MICROANATOMICAL CHANGES IN THE SPLEEN OF GIRIRAJA BIRDS *(Gallus domesticus)**

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Abstract

Age wise microanatomical studies were carried out on the spleen of post-hatch Giriraja birds. Histological, histometrical and histochemical changes were recorded from day old to involutary period. The differentiation into stromal and cellular constituents of red and white pulp were studied. The presence of germinal centres was confirmed and its age wise average numbers were recorded. Macrophages and periarteriolar macrophage sheaths were also studied.

Key words: *Involution, Giriraja, spleen, histology.*

Giriraja is a synthetic dual purpose coloured strain of chicken developed at University of Agricultural Sciences, Bangalore, by crossing the exotic broiler breeds White Plymoth Rock, Red Cornish and New Hampshire (Pirani *et al.,* 2007). Spleen is a round, reddish brown secondary lymphoid organ lying close to the right side of the junction between the proventriculus and gizzard (Hodges, 1974). Studies on the involutary changes in the spleen of chicken are scanty and some controversies regarding the functional classification of avian spleen and the presence/ absence of germinal centres in spleen still remain. The present study was undertaken to investigate the age related histological, histochemical and histometrical variations in the chicken spleen.

Materials and Methods

A total of 72 birds were reared separately at the UAS poultry farm, Bangalore from day old to 24 weeks. Spleen was collected from 6 birds each every alternate week. Tissue pieces from the spleen were fixed in fixatives namely 10% neutral buffered formalin, Bouin's fluid, lymphatic tissue fixative and Zenker's acetic fluid. The tissue pieces were processed for paraffin embedding, sections were cut at 4µm thickness and were stained by hematoxylin and eosin, Van geison's stain for collagen fibers, Masson's trichrome method, method for reticulin (Kiernan, 1981) Weigert's elastic stain and methyl green pyronin method for the demonstration of DNA and RNA (Luna, 1968).

The paraffin sections were also subjected to periodic acid Schiff's reaction, alcian blue method (for mucosubstances at pH 2.5) and toluidine blue method (for metachromasia) (Luna, 1968). Cryostat sections of 12µm thickness were obtained from fresh tissues and were used for Gomori's alkaline phosphatase cobalt method, Gomori's acid phosphatase method and oil red O in propylene glycol method for lipids (Singh and Sulochana, 1978).

For micrometry three sections from proximal, middle and distal parts of the spleen were collected from birds of all age groups and measurements were carried out with an Erma ocular micrometer. The capsule thickness of the

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spleen (average of 20 readings) and number of germinal centers (per 10 microscopical fields) were recorded. The data was analyzed statistically (Snedecor and Cochran, 1994)

Results and Discussion

The adult chicken spleen showed the following histological features. The spleen was enclosed by a thin fibrous capsule. Trabeculae were less developed and the parenchyma looked uniform. The stroma consisted of reticular fibres which were particularly dense around arteries. The parenchyma was not organized into red and white pulp which concurred with the observations of King and McLelland (1983). The red pulp consisted of cord like arrangement of reticular cells interspersed with venous sinuses and blood cells. The white pulp consisted of lymphocyte clusters, lymphoid follicles and white blood cells. The predominance of lymphoid cells varied with the activity of the nearby germinal centres.

Germinal centres were observed in the white pulp from the 28th day onwards (Plate 1). However, Indu *et al.,* (2000) reported that (in ducks) germinal centres appeared by 95 days only. The germinal centres showed a pale circumscribed portion containing dividing lymphocytes. Macrophages with dark staining inclusions, plasma cells and dendritic cells were also seen in the germinal centres.

Marginal zone covering the white pulp as seen in the mammalian spleen was not observed in chicken. Periarteriolar macrophage sheaths (PAMS) were detected when stained using the Prussian blue reaction. Macrophages had large vesicular nucleus with pigments. Blue and Weiss (1981) stated that in the spleen, PAMS are the major sites of clearance of blood borne particles. The macrophages were also slightly alcial blue positive indicating their secretory nature. Hemosiderin content of macrophages was identified in all the age groups studied. The macrophages also gave a positive reaction to lysosomal enzyme acid phosphatase. Maximov and Bloom, (1957) reported similar findings. The spleen also disposed off effete erythrocytes by phagocytosis within macrophages of red pulp (Tizzard, 1987).

In the day old stage, the parenchyma was not differentiated into red pulp and white pulp. The development of white pulp was

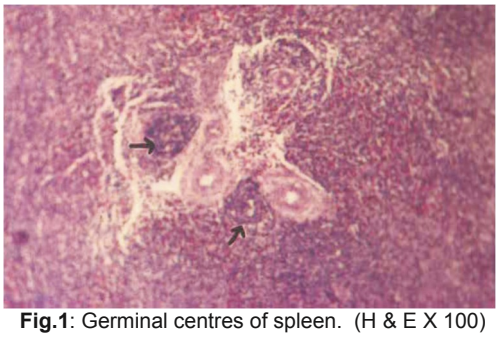

Fig.1: Germinal centres of spleen. (H & E X 100)

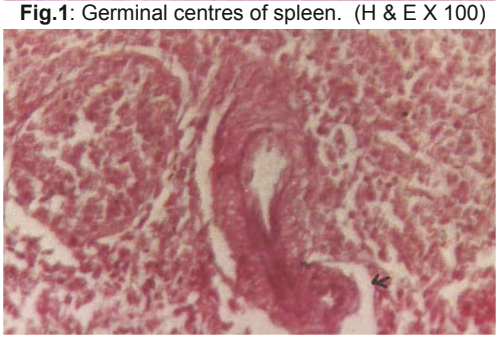

Fig 2: Blood vessels with depleted PALS around it. (H & E X 100)

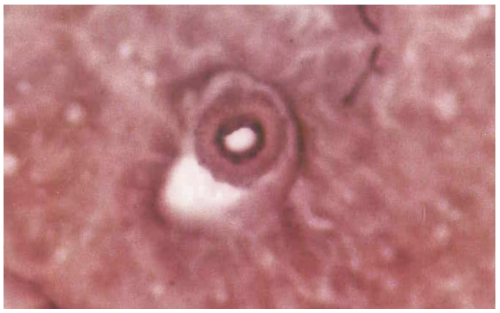

Fig 3: Perivascular spaces with alkaline phosphatise reaction. (Alkaline Phosphatase X 100)

scant and was observed by 21 days. Indu *et al.,*(2000) reported the development of white pulp in ducks by 15 days itself. White pulp was seen as lymphatic aggregations around larger blood vessels. Similar observations were made by Hashimoto and Sugimura (1980) in ducks. The red pulp was predominant immediately after hatching. There is an outpouring of heterophills from spleen followed by massive increase in number of lymphocytes 48 hours after hatching. The chicken spleen is considered to be involved in granulopoesis and erythropoiesis during embryonic development and has no role in embryonic lymphopoesis (Tizzard, 1987). Lymphoid system of spleen has its major development after hatching when antigen challenge begins. Venous sinuses were apparent at day one and were well defined by one week. Blood vessels were numerous even

Table 1. Comparison of splenic weights, proportions, capsule thickness and germinal centers

Parameter studied	Age in weeks															
	0	1	2	3	4	5	6	8	10	12	14	16	18	20	22	24
Body weight (g)	45.08 ±6.1	135.83 ±23.2 **	325.09 ±45.8 **	625.5 ±54.3 **	966.6 ±76.8 **	1566.6 ±220 **	950 ±74 **	1225 ±113.2 *	1625 ±293.1 *	1725 ±313.2 ns	1900 ±343.2 ns	2130 ±431.1 *	2975 ±55.8 *	3000 ±398.7 ns	3183 ±415.6 ns	2930 ±564.7 ns
Splenic Weight (mg)	0.016 ±0.00 9	0.116 ±0.02 **	0.265 ±0.0 4**	0.531 ±0.04 **	1.12 ±0.03 **	1.72 ±0.04 **	1.443 ±0.03 **	2.820 ±0.8 *	2.34 ±0.5 *	2.71 ±0.4 *	3.63 ±0.6 *	3.50 ±0.6 ns	4.19 ±0.6 *	3.60 ±0.5 *	4.24 ±0.6 *	4.5 ±0.5 ns
Proportion of body weight of spleen	0.036 ±0.00 1	0.085 ±0.004 **	0.081 ±0.003 *	0.084 ±0.002 *	0.116 ±0.01 **	0.109 ±0.02 ns	0.15 ±0.03 *	0.23 ±0.04 *	0.144 ±0.05 *	0.157 ±0.02 ns	0.191 ±0.03 *	0.164 ±0.02 *	0.141 ±0.04 *	0.12 ±0.02 *	0.13 ±0.02 ns	0.15 ±0.02 *
Capsule thickness of spleen (µm)	10.9± 1.7*	12.9± 1.3*	14.4± 1.5*	19.7± 2.4*	30.2± 2.6**	31.09± 2.1ns	32.05± 3.1ns	33.3± 2.1ns	33.5± 3.1ns	34.7± 2.5ns	34.9± 2.9ns	35.01± 1.1ns	36± 1.3*	53.2± 7.2**	Not constant	
Germinal centres in 10 fields(100X)	0	0	0	0	2±0.2	6±0.4 **	11±0.8 **	14±0.6 **	19±0.9 **	24±0.9 **	20±0.8 **	16±0.6 **	17±0.7 *	19±0.8 *	16±0.5 **	14±0.4 **

** denotes highly significant date with p> 0.01, * denotes significant result with p> 0.05 and ns- denotes non significant results. n= 6 observations per week

at day one stage and possessed all the tunics. Lymphatic aggregations were seen around larger blood vessels alone. Capsule was well developed at day one. Other cells seen in the day old spleen consisted of reticular cells, lymphocytes, lymphoblasts, heterophills and macrophages.

By the first week there was a tendency towards formation of periarteriolar lymphatic sheaths (PALS) around large arteries though well defined PALS were seen only by 4 weeks of age. By 4 weeks, two distinct parts of white pulp, the germinal centers and PALS were identified.

In the involutary period (eight weeks), there was atrophy of white pulp. There was however little or no change in spleen follicles, a finding in agreement with the observations of Hecser *et al.* (1992). As age advanced, the blood vessels showed empty spaces or depleted PALS around them (Plate 3). By about 22 weeks there was an increase in sinusoidal spaces.

The capsule, germinal centres and perivascular spaces including venous sinuses showed mild alkaline phosphatase (Plate 4) and moderate acid phosphatase activity. Ruskanen *et al.* (1977) however stated that the spleen of chicken did not show any areas of specific location for acid and alkaline phosphatases. Acid phosphatase activity was diffuse in the splenic stroma and was probably shown by phagocytic cells. Alkaline phosphatase activity was also seen in the capsule. Results of histometrical studies are depicted in table 1.

The capsule thickness showed the most drastic increase in size during the fourth and twentieth week. It gradually increased from 11 µm at day one to 53 µm by 20 weeks. Germinal centres were first observed at 4 weeks and later their number increased significantly by 12 weeks and remained at a moreover constant value thereafter.

Due to the absence of a well defined capsular trabecular system and predominance of lymphatic tissue in adult stage, the spleen of the chicken can be classified as defensive spleen based on the classification by Banks (1969). Whether or not the spleen serves as a reservoir of blood in chicken is a matter requiring further detailed study.

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