and Pigeon)

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Abstract:

In this study, we identify the time of formation of the respiratory system in pigeons and chickens, and we investigate the embryonic formation of the trachea for both of them, in addition to identifying the size of the trachea in chickens and pigeons. The current study relied on (120) chicken and pigeon eggs, divided into two groups (60 pigeons and 60 chickens), which were obtained from local markets in Salah al-Din Governorate. Their weights were approximately are close. Finally, comparison is made between the trachea and lung in embryos and after hatching, to identify the similarities and differences in both types during the embryonic period and the period after hatching.

Keywords: Avian**,** pigeon, decoupling, Fixation, Embryological, Histological, and Respiratory system.

مقارنة التطور النسيجي والجيني للجهاز التنفسي في نوعين من الطيور (الدجاج والحمام)

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قسم الطب البيطر/ كلية الطب البيطري / جامعة تكريت / تكريت

بحث مستل من رسالة ماجستير للباحث الاول

مستخلص

تم في هذه الدراسة التعرف على زمن تكوين الجهاز التنفسي عند الحمام والدجاج، كما قمنا بدراسة التكوين الجنيني للقصبة الهوائية لكل منهما، بالإضافة إلى التعرف على حجم القصبة الهوائية في الدجاج والحمام. اعتمدت الدراسة الحالية على (120) بيضة دجاج وحمام، مقسمة إلى مجموعتين (60 حمامة و60 دجاجة)، تم الحصول عليها من الأسواق المحلية في محافظة صلاح الدين. وكانت أوزانهم متقاربة تقريبًا. وأخيراً تم إجراء مقارنة بين القصبة الهوائية والرئة في الأجنة وبعد الفقس للتعرف على أوجه التشابه والاختلاف في كلا النوعين خلال الفترة الجنينية وفترة ما بعد الفقس.

1. Introduction

Avian birds are an excellent biological indicator of ecosystem health, as they play an important role in ecological balance through biological control and natural selection. The class of birds belongs to the animal kingdom, the phylum Chordata, and the number of species belonging to it is estimated at 9,990 species distributed in 28 orders [1].

The pigeon is considered one of the most familiar and closest types of birds to humans. It is one of the oldest domesticated animals in the world, as it is found on all continents, especially the continent of Asia. It is raised as a source of food, decoration, and sport in addition to laboratory purposes. Chickens are also a representative model for studying embryonic development. In birds, but there are important differences between this category of species. An example of one of the oldest groups of birds in existence [2].

Among air-breathing vertebrates, the avian respiratory system, the pulmonary air sac system, is the most structurally complex and functionally efficient. The anatomy of the respiratory system in domestic bird species including chickens and pigeons here plays a major role in avian breathing, which varies Essentially about breathing in mammals during inhalation and exhalation [3]. There are many distinct differences in shape and physiology between the air sac of the avian lung and the bronchoalveolar lung of mammals [4]. As a result, the diffusion capacity (conductivity) of the bird's lungs for oxygen is remarkably efficient. The most important adaptive improvements are: (1) lung rigidity that allows intense partitioning of reciprocal tissues (parenchyma) leading to the formation of very small peripheral respiratory units and thus a wide respiratory surface; (2) a thin blood gas barrier enabled by trapping pneumocytes (particularly type II cells) and connective tissue elements in the atria and the fundus, i.e. away from the respiratory surface of the capillaries; (3) Physical separation (decoupling) of the lung (gas exchanger) from the air sacs (mechanical ventilation), allowing continuous, unidirectional ventilation of the lung [5]. However, many fundamental aspects of the evolution, and even the structure and function of the avian respiratory system remain uncertain. Many anatomical structures and

aerodynamic mechanisms have been described in an attempt to explain the proposed mechanism of breathing [6]. It has been found that in response to existing levels of hypercapnia and hypoxia, the increase is less in spontaneously breathing pigeons and chickens than in other poultry. The primary effect of excessive hypoxia on chicken heart rates is bradycardia [7].

The study aims to identify the time and stages of the formation of the respiratory system and investigate, as well as to determine the size of the trachea pigeons and chickens, the formation of lung for both of them, and the formation of the air sacs and alveoli.

2. Methodology

2.1. Experiment animals

The current study was based on chicken and pigeon eggs, which numbered (120) and were divided into two groups (60) pigeons and (60) chickens. It was received from local markets in Salah al-Din Governorate. Their weights are close, after examination to ensure the safety of the eggs by one of the doctors. The veterinarians housed her in the animal house under controlled conditions in terms of light and temperature reached before the incubation process began.

2.2. Experiment design

After placing the eggs in the incubator, samples were taken for both species to detect the development of the respiratory system, and all animals were sacrificed 60 days after the experiment began. The samples were divided into two groups: A group of pigeons for histological and embryonic examination, and a group of chickens for histological and genetic examination.

60 birds of each species were divided into two groups: a genetic study group before hatching and another histological group after hatching. The embryonic study group included collecting the sample (eggs) before hatching for pigeons and chickens at one day old and 7, 8, 10, 12, 14, 16, 18 and 20 days before hatching. As for the histological study group, it includes collecting the sample (birds) one, 10, 20, 30, and 40 days after hatching.

2.3. Anesthesia and anatomy

After reaching the hatching stage, the animals were anesthetized by placing cotton soaked in the anesthetic chloroform in a glass container with a tight lid. Then the animals were placed inside the container until they stopped moving, and they were dissected ac-

cording to the method (AL Sayed, 2008). After anesthesia, they were transferred to the dissection dish and placed on a plate. The dorsal side was securely fixed from its front and back ends with pins, and then the following steps were performed:

1. Remove the feathers from the ventral side of the bird, from the lower jaw area to the mandible area.

2. Make a longitudinal incision from the compound to the neck area.

3. The thorax cage was raised and shifted to the right and left sides to reveal the internal organs clearly, and it was washed with running water to remove the remains of blood and visceral fluids.

2.4. Embryological study

A total of 60 embryos were collected. Eggs were collected daily from date to, from different nests, and transported to the center affiliated with the University of Tikrit, where they were disinfected with a 0.1% sodium hypochlorite solution and incubated at an adjustable temperature of 37.5°C and an average relative humidity of 45%. Age "0" was defined as the time at which eggs was laid in incubation. Embryos and fetuses were collected from the first day of incubation to the twentieth day of development.

2.5. Histological study

Glass slides were prepared according to the method of 2010, Stevens and Bancroft. Histological sections were prepared according to the method mentioned in the Life Sciences Laboratory - College of Science / Tikrit University, as follows:

1. Fixation: The target organs was fixed immediately after dissecting the bird embryos for the studied groups with 10% formalin fixative (90 ml of water + 10 ml of 40% formaldehyde) for 24 hours.

2. Washing: The samples was washed with running water to remove the residue of the stabilizer for half an hour.

3. Dehydration: The samples was passed through increasing concentrations of ethyl alcohol (30-50-70-80-95-100%) for the purpose of gradually withdrawing water from them. The duration of each pass was (30) minutes, and the absolute alcohol step (100%) was repeated twice to complete the final removal of water.

4. Clearing: The samples was placed in xylene for (30) minutes in two stages for the purpose of making the tissue more transparent.

5. Infiltration: The samples was placed in a mixture of xylene and molten paraffin wax at 58ºC in a ratio (1:1), for (15) minutes, then passed the molten wax for half an hour with three passes to ensure even penetration of the wax.

6. Embedding: The samples was quietly buried in molten wax inside an L-shaped iron mold. The information (name of the group and type of member) was written and stuck to one side of the mold using tweezers. A hot needle was passed near the form to get rid of bubbles that might be present around the sample. The sample was left The sample cooled and solidified, then it was separated from the molds.

7. Trimming and sectioning: The wax molds containing the models was trimmed with a sharp blade, then the mold was placed on a stand mounted on a rotary microtome, and cut to a thickness of 5 micrometres. Then the ribbons containing the histological sections were transferred to a water bath at a temperature of (40) °C for the purpose Spread the tissue and prevent cells from accumulating on top of each other. Then the sections was placed on glass slides marked with a diamond pen (the type of member and the name of the group), after wiping them with Mayer's albumen loading medium prepared by adding (1) ml of egg white and (1) ml of glycerol (they were mixed In a beaker, then filter the mixture several times using several layers of medical gauze, and collect the filtrate in a tightly capped bottle and place it in the refrigerator until use after adding (1 gram of thymol crystals to it to prevent rotting), then place the slices on a Hot Plate at Temperature 40ºC, and left to dry.

8. Staining: Harris Hematoxylin & Eosin stain was used according to the method of Luna (1968), and the histological sections was colored according to the method of Humason (1997) as follows:

- I passed the glass slides with xylol twice for three minutes each time to remove the wax from them.
- I then went through a series of descending concentrations of ethyl alcohol (30-100%) for (3) minutes for each concentration.
- The sections was colored with hematoxylin solution for (5) minutes, then washed with running water for (3) minutes to distinguish the color and remove excess dye.
- They was then colored with eosin dye

for (15) seconds, then washed with water and immersed in containers for (5) seconds to distinguish the color.

- I then went through a series of ascending concentrations of ethyl alcohol (30-100)% for (2) minutes for each concentration.
- They was then placed in pots on xylol for (5) minutes for the purpose of softening.

9. Mounting: The slides was covered with loading medium Dibutylphthalate Polystyrene Xylene (D.P).

2.6. Histological examination and microscopic photography

An optical microscope designed for imaging, Microscope Light, will be used to examine and photograph tissue slides. The magnification power of 100X, 400X, and 1000X is used to determine the histological structure of the lung in pigeons and chickens and the histological changes.

After recording the notes, the clips were photographed using a Sony camera, and the images were taken using a 2400 HP color printer.

Microscopic histological measurements was calculated using a program to perform image metric measurement. Calibration and measurements taken in photographs was made before measuring with a 100-micrometer image of gradations with the same magnification power used for photography and digital cameras.

3. Results and Discussion

First: the appearance of the lungs and trachea of pigeons and chickens

3.1. Location and shape of the lung and trachea of pigeons and chickens

The trachea has been seen in both pigeons and chickens and is pale pink in colour. This organ is a long tube whose function is to transport respiratory gases from the upper respiratory tract to the respiratory organs – the lungs and air sacs or from the air sacs and lungs to the upper respiratory organs. Average-sized tracheas range in size from 15 to 18 cm. It is permanently open through 108 to 125 cartilage rings.

The bright pink lungs are formed at the end of the bronchi. The lungs are located on the sides of the chest, intertwined between the ribs, and their tissue is not elastic. The body has air sacs that derive their beginning from the lungs and bronchioles. The benefit of these sacs is to supply the lungs with air to help speed up the oxidation nec-

essary for flight. Each lung is covered from the inside by a thin, transparent membrane that blocks it from the rest of the body's internal organs. It does not have a diaphragm as in mammals. The respiratory system consists of the nose, throat, trachea, bronchi, and lungs, and a number of air sacs are connected to the lungs. As in the figure 1, and 2.

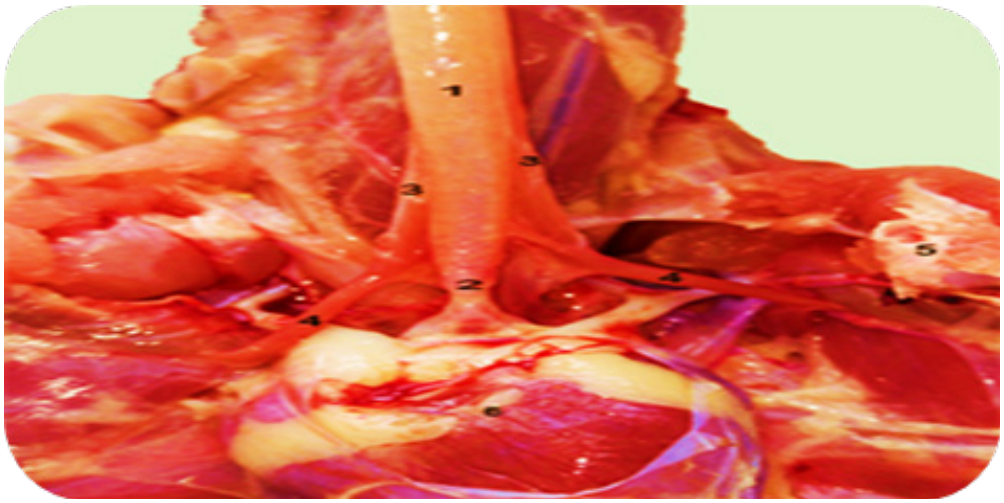

Figure.1 the location of the trachea in poultry.
1. Trachea. 2. Eardrum. 3. Tracheal muscles. 4. Sternotracheal muscles. 5. Cutting. 6. Heart

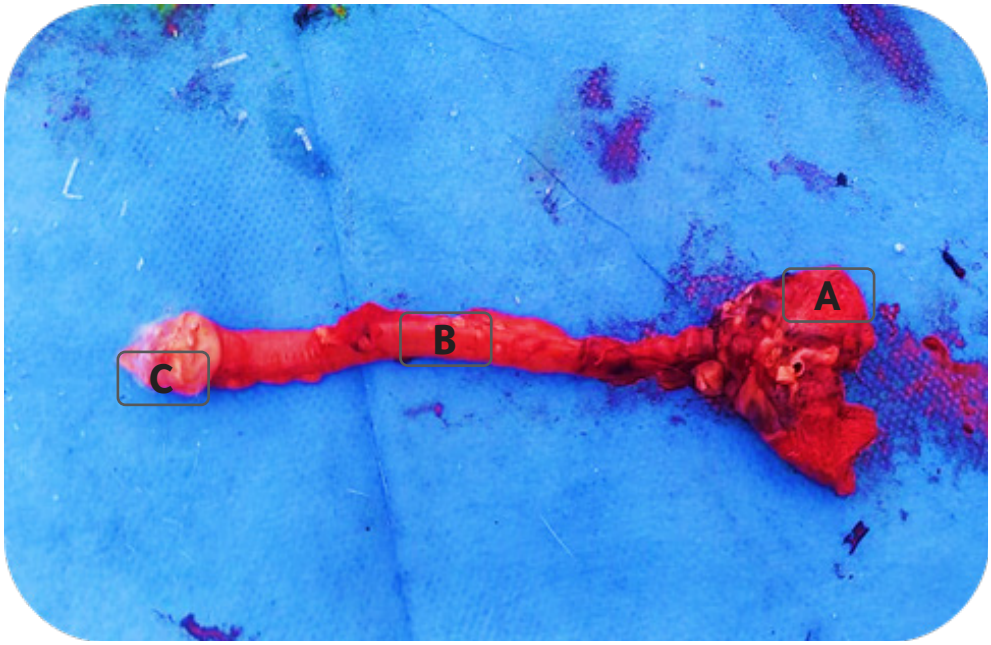

Figure. 2 Lung (A), trachea (B) and larynx (C) in poultry

3.2. ***Trachea and lung in both pigeon and chicken embryos at 7 days of age***

The results of the current study showed that in a 7-day-old pigeon embryo, the lung tissue contained soft mesenchymal tissue and contained star-shaped mesenchymal cells, in addition to the presence of a primitive trachea lined with simple cubic cells surrounded by a row of phagocytic cells and a few white blood cells, as shown in Figure.3. The lung also contained some alveolar ducts surrounded by interstitial connective tissue, in which there was an infiltration of many cubic cells, leading to the presence of cubic epithelial cells with large spherical nuclei in the wall of the alveoli. The lung tissue also contained alveolar cavities continuous with each other, and around them were the alveoli containing simple squamous epithelial cells with cells. The walls of the alveoli are cuboidal and continuous with the interstitium containing white blood cells, and the lung tissue is surrounded on the outside by a thin membrane of loose connective tissue, Figure.4.

While the larynx in pigeons contained pseudocolumnar epithelial cells and also contained numerous rows of dark-layered nuclei surrounded by wide-form melanocytic tissue. Figure.5.

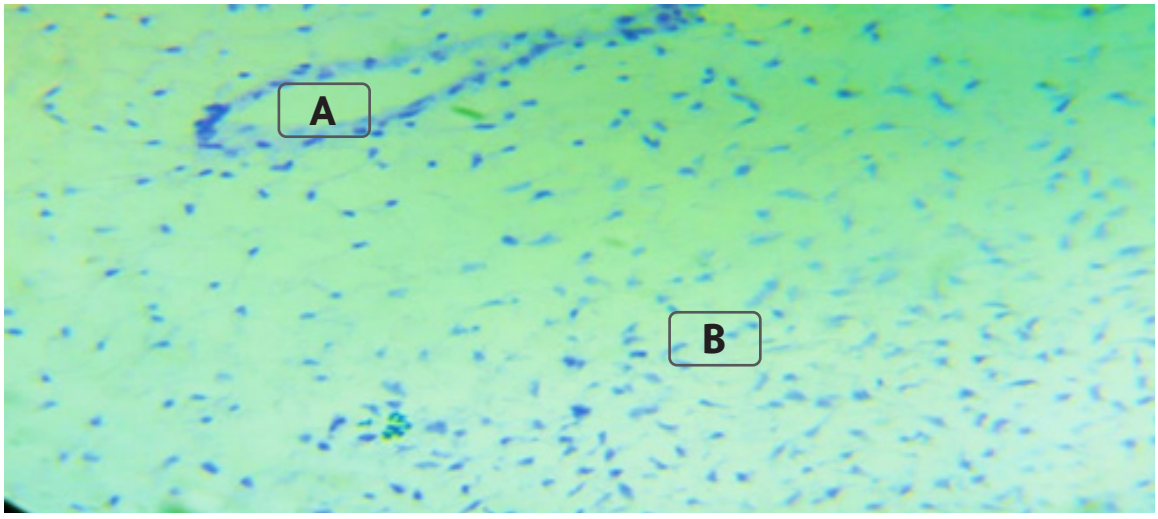

Figure. 3 Pigeon lung tissue
(A) Budding pneumonia (B) Mesenchymal tissue (E X40)ȣ H)

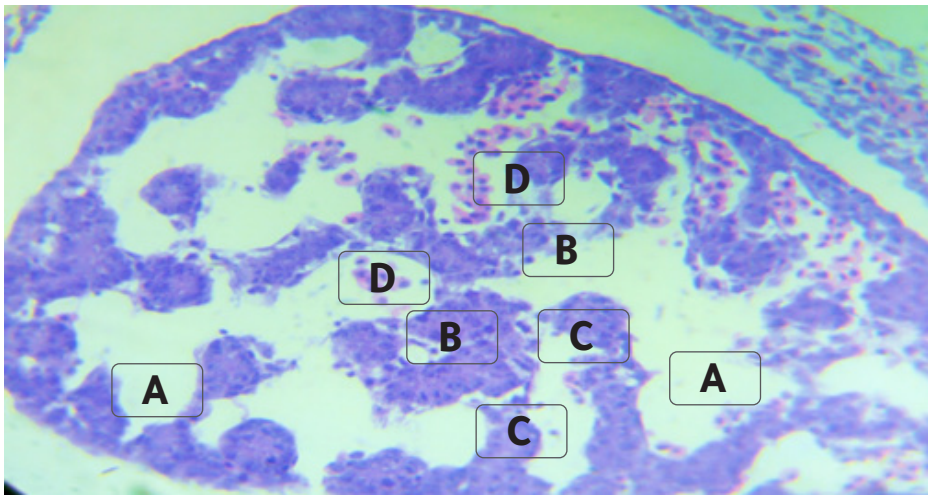

Figure. 4 Pigeon lung tissue (A) Alveolar cavities (B) Squamous and cuboidal epithelium (C) Interstitium between alveoli (D) White blood cells (E X40)Ꞓ H)

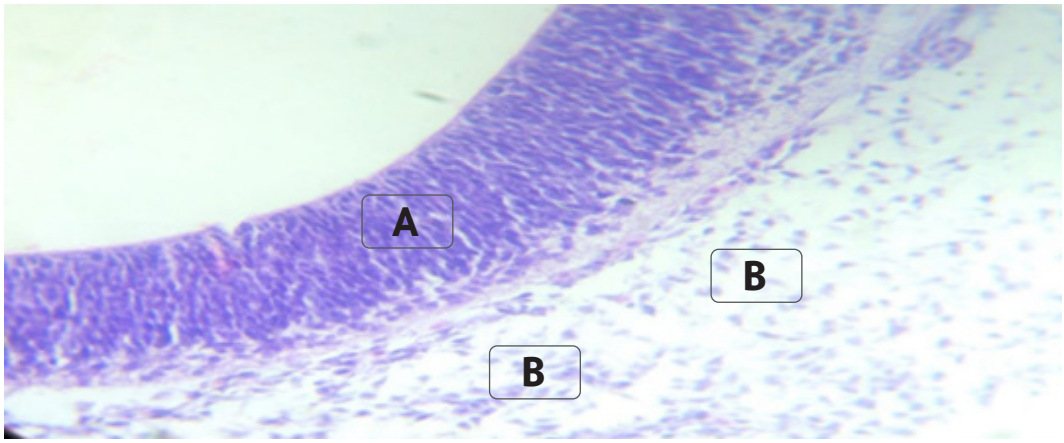

Figure. 5 Larynx of the pigeon fetus (A) pseudocolumnar epithelium (B) main page contains mesenchymal tissue and white blood cells (E X40)Ꞓ H)

It was observed that the lung tissue of the seven-day-old chicken fetus contains mesenchymal tissue and contains large numbers of mesenchymal cells with fibroblasts and white blood cells surrounding the lining of the bronchioles.

3.3. Trachea and lungs in 10-day-old pigeon and chicken embryos

The pigeon's larynx contains a crescentic epithelial membrane surrounded by hyaline cartilage, surrounded on the outside by perichondrium, and part of the loose colloidal fibers that make up the underlying layer beneath the mucus., Figure.6.

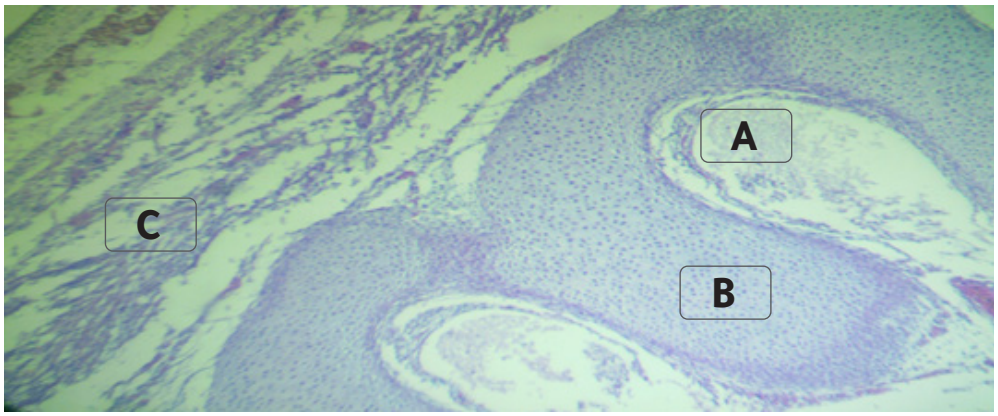

Figure. 6 Laryngeal tissue in the pigeon fetus (A), respiratory epithelium (B), hyaline cartilage (C), submucosa, which contains loose colloidal fibers (E X40)ʒ H).

While pigeon lung tissue contained mesenchymal tissue containing stellate and spindle mesenchymal cells, and the mesenchymal interstitial tissue appeared in a soft, pale-coloured form surrounding large numbers of bronchial canals lined with simple epithelial cuboidal cells, with a blood portion between those bronchioles. Figure.7.

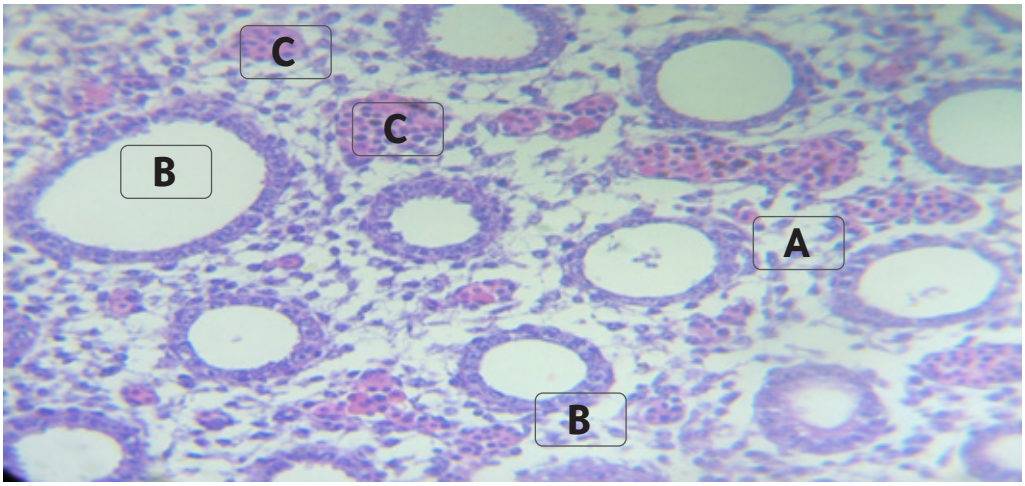

Figure. 7 Lung tissue in a pigeon fetus
(A) mesenchymal cells (B) bronchiolar ducts (C) blood islands (E X40)ʒ H)

The tracheal cavity in chickens contains a network of elastic fibers with fibroblasts and white blood cells. This is the singing organ in birds and chickens, and around it is the hyaline tracheal cartilage containing chondrocytes and surrounded by the ground substance of the cartilage, where the chondrocytes appeared individually and the cell nuclei appeared dark, Figure.8.

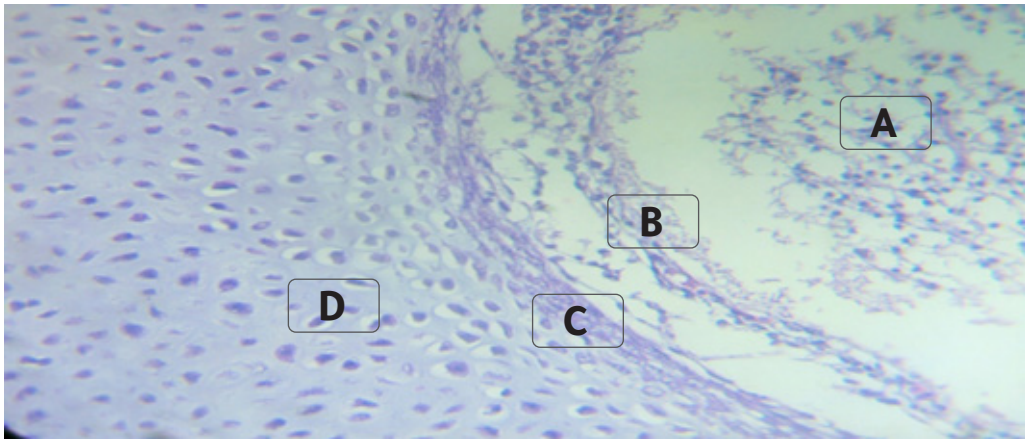

Figure. 8 Trachea in the chicken embryo (A) The coccygeal organ at the base of the trachea (B) Elastic fibers with fibroblasts (C) Periosteum (D) Hyaline cartilage (E X40)، H)

3.4. Trachea and lungs in 12-day-old pigeon and chicken embryos

The larynx of the pigeon's respiratory tract contains a stratified, columnar, ciliated epithelium found in the form of muscles resting on the basal section, and the underlying layer is composed of colloidal fibrous tissue infiltrated by numbers of white blood cells extending along the submucosal layer Figure.9.

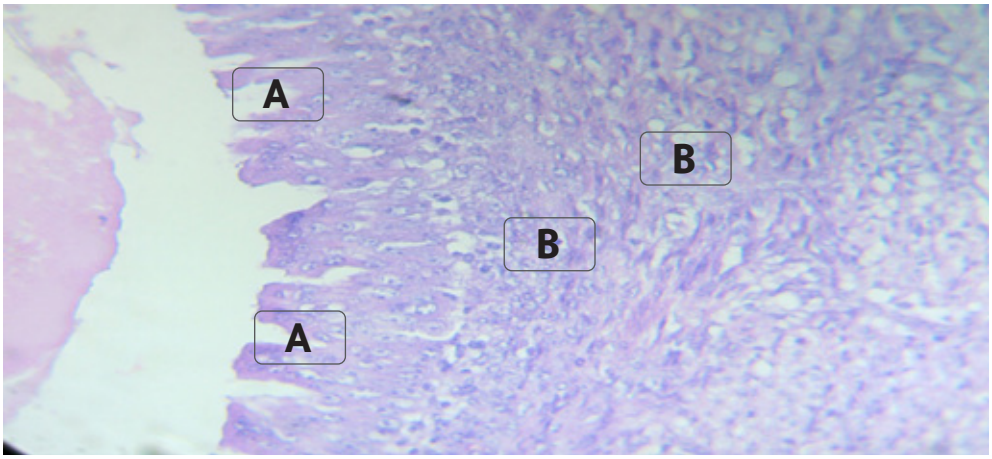

Figure. 14 Larynx in the pigeon fetus (A) Pseudostratified columnar epithelium (B) The underlying submucosal page contains colloidal fibers with infiltration of white blood cells (E X40)، H).

While the lung tissue in pigeons contains a trachea lined with respiratory epithelial cells and surrounded by mesenchymal tissue, and around the wall of the trachea, large numbers of white blood cells and mesenchymal cells were found around the white blood cells, Figure.10.

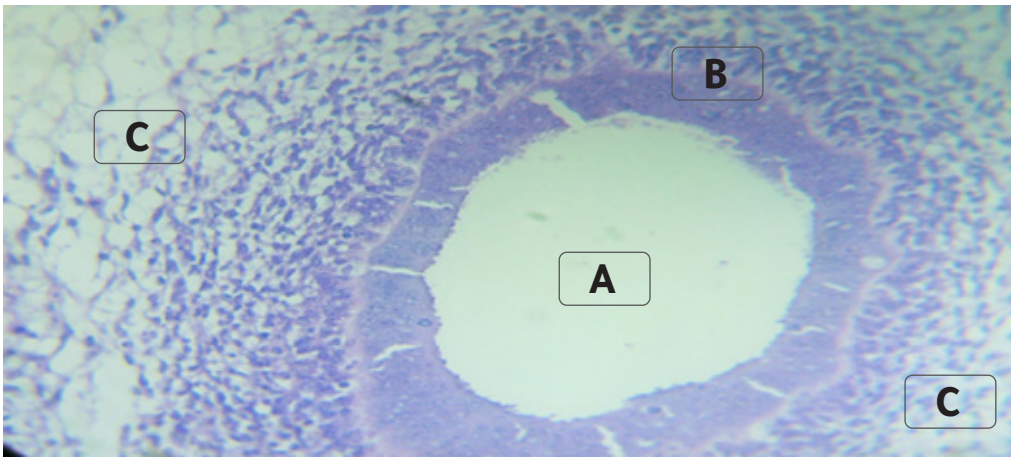

Figure. 10 Lung tissue in a pigeon fetus, (A) trachea lined with epithelium (B) white blood cells (C) mesenchymal tissue (E X40)ß H)

The lung tissue in chickens also contains soft connective tissue fibers composed of colloidal and elastic fibers, surrounded by fibroblasts and capillaries devoid of blood, the presence of white blood cells and a large number of phagocytic cells, in addition to the presence of mesenchymal tissue adjacent to the rest of the lung tissue, Figure.11.

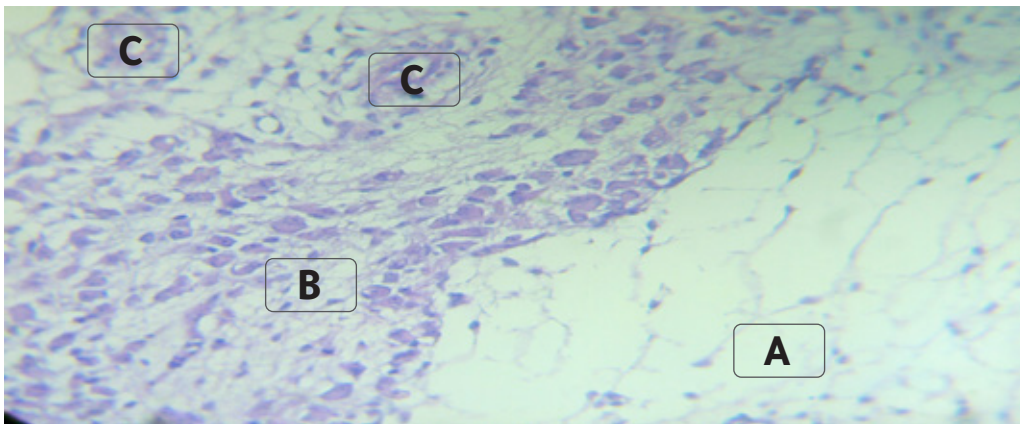

Figure. 11 Lung tissue in the chicken embryo (A) Mesenchymal tissue (B) Loose fibrous tissue containing white blood cells with macrophages (C) Capillaries (E X40)ß H)

While the chicken trachea contains epithelial tissue, and beneath it is the hyaline cartilage attached to the perichondrium, and the underlying layer contains connective tissue disassembled into fibroblasts and fibroblasts, Figure.12.

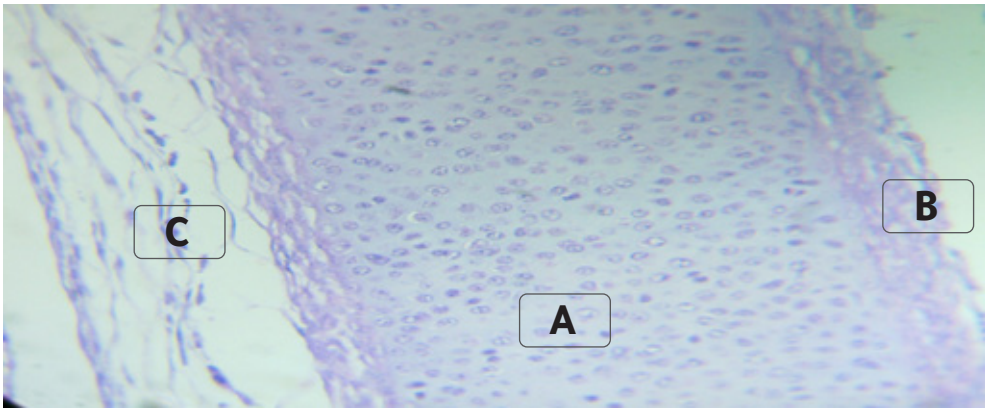

Figure. 12 Trachea in the chicken embryo (A) hyaline cartilage (B) perichondrium (C) loose connective tissue containing fibroblasts (E X40)، H)

3.5. Trachea and lungs in 14-day-old pigeon and chicken embryos

It appeared that there were many bronchioles in the pigeons, as well as bronchioles inside the lung tissue, lined with simple columnar epithelial cells in the bronchioles, and pseudostratified columnar epithelial cells in the bronchioles surrounded by hyaline cartilage and between the bronchioles. There were large numbers of alveoli, and the interstitial tissue was found in a loose form with infiltration and no numbers of cells. White blood as in Figure.13.

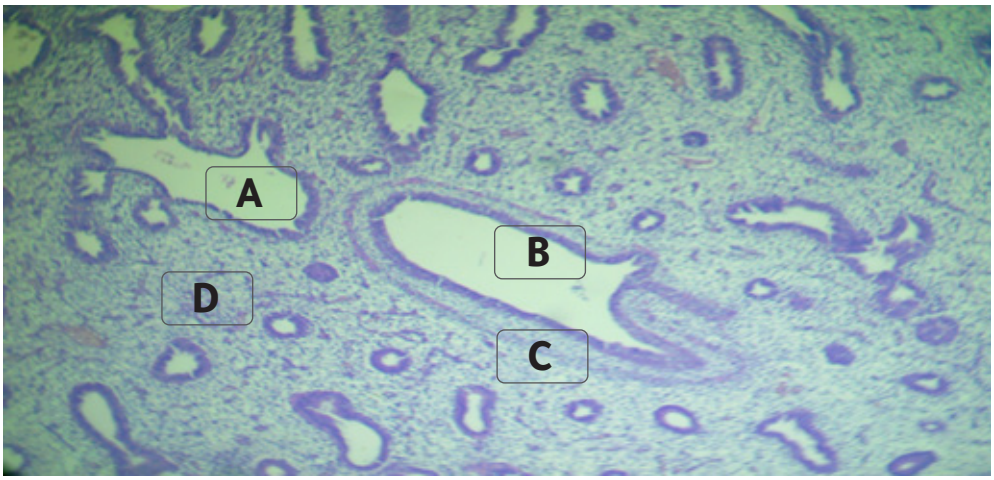

Figure. 13 Lung tissue in a pigeon fetus (A) Bronchioles (B) Trachea (C) Hyaline cartilage (D) Soft interstitial tissue containing white blood cells (E X40)، H)

While the trachea in pigeons contained masses of hyaline cartilage on the main page surrounded by soft fibrous tissue with the presence of some

small blood vessels, and the pieces of hyaline cartilage were surrounded by perichondrium, and these cartilages were surrounded from the outside by smooth muscle fibers in the form of bundles, surrounded from the outside by soft connective tissue containing white blood cells and vessels. Bloody as shown in Figure.14.

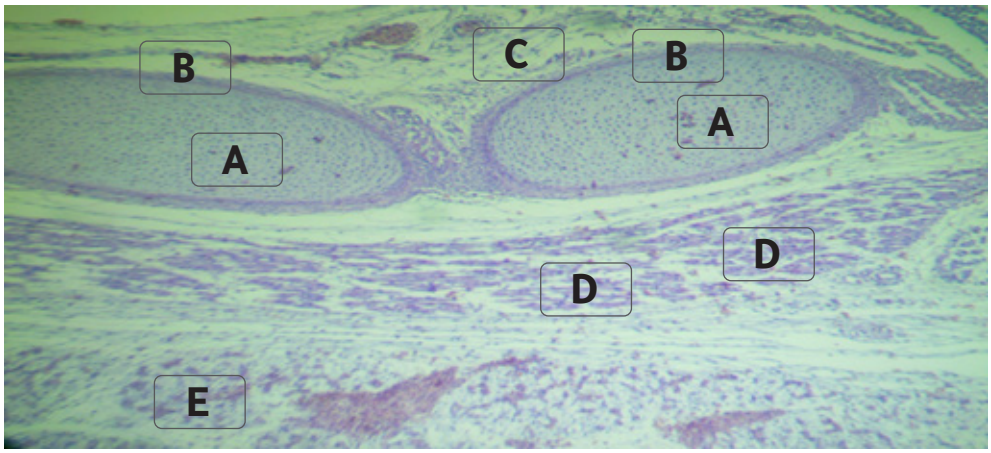

Figure. 14 Trachea in a pigeon fetus (A) Section of hyaline cartilage (B) Periosteum (C) Primary page with capillaries (D) Smooth muscle fibers (E) Blood vessels with white blood cells (E X40)ة H)

3.6. ***Trachea and lungs in 16-day-old pigeon and chicken embryos***

The lining of the trachea in pigeons was composed of stratified, false, ciliated columnar epithelial cells with the presence of some mucus-secreting mucus cells. The main page contained masses of hyaline cartilage, and these masses were surrounded by loose connective tissue that contained white blood cells and macrophages in addition to some blood capillaries. Figure.15.

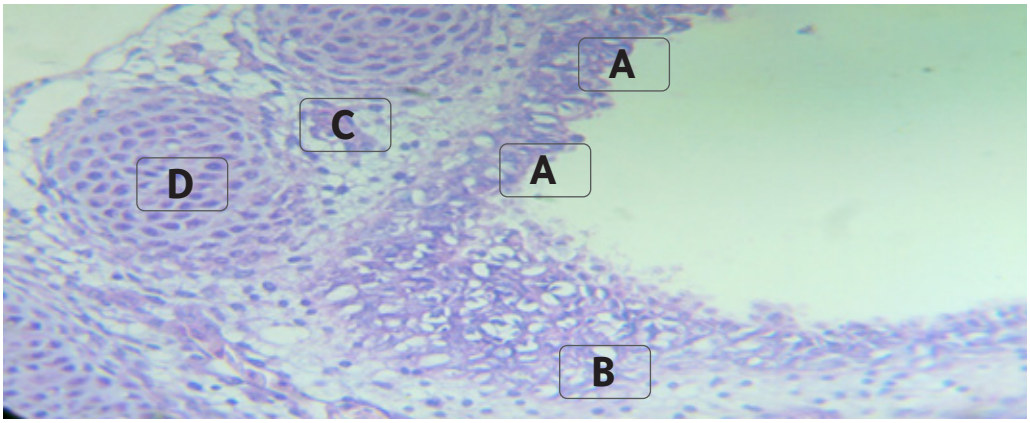

Figure. 15 Trachea of the pigeon embryo (A) Pseudostratified columnar epithelium (B) Goblet cells (C) Capillaries (D) Hyaline cartilage (E X40)ة H)

The lung tissue in pigeons contains blood vessels, some of which are wide-bore and filled with blood, and white blood cells spread abundantly around the bronchioles, in addition to the presence of a number of blood capillaries in the interstitial tissue, as shown in Figure.16.

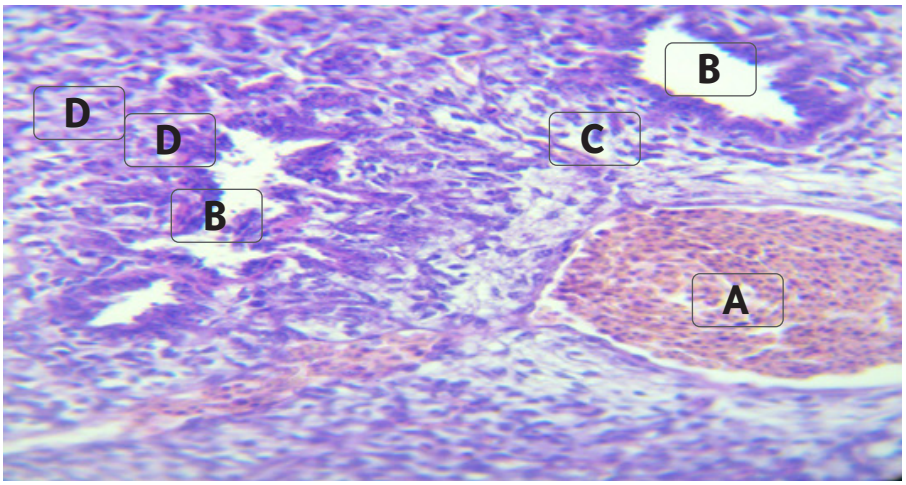

Figure. 16 Pigeon fetal lung (A) A blood vessel filled with blood in the lung tissue (B) Bronchiole (C) Interstitial tissue containing white blood cells (D) alveoli (E) Mesenchymal tissue (E X40)ȣ H)

3.7. ***Trachea and lungs in 18-day-old pigeon and chicken embryos***

The wall of the larynx in pigeons contains many deep, branched grooves lined with pseudostratified columnar epithelial cells with the presence of simple columnar cells in other areas of the epithelial membrane. The underlying submucosal page contained soft fibrous tissue containing white blood cells and fibroblasts, Figure.17.

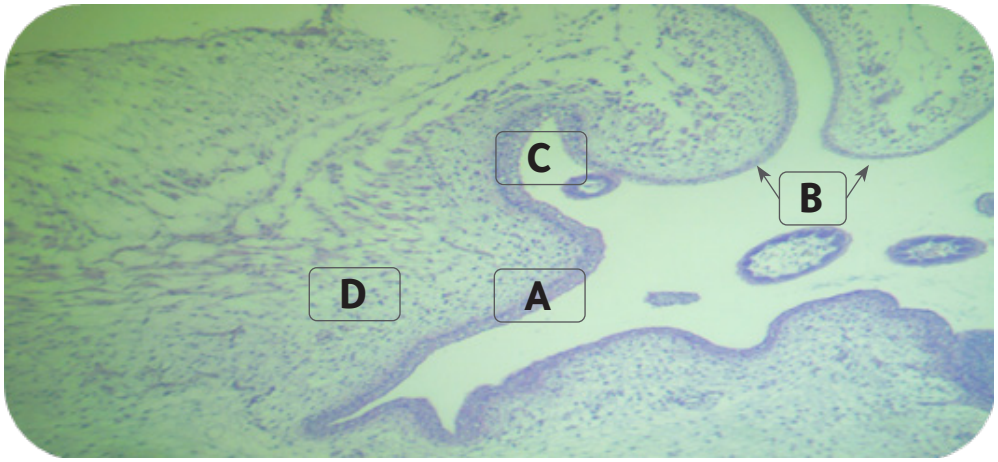

Figure. 17 Larynx in the pigeon embryo, (A) false stratified epithelial cells (B) laryngeal folds (C) laryngeal grooves (D) main page containing soft tissue with white blood cells (E X40)ȣ H)

While The lung tissue contains bronchioles lined with cubic cells and bronchioles lined with columnar cells with narrow cavities for these bronchioles that are closely packed with each other, in addition to their presence independently and not continuous with each other, Figure.18.

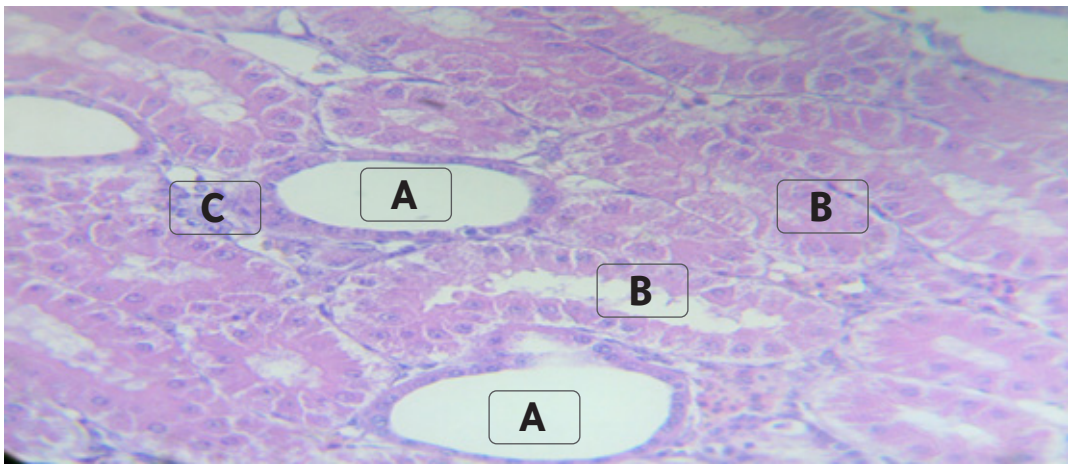

Figure. 18 Lung in the pigeon fetus,
(A) The bronchioles are lined with cuboidal cells (B) The bronchioles are lined with cuboidal cells
(C) White blood cells around the bronchioles (E X40)ة H)

4. Conclusions

The trachea in both pigeons and chickens appeared in a pale pink color. This structure is a long tube whose function is to transport respiratory gases from the upper respiratory system and then outside the body - the lungs and air sacs, or from the air sacs and lungs to the upper respiratory organs. The average size of the trachea ranged between 15 and 18 mm. It is permanently open through 108 to 125 cartilage rings

The current study agrees with [8] in terms of the morphology of other respiratory organs. The lungs were small, compressed, and non-expandable, and they were found to be bright red in color. It has been observed that the trachea in both quails and pigeons is located in the ventral part of the esophagus along the neck. There were complete tracheal rings consisting of the trachea, not C-shaped as in mammals. It has been observed that the right and left bronchi originate from the trachea in both species and enter the left and right lobes of the lungs, respectively.

Chicken embryos have been widely

used by many authors in research on embryonic events in birds [9-10]. However, there is still a data gap in research on the morphological characteristics of the respiratory system during embryonic and ontogenetic development in pigeons and chickens, which limits, in part, the discussion of our results for comparison with available data.

The present study thus reveals some notable historical differences in various structural components of the respiratory system compared to other bird species. However, many of these morphological characteristics are similar to those of other birds, taking into account the difference in incubation times [11].

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