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SOME HISTOLOGICAL AND HISTOCHEMICAL STUDIES ON THE SPLEEN OF QUAIL DURING PRE – AND POST-HATCHING PERIOD

A THESIS

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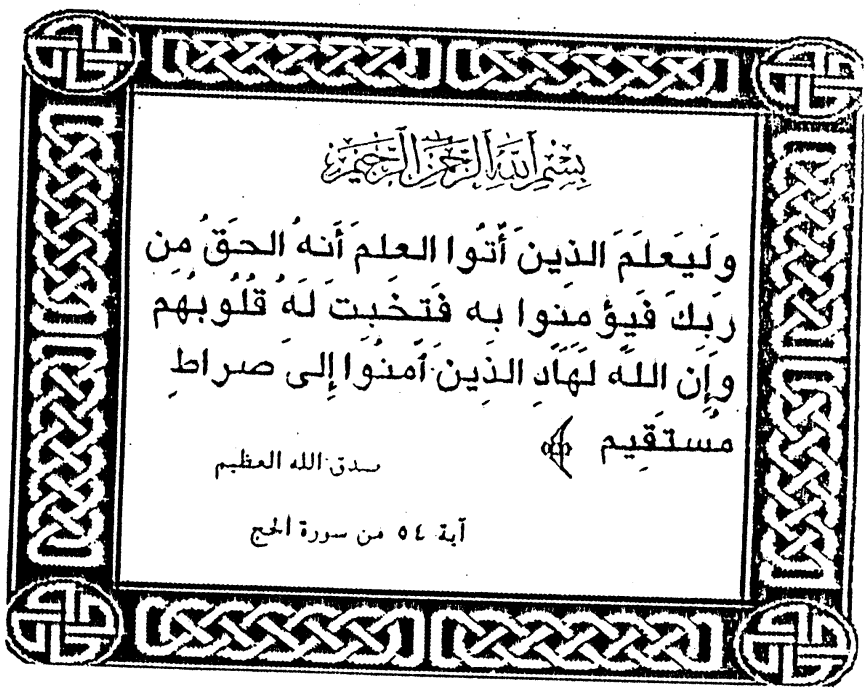
بسم الله الرحمن الرحيم
وليعلم الذين أتوا العلم أنه الحق من ربك فيؤمنوا به فتخبت له قلوبهم وإن الله لهاد الذين آمنوا إلى صراط مستقيم
صدق الله العظيم
آية ٥٤ من سورة الحج

TO

THE MEMORY OF
MY PARENTS

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INTRODUCTION

Introduction

Formerly the quail (coturnix coturnix) is regarded as a migratory species but now it is domesticated all over the world. Quail is used primarily as laboratory animal and is recommended to interested investigators.

Also of special interest to the quail is the short life cycle (16 to 17 days are required for incubation and approximately 42 days from hatching until sexual maturity) and the large number of eggs that obtained per female in one year " up to 300 or more " ***(Sainsbury, 1992)***.

In birds the embryonic development and post-hatching structure of the primary lymphoid organs, thymus and bursa of Fabricius, are subjected to numerous studies and investigations ***(Ackerman and Knouff, 1959; Fennell and Pearse, 1961; Ackerman, 1962; Cooper et al., 1965).***

In contrast little attention has been paid to the secondary lymphoid organs. The spleen is considered the main secondary lymphoid organ in both mammals and birds. It serves as a complex filter interposed in the circulation to clear the blood of particulate matter and senescent blood cells. It is also concerned with immune defense against blood- borne antigens. These besides its vital role as a hemopoietic organ in the amniote embryos***(Hodges, 1974 and Fawcett,1986).*** Despite of their vital functions to our knowledge, few studies have been devoted to clarify its structure specially during pre-hatching period.

Few of these studies were conducted on the cytodifferentiation of the spleen *(Romanoff,1960; Delanney and Ebert, 1962; Ogata et al., 1977; Yassine et al .,1989)* while much more studies were carried out post-hatching *(Hodges, 1974; Sugimura and Hashimoto, 1980; King and Mclelland, 1981; Olah and Glick ,1982; Bareedy et al .,1986; Nasu et al .,1992).*

So the aim of this study is to **1)** Assess the changing hemopoietic population in the spleen of quail in order to obtain information on the role of the spleen in the developing blood-forming system **2)** Determine how differentiating vascular anatomy, evaluated from the aspect of the reticulum, relates to the switch from hemopoietic to immune function **3)** Clarify the mode of origin and pattern of differentiation and organization of the cells appearing in the spleen of quail embryo .

REVIEW OF LITERATURES

Review of literature

Pre-hatching development of avian spleen

The splenic primordium becomes recognizable at the 4th day of incubation as slight bulge of the dorsal mesentery capping the dorsal pancreatic bud on the left side of the body . Until 5th day of incubation (E5) the splenic and pancreatic mesoderm are continuous while at E6 the duodenal loop grow out and the spleen separate from the pancreas *(Romanoff, 1960; Yassine et al.,., 1989)*. The primordium first appear as a loose network of mesenchymal cells then prevaded by a sinusoid network which fills with hemocytoblasts from the day 8 of incubation *(Romanoff ,1960; Delanney and Ebert, 1962).*

The reticular fibers become visible in the chick embryo at 11th day of incubation, they were short filaments, uniformly distributed over the whole organ, slightly more abundant around large sinusoids and arterioles. At 15th day of incubation, the fiber net work become organized as multilayered rings of fibers around arteries , then become recognizable as a network around the different types of vessels at E18 *(Yassine et al., 1989).* The authors added that the arterial system is the most heavily invested with reticular fibers. The wall of central arteries is circled by a heavy basket of interwoven fibers, the ellipsoid which harbars specialized macrophages "ellipsoids associated cells " are sustained by a special pattern of reticular fibers as a heavy reticular ring delineates the lumen, presumably

immediately surrounding the endothelium, and the rest of the spleen is prevaded by a network of very fine fibers.

As early as 5th day of incubation, the mesenchymal network of the splenic primordium becomes progressively interspersed with basophilic cells (whose ultrastructural aspect is clearly distinct from that of the mesenchymal cells) which will undergo hemopoiesis *(Yassine et al.,1989).* From 10th to 14th day of incubation *(Delanney and Ebert, 1962)* or from 9th to 13th day of incubation *(Yassine et al, 1989)* the basophilic cells are very abundant and erythropoiesis starts and picking up rapidly. A round 13th day of incubation the granulopoiesis begins in the red pulp and becoming the dominant process from 15th day of incubation as erythropoieses decreases *(Romanoff , 1960;Delanney and Ebert, 1962; Yassine et al., 1989)* . Small lymphocytes do not appear in the white pulp until 15 to 17 days of incubation *(Romanoff; 1960).*

In the quail the 1st hemopoietic progenitor were detected early as E7 and their number peaked at 10th day of incubation but they disappeared definitively before hatching while in the chick some were still present at 3rd day after hatching *(Nicolas –Bolnet et al., 1991).*

On the embryonic day 18 , the stromal anatomy is similar to that of the adult spleen *(Yassine et al., 1989)* , the development of the ellipsoid is completed and the penicillary capillaries become fenestrated *(Kasai et al., 1995).*

Post-hatched avian spleen

Just after hatching dramatic changes take place in the cell content of the spleen *(Hodges, 1974)*. Over a very short period of time the granulocytopoietic functions are reduced and the organ becomes predominantly a lymphocyte – producing and an erythrocyte-destroying organ *(Danchakoff, 1961; Lucas and Jamroz, 1961)*. In the 24 hrs immediately after hatching there is an outpouring of heterophils from the spleen followed by a massive development of lymphocyte and monocyte *(Lucas and Jamroz,1961)*, and by 48 hrs post-hatching lymphocytes are the dominant cells of the organ .

General structure of the avian spleen .

The avian spleen is enclosed in a thin fibrous capsule limited externally by a flattened layer of peritoneal mesothelium *(Hodges, 1974; Bareedy et al., 1986; Nasu et al., 1992)*. This capsule consists of an elastic fiber network in association with fine collagen fibers , a few muscle fibers *(Hodges 1974; Bareedy et al.,1986)*. Fibroblasts *(Hodges, 1974)* and few reticular fibers *(Bareedy et al., 1986)*.

Although some investigations that carried out on the avian spleen stated that no true trabeculae can be observed *(Lucas et al., 1954; Malewitz and Calhoun, 1958; Hodges, 1974)* others found that the thin capsule of the spleen extended from the hilus into the organ as poorly developed

highly branched trabeculae (*Taliaferro and Taliaferro, 1955; Bradley and Grahame, 1960; Bareedy et al., 1986; Nasu et al.,1992).*

The underlying framework of the splenic tissue consists of a network of reticular cells and reticular fibers . This reticulum is particulary dense around the arteries of the white pulp and in the capsule *(Bradley and Grahame, 1960; King and Mclelland ,1981*). Superimposed upon this framework are the two types of splenic tissue ,the white and the red pulp *(Hodges, 1974).* Although white pulp exists as islands enclosed by red pulp the distinction between the two type of pulp is not marked as in mammals *(Thorbecke et al., 1957; Fukuta et al., 1969;Hodges, 1974; Miyamoto et al., 1980; Nasu et al., 1992).*

The amount of white and red pulp differs among species of birds and may reflect the function of the spleen . In pigeon the spleen has a lot of red pulp. So this may reflect its function as a storage organ for blood . Contrary to the spleen of pigeon, the white pulp of geese exceeded the red pulp, in doing so, it reflects its function as an immune producing organ than to be a storage one *(Bareedy et al., 1986).* In duck spleen the red and white pulps occupied about 54% and 38 % of the spleen respectively, and the remainder was occupied by trabecular and vascular tissue *(Sugimura and Hashimoto, 1980)* while about 85 % of the substance of the chicken spleen is divided about equally between white and red pulp *(Lucas et al., 1954;*

King and Mclelland, 1981). The remainder of the total volume being taken up by blood vessels, connective tissue and other miscellaneous tissue

Splenic white pulp and associated vasculature

Olah and Glick (1982) stated that the splenic arteries entering the spleen at the hilus are surrounded by trabecular connective tissue that imparts it the name of trabecular arteries . In contrast to numerous muscle layers of the trabecular artery , the centeral artery "CA" possesses a single muscle layer along its entire length and is surrounded by a periarterial sheath (PALS). The central artery then makes a right angled curve and becomes the penicillary capillary (PC) . Unlike the common capillaries, where usually only one or sometimes two endothelial cells can be seen in a cross section. The PC shows at least three and frequently five endothelial cells. These high endothelial cells were surrounded by thick basal lamina that was disrupted by endothelial cell processes which form a channel through the basal lamina from the lumen of the P.C to the ellipsoid.

In regard to the structure of the splenic white pulp, *Olah and Glick (1982)* described the splenic white pulp of the chicken to be formed from the periarterial lymphoid tissue, ellipsoid cells, ellipsoid-associated cells, germinal centers and peri-ellipsoid white pulp. While the other studies that were carried out on the duck spleen by *Sugimura and Hashimoto (1980)* divided the white pulp into four elements; periarterial, perivenous, periellipsoidal lymphoid tissues and germinal centers.

There is a general agreement that the spleen of birds has two distinct areas controlled respectively by the thymus (T) and the bursa of fabricius (BF). At present, it is believed that T-dependant areas are represented by the periarterial lymphoid tissue while the BF- dependant area is represented by the periellipsoidal tissues, germinal centers and cells in the plasma cell line (marginal zone) in the chick spleen *(Cooper et al., 1966;Hoshi, 1972;Hoffman-Fezer et al., 1977; Boyd and Ward 1978; Olah et al., 1985)* while *Romppanen and Sorvari (1981)* described the periarterial lymphoid tissue as a B-dependant compartment. *Suginura and Hashimoto (1980)* explained some differences where they showed the presence of aT- and BF-independant area in the duck spleen. This area is represented by the perivenous lymphoid tissue . In the chicken spleen this area was considered to be a T-dependant *(Hoshi and Mori ,1973),* both T-dependant and BF-dependant *(Hoffmann-Fezer et al.,1977)*and B-independant *(Romppanen and Sorvari ,1981).*

The periarterial lymphoid tissue surround the central artery and consists of densely packed small lymphocytes , several medium-sized and large lymphocytes as well as few macrophages and usually ends where the central artery makes a right angled curve and becomes the penicilliform capillary *(Olah and Glick ,1982).* This compartment occupied about 2.3 % of the duck spleen *(Sugimura and Hashimoto, 1980)* .

Ellipsoids are the extra-vasculature sites surrounding the entire length of the penicilliform capillary (P.C) (***Olah et al., 1994 and Kasai et al., 1995).*** The first author stated that the original name given to the splenic ellipsoid region as Schweigger-Seidel Sheath (S.S.S) "Kapillarhulse" or capillary sleeve would be a more appropriate term than ellipsoid at least in the chicken because the sheath covers the entire length of the pencilliform capillary from the central artery, including the branching area and in the section plane through this region the shape of the sheath is highly irregular, staghorn-shaped. Following the individual penicilliform capillary the uniform sheath continues as a sleeve, where it looks like an "ellipsoid " which is actually only an (arm) of the staghorn-shaped sheath what means that the actual shape of the S.S.S is never ellipsoid.

The studies that were carried out by ***Olah and Glick (1982) and Olah et al., (1994)*** proved that the avian S.S.S was built up from 2 types of cells, one of them lined up in one or two layers and surrounded the thick basal membrane of the cuboidal shaped endothelial cells of the P.C. This type of cells was belonging to the normally nonphagocytic splenic reticular cells . No intercellular substance exists between these cells and the subendothelial c.t of the P.c that may have a few small branches invading the ellipsoid .The other type of cells were sitting on the surface of the S.S.S but their processes penetrated it and form a 3-dimensional network among the non-phagocytic cells. Olah and Glick (1982) or ellipsoid-associated

nonlymphoid cells (EANCs) by Jeurissen et al., (1992) named these cells. The study of the 1st authors showed that a few (EACs) may be recognized inside the ellipsoid but the majority is located at the surface of ellipsoid. Those within the ellipsoid are generally ovoid in shape and rarely contain cytoplasmic granules. But generally the authors described a unique feature, which distinguish the EACs from other cells. This feature is represented in the nuclear structure of these EACs where a portion of heterochromatin appears as clumps along the nuclear membrane, while the remainder is evenly dispersed through the nucleus. Two morphologically different types of EACs located on the surface of the ellipsoid as well as are also described by the authors one of these is round or moderately elongated; the other is extremely elongated or spindle-shaped with one or two long processes and frequently seem to be detached from the ellipsoid. Cytoplasmic differences also exist , the round EACs have an endoplasmic area with organelles specially a well–developed Golgi apparatus and large number of small or medium sized electron dense granules , and a thin ectoplasmic zone without organelles . These round or ovoid EACs elaborate active Golgi zones, which may suggest that they are immature forms. The Golgi region of the spindle-shaped EACs does not show vesicles filled with an electron–dense substance .The granules are larger and their number is reduced. These cells reveal inactive Golgi zones, which may suggest that they are mature stage.

Many authors described the EACs as precursor cells of the interdigitating and follicular dendritic cells of the T-dependant PALs and germinal centers, respectively *(Olah and Glick, 1982; Gallego et al., 1993; Delcacho et al.,1995)* where these cells migrate from their transient site at the periphery of the ellipsoid to the PALs and germinal centers, where it may function as an interdigitating cells or as follicular dendritic cells , depending on its location .

The ontogenic study that were carried out on the EACs by *Gallego et al., (1993)* stated that by 1 week of age the EACs formed a large cellular mass that consists of elongated and oval cells . They arranged in 3 to4 ring-shaped layers and occasionally appeared close to the endothelial cells of the P.C but between 2-4 weeks of age numerous EACs were present in the red pulp . They added that by 4 week of age the ellipsoid and EACs complex were fully developed .

The S.S.S and P.C can be followed until the red pulp where they are suddenly ceased . Also the S.S.S grouped or form rosette-like patterns, which may indicate that the splenic white pulp is highly, organized *(Olah et al, 1994).*

The outer border of the ellipsoid is well delineated , but there is no limiting structure between the ellipsoid and the surrounding white pulp . Mobile cells like small lymphocytes and red blood cells are occasionally present in the in the ellipsoids *(Olah and Glick ,1982).*

The periellipsoid white pulp (PWP) or periellipsoid lymphoid tissue accumulated around the sheathed capillary and possesses few small lymphocytes, plasma cells ,macrophages with the majority of cells being young blast like cells, also EACs may appear in the PWP but the mitotic divisions are rarely observed in them *(Olah andGlick. 1982).*In addition to the blast cells, pyroninophilic cells are also present within the PWP.

These pyroninophilic cells seems to be primitive reticular cells *(Ham,1987)* and not large and medium sized lymphocyte, where the white pulp has reticular fibers ensheathed by reticular cells as a fine supporting system *(Olah and Glick ,1982).* Also the PWP may contain medium sized lymphocyte *(Ogata et al., 1981)* or medium and large sized pyroninophilic lymphoid cells around the ellipsoid sheath(**Sugimura and Hashimoto ,1980)** , the later authors added that these PWP occupied the greatest part of the white pulp about 32.6 % of the duck spleen .

The perivenous lymphoid tissue (P.V.L) occurred discontinuously along the course of large veins in the red pulp *(Fukuta et al.,1969;Sugimura and Hashimoto 1980; Ogata et al.,.,1981; Bareedy et al.,1986)* and consists mainly of small lymphocytes as did the PAL tissue. Moreover *Ogata et al., (1981)* added that this structure usually present as nodular lymphoid masses outside a thin layer of c.t or under the endothelium of veins .This portion of the white pulp account for 2.5% of the duck spleen *(Sugimura and Hashimoto, 1980).*

King and Mclelland (1981) stated that the focal accumulations of specialized cells formed within the secondary lymphoid tissue in response to stimulation by antigen are refereed to as nodules or follicles . These accumulations often have a pale circumscribed portion called the germinal centers in which are many dividing lymphocytes. They also added that the light staining of the germinal center is due to the presence of numerous immature lymphocytes, while the dark staining inclusion bodies which are often present in the macrophages in the centers appear to be the nuclei of the cells which die soon after they enter mitosis.

Generally the germinal centers have been considered as variable structure where in the chicken they are absent at hatching and appear at about 4 to 5 weeks old *(Cooper et al., 1965),* as the germinal centers in the spleen appear mainly in association with antigenic stimulation *(French et al.,1969;Makinodam et al.,1969; white et al., 1970; Nagy and Feher 1972; Anderson 1973; Nagy et al.,1975; white et al.,1975).*

The germinal centers might be formed by hemocytoblasts which appeared at the periphery of ellipsoid in the chicken spleen after antigen injection and then migrate into PALs and PVL tissue forming the germinal centers *(Anderson, 1973; Ogata et al., 1981).Olah and Glick (1982)* described the germinal centers to be formed by the migration of the EACs to the border of the PALs.

The germinal centers are located at the beginning of PALs but not enclosed in it *(Lucas et al., 1954;Nagy, 1980;Olah and Glick, 1982)* from this location the later authors concluded that the distribution of germinal centers in the chicken spleen is not random but rather confined to sites close to the hilus.

Suginura and Hashimoto (1980) and Ogata et al., (1981) described the germinal centers to be accumulation of large, medium and small-sized lymphocyte in the PALs and PVL tissue and surrounded by a thin fibrous capsule. The former authors added that these centers occupied about 0.1% of the 7 weeks old duck spleen and the fibrous capsule located normally in the PALs but occasionally in the PVL tissue.

In addition to the lymphocyte the germinal centers of the chicken spleen has secretory cells that may be identical to the mammalian follicular dendritic cells *(Olah and Glick ,1979; Kroese et al., 1982).*

The chicken follicular dendritic cells were described by several studies *(Eikelenboom et al., 1983; Jeurissen, 1993;Gallego et al., 1995)*. Moreover the latter authors identified 2 morphologically distinguishable types of follicular dendritic cells in the chicken splenic germinal centers . These were respectively follicular denderitic cells (FDCs) with long and convoluted filiform cell processes and F.D.Cs with thick beaded dendrites.

Ogata et al., (1981) described the formation of two types of germinal centers, type I with many follicular denderitic cells, while type II without .

The formation of the germinal centers in the chicken spleen depends not only upon the bursa of fabricious *(Jankovic and Isokovic, 1964; Cooper et al., 1966; Sugimura and Hashimoto 1976)* but also upon the thymus *(Hoshi and Mori, 1973; Hirota and Bito, 1978;Ogata et al., 1981).* These centers also pass a sequence of developmental changes and ultimately to involute and disappear *(Raviola ,1975).* But germinal centers in pigeon and goose spleen were difficult to be seen *(Bareedy et al.,1986)* .

At the junction of red and white pulp is the **marginal zone** in which both T and B-cells occur *(Ford,1975).* This marginal zone may be clear as in goose spleen *(Bareedy et al., 1986)* or absent as in the chicken spleen *(Olah and Glick,1982)* .In the chicken the functional equivalent of the mammalian marginal zone may be the EACs *(Olah and Glick ,1982)* or the complex formed by ellipsoid cells , the periellipsoid B-cells sheath and the surrounding macrophages *(Jeurissen et al.,1992).*

The red pulp of the spleen is a loose spongy tissue composed of ramifying cellular cords surrounded by venous sinuses *(Hodges, 1974)* . The splenic cords consists of reticular cells within which are found lymphocytes, macrophages, all types of circulating blood cells *(Hodges ,1974; King and Mclelland ,1981; Bareedy et al., 1986)* and plasma cells *(Taliaferro and Taliaferro ,1955; Thorbecke et al.,1957; Lucas and Jamroz ,1961; Warner and Szenberg, 1964).*

The venous sinus of the spleen has irregular shape ***(Fukuta et al., 1969; Hodges, 1974)*** or cylindrical shape ***(Nasu et al., 1992)***within the red pulp. The luminal wall of the sinus is lined by flattened elongated cells "Littoral cells" ***(Hodges, 1974;Nasu et al., 1992)***or single layer of rod shaped cells ***(Miyamoto et al., 1980)***. Small oval gaps were present between the endothelial cells ***(Nasu et al., 1992).***

MATERIALS AND METHODS

Materials and methods

In the present study ,eggs ,chicks and mature birds of the Japanese quail (coturnix coturnix Japonica) were collected from the farm of the faculty of agriculture in Kafr-EL-sheikh governorate . The collected materials were chosen to cover mostly the whole life of the bird.

For the incubation period 200 eggs were collected and incubated in the farm incubator at 37.5 C (99.5F) and 87% relative humidity .These conditions continued until 15th day of incubation as the temperature was reduced to 37C (98.6F) and relative humidity was increased to 90%.

For the pre-hatching period, the eggs (table1) were labeled according to the day of incubation as E0,E1,E2.....to E17 with E0 represent the 1st day of incubation. From E1- E10 the whole embryos were taken and processed for histological studies, while from E11 to E17 the embryos were large enough to be opened and the spleen was extracted and processed alone .These collecting materials were quickly fixed in Bouin's solution (Bouin, 1897).

For post-hatching period (table 2) the chicks were collected directly at the first day post-hatching and then collected every week until 8 weeks of age (approximately the age of maturity), then the mature birds were collected again at 6 month and one year as a senile birds *(Sainsbury, 1992).*

For the chicks and mature birds, the spleens were obtained as soon as possible after slaughtering of these birds and were quickly transferred to

Bouin's fluid , zenker formol *(Helly, 1903)* and Carnoy's fluid *(Carnoy ,1887)* for fixation, then the specimens were processed and embedded in paraffin wax . Serial and step serial sections of 3-5 micrometer thick were prepared from the whole embryos of E1 to E10 of incubation , while the spleens were sectioned at 3-5 micrometer . Following the sectioning of the specimens the following, staining procedures were adopted .

- Harris haematoxylin and eosin *(Harris,1900)* for general features of the cells .
- Crossman's trichome *(Crossman,1937)* for identification of muscle and collagen fibers.
- Gomori's reticulin *(Gomori,1937)* for identifications of reticular fibers .

For the electron microscope sections, less than 1m.m (millimeter) thick sections were removed from different areas of the spleen of the 8 weeks age quail then fixed in 4% buffered glutaraldehyde and then cut into smaller pieces which were further fixed for 3-4 hours in fresh 4% glutaraldehyde. The tissue sample were washed in two buffers overnight and post fixed in 1% buffered osmium tetroxide. Following fixation, the tissues were prepared and semithin sections of 1 micrometer were made and stained with toluidine blue for orientation, then the thin sections were counter stained with uranyl acetate and then examined with E.M of Zagazig University. This method was applied as reported by *Hayat, (1986).*

Table (1) : *The available samples in the pre-hatching study*

Day of incubation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
No. of eggs	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

Table (2) :The available samples in the post-hatching study

Age of bird	1st day	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week	6 month	1 year	*Total No.*
No. of samples	5	5	5	5	5	5	5	5	5	5	5	*55*

RESULTS

Results

Development of the spleen during pre-hatching period.

The primordium of the spleen of quail was firstly identified at the 5th day of incubation (E 5) as spherical mass of condensed cells beside the gastric primordium and ventral to the gonad and mesonephros in a close association with the wall of the primordium of the proximal part of intestine (Fig.1).

This splenic primordium consisted of condensation of fusiform or roughly round progressed mesenchymal cells with large nucleus and prominent nucleolus. Between these mesenchymal cells, very few cells with darkley stained basophilic nuclei and acidophilic cytoplasm, were present. Some cells showed mitotic figures (Fig. 2).

At the periphery of the splenic primordium, there were blood spaces that lined by elongated cells with large elongated nuclei (Fig. 3).

By the end of the 6th day of incubation, the primordium separated from the wall of the primordium of the proximal part of intestine and formed an isolated organ of ovoid shape and containing 2 large peripheral blood spaces (Fig. 4). These large blood spaces were lined by elongated cells. Some of the mesenchymal cells surrounding the blood spaces began to acquire an elongated form (Fig. 5).

From 7^{th}. To 12^{th}. day of incubation the splenic primordium changed its developmental behaviour where it became more vascular and started to acquire the spongy structure characteristic of the red pulp as several clefts began to appear among the cells. Some of these clefts joined to form a network of spaces. (Fig 6).

The mesenchymal cells bordering these spaces flattened and gave the spaces a smooth internal surface. The first hemopoietic cell progenitors (lymphoid hemocytoblast or erythroblast) began their differentiation (Fig 7).

Toward the end of the 10^{th} day of incubation there was a massive increase in the number of the hemopoietic cells (Fig.8)

With the end of 12^{th} day of incubation the developing spleen entered another stage of development where it became more cellular with reduction in the size of the blood spaces except for very few spaces that found under the ill-developed capsule. Some of these spaces still filled with hemopoietic cells (Fig. 9)

During this period (E 7 - E 12) the hemocytopoietic progenitors peaked only for one day (11^{th} day of incubation) and the arterial blood vessels started to appear, these grow in from the periphery of the organ and then extended to the center. Firstly these arterial blood vessels were lined by cuboidal cells(Fig. 10,11).

The appearance of the arterial blood vessels were accompanied by the appearance of the main venous blood vessels and blood capillaries. The venous blood vessels was large and occupied the center of the spleen. This central vein was divided by a narrow constriction into 2 parts, one of them has a side branch. The (2) divisions and its branch lined by elongated cells. (Fig. 12).

The blood capillary lined by high cuboidal cells and surrounded by 2 layers of elongated squamous cells. (Fig.13).

The peripheral counter of the spleen was limited by a layer of fibroblasts. (Fig. 14).

The reticular fibers is firstly identified by means of their argyrophilic properties at the 12^{th} day of incubation as very fine filaments in the overall aspect of the organ but specially concentrated around the different types of vessels (Fig. 15).

After the hemocytopoietic cells approached its peak at the 11^{th} day of incubation, the process of the hemopoiesis began to decrease and some of the cells in the erythoid lineage became mature and passed to the veins to be engaged within the circulation. These mature cells were oval and nucleated (Fig. 16).

In the period between 14 and 17 day of incubation the red and white pulp became differentiated but without a clear distinction.

There were more numerous capillaries (penicilliform capillaries), most of them filled with blood. The hemopoiesis decreased suddenly and tend to be ceased (Fig. 17,18).

The arterial wall became relatively more thicker than the previous and its lining cells acquired the elongated from of the endothelium. Some cells in the periarterial tissue showed mitotic figures (Fig. 19).

Toward the end of incubation, the spleen became well established and approached the mature form. It covered externally with a well developed capsule, The arteries and veins acquired mature shape and the red and white pulp were identified but intermingled to each other (Fig. 20). The subcapsular blood spaces became more differentiated and lined by endothelial cell layers (Fig. 21).

The most characteristic feature of the organ at this period is identification of the 1st Lymphocyte at the 16th day of incubation. (Fig. 22).

The reticular fibers became more abundant specially around different types of vessels but in the tissue in between, the fibers are relatively fewer and uniformly distributed (Fig. 23).

Development of the spleen during the post-hatching period

During the 1st 3 weeks, the post-hatched spleen was more established than the pre-hatched one and consisted of the usual white and red pulp that were intermingled to each other without clear demarcation. The white pulp was darkly stained diffuse and nodular basophilic areas except for a very small regions that constitute the exact site of the penicilliform capillaries which appeared as lightly acidophilic areas of a central lumen. The red pulp occupied the remained areas between the white pulp (Fig. 24).

The whole organ was covered externally by a relatively thick capsule formed of fibroblasts, few smooth muscle cells, few collagen fibers and abundant reticular fibers (Fig. 25, 26).

The parenchymal elements of the organ was superimposed upon a highly specialized network of reticular fibers. The venous system was surrounded by a relatively thin layer of reticular fibers from which a very fine fibers were widely distributed to the surrounding tissue. The penicilliform capillaries were characterized by a special pattern of reticular fibers where there was a heavy reticular ring delineates the lumen and another ring immediately surrounding the endothelium. These 2 rings were connected by thin ray - like fibers in a form of basket (Fig. 27).

The arterial system was the most heavily invested with reticular fibers where the wall of central arteries was circled by a heavy ring of interwoven fibers(Fig. 28).

During the 1st 3 weeks post hatching the white pulp formed of diffuse lymphoid tissue distributed around the different types of the blood vessels (Fig. 24).

Around the central arteries the periarterial lymphoid sheath (PALS) formed of medium and large sized lymphocyte. Very few small lymphcytes and lymphoblasts were observed. Besides the lymphocyte some of the visitant cells such as macrophages, plasma cells and some of the blood cells were also encountered within this sheath. Macrophage-like cells with large eccentric nucleus and basophilic foamy cytoplasm were also present (Fig. 29, 30).

The perivenous lymphoid tissue surrounding the central vein; was also formed as the PALS was done but the visitant cells specially that of the blood type were more numerous in the perivenous than in the periarterial tissue. The central vein was lined by 2 types of cells, one of these types was elongated with elongated oval nucleus, the other type was cuboidal with large spherical nucleus and prominent nucleolus (Fig. 31).

The most characteristic feature of the quail spleen is the presence of large number of the penicilliform capillaries or ellipsoid. (Fig. 24). This was a unique structure where it has several shapes according to the level of section as it was circular, elongated, U - shape or Y - shape. (Fig. 32, 33,34). It was lined by high cuboidal cells. These lining cells were surrounded by 2 discontinuous layers of the splenic stromal cells (Fig. 35, 36).

At the surface of the circular profile of the penicilliform capillaries, there was another type of cells. These were large, with large eccentric nucleus and basophilic cytoplasm and similar to the macrophage-like cells that were observed in the PALS. These cells were surrounded by a ring of lymphocytes or lymphocytes and plasma cell (Fig. 37).

The ultrastructure of the penicilliform capillaries showed no cell junction between the lining high cuboidal cells. These cells were surrounded by a thick basal lamina that may enclose the 1st layer of the reticular cells (Fig. 38).The ultra-structure of the macrophage - like cell or the ellipsoid - associated cells as named , showed that it has the ability for both endocytosis and phagocytosis and surrounded in both cases by lymphocyte arranged in a circular pattern (Fig. 39, 40).

Towards the end of the 3rd week of age the lymphoid tissue surrounding the blood vessels showed a circumscribed structure similar to the lymphoid nodules. These nodules were firstly associated with the central arteries and veins (Fig. 41,42).

The lymphoid nodule associated with the arteries appeared to be more demarcated than that associated with the veins where it was completely surrounded by a reticular capsule of interwoven fibers. Very few of these fibers penetrated the periphery and the center of the nodules. An eccentric vascular channels were also observed associated with these nodules (Fig. 43, 44).

The nodules associated with the vein appeared without capsule except for the part closed to the wall of the vein (Fig. 45).

With advanced age the number of the lymphoid nodules increased and a 3rd type of lymphoid nodules (similar to that associated with the arteries) appeared within the periellipsoid white pulp but not associated with either arteries or veins. The total number of the 3 types of the lymphoid nodules is peaked at about 7 - 8 week of age (Fig. 46, 47).

The distribution of cells within the lymphoid nodules acquired a unique form as the lymphocytes wrapped in a circle or ring around cells whose feature is similar to the ellipsoid - associated cells that were seen at the surface of the ellipsoid and in the PALS. The

majority of cells within the lymphoid nodules were large - sized lymphocyte but considerable number of lymphoblast and few number of small lymphocyte were also observed (Fig. 48, 49, 50). Some cells of the lymphoid nodules showed mitotic figures (Fig. 51).

This unique form observed in the lymphoid nodules were also found in the surrounding white pulp but the central cells were granulocytes (Fig. 52).

By the end of the 6^{th} month the lymphoid nodule began to involute and their number decreased again. The involution was observed firstly in the nodules associated with the vein followed by that associated with the arteries and later to the nodules in the periellipsoid white pulp (Fig. 53). With the decrease in the number of the lymphoid nodules there was an increase in the number of the plasma cells through out the whole organ (Fig.54).

The arterial system of the spleen showed another unique feature in some of their elements as it showed valve - like structure or arterial bolsters. In section such arterial bolsters resembled a bicuspid valve guarding the opening of the artery. These valve-like arterial bolsters formed from the hyperplasia of the intimal endothelium with some fibrous elements from the lamina subendothelials (Fig. 55, 56). No marginal zone can be observed in the quail spleen.

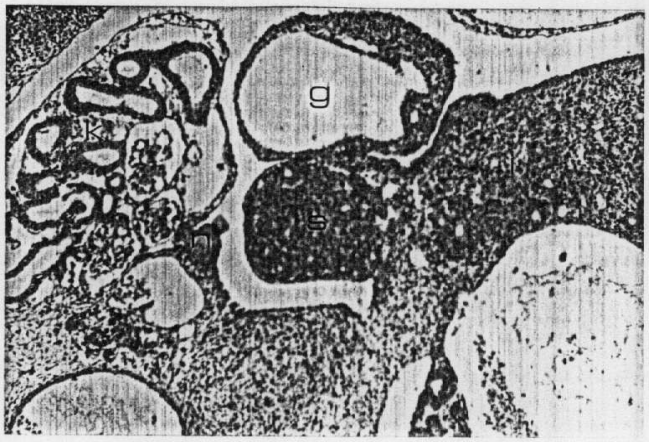


Fig.(1): 5th day of incubation, splenic primordium (s) in relation to the gastric primordium (g), mesonephros(k). Gonad (n) and intestinal primordia(i) (cross section)
H&E stain X100.

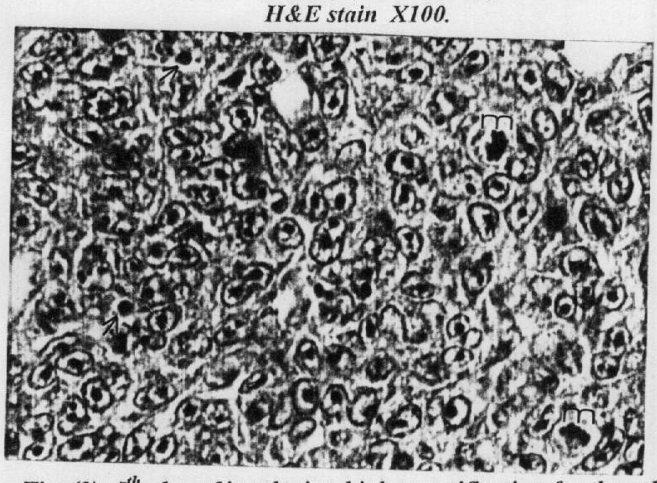


Fig. (2): 5th day of incubation,high magnification for the splenic perimordium showing the presence of some cells with darkly stained basophilic nuclei and acidophilic cytoplasm between the mesenchymal cells (arrows). Some cells showing mitosis(m).
H&E stain X1000.

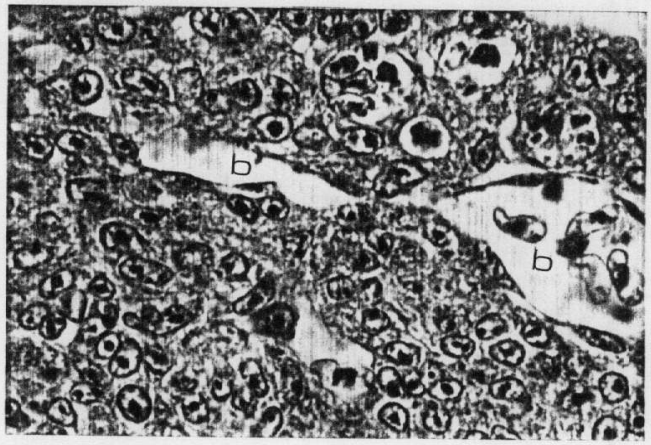


Fig .(3): 5th day of incubation,high magnification for the splenic primordium showing blood spaces(b) lined by elongated cells H&E stain X1000.

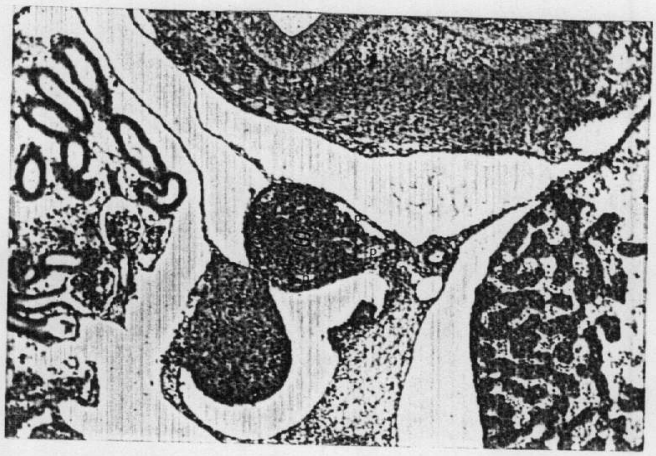


Fig. (4) :6th day of incubation , the spleen as isolated organ(s) with peripheral blood spaces (p). H&E stain X100.

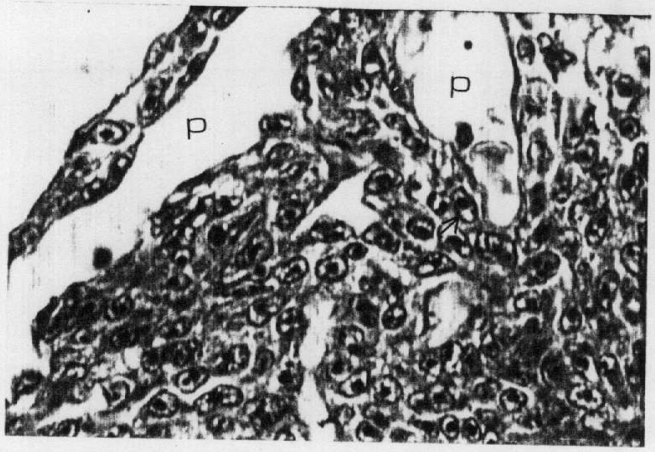


Fig. .(5) :6th day of incubation,high magnification of the spleen showing large blood spaces(P) lined by elongated cells and surrounded by flattened mesenchymal cells (arrow).
H&E stain X1000.

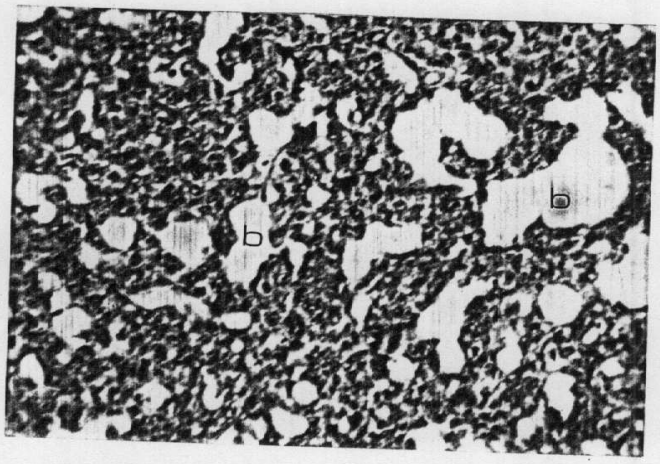


Fig. (6) : 7 th day of incubation , section of the spleen in quail chick embryo showing the vascular stage of the splenic primordium with more blood spaces (b).
H&E stain X400.

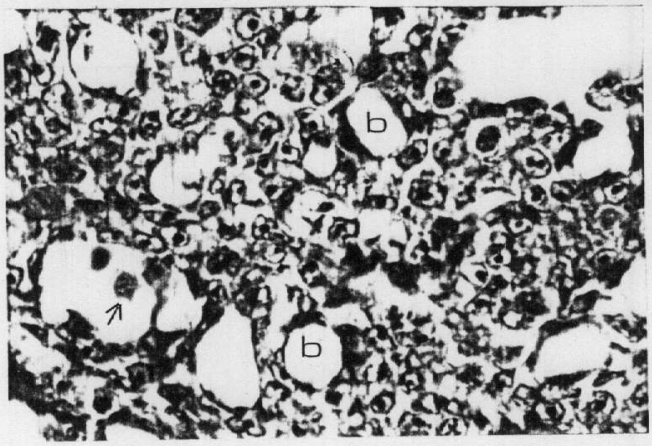


Fig.(7) : 7th day of incubation section of the spleen showing smooth internal surfaces blood spaces(b) and differentiated hemocytoblast (arrow). H&E stain X1000.

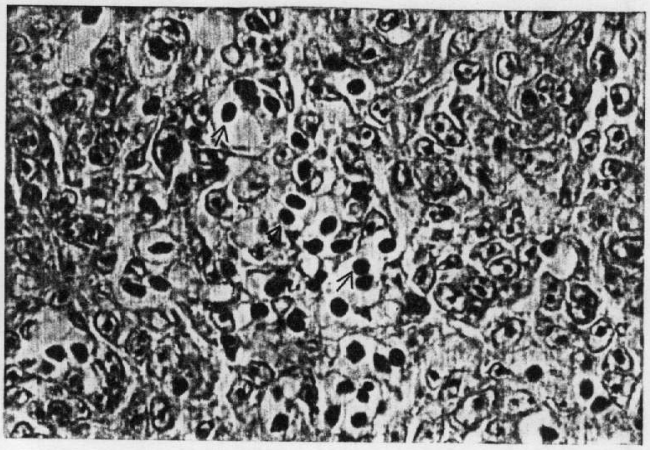


Fig.(8) :10th day of incubation , section of the spleen showing massive increase in the hemopoietic cell progenitors (arrows). H&E stain X400.

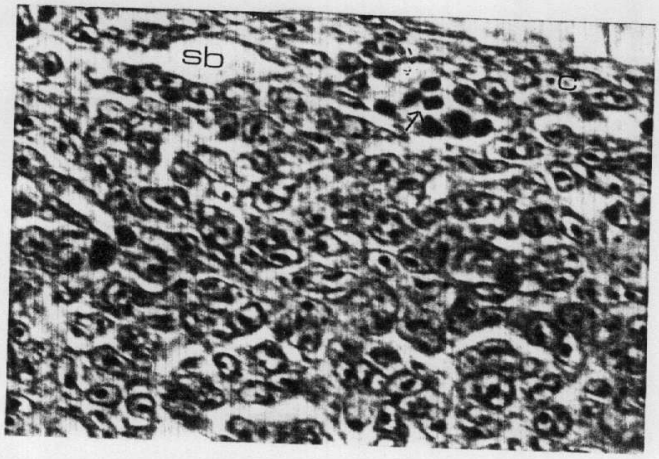


Fig.(9) : 11th day of incubation , section of the spleen showing ill-developed capsule(c) Subcapsular blood spaces (sb), some of them contain hemopoietic progenitors (arrow).
H&E stain X1000.

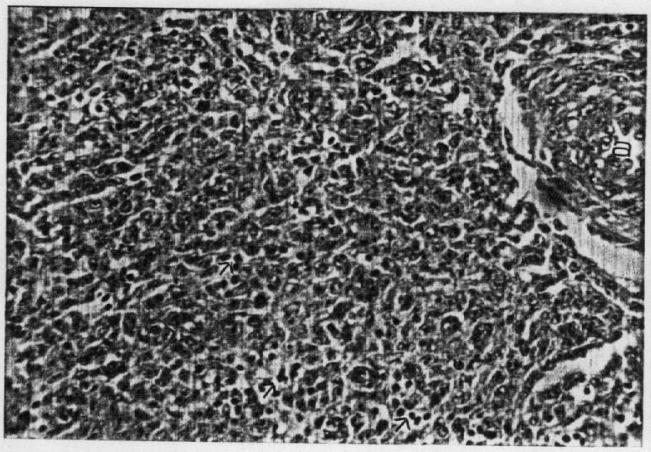


Fig.(10) :11th day of incubation, section of the spleen showing the peak of hemopoiesis (arrows). The arterials vessels (a) began to appear.
H&E stain X400.

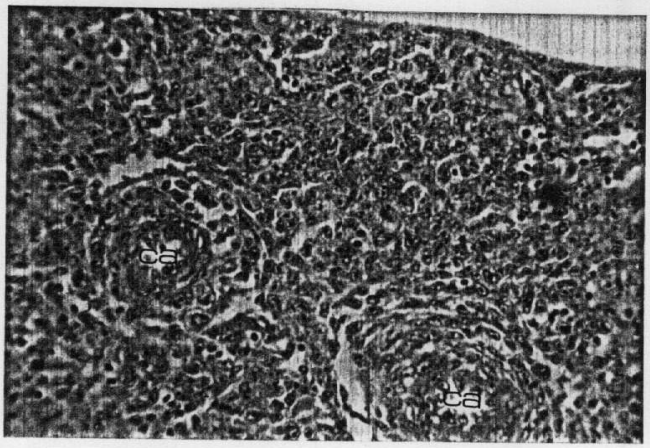


Fig. (11) : 11th day of incubation, section of the spleen showing the central arteries (ca) were surrounded by several layers of elongated cells . H&E stain X400.

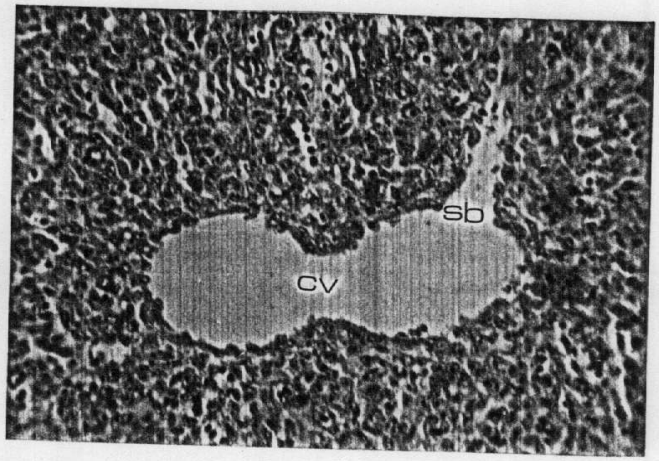


Fig. (12) :12th day of incubation ,section of the spleen showing central vein (cv) with one side branch (sb). The vein and its branch lined by elongated cells. H&E stain X400.

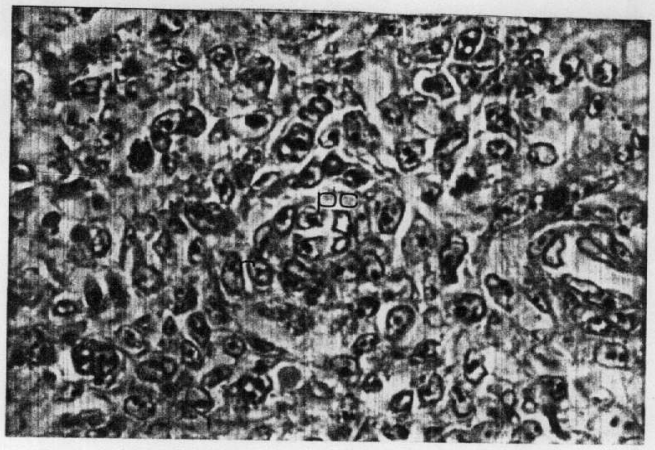


Fig. (13) :12th day of incubation , section of the spleen showing the penicilliform capillary (pc) lined by high cuboidal cells and surrounded by 2 layers of stromal cells (r).
H&E stain X1000.

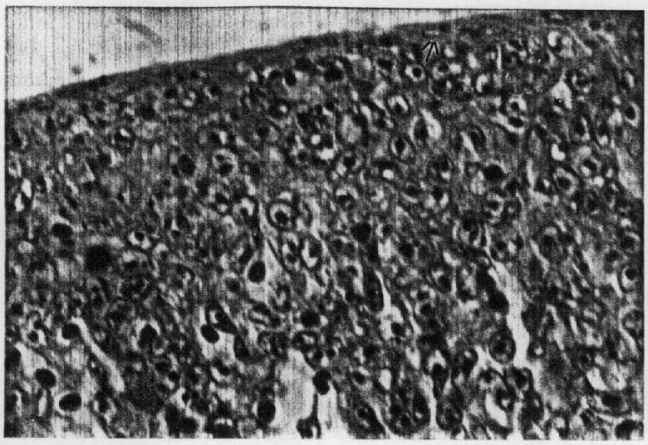


Fig. (14) :12th day of incubation the spleen demarcated by a layer of fibroblasts (arrow).
H&E stain X1000.

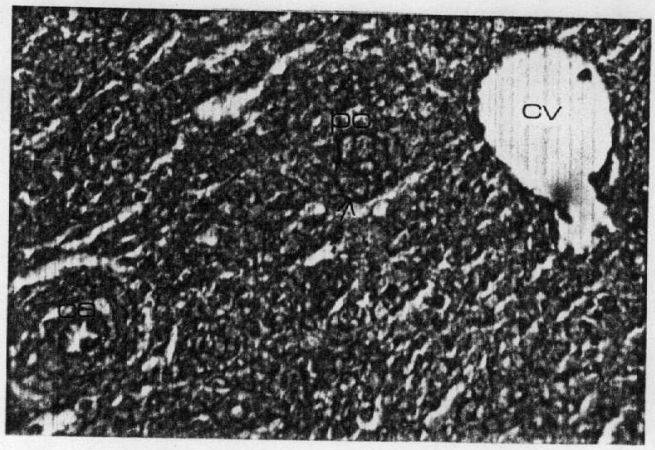
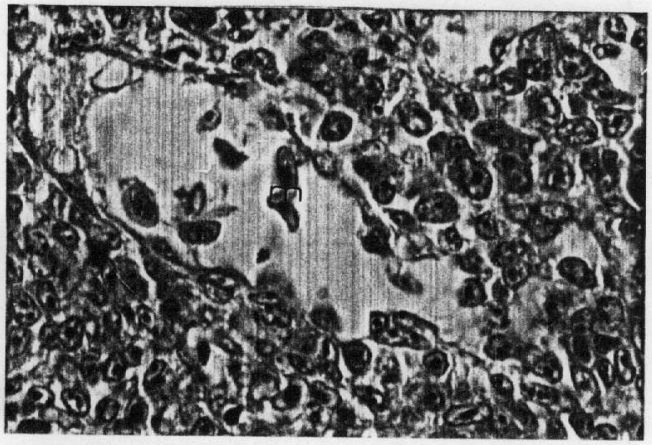


Fig. (15) :12th day of incubation ,section of the spleen showing the 1st identification of reticular fibers(arrows) around central artery (ca), penicilliform capillary (P.C) and central vein (cv). Gomori's reticulin stain X400



Fig(16): 13th day of incubation ,section of the spleen showing Some mature erythrocyte (m) and immature one. (Erythroblasts) (I). H&E stain X1000.

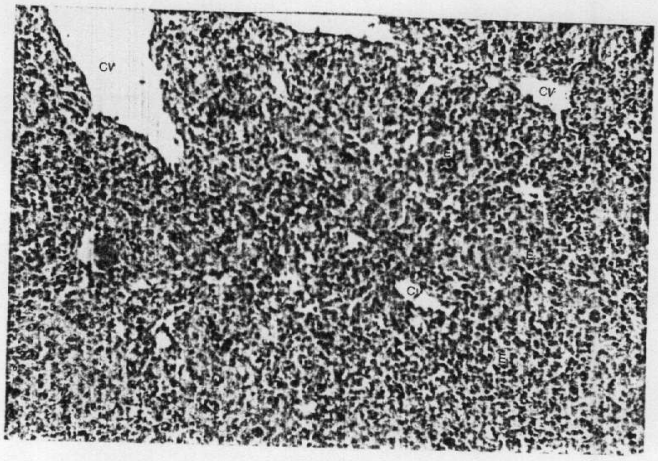


Fig. (17): 14th day of incubation , section of the spleen showing differentiated red and white pulp but without a clear distinction. More ellipsoids (E) or penicilliform capillaries. More established central vein (cv). H&E stain X100.

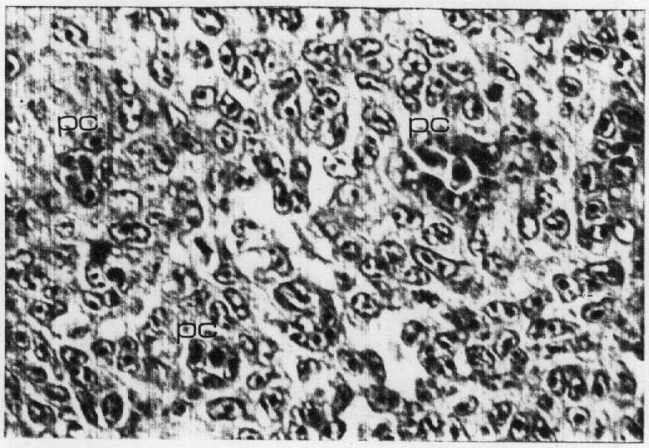


Fig. (18): 14th day of incubation , section of the spleen showing more developed penicilliform capillaries (pc). H&E stain X1000.

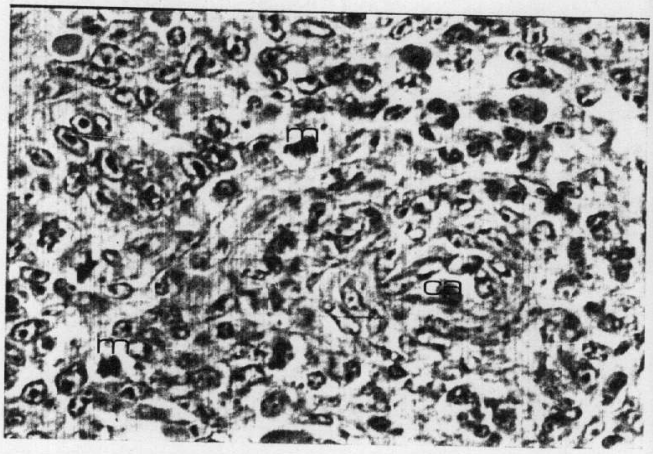


Fig. (19): 14th day of incubation , some cells in the PALs surrounding the central artery (ca) showing mitotic figures (m).
H&E stain X1000.

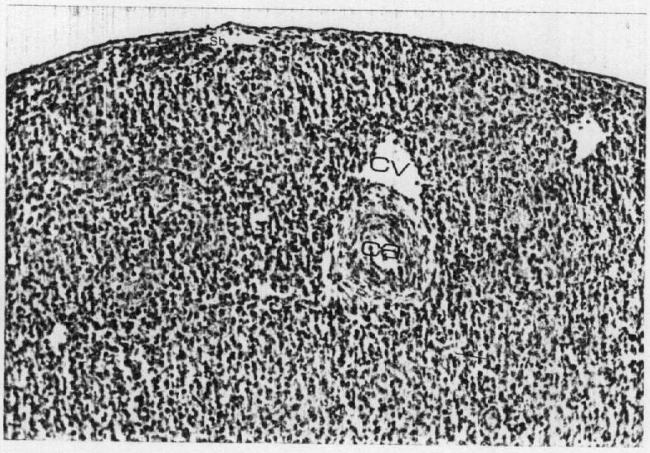


Fig. (20): 15th day of incubation , well established spleen with well-developed capsule, subcapsular blood space (sb), central veins (cv), central artery (ca)
H, E stains X100.

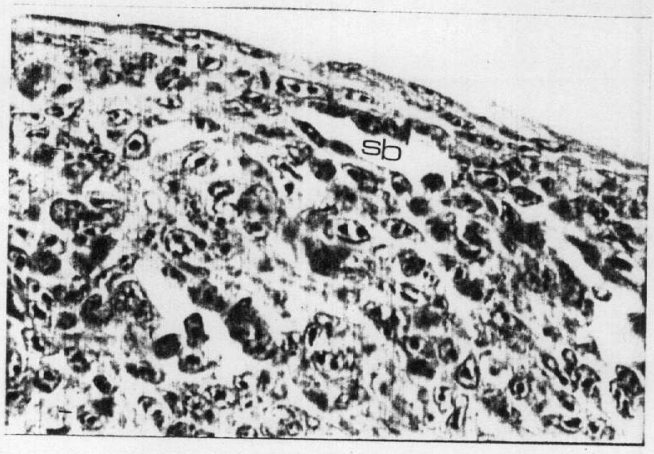


Fig. (21) : 16th day of incubation ,section of the spleen showing well developed subcapsular blood space (sb) under the capsule . H&E stain X1000.

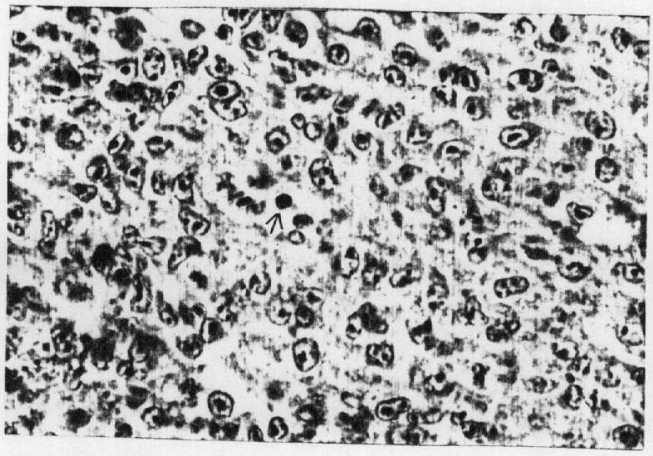


Fig.(22) : 16th day of incubation , section of the spleen showing the identification of the 1st lymphocyte (arrow). H&E stain X1000.

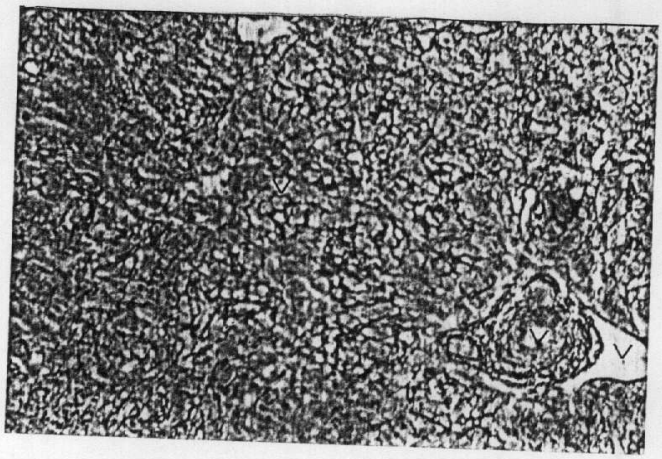


Fig.(23) :17th day of incubation, the reticular fibers become more abundant specially around different types of vessels (v). Gomori's reticulin stain X 100.

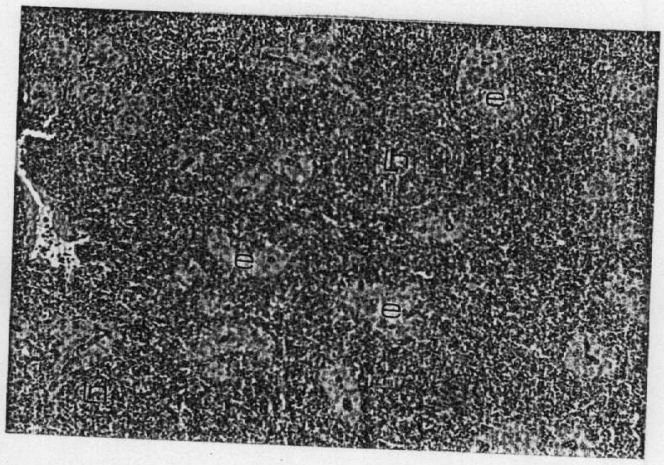


Fig.(24) :Post-hatched spleen-over all view of the spleen .Ln (lymphatic nodules), e. (ellipsoids or penicilliform capillaries). H&E stain X100.

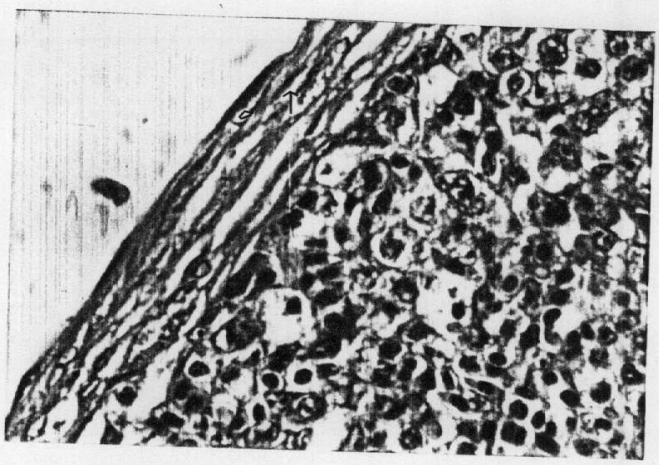


Fig.(25) :Splenic capsule with smooth muscle (arrow) .Fibroblasts (arrow head) and collagen fibers (faint green) .
Crossman's trichrome stain X1000.

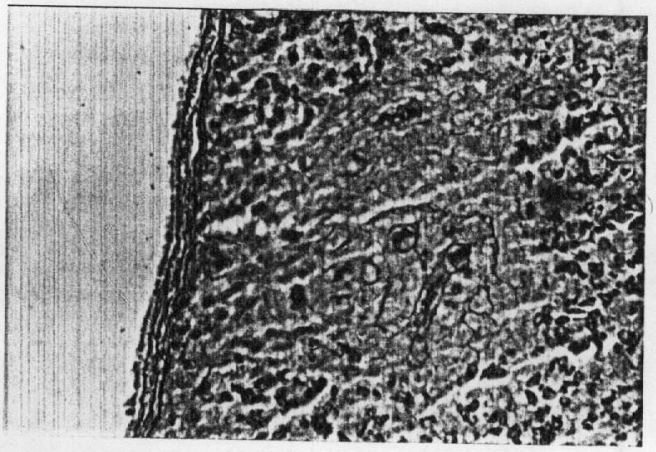


Fig. (26) : Reticular fibers in the splenic capsule
Gomori's reticulin stain X400

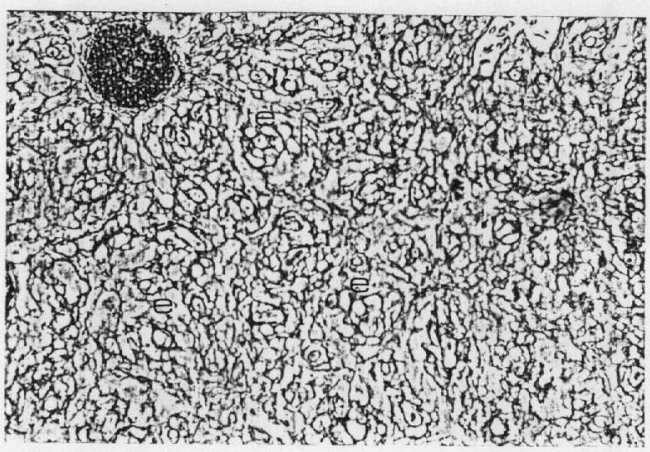


Fig.(27): post- hatched spleen- over all view- reticular fibers around the ellipsoids (e) and central vein (v).
Gomori's reticulin stain X100

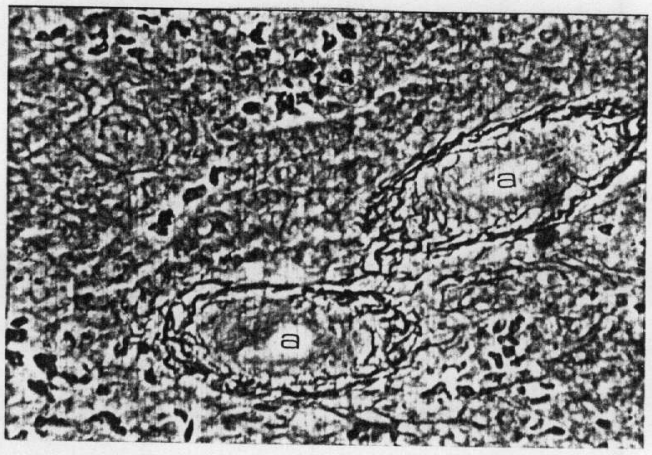


Fig. (28) : dark interwoven reticular fibers around the central artery(a).
Gomori's reticulin stain X400

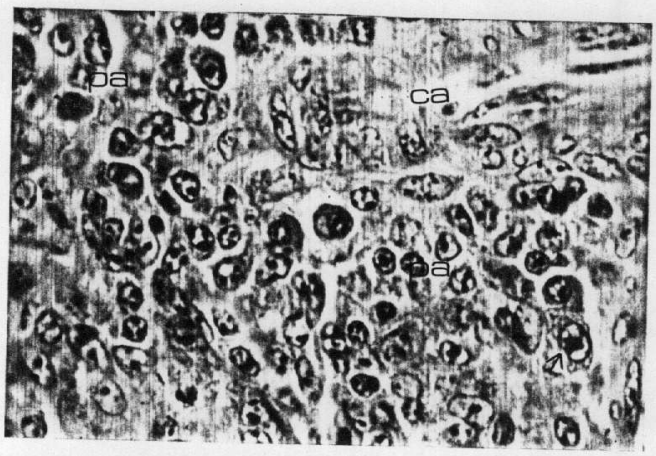


Fig. (29) :The periarterial lymphoid sheath (pa) surronding the central artery (ca) some of the macrophage-like cell (arrow) present within this sheath. These cells have large nucleus and basophilic cytoplasm. H&E stain X1000.

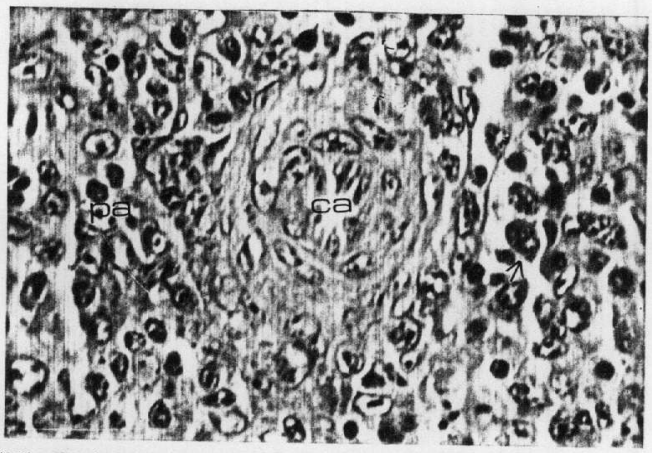


Fig.(30) : periarterial lymphoid sheath (pa) surrounding the central artery (ca). Some macrophage-like cells present within this sheath (arrow) . H&E stain X1000.

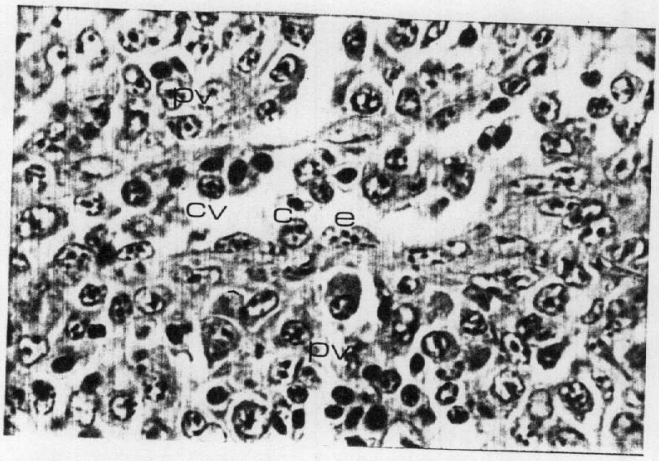


Fig.(31) : Perivenous lymphoid tissue (pv) surrounding the centeral vein (cv) .This vein lined by elongated (e) and cuboidal (c) cells H&E stain X1000 .

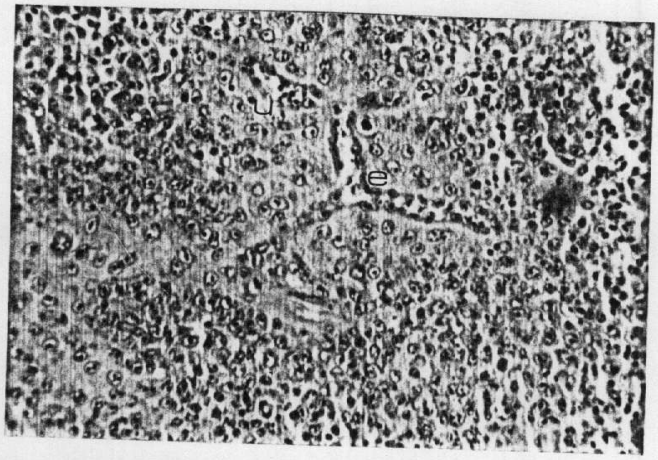


Fig.(32) : Different shapes of ellipsoid, either Y-shape (e) or elongated (u) . H&E stain X400.

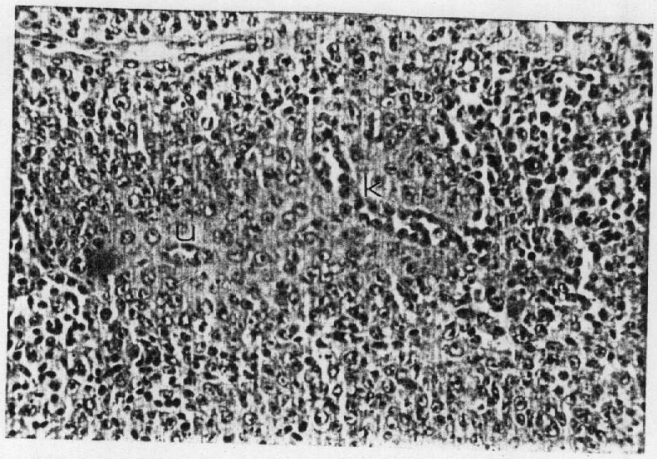
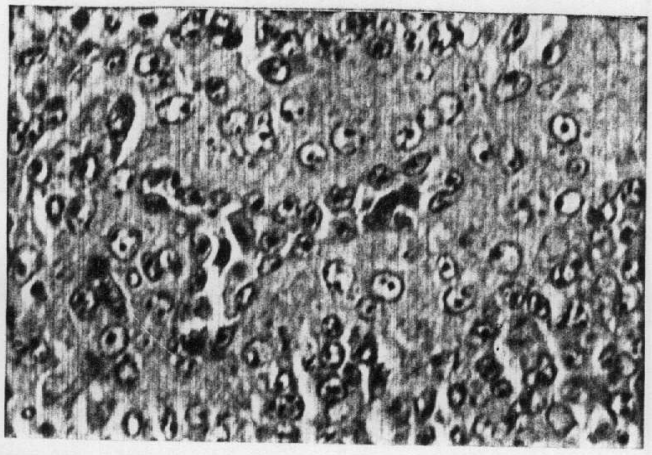


Fig.(33) : Different shapes of ellipsoids , either U-shape (k) or circular (u) . H&E stain X400.



Fig(34) :High magnification for (Y) shaped penicilliform capillary showing the lining cuboidal cells H&E stain X1000

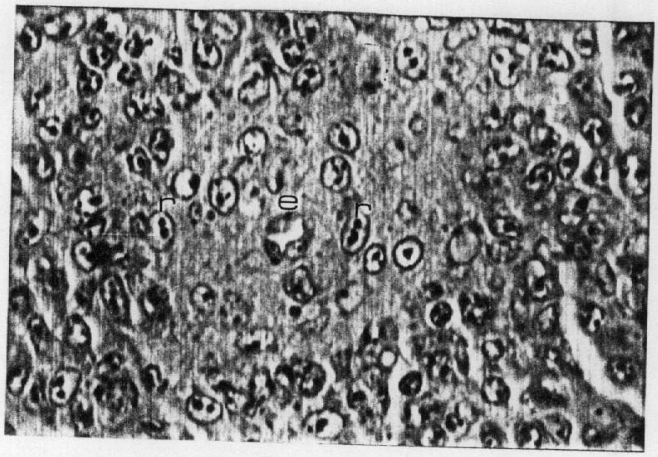


Fig.(35) :Lining cells of the ellipsoids or penicilliform cappilary (e) and the surrounding stromal cells (r) .
H&E stain X1000.

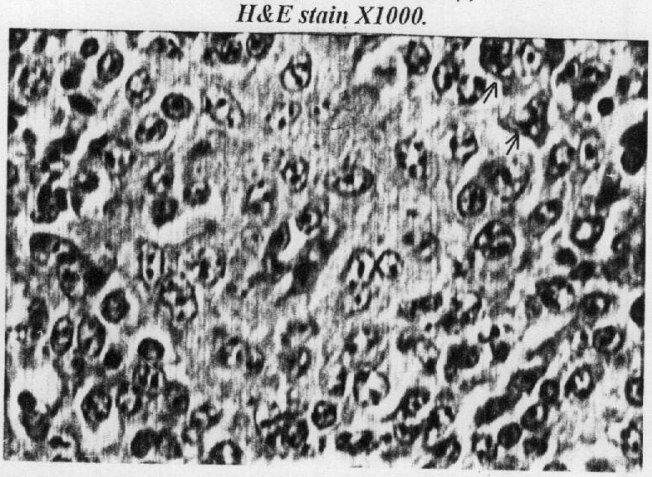


Fig.(36) :Presence of the macrophage -like cells or ellipsoid associated cells (EAC) at the periphery of the circular profile of the ellipsoid (arrows) .
H&E stain X1000.

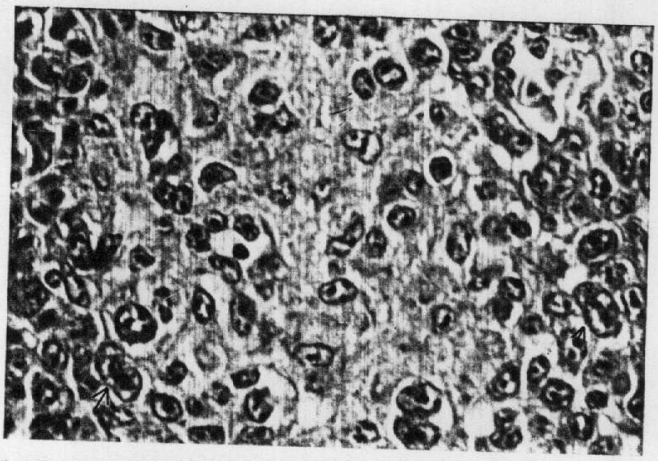


Fig.(37) : Presence of EACs surrounded by lymphocyte and plasma cells at the periphery of the circular profile of the ellipsoid (arrows) H&E stain X1000.

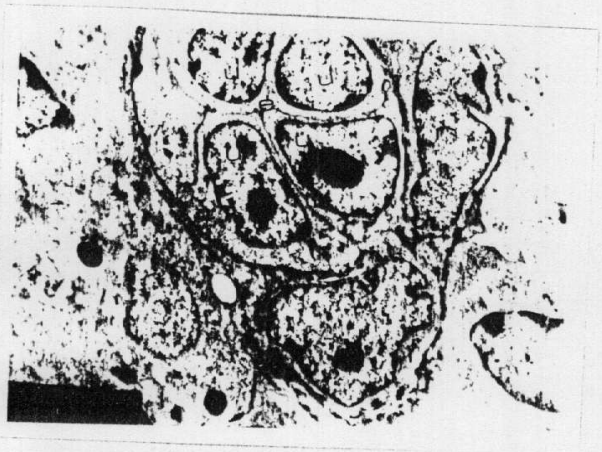


Fig.(38) : Ultrastructure of the ellipsoids (e) with its lining epithelium (u) and surrounding reticular cells (r). E.M X 5000.

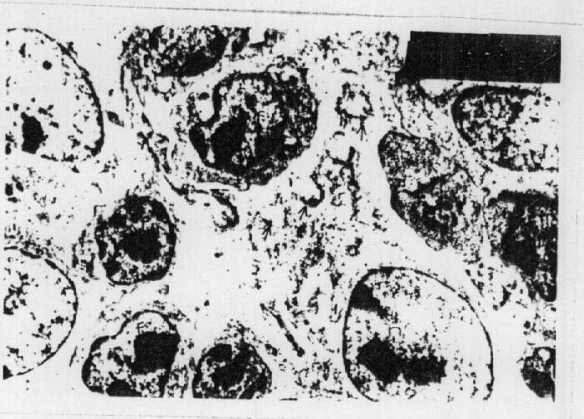


Fig.(39) : Ultrastructure of the EAC showing endocytotic activity along its cell membrane (arrows) and its large nucleus (n) .This cell surrounded by a ring of lymphocytes (f).
E.MX 4000.

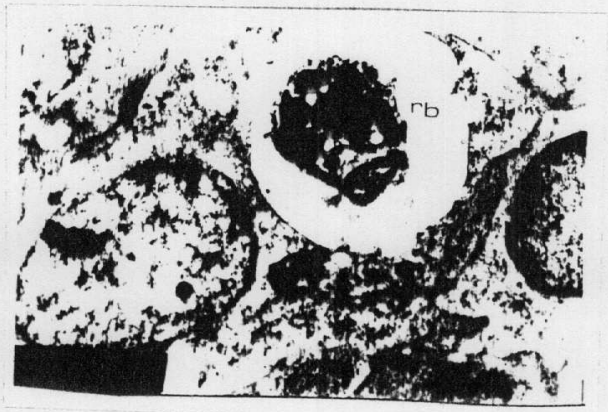


Fig.(40) : Ultrastructure of EAC showing the phagocytic activity (residual body) (rb) and its nucleus (n).
E.MX 5000.

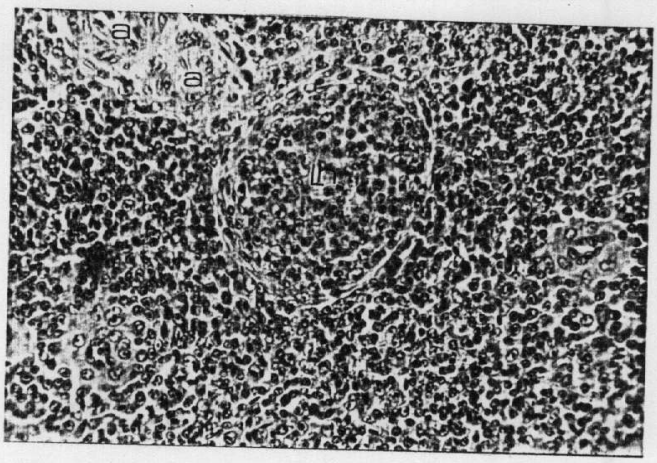


Fig.(41) : Showing lymphoid nodule (Ln) associated with centeral artery (a)

H&E stain X400.

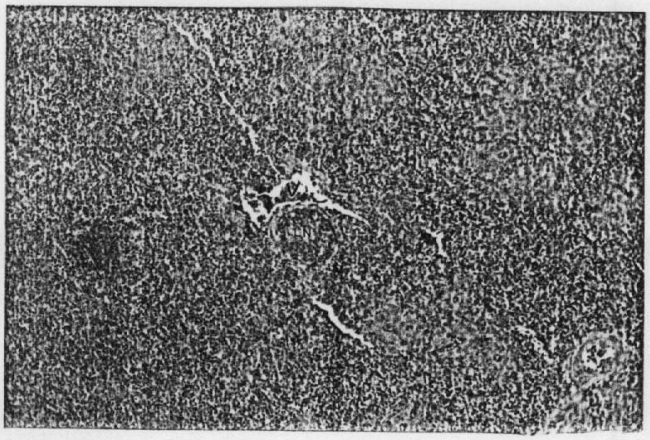


Fig.(42) :Showing lymphoid nodule (Ln) associated with vein (v) .
H&E stain X100.

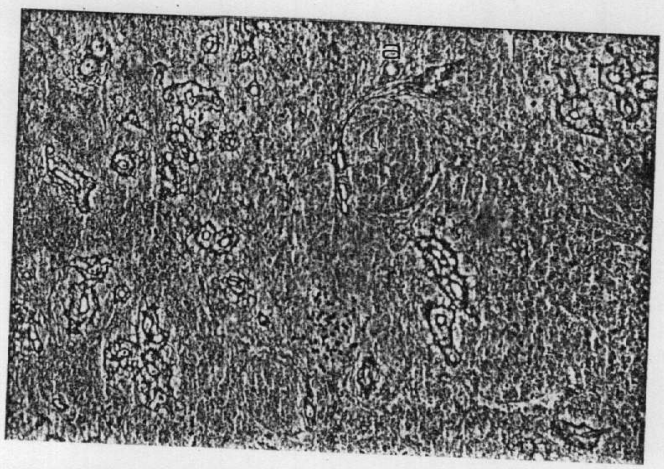


Fig.(43) : Showing lymphoid nodule (Ln) associated with artery (a) and surrounded by complete capsule of reticular fibers . Gomori's reticulin stain X 100.

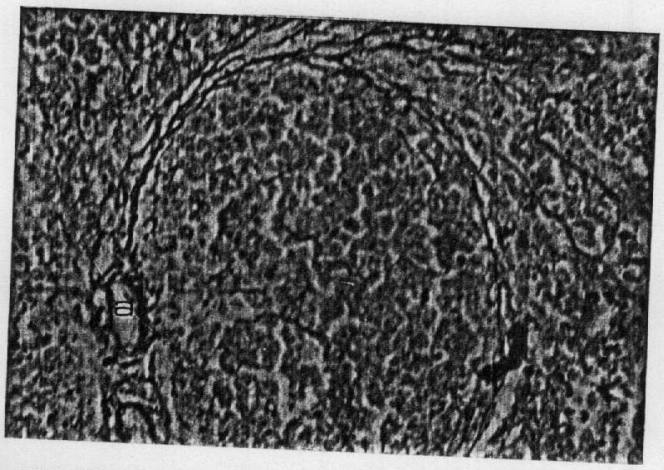


Fig.(44) :Showing lymphoid nodule associated with eccentric arterial channel (a) and surrounded by capsule of reticular fibers .very few of these fibers penetrated to the periphery and the center of the nodule . Gomori's reticulin stain X1000.

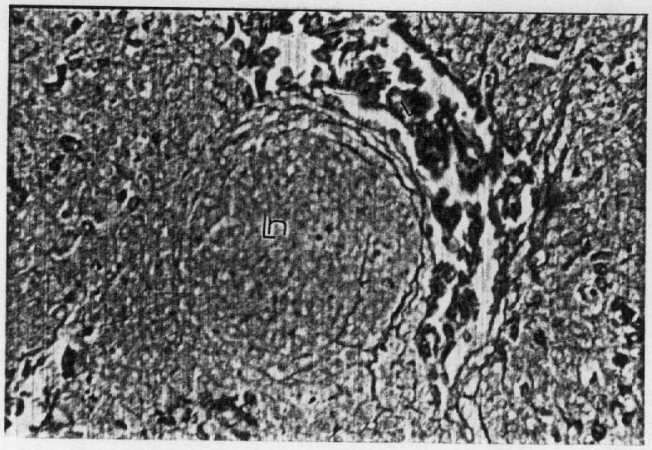


Fig.(45) :Showing lymphoid nodule (Ln) associated with central vein (v) but without capsule.
Gomori's reticulin stain X400

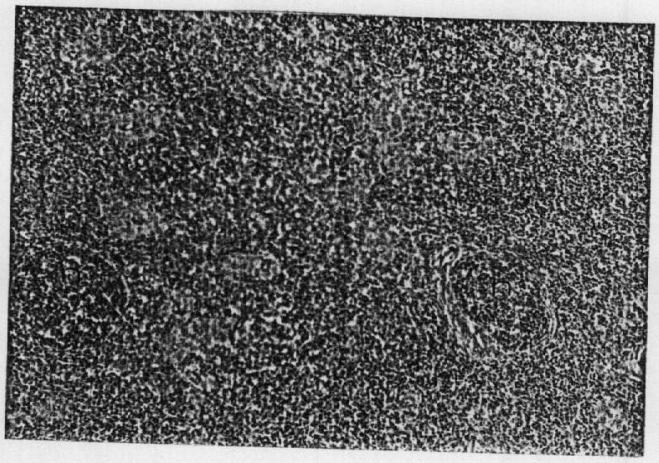


Fig. (46): Showing increasing in the lymphoid nodules (n) after the 3rd week of age.
H&E stain X100.

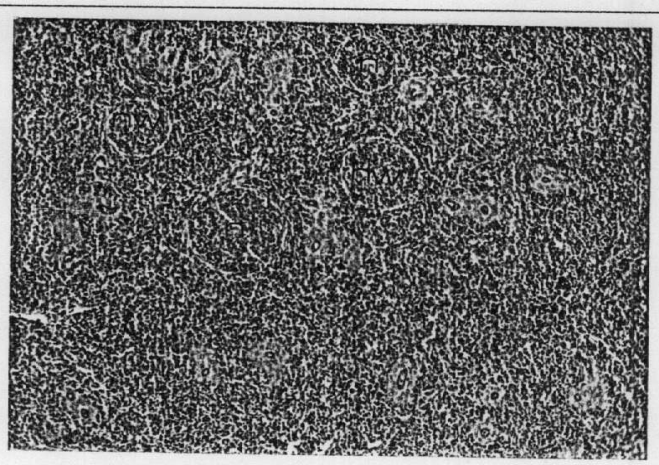


Fig.(47):Showing the peak of the lymphoid nodules (n) at 7th to 8th week of age and appearance of 3rd type of nodules (nw) in the periellipsoid white pulp.
H&E stain X 100.

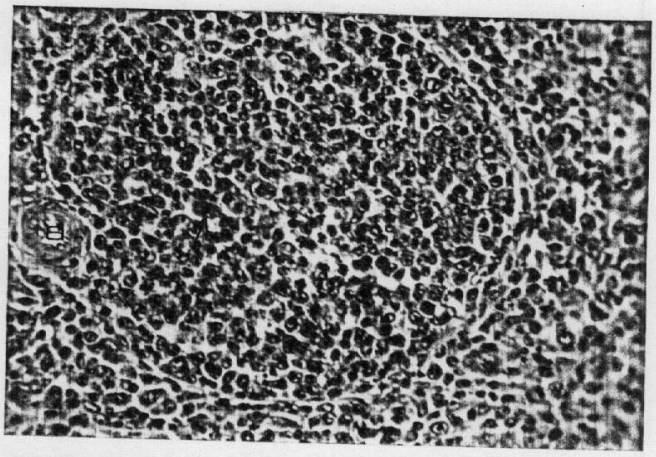


Fig.(48) :Showing lymphoid nodule associated with arteriole (a) and the circular arrangement of lymphocytes around the macrophage-like cell (arrow).
H&E stain X400.

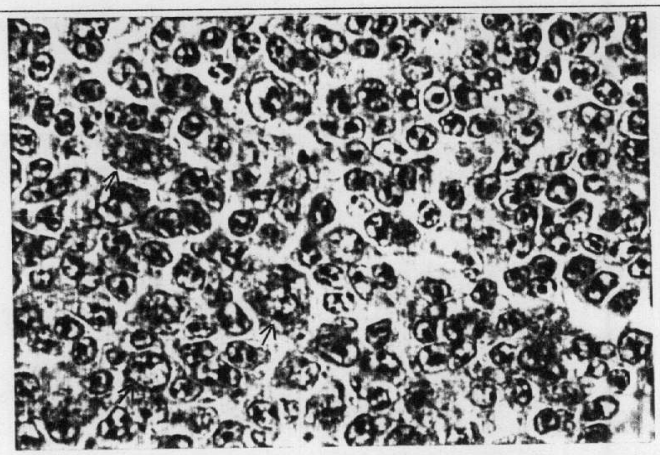


Fig. (49): Showing the structure of lymphoid nodule. The circular arrangement of lymphocyte around the macrophage-like cells (arrows). H&E stain X1000.

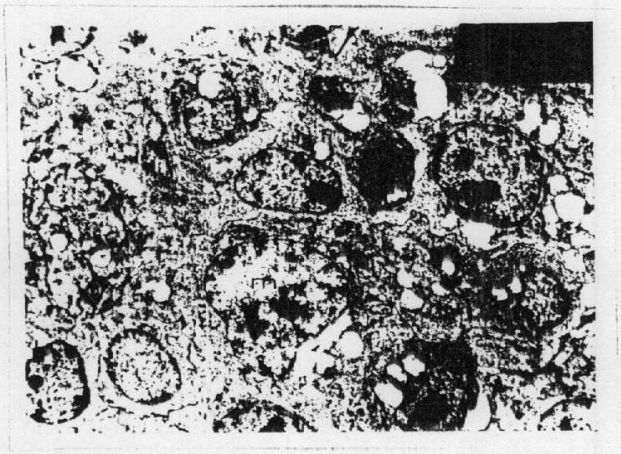


Fig.(50):Electron-micrograph showing the circular arrangement of lymphcyte around the macrophage-like cell (m) within the nodule. E.M X2.700.

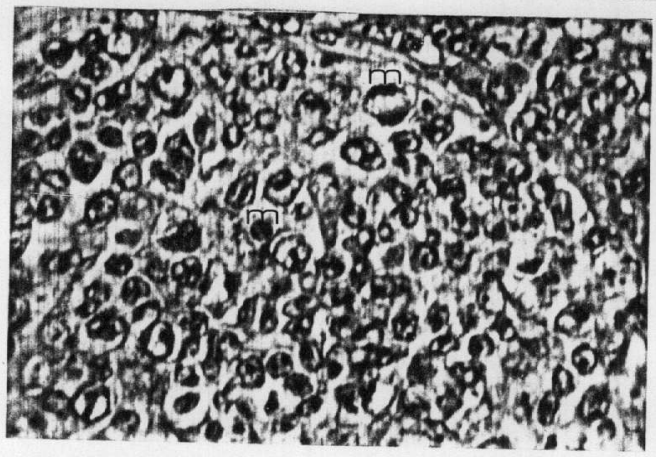


Fig. (51): Showing mitotic figures (m) in some cells of the lymphoid nodule .

H&E stain X1000.

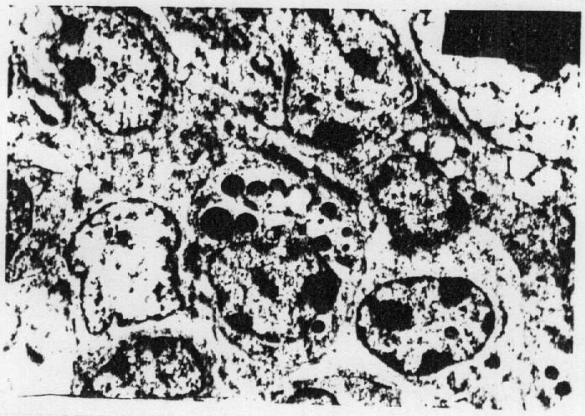


Fig.(52) :Electron-micrograph showing the circular arrangement of the lymphocyte around granulocyte(g) .
E.MX 4.000.

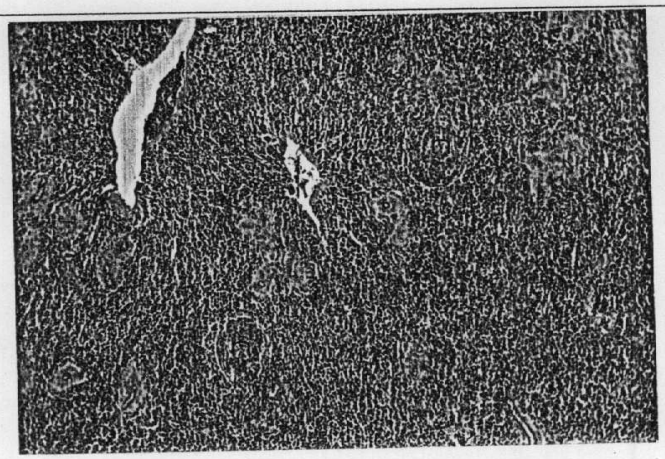


Fig.(53) : Section of the spleen of quail at the 6th month old showing reduction in the number of lymphoid nodules (n) .
H&E. stain X100.

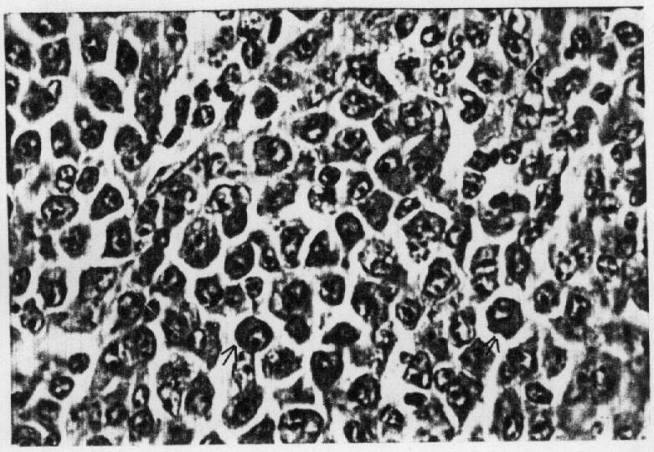


Fig.(54) : Section of the spleen of quail showing increase in the plasma cells (arrows)
H&E stain X 1000.

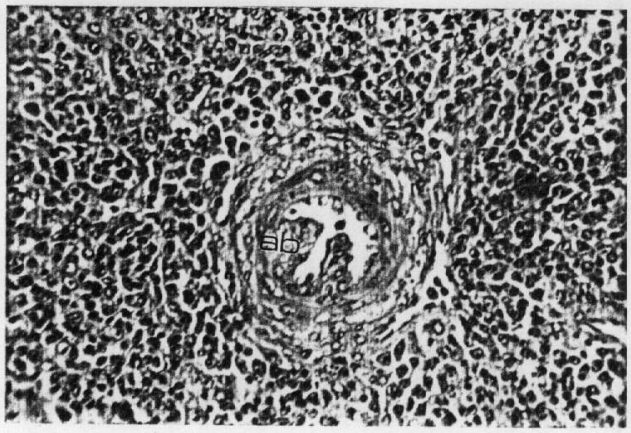


Fig.(55) :Showing the persence of the arterial bloster (ab) in the central artery .
H&E. stain X400.

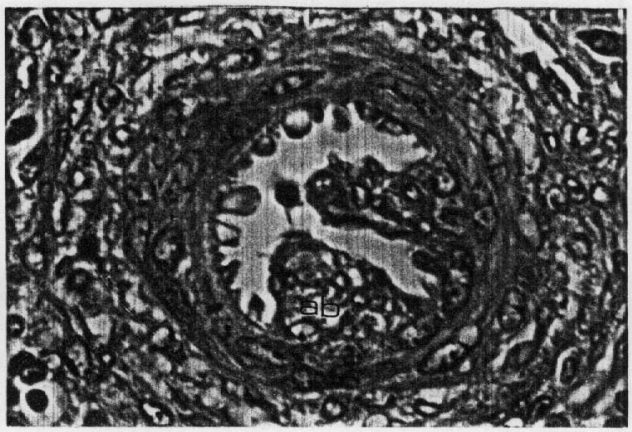


Fig.(56) : Showing the structure of the arterial bloster (ab).
H&E. stain X1000.

DISCUSSION

Discussion

Our investigation of the developing quail spleen aimed to understand the mechanisms underlying the functional switches that occur during the development of this organ. Information on the microanatomy of the spleen and the cellular composition of the hemopoietic population during ontogeny is scarce in the literature. Thus it seemed important to determine first the pattern of splenic differentiation and second its post-hatching microanatomy.

In this study the splenic primordium was well recognized at the 5th day of incubation in contrast to the primordium of the chicken spleen which was identified slightly early at the 3rd or 4thday of incubation *(Romanoff, 1960 ; Yassine et al.,1989).*

The condensation of the mesenchymal cells within the quail primordium indicate that the 5th day (in which the primordium is identified) is not the 1st day of the development and the primordium may be appeared earlier where the primordium firstly appears as a loose network of mesenchymal cells *(Yassine et al 1989).*

The position of the splenic primordium beside the gastric primordium describes why the adult spleen occupies the junction between the proventriculus and gizzard *(Hodges, 1974 ; King and Mclelland, 1981).*

The present study revealed that, the primordium consists of condensation of mesenchymal cells with some blood spaces. At the

early stages of development, the spaces are small and peripheral, then enlarges and extends to occupy the whole aspects of the organ. These blood spaces may represent the precursors for the venous blood sinuses of the red pulp which indicates that the red pulp is developed more earlier than white one . Such result is also stated by ***Romanoff (1960).***

On the other hand, the early function of the spleen as hemopoietic organ is closely related to this finding, because the outpouring of the hemopoietic cells within the circulation requires a venous blood sinuses or spaces connected to the venous circulation ***(King and Mclelland , 1981).*** Moreover the change in the developmental behavior of the primordium in the period between 7th. to 12th. day of incubation where it becomes more vascular can be attributed to the increasing in the hemopoiesis at this stage of development.

Our study showed that, very few cells with darkly stained basophilic nuclei and acidophilic cytoplasm, are found in between the mesenchymal cells at the 5^{th} day of incubation. This may explain the early beginning of the hemopoiesis because these cells may represent the precursors of the hemopoietic cells that appeared later. This also confirms the beginning of the primordium more earlier than the 5^{th} day of incubation ***(Yassine et al. 1989).***

In the present study the 1st. hemopoietic progenitors were detected early as E7 and their number peaked at E 11 but they disappear definitively before hatching at about E14. However ***Nicolas -***

Bolnet et al. (1991) Showed that the peak of hemopoeisis in quail was encountered at E10 and the process of hemopoiesis in chicken continued until the 3rd day after hatching. This short period of hemopoiesis in quail indicate that the hemopoietc function of the quail spleen is not the main function of this organ .

The appearance of the arterial blood vessels accompained by the appearance of the penicilliform capillaries with the peak of hemopoiesis at E11 may be attributed to the preparation of the organ to be shuttled from the hemopoietic function to the immune function immediately after this stage of development and this can be explained by the formation of the white pulp around these vessels after the decreasing of hemopoeisis.

Our study also revealed that, the reticular fibers are observed around the 12th day of incubation in close association with the arterial blood vessels and penicilliform capillaries. These fibers are clearly involved in organizing compartments of the spleen microenvironment. On the other hand detection of the reticular network indicated that the spleen- specific arterial system differentiate through a late - occurring, rapid process, since the stromal framework, which sequesters subpopulations of immune cells organizes between E 14 and E 17. Moreover stromal anatomy at E 17 is similar to that described in the adult spleen *(Olah and Glick, 1982; paye and powell, 1984)* .Interstingly, differentiation of the reticular network and vascular anatomy occurs at the time when the embryonic function of the spleen, i. e. Hemopoiesis, is dwindling away and slightly earlier to the 1st. observation of the lymphocytes. These finding are nearly similar

to that described by *Yassine et al., (1989)* but they showed the stromal framework of the chicken spleen to be organized between E 15 and E 18. From this finding we can conclude that the stromal anatomy, probably responsible for the shuttling or transition of the spleen from a hemopoietic organ to an immune organ. This developmental pattern thus fits well the idea that the complementary localization of lymphocytes in the spleen are indicated by the stromal microenvironment *(Van vliet et al., 1986)*.

The early ceasation of the hemopoiesis and the early establishment of the white pulp with its great ratio as indicated by its basophilia may reflect the main function of the quail spleen as an immune organ. In this aspect it is similar to the spleen of geese but contrary to the spleen of pigeons which is considered as a storage organ *(Bareedy et al., 1986)*.

The basic structure of the quail splenic tissues consisted of 2 areas, the white pulp and the red pulp. However, the 2 could not be distinguished as clearly as in the case of the mammalian spleen. This finding is the same to that observed in the chicken *(Fukuta et al., 1969 ; Hodges, 1974 ; Miyamoto et al., 1980)* and doves *(Nasu et al. 1992)*.

The present study revealed that white pulp, during the 1st 3 weeks post-hatching, is represented by the diffuse areas around the different types of vessels and after this age a nodular structure is added to this pulp. The PALS surrounding the central arteries

consisted mainly of medium and large lymphocytes beside few small lymphocytes, and lymphoblasts. In chicken the PALS appeared to be formed mainly of densely packed small lymphocytes, several medium - sized and large lymphocytes *(Olah and Glick, 1982).*

The perivenous lymphoid tissue surrounding the central vein, was also formed as the PALS was done. This finding is similar to that of *Ogata et al., (1981).*

The appearance of the penicilliform capillaries in association with the arteries may indicate their relation, as the central arteries bifurcate into smaller branches until ended with these capillaries *(Olah and Glick, 1982).*

Our investigation showed clearly that, the capillaries are lined by 3 - 5 high cuboidal cells, instead of the endothelium lining the ordinary capillaries. It is also surrounded by 2 layers of reticular cells and discontinuous layer of macrophage - like cells or ellipsoid associated cells (EACS) as named by *Olah and Glick (1982).* This structure of the penicilliform capillaries (P.C) reflects the high organization of these vessels to perform its function. This finding may be realized by the presence of the arterial blosters in the central arteries of the spleen. The arterial bolsters have been described in the nasal mucosa and others organs of birds by *Mark (1952)* and in the nasal mucosa of the albino rat by *Taher (1976).* These investigations discussed the arterial blosters to have a regulating

mechanisms that control the circulation of blood and also blood pressure. The blood flow in a given organ is effectively regulated by changing the caliber of the small arterioles and capillaries *(Olah and Glick 1982)*. This can be represented by the following formula:

Velocity = blood flow / area of the conduct

This formula suggests that if the lumen of the central arteries or its collateral branches narrow due to the presence of these blosters with narrowing in the lumen of the P.C. due to the presence of high cuboidal cells, the velocity and pressure of the blood increases, causing the filtration through the P.C. Thus one can conclude that if this filterate contains any antigens it will be captured by the E.A.Cs that bordering the P.C. The filteration may be occured through the lining cells where there was no cell junction in between as this study showed or through a channel between the lining cells as stated by ***Olah and Glick (1982).***

However, there is agreement that intravenously injected tracers (dyes, carbon, bacteria and malaria parasites) promptly leave the circulation in those animals which have an ellipsoid, and only a very small amount of the tracers passes into the red pulp *(Blue and Weiss, 1981)*. These observations suggest that the endothelial lining of the P.C. should have special structural features which allow the tracer to leave the circulation.

According to our study, the quail spleen has no marginal zone. The absence of marginal zone in the chicken spleen has been described by

Olah and Glick (1982). However, the study carried out by ***Bareedy et al., (1986)*** showed a marginal zone in both pigeon and geese spleen. In the chicken the functional equivalent of the mammalian marginal zone may be the EASc ***(Olah and Glick, 1982)*** or the complex formed by ellipsoid cells, the periellipsoid B - cells sheath and the surrounding macrophages ***(Jeurissen et al., 1992).***

The phagocytosis, antigen presentation and migration of the EACs have been described in the chicken ***(Olah and Glick 1982 ; Gallego et al 1993 ; Delcacho et al., 1995).*** In our study the presence of the EACs around the P. C. and in the PALS as well as a similar cells in the lymphatic nodules may indicate its migration. Moreover the presence of endocytosis and residual bodies along the cell membrane and within the cytoplasm of the EACs, respectively, may reflect its function as phagocytic cells. Further more the occurrence of lymphocyte ring and plasma cells around these cells may give an indication for another function as antigen - presenting cells.

Weiss (1962) also observed 2 types of cells in the dog ellipsoid, one of them was capable of phagocytosis while the other was not. The former cells may be similar to the EACs while the latter may correspond to the ellipsoidal reticular cells of the quail.

Our finding showed the appearance of 3 types of lymphatic nodules within the quail spleen. These nodules are associated with arteries, veins and periellipsoid white pulp. The 1st is capsulated by a layer of reticular fibers and showed an eccentric vascular channel. The

2nd is not capsulated as the cap of the reticular fibers that appeared around the part closed to the vein tend to be the reticular fibers of the venous wall itself and not a capsule.

The 3rd type appeared later with increasing in the number of the nodules at about 7th - 8th week of age and tend to be similar to the 1st type. All of these nodules are primary due to the absence of germinal centers, because there is no germinal centers could be detected on them. As the germinal centers in the spleen appear mainly in association with antigenic stimulation (*French et al., 1969; Makinodam, et al., 1969; white et al., 1970; Nagy and Feher, 1972; Anderson, 1973; Nagy et al., 1975; White et al., 1975).*This may be explained that , this bird may be antigenically free during the period of the present study . On the other hand , the absence of the germinal centers may be attributed to its occurrence in periods away from the time in which our samples were taken . In contrast to our study the presence of germinal centers in birds have been described by many investigators *(Anderson, 1973; Ogata et al., 1981; Olah and Glick, 1982).* The absence of germinal centers in the quail spleen is identical to that observed in pigeon and geese *(Bareedy et al; 1986).*

The lymphatic nodules appeared suddenly at the 3rd . week of age, approached the peak at 7th - 8th week and then involuted again. The involution of the nodules is similar to that occur for the germinal centers *(Raviola, 1975).* This may indicate that the nodules and germinal centers have the same developmental behavior.

The present study showed the presence of unusual finding in the spleen represented in the subcapsular blood spaces. These sinuses were lined by endothelial cell layer. The exact function for the presence of these sinuses is unknown but their lining endothelium may be capable of taking up small amounts of particulate matter by endocytosis like that occurs in the sinuses of the lymph node *(Fawcett, 1994).*

ENGLISH SUMMARY

Summary

In the present study, eggs, chicks and mature birds of the Japanese quail (conturnix conturnix Japonica) were collected to cover mostly the whole life of the bird. The collected materials were transferred to the fixatives then processed and embedded in the paraffin wax. After the sectioning of the specimens, it was stained as usual with different stains and examined under the light microscope. Some specimens were processed and examined under the electron microscope.

The results obtained can be summarized as follows :-

A-Pre-hatching spleen:-

1-The first identification of the spleen development was noticed in quail embryo at the 5^{th} day of incubation as spherical mass of condensated mesenchymal tissue with some cells belong to the hemopoietic series.

2-Some blood spaces were observed in this stage.

3-As development proceed, The organ enter a transient vascular stage between 7^{th} to 12^{th} day of incubation and then return again to the high cellularity.

4- The 1^{st} hemopoietic cell was detected at the 7^{th} day of incubation. These cells reach the peak at 11 day of incubation, then decreased and completely ceased at 14^{th} day of incubation.

5- The 11th and 12th day of incubation were characterized by the appearance of the 1st arteries, large veins, and capillaries. In addition to the appearance of the 1st reticular fibers.

6-After ceasation of hemopoiesis, the organ shuttled to the immune function and the 1st lymphocyte could be detected at the 16th of incubation.

7-Slightly before hatching the organ become well established where the white and red pulp become differentiated but without a clear demarcation.

8-The organ was characterized by the presence of subcapsular blood sinuses.

B-Post-hatching spleen: -

1-After hatching the organ was more developed than pre-hatching one , it was covered by well developed capsule formed of few smooth muscles, collagen fibers, and reticular fibers.

2-The white pulp formed of the areas surrounding the arteries, veins and penicilliform capillaries.

3- The penicilliform capillaries showed usual lining, as it was lined by high cuboidal cells instead of the usual endothelium. This besides the presence of macrophage-like cells at the periphery of the circular profiles of these capillaries.

4-At the end of the 3rd week of age a nodular form of the lymphoid tissue appeared but without germinal centers. These nodules peaked up at 7th – 8th week of age and then involuted again .

5-After the involution of the lymphoid nodules the macrophage and plasma cells increased within the white pulp.

6-The spleen of quail showed no germinal centers in any stage of development.

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ARABIC SUMMARY

الملخص العربي

الملخص العربى

بعض الدراسات الهستولوجية والهستوكيميائية على طحال السمان قبل وبعد الفقس

فى هذه الدراسة جمعت عينات السمان اليابانى (البيض والكتاكيت والطيور البالغة) لتغطى معظم حياة الطائر . نقلت العينات إلى محاليل التثبيت و تم اعـــداد شـــرائح منها صبغت بالصبغات المختلفة وفحصت تحت المجهر الضوئى . بعض العينـــات تم إعدادها وفحصت تحت المجهر الإلكترونى ، ولخصت النتائج كالآتى :-

(أ) طحال ما قبل الفقس :-

١- بدأ النمو الأول للطحال فى اليوم الخامس من فترة التحضين حيث ظهر ككتلة كروية من خلايا مكثفة للنسيج الحشوى الوسطى مع بعض الخلايا التى تنتمى لخلايا تكوين الدم .

٢- تم ملاحظة بعض الفراغات الدموية أثناء هذه المرحلة من النمو .

٣- مع تقدم النمو دخل الطحال مرحلة وعائية مؤقتة استمرت بين اليوم السابع واليوم الثانى عشر من فترة التحضين ثم عاد إلى المرحلة الخلوية مرة أخرى.

٤- كان أول ظهور حقيقى لخلايا تكوين الدم فى اليوم السابع من فترة التحضين ووصلت هذه الخلايا إلى أعلى معدل لها فى اليوم الحادى عشر من هذه الفترة ثم تراجعت مرة أخرى إلى أن انتهت تماما فى اليوم الرابع عشر .

٥- يعتبر يومى الحادى عشر والثانى عشر أكثر فترات النمو تقدما حيث تم أول ظهور للشرايين والأوردة والشعيرات الدموية (الشعيرات القلمية) هذا بالإضافة إلى ظهور الألياف الشبكية .

٦- بعد توقف عملية تكوين خلايا الدم تحول الطحال إلى وظيفته المناعية وتم التعرف على أول خلية ليمفاوية فى اليوم السادس عشر من فترة التحضين .

٧- أصبح الطحال قبل الفقس بقليل عضوا كامل النمو حيث تم التمييز بين اللب الأبيض (النسيج اليمفاوى) واللب الأحمر ولكن بدون حد فاصل بينهما .

٨- تميز طحال السمان بوجود تركيب غير عادى بالنسبة للطحال ممثلا فى وجود جيوب دموية تحت المحفظة .

(ب) طحال ما بعد الفقس :-

١- بعد الفقس أصبح الطحال أكثر تطورا من نظيره قبل الفقس حيث أصبح مغطى بمحفظة كاملة تتكون من بعض الألياف العضلية الملساء والألياف الشبكية والألياف المرنة .

٢- تكون اللب الأبيض من المساحات المحيطة للأوردة والشرايين و الشعيرات القلمية .

٣- أظهرت الشعيرات القلمية تركيب فريد حيث كانت مبطنة بخلايا مكعبة بدلا من الخلايا الحرشفية المبطنة للشعيرات الدموية العادية هذا بالإضافة إلى وجود خلايا تشبه الخلايا الأكولة عند أطراف المقطع الدائرى لهذه الشعيرات.

٤- مع نهاية الأسبوع الثالث بعد الفقس ظهرت فى اللب الأبيض بعض الحويصلات الليمفاوية بدون مراكز جرثومية ووصلت هذه الحويصلات أعلى معدل لها فى الأسبوعين السابع والثامن ثم اضمحلت .

٥- بعد اضمحلال الحويصلات الليمفاوية إزدادت نسبة الخلايا الأكولة وخلايا البلازما فى اللب الأبيض .

٦- لم يظهر طحال السمان مراكز جرثومية فى أى مرحلة من مراحل النمو .

قسم التشريح و الهستولوجيا
كلية الطب البيطري بكـــفر الشـــيخ
جامعة طنطا

قرار لجنة الحكم والمناقشة

توصى لجنة الحكم والمناقشة بجلستها في يوم الاثنين الموافق ١٩٩٩/٨/٢ بترشيح السيد ط.ب./ احمد شوقي إسماعيل المعيد بقسم الهستولوجيا بكلية الطب البيطري جامعة المنصورة للحصول على درجة الماجستير في العلوم الطبية البيطرية (هستولوجيا).

اسم عضو اللجنة	التوقيع
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أ.د على عبد القادر منصور أستاذ التشريح والأجنة رئيس قسم التشريح والهستولوجيا ووكيل الكلية لشئون الدراسات العليا والبحوث كلية الطب البيطري - كفر الشيخ .	.....
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تحريرا في ١٩٩٩/٨/٢.

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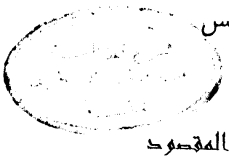
بعض الدراسات الهستولوجية والهستوكيميائية على طحال السمان قبل وبعد الفقس



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للحصول على درجة الماجستير فى العلوم الطبية البيطرية
(الهستولوجيا)

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كلية الطب البيطرى - قسم التشريح
والهستولوجيا

١٩٩٩