

PRE & POST-HATCHING DEVELOPMENT OF THE THYMUS GLAND IN CHICKEN

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ABSTRACT

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The present work aimed to clarify the microscopic details of most developmental stages of thymus gland in both pre and post-hatching life. Therefore eighty chicken embryos in addition to sixty four post hatched birds were subjected to the investigation. The collected glands were fixed and processed to be examined by light and electron microscopy. The thymus primordium appeared as bilateral cellular masses at the 4th day of incubation just beside the jugular vein. Later on the thymus gland became lobulated and surrounded by primitive capsule in addition to appearance of lymphopieotic cells. At the 12th day of incubation demarcation between the thymic cortex and common thymic medulla could be recognized. The thymic lobule of the 16th day of incubation showed over crowded cortex contained large number of thymoblasts and few reticular cells while medulla contained few thymoblasts and numerous reticular cells as well as acidophilic masses. At the day nineteen of incubation up to the hatching day showed the fully developed form of the thymus gland. In one day-old chick, the thymus gland showed well developed stroma and cortex contained a huge number of mature cells (small thymocytes) and few reticular cells as well as medullary thymic corpuscles. At two months the gland showed various degrees of degeneration in both thymocytes and reticular cells while at five month the gland showed marked losing in the cortical architecture and appearance of myoid cells in the medulla. The thymus gland of one year-old chick showed a complete loss of the cortical tissue with massive degeneration of the thymic remnants.

Key words: *Pre & Post-hatching, Thymus, Chicken.*

INTRODUCTION

The immune system of the chicken is very helpful in preventing disease and helping to insure maximum productive potential is realized. We must learn how to take advantage of all parts of the system when designing health programs (Gary and Richard, 1991). Nowadays, there is a progressive need to meat and egg in order to overcome the massive increase of human population. Therefore, the development of poultry industry and production became one of the big national aims. The research institutes have to offer complete informations about the development, ideal methods for management, vaccination and disease-control of poultry. The thymus gland is one of the primary lymphoid organs in birds responsible for differentiation and maturation of T-lymphocytes (cellular immune responses) (Mazzone *et al.*, 2003). During chicken embryonic development, T-cell precursors are processed by the various reticuloepithelial cells and humoral factors that make up the thymic microenvironment (Bodey *et al.*, 2000). This study aimed to give a complete picture about the age-dependant changes occurred in the thymus gland of chicken including pre and post-hatching life with the aid of light and TEM microscopy and point out

the function of the gland in correlation to its microscopical structure.

MATERIALS and METHODS

For light microscopic examination:-

During the pre-hatching period, the healthy developing Baladi chicken embryos were taken from eighty fertilized eggs incubated at $38.5 \pm 0.2^{\circ}\text{C}$ and 50-60% relative humidity from the hatchery unit of Al-azzab farm in Fayom governorate. Four embryos were collected daily (beginning from the second day of incubation up to hatching at the 21st day). The embryos at early stages of development (up to the 8th day) were fixed and embedded undissected. From ninth up to fifteenth day of incubation the anterior halves were obtained. After fifteen day the thymus gland could be easily dissected from the embryos. During the post-hatching period, samples from thymus gland were collected from sixty-four slaughtered healthy chickens of both sexes in different ages at intervals from newly hatched up to 1 year-old. The collected specimens were immediately immersed in 10% Neutral buffered formalin and Bouin's fluid then processed to be stained with Harris Hematoxylin and Eosin, Gomori's reticulin method, Crossmon's trichrome stain, Periodic acid Schiff

technique (PAS) and Alcian blue method (PH 2.5) as outlined by Bancroft and Gamble (2008).

For electron microscopic examination:-

Six samples from thymus gland were collected; three samples during pre-hatching period at the 12th, 17th and 19th day of incubation and another three samples during post-hatching period at the 1st month, the 2nd month and the 8th month of age. The collected specimens were cut into very small pieces and fixed in 4% glutaraldehyde in phosphate buffer solution then washed three times in the same buffer (Hayat, 1986) then post-fixed in 1% osmium tetroxide in phosphate buffer then embedded in epoxy medium. Semi-thin sections were obtained and stained by toluidine blue then examined by the light microscope (Richardson *et al.*, 1960). Ultra-thin sections were obtained, mounted on copper grids and contrasted with 5% uranyl acetate followed by lead citrate stain (Reynolds, 1963), examined by transmission electron microscope JEOL (JEM-1400 TEM 80 kv).

RESULTS

Pre-hatching development:-

Examination of step serial sections of the neck region of 4 days-old embryos revealed the first appearance of the thymus primordia. The latter appeared as bilateral cellular masses of undifferentiated epithelial cells surrounded by mesenchymal tissue on both sides just beside the jugular vein (Fig.1). As the development advanced, the thymus gland showed partial lobulation as the fibroblasts of the primitive capsule proliferated into the gland tissue forming short incomplete septa at the 8th day of incubation (Fig.2). The gland in this stage showed the first appearance of lymphopieotic cells. The latter had spherical shape with large, spherical euchromatic nuclei surrounded by thin rim of cytoplasm. (Fig.3). The chick embryo at the 12th day of incubation showed well developed capsule and septa consisted mainly of fibrous tissue and many fibroblasts. In this stage demarcation between the thymic cortex and common thymic medulla could be recognized (Fig.4). The thymic lobule formed mainly from an outer cortex and an inner medulla. The thymoblast characterized by large spherical nucleus and thin rim of basophilic cytoplasm, while the reticular cell appeared larger in size with spherical nucleus and abundant acidophilic cytoplasm (Fig.5). Ultrastructurally, the thymoblast showed large euchromatic nucleus surrounded by thin rim of electron dense cytoplasm containing few organelles (Fig.6). The thymic lobule of the 16th day of incubation showed over crowded cortex contained large number of thymoblasts and few reticular cells. In contrast to thymic medulla which contained few thymoblasts and numerous reticular cells as well as acidophilic masses (Fig.7). At the day nineteen of incubation up to the hatching day showed the fully developed form of the thymus gland; separated cortex and common medulla. The whole gland enclosed by

fatty tissue (Fig.8). The thymic lobule in this stage contained large number of thymoblasts and few reticular cells as well as fibroblasts which condensed mainly at the corticomedullary junction (Fig.9). Ultrastructurally, the thymoblast possessed large central euchromatic nucleus surrounded by thin rim of electron dens cytoplasm contained large number of mitochondria than before (Fig.10). The parenchyma of the thymus gland during this period appeared to be supported by a network of reticular fibers mainly condensed in the capsule, septa and few fibers distributed all over the parenchyma (Fig.11).

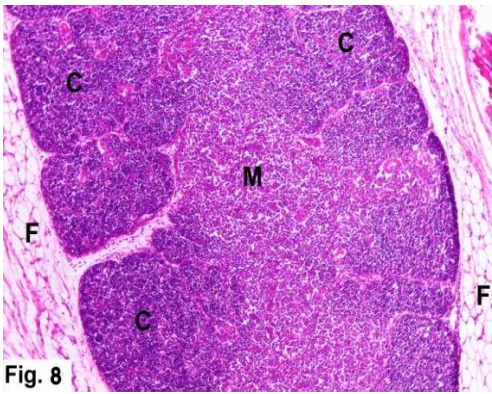
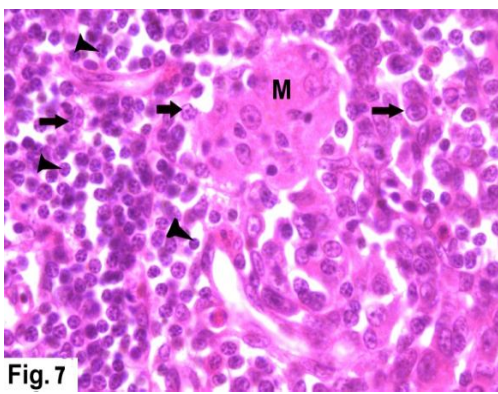
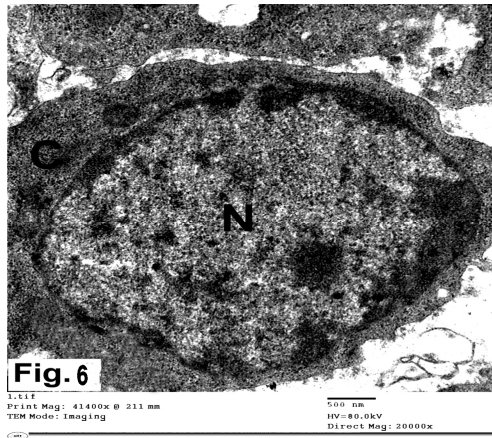
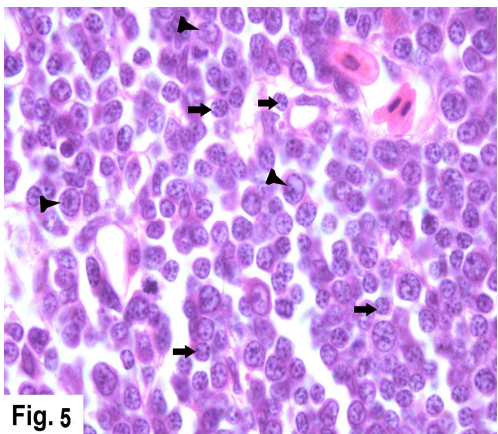
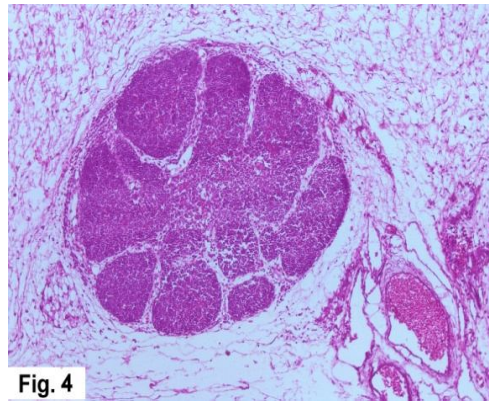
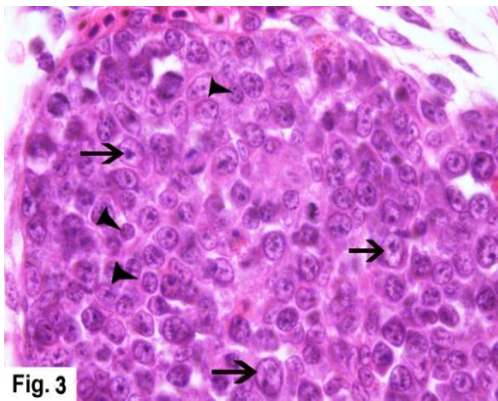
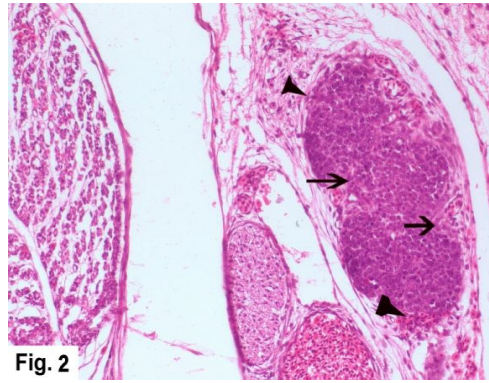
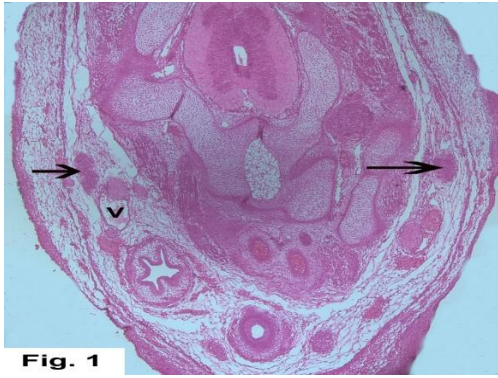
Post-hatching development:-

In one day-old chick, the thymus gland showed well developed stroma represented by a well developed capsule formed mainly of collagen bundles and more developed septa, which appeared loose in structure (Fig.12). The thymic cortex in one day-old chick contained a huge number of mature cells (small thymocytes) and few reticular cells. A post capillary venule lined by cuboidal cells, were frequently noticed at the corticomedullary junction (Fig.13). The medullary epithelial reticular cells were clearly appeared as large cells with vesicular spherical nuclei and acidophilic cytoplasm. Also, thymic corpuscle which appeared as hyalinized acidophilic center surrounded by concentric layers of epithelial reticular cells could be noticed in the medulla (Fig.14). On reaching one month of age, the chicken thymus's cortex became thinner than the thymic medulla. The thymocytes formed the main cellular elements of the cortex. Other immunologic cells as macrophage and eosinophiles occurred mainly at the corticomedullary junction (Fig.15). The thymocytes appeared as rounded cell with large central euchromatic nucleus enclosed by abundant cytoplasm contained gaint mitochondria and lysosomal body (Fig.16). The predominant cells in the thymic medulla were the epithelial reticular cells which appeared as star shaped cell contained spherical euchromatic nucleus, many mitochondria and vacuoles as well as many large cytoplasmic processes connected with other cells forming a continuous network (Fig.17). The chicken at two months of age showed various degrees of degeneration in both thymocytes and reticular cells. The former showed more condensed nuclei with less basophilic cytoplasm. The reticular cells tended to be collected with each other; the cytoplasm became more acidophilic and vacuolated, with more condensed nuclei (Fig.18). The thymus gland at five month old chick showed marked losing in the cortical architecture and numerous extra cellular vacuolation. Accumulation of abundant acidophilic myoid cells in the thymic medulla (Fig.19). As the development progressed, the thymus gland of five months-old chick showed several myoid cells with strong acidophilic cytoplasm and elongated nuclei with massive degenerative changes in both reticular cells and thymocytes (Fig.20). The thymus gland of one

year-old chick showed a complete loss of the cortical tissue with massive degeneration of the thymic remnants.

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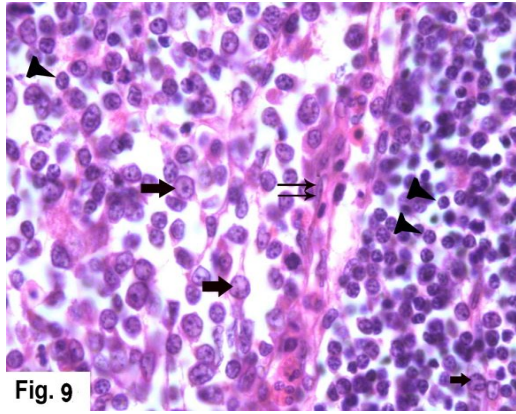


Fig. 9

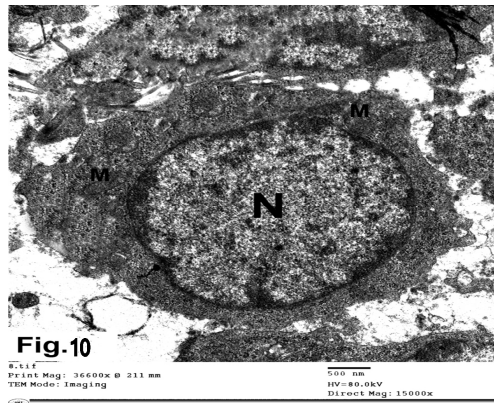


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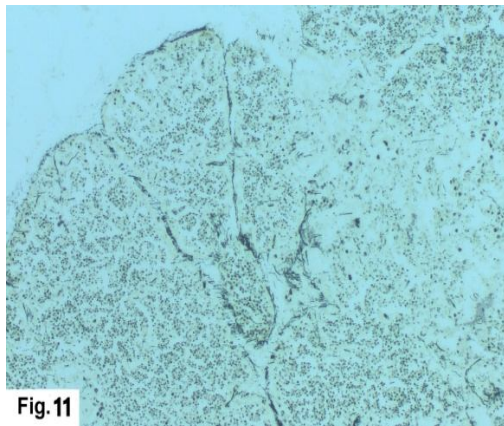


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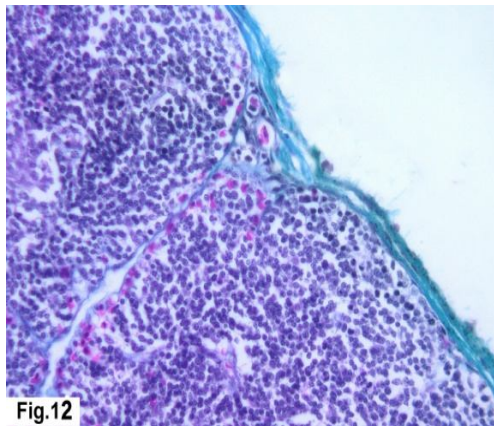


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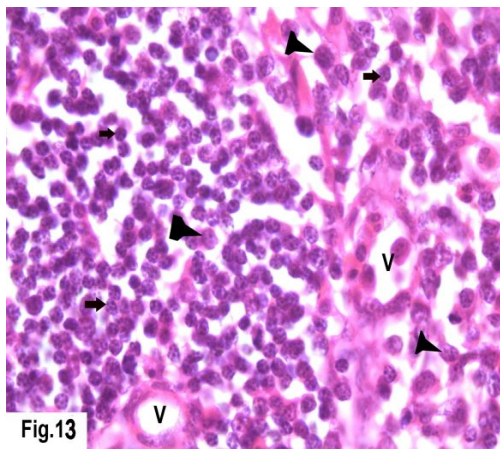


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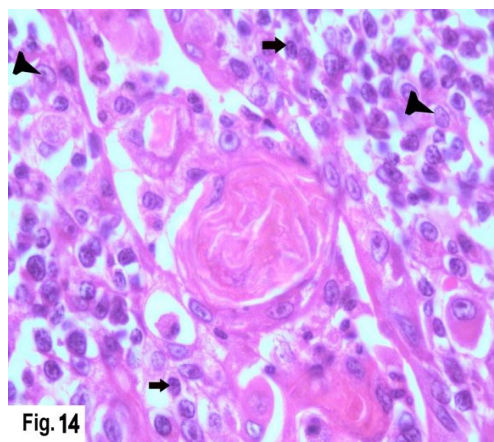


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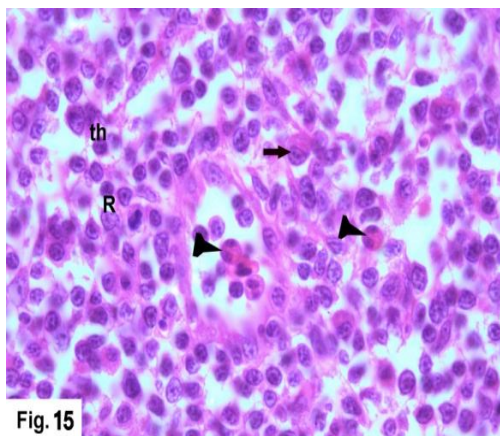


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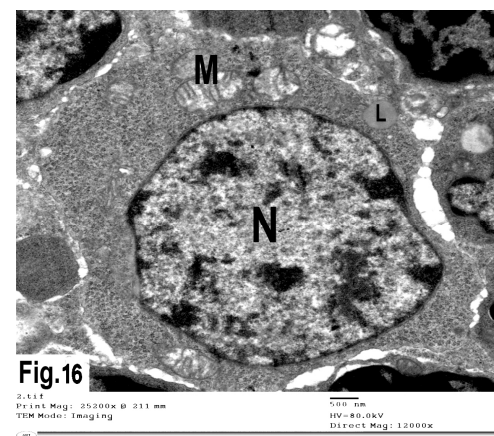


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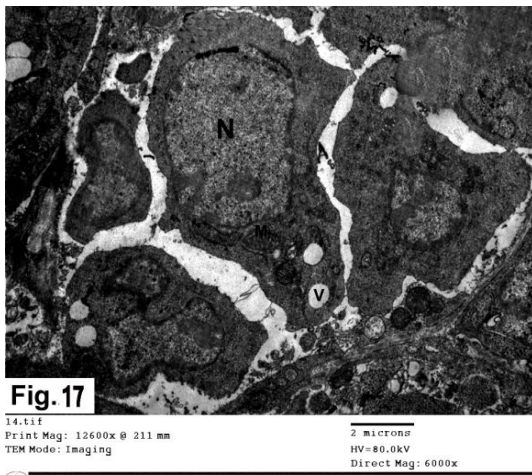


Fig.17

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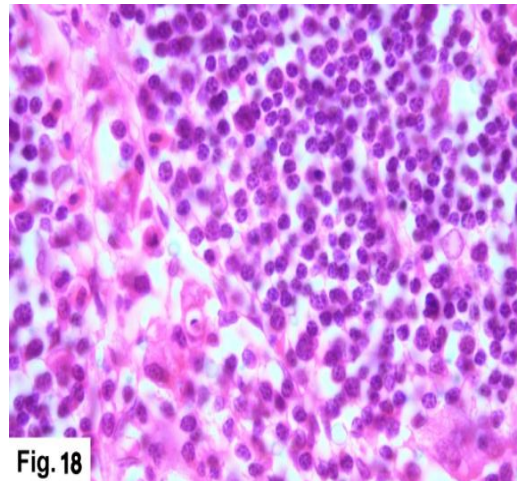


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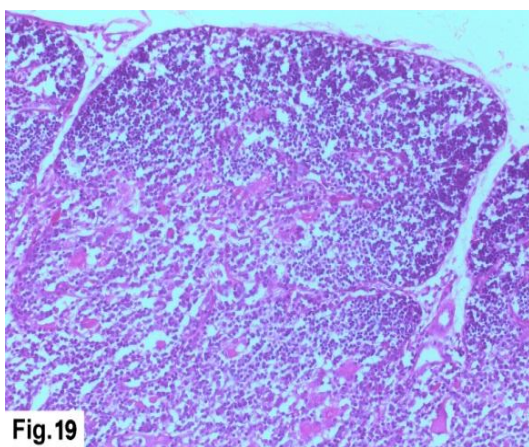


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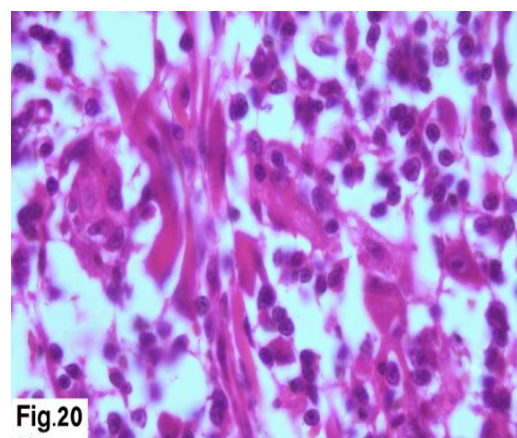


Fig.20

DISCUSSION

There is a general agreement among most embryologists that the lymphatic organs acquired the fully developmental stage before hatching (Edwards *et al.*, 1975; Le Douarin *et al.*, 1976 and Le Douarin *et al.*, 1984). The authors attributed such characteristic phenomenon to the fundamental role of these organs in the early post-hatching period.

Pre-hatching development:-

The present study revealed that the thymic primordia could be firstly detected on either sides of the neck at the day four of incubation just beside the jugular vein as recorded by Yang *et al.* (2001). Regarding the endodermal origin of the gland parenchyma, most embryologists take the same line of Yang *et al.* (2001) and Rezzani *et al.* (2008) in chicken. On the other hand, the connective tissue stroma of the gland and its blood vessels showed some controversial origins, while many authors, as Erik & Gunnar (1973) and Yang *et al.* (2001) considered our

opinion about the mesodermal origin, others explain the ectodermal origin of the gland stroma and blood vessels as Couly *et al.* (1995). Generally, the migratory hemopoietic cells as well as medullary dendritic cells are discussed as mesodermal origin (Le Douarin & Jotereau, 1975 and Yang *et al.*, 2001). Dieterlen & Le Douarin (2004) recorded that, the first wave of cell precursors that colonize chicken thymus apparently comes from the intraembryonic mesenchyme of both aortic and paraortic regions in 4-to-7 days-old chicken embryos.

Erik & Gunnar (1973) in chicken agreed with the results of this study that, in the 8th day of incubation the developing thymus gland showed the beginning of partial lobulation. Blackburn & Manley (2004) confirmed our results, that, the gland possessed first appearance of lymphopoietic cells at eight days-old embryos which originated from the blood lymphopoietic cells. Moore & Owen (1965) postulated the extrinsic origin of the lymphoid stem cells of the thymus that, the thymic endoderm or thymic

mesenchyme showed that none of these rudiments are able to differentiate into lymphocytes.

The present investigation revealed a marked demarcation between cortex and medulla at the 16th day of incubation as reported by Yang *et al.* (2001).

Regarding the large homogenous acidophilic masses detected in 16 days-old chick embryos was also recorded by Itoi *et al.* (2001). While, Yang *et al.* (2001) in chicken recorded the first appearance of these masses at 12th day of incubation. In our opinion which could be augmented by that of Coltey *et al.* (1987) and Yang *et al.* (2001), these thymic corpuscles considered as a grave for complete isolation of necrosed and degenerated developing cells, which fail to reach their full maturation and programming. Three reasons could support our previous opinion, firstly, the appearance of already degenerated and necrosed cells in the center of these corpuscles as also mentioned by Yang *et al.* (2001). Secondly, the presence of concentric arrangement of epithelial reticular cells confirmely around these degenerated cells as also recorded by Kendall, (1980). Thirdly, the appearance of these corpuscles as the gland became functioning then increased in size and number with the advanced age either pre or post-hatching as also discussed by Payne (1971). Bodey *et al.* (2000) indicated that the thymic corpuscles, constituting multicellular components of the nonlymphocytic, cellular micro-environment of the thymic medulla, and participating in the physiological activities of the prenatal and adult thymus.

The thymus gland at the day nineteenth up to full term of incubation showed the fully developed form. The thymic lobule in this stage contained large number of thymoblasts and few reticular cells. These findings were also described by Gary & Upendra (1981).

Post-hatching development:-

The thymus gland just after hatching enclosed by thin thymic capsule consisted mainly from collagen and reticular fibers, the same findings were obtained by Sultana *et al.* (2011) and Song *et al.* (2012).

In our study the thymus gland just after hatching clearly showed outer cortex contained a huge

number of mature cells (small thymocytes) and few reticular cells in contrast to the thymic medulla which contained lesser population of the thymocytes and numerous epithelial reticular cells, this cellular distribution giving the cortex more darker appearance than the medulla. Same findings were stated by Elewa (2005) and Song *et al.* (2012).

The main immunologic cells of thymic cortex were the thymocytes which accept the ability for neutralizing the invading organism which augmented by the ultrastructural finding that, containing mitochondria and lysosomal body (Raymond *et al.*, 1965). The thymic medulla appeared more lighter due to much lesser cellular contents which possessed numerous reticular and few thymocytes. Same findings were recorded by Ciriaco *et al.* (2003); Sultana *et al.* (2011) and Song *et al.* (2012). The predominant cells in the thymic medulla were the epithelial reticular star shaped cells forming the main support of the thymic parenchyma. The same description of the reticular cell explained by Kendall (1981), Sultana *et al.* (2011) and Song *et al.* (2012).

The thymus gland of studied chicken suffered from different involutive changes. Many authors described these changes and decided that these involutive changes accompanied the onset of sexual maturity (Franchini & Ottaviani, 1999 and Buker, 2008).

Regarding the appearance of myoid cells, they observed especially in thymic medulla and described as epithelio-reticular cells undergoing hyaline degeneration (Hoffman, 1973). Raviola & Raviola (1967) suggested that myoid cells played a mechanical role in facilitating lymphocyte passage through the thymus by their spasmodic contraction.

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تطور الغدة الزعترية ما قبل وبعد الفقس في الدواجن

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أجريت الدراسة على ٨٠ بيضة مخصبة لمرحلة ما قبل الفقس وكذلك ٦٤ من الدجاج النامي والدجاج الناضج تم جمعها من وحدة تفريخ مزرعة العزب في محافظة الفيوم. تم تجهيز العينات المراد دراستها بالطرق الهستولوجية المختلفة وإعدادها للفحص يكلا من المجهر الضوئي والمجهر الإلكتروني النافذ وكانت النتائج التي تم الحصول عليها كالتالي:- ظهرت براعم الغدة الزعترية على جانبي الرقبة لأجنة الدجاج في اليوم الرابع من التحضين بجانب الوريد الوداجي ومع تطور العمر عند اليوم الثامن أظهرت الغدة الزعترية النامية بداية التفصص الجزئي للغدة وأول ظهور للخلايا الليمفاوية أظهرت النتائج كبسولة أولية مغلقة للغدة يخرج منها حواجز قصيرة تقسم الغدة إلى فصيصات، كل فصيص له قشرة منفصلة والنخاع مشترك. تحتوى القشرة العديد من الخلايا الليمفاوية مع عدد قليل من الخلايا الطلائية الشبكية على عكس النخاع. في اليوم السادس عشر من التحضين ظهرت ترسيم واضح بين القشرة والنخاع لفصيصات الغدة وظهرت كتل كبيرة من الأجسام المتجانسة والتي تعرف بكريات الغدة الزعترية. ظهر الشكل الكلاسيكي للغدة الزعترية عند اليوم التاسع عشر حيث تكونت من فصيصات كل منها يحتوى على قشرة مفصولة عن بعضها بحواجز ليفية ونخاع مشترك وكانت الخلايا الليمفاوية أكثر تواترا في القشرة. في اليوم الأول بعد الفقس ظهرت الغدة الزعترية محاطة بكبسولة متطورة تكونت من الألياف الغروية يخرج منها حواجز ليفية تقسم الغدة إلى فصيصات. أظهرت النتائج النموذج الكلاسيكي لكريات الغدة الزعترية في النخاع والتي ظهرت على شكل كتلة وسطية متجانسة من الأجسام المحبة للأحماض محاطة بعدة طبقات من الخلايا الطلائية الشبكية عند عمر شهر بعد الفقس. وأظهرت الغدة الزعترية بعد شهرين من عمر الدجاج درجات مختلفة من التدهور في كلا من الخلايا الليمفاوية والخلايا الشبكية. والتي ازدادت تدريجيا مع تقدم العمر عند خمسة أشهر فقدت الغدة مكونات القشرة وظهر العديد من الفجوات بين الخلايا إضافة إلى ظهور العديد من الخلايا شبه العضلية في نخاع الغدة الزعترية.