

**GROSS ANATOMICAL AND HISTOMORPHOLOGICAL  
STUDIES ON THE SPLEEN OF CAMEL  
(*CAMELUS DROMEDARIUS*)**

**By**

**ASHWANI KUMAR**

**Thesis submitted to the Chaudhary Charan Singh  
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of the requirements for the degree of:**

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*TO MY*

*GRAND PARENTS*

## CERTIFICATE - I

This is to certify that this thesis entitled. "Gross anatomical and histomorphological studies on the spleen of camel (*Camelus dromedarius*)", submitted for the degree of M.V.Sc. in the subject of Veterinary Anatomy and Histology of the Chaudhary Charan Singh Haryana Agricultural University, is a bonafide research work carried out by Ashwani Kumar under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

Handwritten signature of S.K. Nagpal in black ink, with the date 29.X.97 written below it.

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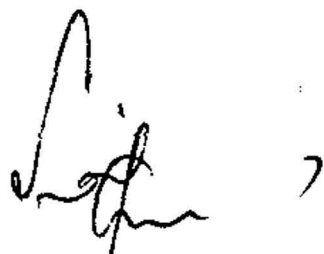
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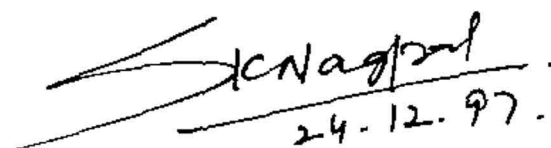
## CERTIFICATE - II

This is to certify that this thesis entitled, "Gross anatomical and histomorphological studies on the spleen of camel (*Camelus dromedarius*)", submitted by Ashwani Kumar to the Chaudhary Charan Singh Haryana Agricultural University, in partial fulfilment of the requirements for the degree of M.V.Sc., in the subject of Veterinary Anatomy and Histology, has been approved by the Student's Advisory Committee after an oral examination on the same.



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DEAN, POSTGRADUATE STUDIES

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*Ashwani*  
29/10/97  
[ASHWANI KUMAR]

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## INTRODUCTION

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The word 'Camel' is derived from the Greek word "Kremal", probably taken from the Sanskrit word "Kreluk" which means "throw away legs". In Hindi, it is called as "Oont" and in Persian "Ustar", the words probably derived from the Sanskrit word "Ustra". The word "Dromedary" used for one humped camel literally means "runner".

The camel - nature's wonderful and magnificent creature has its origin probably in North America while it has its focus of domestication in South Arabia from where it migrated and distributed to other global areas.

India has the third highest camel population in the world. Out of total world population (17.019 million), India has 1.5 million (FAO, 1992). Of this, the state of Rajasthan accounts for about 70 per cent followed by Haryana (11.2%), Gujarat (7%), Punjab (5.9%) and the rest in Madhya Pradesh and Uttar Pradesh (Khanna *et al.*, 1990).

The camel by virtue of having many morphological, anatomical and physiological peculiarities, has well adapted to the harsh hot desertic conditions. It has remarkable ability to withstand scanty feed and water supplies and high ambient temperatures.

The spleen, the largest lymphoid organ, has multiple functions - blood cell formation, haemoglobin and iron metabolism, red blood cell

destruction, blood filtration, blood storage, and immune response. The structure of the spleen and the relationships between the red and white pulp depend largely on the distribution of the blood vessels and differ markedly in different animal species. According to Bloom and Fawcett (1975), animals with a large blood volume (horse, ruminants, carnivores) have scanty white pulp and a robust connective and muscular tissue. According to Turner and Hodgetts (1959), the spleen could be cause of the variations in hematocrit-blood can be trapped in an enlarged spleen or released into the circulation, greatly affecting the hematocrit. Yagil (1985) even suggested that desert species of animals have larger spleens than animals in temperate climates.

Consequent to the paucity of literature on the various gross anatomical and histological aspects of spleen of camel, the present study was undertaken with the following objectives:

1. To study the topographic anatomy of spleen
2. To demonstrate the arterial pattern
3. To study the histomorphology of spleen at different anatomical sites
4. Micrometry of different segments of spleen

## REVIEW OF LITERATURE

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Srinivasan (1952) reported the double spleen in a dog which was developed from an accumulation of mesenchymal cells beneath the peritoneal epithelium on the left side of the dorsal mesogastrium. It was projected above the omental surface. Multiple spleens or partially subdivided spleens were observed resulted from the continuance of the multiple hillocks or from the exaggerations of temporary incisures that appeared at about 3 or 4 months.

Hegazi (1953) described that the spleen of the camel differed greatly in structure and position from that of other domesticated animals because of its crescent shape and presented a body, a head and a tail. It was situated in the left dorsal part of the abdominal cavity just above the posterior half of rumen, extended obliquely from third lumbar transverse process to the seventh one. It was soft, elastic and dark greyish colour tinged with violet. It presented parietal and visceral surfaces, cranial and caudal borders along with its proximal and distal extremities. The spleen of an average 1 lb weight, measured 40 cm in length with a width of 9.0 cm at middle of its extent.

Purohit and Rathor (1958) reported the spleen of the camel was dark grey in colour, strongly curved and sickle shaped. It was located on the left side of rumen and extends from third to seventh lumbar transverse

processes. The average weight length and width was about 2 lbs, 21 inches and 8 inches, respectively.

McLeod *et al.* (1964) stated that the spleen of the bovine was long and narrow with rounded extremities having red or purple colour. It was located on the left side of the abdomen between the diaphragm and rumen extending from upper part of last two ribs to the sixth rib. Its parietal surface was convex and visceral surface was concave. It was sixteen to twenty inches long, four to six inches wide and an inch in thickness. The hilus was an oval depression on the upper fourth of the visceral surface near the anterior border. The blood supply to the spleen was by the splenic artery which was the branch of right ruminal artery. It passed downward in the substance of the organ and giving off numerous branches at right angle. The nerve supply to the spleen was by the large splenic plexus arising by a number of fibre bundles from the right and left caelia consentric ganglia.

Raghavan (1964) described the spleen as the largest ductless gland in the body being situated on the left face of the rumen. It was elliptical in ox, scythe shaped in horse and human foot shaped in dog. The average length width and weight were 50 cm, 15 cm and about 1 kg in ox and 50 cm, 20-25 cm and 1-1.2 kg in horse, respectively. The hilus was present on a longitudinal ridge in case of horse and dog.

Miller (1965) stated that sigmoid shaped and grey-brown coloured spleen of dog was located in the left hypogastric region, approximately parallel to the greater curvature of the stomach. Its position depended on

the fullness of stomach, to which it was loosely attached. It was having two extremities, two surfaces and two borders. Its free ventral extremity was lying on the floor of the abdomen, frequently extending across the mid-ventral line to the right side.

May (1970) described that the triangular shaped spleen of sheep was located above the dorsal sac of the rumen under the vertebral ends of the last four ribs of the left side. The hilus was a rounded depression near the cranial basal angle on the visceral surface. The nutritive blood was supplied by the splenic artery.

Cardinet and Hartke (1972) studied that the splenic artery of the dog was originated as a primary branch of coeliac artery or cranial mesenteric artery. The splenic artery supplied the spleen and gave rise to the left gastroepiploic artery and gastric branches which anastomosed with the left gastric artery, a primary branch of coeliac artery.

Nickel *et al.* (1979) reported that, the position of the spleen depended on the fullness of the stomach in dog, pig and horse. It was a laterally flattened, enlongated organ with parietal and visceral surfaces, cranial and caudal borders and dorsal and ventral extremities. In the carnivores the ventral extremity was little wider than the dorsal. The spleen of pig was of uniform width and on cross section it was triangular. The spleen of the ox was elongated oval and triangular in the sheep and more rectangular in the goat and coma shaped in horse; its dorsal end was wide and ventral end was pointed and bent cranially. The average length, width and weight in canine was 9.7-24 cm, 2.5-4.6 cm and 8-147 gm, respectively. The



spleen of pig was 24-45 cm long, 3.5-12.5 cm wide and 90-335 gm weight. The spleen of horse was 40-67 cm long, 17-22 cm wide, and 950-1680 gm weight. The spleen of ox was 41-50 cm long, 11-14.5 cm wide and weight 665 to 1155 gm. Whereas, the length, width and weight of the spleen was 8.5-14 cm, 6-11 cm and 46-133 gm in sheep and 9.4-12.4 cm, 6.5-7 cm and 70 gm in goat, respectively. The long groove like hilus was on the visceral surface of the spleen in the carnivores, pig and horse and was marked by the splenic vessels, nerves and the attachment of the gastrosplenic ligament. The hilus of the ruminant spleen was a small indentation on the dorsal end.

Ellenport (1975) reported that the soft, highly vascular and bright red to dark purple coloured spleen was located in the left hypogastric region in domestic animals. It was supplied by the splenic artery and was drained by the splenic vein. Most of nerve fibres were postganglionic sympathetic fibres.

Getty (1975) stated that the spleen was elongated and elliplical in ox, triangular in sheep, long and narrow in pig and falciform in dog. It was located in the left hypochondriac region in horse. Its weight, length and width was 1 kg, 50 cm and 20-25 cm in horse, 900 gm, 50 cm and 15 cm in ox, 100 gm, 12-15 cm and 7.5 to 10 cm in sheep; 350 gm, 60 cm and 8 to 10 cm in pig and 50 gm in dog, respectively. It was having two surfaces, two extremities and two borders in all these domestic animals. The hilus was situated on dorsal 3rd of visceral surface near the cranial border. It was present as a simple impression not a groove in bovine. *The hilus in*

sheep was close to the cranial angle and it was a round depression, not a groove. Whereas, in horse the hilus was a groove near the cranial angle. the hilus of the pig and dog spleen was situated on a longitudinal ridge on the visceral surface.

Smuts and Bezuidenhout (1987) reported that the crescent shaped spleen of camel occupied the left flank and was attached to the dorsocaudal aspect of the rumen and greater omentum. It was having concave and convex borders, visceral and parietal surfaces and dorsal and ventral extremities. Its weight was about one kg in the adult. The hilus extended on the visceral surface of the dorsal extremity and along the concave border. The blood was supplied by the splenic artery of the coeliac artery.

Awal *et al.* (1991a) observed elongated and elliptical shaped spleen of indigenous cattle was exclusively located on the left side of the abdominal cavity in close relation to the greater curvature of the rumen. It had two surfaces, two borders, two extremities, a base and an apex. The mean length, width, thickness and weight were 34.80 cm, 8.85 cm, 1.85 cm and 355 gm, respectively. Thickness and width were highest in the middle and upper third of the spleen whereas, the least values were observed at its apex. The hilus was an oval depression of about 5 cm, on the anterior border of the visceral surface.

Awal *et al.* (1991b) studied that the spleen of indigenous sheep was bluish red in colour and triangular in outline with the angles rounded off being located in the left side of the abdominal cavity in close relation to the dorsal sac of the rumen. It extended obliquely downward and forward from the vertebral end of the last two ribs to about the middle of tenth

intercostal space. The convex perital surface was adherent to the diaphragm whereas the concave visceral surface was related to the dorsal curvature of the rumen. The average weight, length, breadth and thickness were  $50.72 \pm 2.68$  gm,  $9.75 \pm 0.56$ ,  $6.91 \pm 0.53$  and  $0.81 \pm 0.09$  cm, respectively. The hilus in the form of rounded depression was located on the visceral surface close to the angle formed by the dorsal extremity and cranial border.

Smallwood (1992) mentioned that the tongue shaped spleen of ox was positioned against left abdominal wall. The spleen did not present a complete covering of visceral peritoneum, at the nonlinear hilus. However, the hilus of the equine spleen was linear, like that of the pig and had many splenic lymph nodes located along the hilus.

Gupta *et al.* (1976) demonstrated the corrosion casts of human splenic artery which revealed the presence of superior and an inferior splenic segments in 84 per cent cases and an additional middle segment in 16 per cent of cases. These segments were separated by avascular planes.

Gupta *et al.* (1978a) prepared the corrosion casts of the dog splenic artery to observe the vascular segments. Only a dorsal and ventral segments were observed in 97.5 per cent cases and no segmentation was seen in 2.5 per cent cases.

Gupta *et al.* (1978b) observed the presence of two arterial segments, a right and a left in 74 per cent cases, three arterial segments, a hilar, a right and a left one in 10 per cent cases and the absence of arterial segmentation in 16 per cent cases in the goat spleen by the corrosion cast method. The dog and swine spleen were having a dorsal and a ventral

segment in 97.5 per cent of cases and 100 per cent cases, respectively.

Gupta *et al.* (1978c) demonstrated the corrosion casts of splenic artery of swine spleen by injecting red butyl butyrate solution in acetone. The casts were studied for the arterial segments in the spleen on the basis of intrasplenic branching pattern of the splenic artery. The swine spleens were having a dorsal and a ventral segment in each specimen.

Gupta *et al.* (1979a) observed that the corrosion casts of splenic artery of sheep had two primary right and left arterial segments in 70 per cent cases and a visceral and a parietal segment in 2.5 per cent cases. However, there were no anastomoses between the branches of these two primary division. The 27.5 per cent of cases did not show any arterial segmentation.

Gupta *et al.* (1979b) studied corrosion cast of the splenic veins in the goat which revealed the presence of two splenic venous segments - a right and a left one (56.5% cases), whereas, three venous segments - a right, a left and a hilar one were observed in 30.4 per cent cases. The rest of the specimens did not exhibit any venous segmentations. There was no anastomosis among the minor veins of two main intrasplenic tributaries.

Gupta *et al.* (1981) studied the venous segments in the spleen of buffalo and dog by corrosion cast techniques. The buffalo spleen showed the presence of dorsal and ventral venous segments in 85 per cent cases with an additional intermediate in rest of 15 per cent cases. Whereas, the dog spleen revealed only two venous segments - a dorsal and a ventral in all the specimens. There was no anastomosis among the minor veins of dorsal

and ventral intrasplenic veins. The characteristic feature was that each main vein drained a particular segment of the spleen.

Jain and Singh (1986) studied the arterial and venous circulation of spleen in sheep by injecting the radio-opaque material. The splenic artery divided into two primary branches and each primary branch divided into secondary and tertiary branches. There was no intersegmental anastomosis between arteries. The venous drainage of spleen was carried out by numerous venous sinuses which joined to form pulp veins, trabecular veins and finally the splenic vein.

Jain and Singh (1988) studied the arterial and venous organization of the spleen in buffalo calves through roentgenograms. the splenic artery in buffalo calves emanated from the right ruminal artery and it divided into four primary branches. Each primary branch entered the hilus of spleen independently and supplied a particular segment of the organ without any anastomosis between them. The primary branches divided into 2-10 secondary branches. The secondary branches again divided into numerous tertiary branches. The spleen was drained by the numerous venous sinuses which joined to form pulp veins, trabecular veins and finally splenic vein.

Awal *et al.* (1989) reported that the spleen of indigenous cattle was supplied by a single splenic artery originated as a common trunk with the right ruminal artery of the coeliac artery. The splenic artery in the parenchyma divided into acute angles. One of the divisions further branched into ten prominent sub-branches and the other divisions sub-branched into eight small twigs. Interanastomosing of the major subdivisions of the splenic arteries were observed.

Bajpai and Chandra (1995a) observed varying branching patterns in the goat spleen by the technique of angiographic and vinyl acetate casts. The main artery divided into two primary branches which further divided into secondary, tertiary and finer branches without any intercommunications.

Snook (1950) described the splenic vascular channels by means of graphic reconstructions and divided the mammalian spleens into sinusal and non-sinusal types. In sinusal group, an elaborate anastomosing plexus of true sinuses was observed in the red pulp of dog and man. The non-sinusal type of the red pulp of horse, cow and pig was containing few branched primordial veins leading from the pulp meshes into collecting veins. the wide spreaded marginal zone was considered as an important filtration center.

Doggett (1951) reported that a capillary system connecting arterioles to the sinuses existed in the dogs spleen and established a "closed" type of circulation. Each capillary was composed of two continuous segments, arterial and venous. Some capillaries had short venous component and others had a longer venous component.

Hegazi (1953) observed that the spleen of camel had a thick fibrous and muscular capsule and trabeculae. The trabeculae formed a network where the red and white pulp were present. the Malpighian corpuscles were composed of dense lymphoid tissue with reticular fibres around the small arteries. The small arteries branched to form penicilli which later terminated into numerous ellipsoidal capillaries being surrounded by



lamellae of reticular cells and lymphocytes.

Lewis (1957) observed that the arterial branches after entering the dog spleen formed a simple arborizing pattern down to the smallest capillaries without giving rise to any smaller branches. An open type of circulation was observed as arterial capillaries and venous sinusoids opened into the pulp. An intimate relationship existed between the splenic ellipsoids and venous sinusoids.

Snook (1958) observed that the open type of circulation predominated in the rabbits spleen. The red pulp arterial capillaries opened into the reticular tissue of splenic cords. The four types of vascular terminations were found: A cone shaped ampulla of reticular fibres, an oval ampulla lying adjacent to a sinus and in the center of a splenic cord, and a funnel shaped opening. A few direct connections were present between the white pulp capillaries and perimarginal sinuses.

McLeod *et al.* (1964) reported that the bovine spleen was covered by an incomplete serous coat. The thick white capsule contained fibrous and elastic connective tissue and unstriated muscle. Numerous trabeculae from the deep surface of the capsule passed into the substance of the spleen. They formed the irregular spaces by joining each other and constituted the interstitial tissue. The splenic pulp was a soft and dark red tissue occupying the spaces formed by the trabecula.

Raghavan (1964) stated that the spleen of ox had a fibro-elastic and a muscular capsule which gave off trabeculae to form a supporting network. The Malpighian corpuscles were made up of lymphoid tissue surrounding

the smaller arteries. The red pulp was composed of reticular network, red and white blood corpuscles, macrophages or splenic cells and reticular cells.

Miller (1965) reported that fibrous tunic of capsule<sup>s</sup> and trabeculae of dog spleen was rich in elastic and smooth muscle fibres. The lymphatic nodules of white pulp were less than one cm in diameter. The germinal centers of these nodules were lighter in colour than the surrounding pulp. He also mentioned the "open" circulation theory by Mall (1903), "closed" circulation theory and the compromise theory by Kniseley (1936) of the blood circulation.

Greep (1965) reported that the capsule was made up of dense connective tissue and from the internal, surface, a rich network of trabeculae subdivided the organ into communicating compartments. The red pulp was made up of splenic sinuses and splenic cords. The pulp contained a delicate meshwork of reticular cells and extracellular reticulum which was argyrophillic and periodic acid-schiff positive.

Thomas (1967) suggested that the sinus wall of dogs spleen contained three elements, namely, an inner layer of sinus cells, an intermediate layer of basement membrane, and an outer layer of cord limiting cells. the sinus and cord limiting cells were reticular cells, apparently similar to each other in cytoplasmic details.

Dellmann (1971) reported that the capsule surrounding the spleen of domestic animals gave off the trabeculae which incompletely subdivided the organ into lobules containing red and white pulp. The capsule and



trabeculae were constituted by the smooth muscle cells, collagenic and elastic fibres. The red pulp was composed of splenic sinuses and the splenic cords, whereas, the white pulp was composed of splenic nodules and periarterial lymphatic sheaths. He also suggested a combination of "open" and "close" circulation theories.

Nickel *et al.* (1979) reported smooth muscle cells collagenous and elastic fibres as main constituents of the capsule. Numerous trabeculae were accompanied by the vessels and formed a spongy framework which supported the splenic pulp. The white pulp corpuscles were nodular accumulations of lymphoreticular tissue along the course of small pulp arteries. The pulp arteries formed the penicilli which divided into the sheathed arteries and gave rise to the capillaries. The blood was directed into the reticular mesh work of the red pulp (open circulation) being followed by venous sinuses, pulp veins, trabecular veins and lastly to the splenic vein.

Gamble (1974) mentioned the quantitative histological study of the capsule and trabecular component of the spleen was made by Tehver and Grahame (1931) who found that the ratio of capsular and trabecular tissue to pulp in the spleen of the dog was 1:4.41. The capsular and trabecular components and their smooth muscle content were sparse in the rabbit relative to that in the dog.

Bloom and Fawcett (1975) reported that the capsule and trabeculae of the spleen were made up of dense connective tissue, smooth muscle cells and elastic networks. The smooth muscle cells were abundant in

splenic capsule of the horse, ruminants and carnivores. The trabeculae contained a larger number of elastic fibres than the capsule and varying amount of smooth muscle cells. The red pulp was formed by a network of branching and anastomosing tortuous sinuses separated from each other by splenic cords. The periarterial lymphoid sheaths had a loose irregular framework of reticular fibres with associated reticular cells. The germinal centres were eccentrically situated within the sheath and when fully developed, their light region and cap of small lymphocytes were directed toward the red pulp.

Ellenport (1975) reported that capsule, trabeculae, red pulp (venous sinuses) and white pulp (lymphatic follicle) constituted the spleen of domestic animals. The arteries were closely connected with white pulp and veins with the red pulp (Bloom and Fawcett, 1968). The spleen had an open type of circulation. The veins had numerous anastomosis, whereas, the arteries seldom anastomosis (Goss, 1966). Lymphatic vessels were present only in the capsule and large trabeculae. Most of the nerve fibres were postganglionic sympathetic fibres to the smooth muscle of the capsule, trabeculae and splenic vessels in the pulp.

Getty (1975) reported that the spleen of horse had an almost complete serous coat (tunica serosa) to which a capsule of fibrous tissue (tunica albuginea) was attached. The deep face of capsule presented numerous trabeculae which formed a network in the splenic parenchyma. The red pulp was formed by the leucocytes, large splenic cells, red blood corpuscles and pigment. A sheath of lymphoid tissue aggregated around the wall of arteries formed small splenic lymph nodules.

Saigal *et al.* (1977) observed the morphometric changes in the spleen of ageing goats including the per cent white pulp, per cent red pulp, per cent trabecular tissue, size of white pulp follicles and number of white pulp follicles per cm<sup>2</sup> area. Agewise, sexual and cyclic changes in these parameters was presented with the help of statistically analysed data by applying multiple range test and computed the correlation coefficients and regression equations. The interrelationship between different parameters have also been considered. By six months and above fully developed Malpighian follicles with characteristic germinal centre appearing paler which were surrounded by a darker zone (the corona) and well distinguished paler margins (the marginal zones) were mostly observed. The follicles in the spleen of male goats appeared more developed and were much larger in size than those in the females of nearly the same age. In the goats over one year of age the corona became less distinct. Beyond one and a half year the marginal zone was merged with the red pulp. With the advancing age towards 5 years and over, there appeared no clear demarcation between red pulp and white pulp.

Dellmann and Brown (1987) mentioned that the spleen was surrounded by a thick connective tissue capsule invested by the peritoneum. The capsule had two ill defined layers of connective tissue and smooth muscle. The horse had the thickest and the dog had the thinnest splenic capsule. The pig and ruminants had moderately thick splenic capsule. Trabeculae were composed of collagen, elastic and smooth muscle cells along with arteries, veins, lymph vessels and nerves. The red pulp was

composed of pulp arterioles, sheathed and terminal capillaries, venous sinuses and splenic cords. The spleens of the horse, dog and pig had abundant lymphatic nodules and periarterial sheaths, but the ruminants spleens had less abundant lymphatic tissue. The pericapillary macrophage sheaths were large and abundant in the pig and smaller in the horse and dog and were narrow in ruminants.

Ahmed *et al.* (1987) observed that the thick fibromuscular capsule and trabeculae of the spleen of black Bengal goat was composed of collagen, elastic and reticular fibres with smooth muscles. A marginal zone separated the white pulp from the red pulp in which the reticular fibres were found to form a circular ring. The red pulp was composed of fine meshwork of reticular fibres and cells in which the blood cells were enmeshed. Iron pigments were found in the red pulp.

Awal *et al.* (1992) reported a thick fibromuscular capsule covering the spleen of indigenous cattle was composed of collagen, elastic, reticular and smooth muscle fibres. The trabeculae were branched and formed the framework of the spleen. Splenic corpuscles were as an ovoid mass of compact lymphatic tissue and had a fine meshwork of reticular connective tissue containing mainly lymphocytes of various sizes. The spaces between the white pulp and the trabeculae were occupied by the red pulp containing the the meshes of reticulum erythrocytes, leucocytes, plasma cells and a large number of phagocytic cells.

Kraal (1992) found that a variety of cell types were present in the marginal zone of the spleen. such as the marginal, zone macrophages, the

marginal metallophilic macrophages at the inner border and to a lesser extent the marginal zone B cells.

Smallwood (1992) observed the thick fibrous capsule and the numerous trabeculae that extended into the parenchyma of the ruminants spleen. The red pulp was made<sup>up of</sup> reticular connective tissue and venous sinuses. The splenic corpuscles were constituted by the accumulation of lymphoreticular tissue.

Bajpai and Chandra (1995b) observed that the spleen of goat was invested by a connective tissue capsule which varied in thickness from 128  $\mu\text{m}$  to 232  $\mu\text{m}$  and was covered by a peritoneal layer. The capsule comprised of an outer connective tissue layer and an inner layer of interwoven reticular, collagenous, elastic and smooth muscle fibres which also constituted the structural component of trabeculae. The larger trabeculae were occupied by the arteries, veins and nerve bundles with a peripheral layer of smooth muscle fibres.

## MATERIALS AND METHODS

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The present study was conducted on eight apparently healthy adult camels (*Camelus dromedarius*) of either sex. The animals were deeply sedated by slow intravenous injection of 6 per cent aqueous solution of chloral hydrate (w/v) through the left external jugular vein till corneal and pinching reflexes became negative. The left common carotid artery was exposed and cannulated. The animals were completely bled and embalmed with 10 per cent formalin solution according to the Grossman's technique (1959).

After proper fixation the abdominal viscera was dissected out to study the topographic anatomical location of spleen and its relations with other visceral organs. Then the measurements of various physical parameters like weight, length, width and volume (water displacement method) were carried out after its ablation from the abdominal cavity.

### Angiography

Four camels were used for the demonstration of vascularization pattern. The splenic artery was cannulated and the arterial system of the organ was flushed by injecting luke warm heparinized saline solution (100 I.U./100 ml). A radiopaque suspension (20% lead oxide in liquid soap) was injected by steady and constant digital pressure. After satisfactory filling of vessels, the injection material was allowed to settle for about 24

hours in refrigerator. Later, the organ was radiographed at 8 Mas, 50 KVP and 900 mm FFD over Indu X-ray films to obtain the radiographs (arteriographs) depicting the course and branching patterns of the arteries.

### **Microscopic studies**

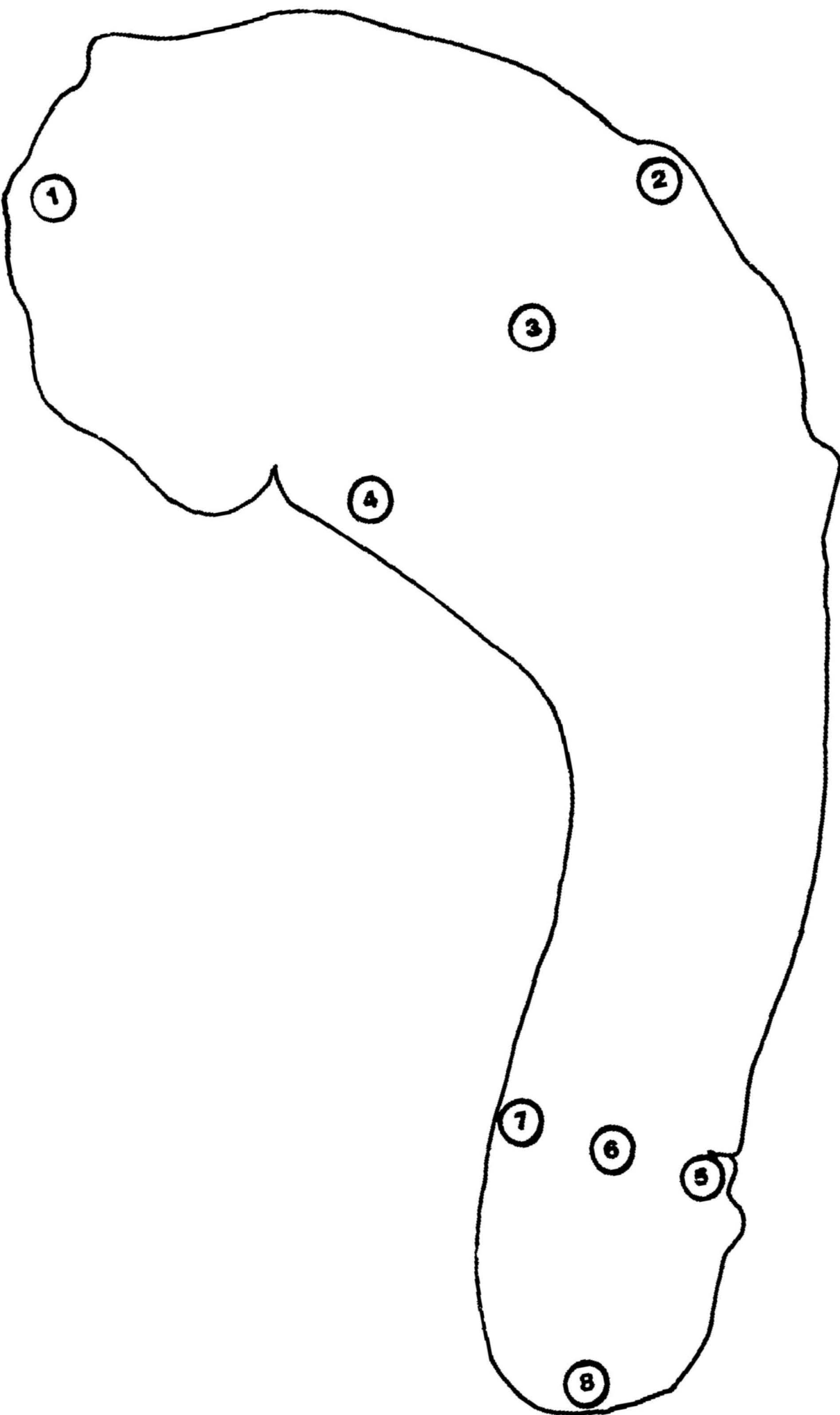
To study the histological architecture the small pieces of tissues were collected from all the eight spleens. From each spleen, the tissues were collected from eight fixed anatomical regions (line diagram) to explore any regional differences. The tissues were processed for light microscopy by using paraffin of 68 to 70°C. The paraffin blocks were sectioned to obtain 5 to 6  $\mu$ m thick paraffin sections which were stained with the following routine histological stains to demonstrate different components of the spleen.

1. Routine Harris haematoxylin and eosin stain (Luna, 1968).
2. Gomori's method for reticulum (Luna, 1968).
3. *Weigerts method for elastic fibres* (Luna, 1968).
4. Crossmann-trichrome method for collagenous fibres (Crossmann, 1937).
5. Bielschowsky method for axis cylinders and dendrites (Luna, 1968).
6. Turnbull blue method for hemosiderin (Luna, 1968).
7. Perl's method for iron (Luna, 1968).
8. McManus method for glycogen (PAS) (Luna, 1968).
9. PAS Alcian blue method for mucosubstances (Luna, 1968).

## Micrometry

The filar micrometer was used to record the observations on capsule thickness, trabecular thickness, diameter of smallest, largest and overall mean diameter of Malpighian follicle. Whereas, disc micrometer net retic<sup>u</sup><sub>A</sub>le was used to determine per cent white pulp, per cent red pulp, per cent trabecular tissue and number of follicles per cm<sup>2</sup> area. These values were subjected to statistical analysis to obtain mean, standard deviation, standard error and "F" value of each region. Duncan's multiple range test was employed to demonstrate the correlation of a single parameter among the different regions. The correlation coefficient presented significant or nonsignificant positive or negative correlation among different parameters.





## RESULTS

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The spleen of the camel differed greatly in structure and position from that of other domesticated animals. It was much smaller in proportion to the body weight and size. It was crescent shaped and presented a body, a head and a tail (Fig. 1, 2). The average weight of the spleen was about 365 g but the weight mainly depended upon the amount of blood contained in it. The average volume was about 470 ml. The average length was about 45 cm and the width was about 14 cm at the widest part of the spleen and the minimum width was 7 cm at the narrowest part. The width decreased gradually toward both extremities. In the fresh state, it was soft and elastic but not yielding and presented a dark greyish colour tinged with violet. It was hard in consistency owing to the large amount of interlobular connective tissue in its substance.

The spleen of the camel (Fig. 1, 2) was situated in the left dorsal part of the abdominal cavity just above the dorsocaudal aspect of the rumen to which the anterior most part was firmly attached by a small triangular area through gastrosplenic ligament. It was extending obliquely from the posterior border of 3rd lumbar transverse process to anterior border of the seventh lumbar transverse process and was reaching upto the pelvic inlet. The dorsal part of the spleen visible from the anterior of fourth lumbar vertebra took a spiral turn which was more laterally directed with its

dorsal border presenting relation with the left kidney. The spleen of the camel presented two surfaces, two borders, two extremities and a hilus. The parietal surface was somewhat convex and was related to the obliquus abdominis internus muscle and to the left sublumbar region. In the centre, it formed the renal impression for the left kidney. The visceral surface was somewhat concave and lay in contact with the dorsocaudal part of the left side of the rumen to which it was attached on its dorso-posterior surface with a small triangular area by the peritoneum. The lateral border was thin, convex, free and was generally irregular outline. There was marked indentations throughout the length of this border. Anteriorly it formed a prominent notch while posteriorly it terminates into a pointed end. It was insinuated between the rumen and the diaphragm. The medial border was concave, short, thickened and rounded upto the hilus, then it became thin upto the posterior extremity. The splenic artery entered the organ at the posterior third of this border. At the hilus the medial border was folded towards the parietal surface making a renal impression for the left kidney. It encircled the lateral convex border of the left kidney.

The anterior extremity was thick, rounded and formed the head of the organ and it was separated from the body by a well defined constricted neck. The posterior extremity which formed the tail was more thinner, flattened, wider and tapering at the end. The hilus was a depression, situated in the lower third of the anterior border. It was wide and blood vessels were placed quite well apart from each other. The splenic branch of the hepatic artery ran along the visceral surface to enter the hilus of the organ about

the middle of its medial border. This artery before entering the hilus bifurcated into two primary branches; a cranial and a caudal one supplying posterior two-third of spleen. Each primary branch was found to supply a definite segment of the spleen. Whereas, the splenic artery nourished the anterior one-third of the spleen and it was the branch of right ruminal artery. The nerves were derived from coeliac plexus of the sympathetic. A purely vascular zone was present in between the cranial and caudal branches, perpendicular to the main axis of the spleen.

The arteriograms (Fig. 3, 4, 5) presented two segments: a cranial and a caudal one on the basis of primary arterial branching pattern. Both the primary branches were supplying the caudal two-third part of the spleen. The cranial part was supplied by the branch of right ruminal artery present above the gastrosplenic ligament. The several secondary branches emerged from the primary branches, which further divided into tertiary branches. These in turn divided into several finer branches. Both of the primary branches entered the hilus independently and supplied a particular segment of the spleen without any anastomosis.

### **Capsule and Trabeculae**

The spleen was covered by a thick fibromuscular capsule. The thickness of capsule varied at different levels of tissues collected from spleen. The outer most layer of capsule was covered by a serous peritoneal coat of the mesothelial cells having squamous to cuboidal cells (Fig. 6). The eosinophilic cytoplasm was very scanty and only the lightly basophilic nuclei were visible. There was a thin layer of connective tissue having a

sparse distribution of connective tissue cells. The capsule was rich in smooth muscle fibres which were arranged in three layers. An outer layer of smooth muscle fibres was oriented longitudinally parallel to the surface adjacent to the connective tissue present just below mesothelium. The smooth muscle fibres were generally present obliquely or transversely along with some fibres running longitudinally in the middle layer. The middle layer was widest as compared to the outer and inner layers. The inner longitudinally arranged layer of smooth muscle fibres was of variable thickness and densely populated with smooth muscle cells.

A mixed population of reticular, collagen and elastic fibres supported the framework of the capsule (Fig. 7, 8, 9). The collagen fibres were densely arranged just above the outer layer of smooth muscle fibres along with few reticular fibres (Fig. 8). The inner most zone was rich in elastic and reticular fibres. However, the connective tissue just below the mesothelium was devoid of elastic fibres. The concentration of reticular fibres in the form of meshwork was maximum in between the inner layer of smooth muscles where these were finner as compared to outer part of capsule (Fig. 7). The reticular fibres formed a uniform layer being arranged longitudinally in the inner most part of the capsule. These fibres continued to the framework of trabeculae, however, their orientation was vertical as compared to the fibres present in the capsule. The thickness of the capsule was slightly more at the point where the trabeculae emanated from the surface. In addition small blood capillaries and nerve fibres of varying number were irregularly distributed in the capsule. A varying PAS reaction was observed in the different components of the capsule.

The mean thickness of capsule at different levels of spleen has been shown in Table .1 Whereas, the overall mean thickness of capsule was recorded highest at the level of 4th region ( $475.41 \pm 29.75 \mu\text{m}$ ) and lowest at the 5th region ( $196.14 \pm 8.45 \mu\text{m}$ ). However, the region 3rd was not significantly different from the fourth. Whereas, the regions 7th, 1st and 6th presented similar type of observations. Similarly, the quantitative measurements made at levels 8th and 2nd were correlated with each other (Table 2). The overall mean value of all the regions was  $310.06 \pm 7.99 \mu\text{m}$ . The correlation coefficient of capsule with trabecular thickness, mean diameter of Malpighian follicles, diameter of smallest follicle, diameter of largest follicle, number of Malpighian follicles per  $\text{cm}^2$  area, per cent white pulp, per cent red pulp and per cent trabecular tissue at different sites had been depicted in Table 3-10. In general, the overall capsule thickness had highly significant correlation with trabecular thickness, mean diameter of Malpighian follicles, number of Malpighian follicles per  $\text{cm}^2$  and per cent white pulp. However, its correlation coefficient was significant in relation to per cent red pulp and insignificant with diameter of smallest and largest Malpighian follicles (Table 11).

The branching and anastomosing trabeculae were emanated from the deeper face of the capsule (Fig. 10). The trabeculae of varying thickness reached to a variable depth and thus dividing the parenchyma of the spleen into different compartments. Some of the trabeculae were uniform with a free pointed end whereas, some of these divided into 2-3 branches and formed an important framework (Fig. 11). The smooth muscle fibres, elastic

fibres and reticular fibres emerged vertically from the capsule and were again oriented along the longitudinal axis of the trabeculae. The arrangements of the smooth muscles in different layers in the capsule assisted smooth muscle of the trabeculae thereby contracting the spleen and to pump out the excess blood in to the circulation at the time of emergency. The smooth muscle fibres were mainly tightly packed and were oriented along its longitudinal axis (Fig. 12). The thin reticular fibres formed a wide meshwork along with few collagenous fibres. The reticular fibres became progressively thinner in the terminal branches of the trabeculae. The elastic fibres of varying concentrations were oriented in different planes in the trabeculae (Fig. 13). At places the elastic fibres were aggregated into larger clumps particularly in the deeper part of the free end of trabeculae. They contained a large number of elastic fibres than the capsule. Only few collagen fibres were present in the trabeculae (Fig. 14). The connective tissue fibres and smooth muscle fibres of the trabeculae were moderately PAS positive (Fig. 15). The large trabeculae contained arteries, veins and were surrounded by smooth muscle fibres. A few nerve fibres and fine blood capillaries were irregularly distributed in the trabeculae. The thickness of trabeculae varied significantly at different levels (Table 1, 2).

The highest observation of mean trabecular thickness was again recorded at the level of 4th region ( $377.71 \pm 11.98 \mu\text{m}$ ) having significant correlation with group 3rd as observed in case of capsule thickness. The least values were observed at the level of 2nd region ( $212.30 \pm 6.52 \mu\text{m}$ ). The interesting feature was that the values decreased gradually in groups



from 7th, 5th, 6th and 1st which were not significantly different with each other (Table 2). The mean thickness of all the eight regions was  $275.57 \pm 4.49 \mu\text{m}$ . The correlation coefficient of trabecular thickness was highly significant with the values of capsule thickness, mean diameter of Malpighian follicles, diameter of smallest follicle, number of Malpighian follicles per  $\text{cm}^2$  area, per cent white and red pulp. An insignificant correlation was exhibited between trabecular thickness and per cent trabecular tissue (Table 11). The statistical values for per cent trabecular tissue were more or less similar at all the levels (Table 12). The levels 7th, 3rd, 5th, 6th, 8th and 4th were not significantly different with each other. Likewise, the levels 6th, 8th, 4th, 1st and 2nd had values which were statistically similar with each other. A maximum of  $12.50 \pm 0.37$  and minimum  $10.96 \pm 0.45$  was observed at the levels 7th and 2nd, respectively. The mean value of all the regions was  $11.85 \pm 0.14$  (Table 13). The correlation coefficient of per cent trabecular area was mostly insignificant with majority of the parameters except the per cent areas where a highly significant positive correlation was observed with the white pulp and highly negative correlation with the per cent red pulp (Table 11).

### White Pulp

The white pulp of the spleen was a lymphoreticular tissue consisting of lymphocytes, plasma cells and macrophages lying in a reticular meshwork being surrounded by the major arterial vessels of the spleen (Fig. 16, 17, 18). The white pulp was divided into two components called as periarterial lymphatic sheaths and lymphatic nodules. The well developed



periarterial lymphatic sheaths were coaxially surrounded the nodular artery (Fig. 19, 20) and it was observed until these vessels became small arterioles. The periarterial lymphatic sheath at its periphery merged with the marginal zone of the nodules. The sheath had a loose, irregular framework of reticular fibres with associated reticular cells (Fig. 21, 22). Around the periphery of lymphatic sheath, the reticular fibres became circumferentially arranged where flattened reticular cells formed the concentric layers and thereby delimiting the lymphoid tissue from the surrounding red pulp. Near the nodular artery, a few elastic fibres were interspersed among the reticular fibres (Fig. 23). The mesh like reticular framework were occupied by lymphocytes, predominantly belonging to the small and medium sized variety. However, the concentration of lymphocytes was variable at different places and generally it constituted a small portion of splenic nodule. Plasma cells and macrophages were only occasionally found but their number was increased towards the periphery of the sheath. The erythrocytes were present at the junction of white and red pulp.

The splenic nodules of different dimensions were observed as an ovoid mass being composed of the aggregation of the lymphatic tissue around the adventitia of the arteries of the splenic parenchyma (Fig. 16, 17, 18, 20, 24, 25). The splenic nodules often found at bifurcation of nodular artery and were randomly distributed in the splenic parenchyma. The lymphatic tissue occurred mainly as splenic nodules and occasionally as periarterial lymphatic sheath. The nodules generally occurred singly and occasionally aggregations of two to three nodules were encountered, having

inbetween the marginal zone differentiating the union between the different nodules. A small portion of red pulp also penetrated inbetween the union between the different nodules (Fig. 26). The nodules had a fine meshwork of reticular connective tissue containing mainly lymphocytes of various sizes. Only few reticular fibres were interspersed in the central part of the white pulp and were coarse, concentrically arranged in the marginal zone (Fig. 21, 22). The cellular component of splenic nodule was mainly contributed by the lymphocytes, plasma cells, macrophages and few RBCs and hemosiderin pigments (Fig. 26, 27, 28, 29).

The lymphocytes were mainly occupied by the round to oval shaped lightly stained nuclei. The nucleolus was mostly centric in position and strongly basophilic. The chromatin material was finely granular and uniformly distributed. The scanty cytoplasm occupied the periphery of the cell and was lightly eosinophilic. Mostly the lymphocytes were aggregated into small irregular clumps (Fig. 27, 28). At places the lymphocytes were arranged in small cords. Some of the lymphocytes also presented the mitotic figures indicating the active proliferative stage. Small vacuolated areas were observed inbetween the aggregations of the lymphocytes being occupied by RBCs and hemosiderin pigment (Fig. 29). The macrophages of different sizes were irregularly distributed inbetween the lymphocytes. At places the clumps of the macrophages were present. The nucleus of macrophage was larger in size, irregular shaped and comparatively less basophilic as compared to that of the lymphocytes. The nucleolus was not distinguishable. The chromatin material was finely granular and uniformly distributed. Only few plasma cells were present in the splenic nodule.

Few collagenous fibres were interspersed in between the reticular fibres of the marginal zone particularly around the blood vessels (Fig. 30). At the periphery of the periarterial sheath and splenic nodules, the lymphoreticular tissue was concentrically arranged. The marginal zone occupies its position just adjacent to Malpighian follicle and was sandwiched inbetween the periphery of splenic nodule and red pulp (Fig. 16, 20, 21). It was constituted by the concentrically arranged reticular cells, macrophages, lymphocytes, blood capillaries and plasma cells. It represented a transitional zone towards the red pulp and closely resembled to the red pulp. It was clearly distinguished by the presence of a thin zone of few layers of loosely arranged fine reticular fibres, along with few collagen fibres encircling the splenic nodule (Fig. 21, 22). The reticular fibres of cords formed a closely knit concentric network and the meshes of the cords had a greater content of small lymphocytes and plasma cells than the rest of the red pulp. The concentration of the lymphocytes was drastically reduced in the marginal zone. The branched reticular cells with many processes were concentrically arranged in this zone. The reticular cells nuclei were elongated and cylindrical in shape with less basophilic chromatin material.

The component of red blood cells was increased along with the number of blood capillaries was increased. It also contained smaller venous sinuses, which were circumferentially oriented around the white pulp. The occurrence of marginal zone was not a constant feature. The arteries associated with these nodules were termed as nodular arteries. One to three

or occasionally more branches of nodular arteries were present in a single nodule as this medium sized muscular artery gave off many branches during its course through the white pulp (Fig. 17, 20, 25). These arteries were generally oriented eccentrically in the nodules and thus the terminology "central artery" used in some text books was not true in this animal. The density and arrangement of reticular framework varied greatly in different parts of the spleen. The comparatively large reticular fibres were coarse, more numerous and densely arranged in the different layers of the nodular arteries. Whereas, these fibres formed irregular meshwork in the small branches of the arteries (Fig. 21, 22). Thick elastic fibres were mainly localized in the tunica intima of the nodular artery whereas, thin fibres surrounded the periphery of the nodular artery (Fig. 23). The collagenous fibres were present only in the peripheral layers of the nodular artery (Fig. 30).

The loose tunica adventitia was surrounded by connective tissue particularly the reticular fibres and a cylindrical sheath of lymphoid tissue. Its tunica media was constituted by three to four layers of smooth muscle cells, which were concentrically arranged and densely packed. Mostly the nodular artery presented the obliterated lumen with concentric arrangement of the smooth muscle cells. The central artery and its branches were having cuboidal or even columnar endothelium which may be so high as to completely efface the lumen (Fig. 19, 20). It branched throughout its course within the pulp and thereby dividing into small capillaries which supplied the lymphoid tissue of the sheath. These capillaries had tall endothelial cells

and a basal lamina being surrounded by the pericytes. Around these blood capillaries the lymphoid tissue of the white pulp and a meshwork of reticular fibres was condensed. The further branching of these capillaries presented a low endothelium and a single layer of basal lamina. The capillaries present in the marginal zone lacked smooth muscle fibres and were surrounded by few reticular cells and a less concentration of the lymphocytes. The different layers of nodular artery were moderately PAS positive (Fig. 31).

The fourth region showed maximum mean diameter of Malpighian follicle with its value  $339.12 \pm 3.54 \mu\text{m}$  followed by levels 5th and 3rd which were not significantly different with each other (Table 14). The mean values further decreased at the levels 7th, 8th, 6th, 1st and 2nd (Table 15). A least value of  $277.55 \pm 5.02 \mu\text{m}$  was observed at the level second. The overall mean value of all the regions was  $308.26 \pm 2.73 \mu\text{m}$ . The mean diameter of splenic nodule showed correlation coefficient which was highly significant with capsule and trabecular thickness, diameter of largest nodule, per cent white pulp and red pulp tissue (Table 11). It was only significant in relation to diameter of smallest splenic nodule and insignificant with number of Malpighian follicles per  $\text{cm}^2$  area and per cent trabecular tissue. The values of diameter of smallest Malpighian follicle were not significantly different at the levels of 5th, 8th, 2nd, 4th and 3rd with their values  $195.79 \pm 5.63 \mu\text{m}$  at the 5th level and  $186.10 \pm 3.10 \mu\text{m}$  at the third level. Further these values in descending order were recorded at the levels of 1st, 6th and 7th which were statistically similar with each other (Table 16). The overall mean value of diameter of smallest follicle of all the regions was  $188.23 \pm 1.42 \mu\text{m}$ .



The dimensions of smallest splenic nodule at different levels had highly significant correlation coefficient in relation to trabecular thickness. A highly significant negative correlation was exhibited in relation to diameter of largest follicle and number of Malpighian follicles. A significant correlation was also observed with mean diameter of follicle whereas, the capsule thickness and per cent red pulp presented positive insignificant correlation with each other (Table 11). However, a negative insignificant correlation occurred with per cent white pulp and trabecular tissue. The maximum value for largest Malpighian follicle was made at the level 6th ( $449.60 \pm 4.03 \mu\text{m}$ ) and minimum value at the level 1st ( $399.49 \pm 3.78 \mu\text{m}$ ). The values at the levels of 6th, 4th, 5th and 3rd; 8th, 7th, 2nd and 1st were not significantly different with each other (Table 16). The levels 5th, 3rd, 8th and 7th had values which were statistically similar with each other. The overall mean value of diameter of largest follicle of all the regions was  $420.13 \pm 10.04 \mu\text{m}$ .

The statistical observations made on diameter of largest follicle showed highly significant positive correlation coefficient with mean diameter of Malpighian follicle, number of Malpighian follicle per  $\text{cm}^2$  area and per cent white pulp. A highly significant negative correlation was observed between the diameter of largest follicle and diameter of smallest follicle along with per cent red pulp. The correlation was insignificant with the thickness of capsule and trabecular and per cent trabecular tissue.

The number of Malpighian follicles per  $\text{cm}^2$  area was maximum in the region 6th having a mean value of  $144.26 \pm 5.09$  which was followed by



regions 7th, 8th and 1st in decreasing order having similar type of statistical observations (Table 15). The values further decreased in the regions 5th, 3rd, 2nd and 4th. The observations recorded in regions 8th, 1st, 5th, 3rd and 2nd were not significantly different with each other. A similar type of pattern was observed between 7th, 8th, 1st, 5th and 3rd and 1st, 5th, 3rd, 2nd and 4th. The overall mean value of number of Malpighian follicles per  $\text{cm}^2$  area in all the regions was  $126.33 \pm 1.93$ . The number of Malpighian follicles per  $\text{cm}^2$  area of splenic parenchyma presented a negative highly significant correlation with the diameter of smallest follicle, capsule and trabecular thickness (Table 11). A highly significant positive correlation was observed only with the diameter of largest follicle. Its insignificant positive correlation with mean diameter of follicles and per cent red pulp and a negative insignificant correlation with per cent white pulp and per cent trabecular tissue was also recorded.

A maximum mean value of  $14.66 \pm 0.30$  per cent for per cent white pulp was observed at the third level of tissues (Table 12). However, this region was not statistically different from the groups 4th and 5th. The values in descending order was followed by the levels 1st, 6th, 8th and 7th. The least value for the same was recorded at the level 2nd ( $10.23 \pm 0.32\%$ ) which was not statistically similar to any of the regions (Table 12). The overall mean value for the per cent white pulp of all the regions was  $12.63 \pm 0.15$  per cent. The correlation of per cent white pulp with mean diameter of follicle, capsule and trabecular thickness was highly significant like that of capsule thickness with these parameters. This type of correlation was

neutrophils and platelets. The number of the lymphocytes and leucocytic cells was drastically reduced, however, at places small clumps were also present (Fig. 35). Small groups of smooth muscle fibres were oriented in varying directions (Fig. 35). In the marginal zone the reticular fibres of the splenic cords formed a closely knit concentric network and the meshes of the cords had a greater content of small lymphocytes and plasma cells than the rest of the red pulp. The collagenous fibres of the trabeculae continued directly into the reticular fibres of the red pulp. The capillaries opened into the mesh work and occasionally, nerve endings were observed in the red pulp. The splenic sinuses were elongated, irregularly contoured slit like vascular channels. These were fairly distributed within the red pulp and mainly present along the longitudinal axis of the trabeculae (Fig. 36, 37). The sinuses permeated the entire red pulp and were numerous around the white pulp. At places the sinuses opened into the red pulp where they branched irregularly (Fig. 12). The sinusoids displayed a unique arrangement of endothelium and basal lamina. The sinuses were lined by the elongated reticuloendothelial cells. These cells were thick in the central nuclear region with tapered ends and had longitudinally oriented nucleus. The occurrence of intercellular gaps was a common feature. The endothelial cells were oriented parallel to the long axis of the sinuses. These cells were active phagocytes and were noticed to have engulfed foreign bodies. The lining cells rested upon the fenestrated basal lamina and were supported by the fine layers of reticular fibres. The cellular elements of circulating blood can easily migrate through the sinus wall by traversing the



also observed in relation to diameter of largest follicle and different per cent areas of splenic parenchyma except per cent red pulp where a highly significant negative correlation was observed. Insignificant negative correlation was exhibited with the diameter of smallest follicle and number of Malpighian follicles per  $\text{cm}^2$  area (Table 11).

### **Red Pulp**

The spaces inbetween the white pulp and the trabeculae were occupied by the red pulp (Fig. 32). It was composed of pulp arterioles, sheathed capillaries, terminal capillaries, splenic sinuses and splenic cords. A meshwork of fine reticular fibres was observed throughout the red pulp (Fig. 33). The reticular framework was composed of reticular fibres and the processes of the reticular cells. In the meshes of reticulum erythrocytes, plasma cells and leucocytes were also observed. The splenic cords of varying thickness were situated between splenic sinuses and distributed irregularly. The loosely arranged reticular fibres formed a network which contained numerous free erythrocytes, reticular cells, plasma cells, macrophages, lymphocytes and other leucocytes and phagocytic cells (Fig. 34). The splenic cords were completely invested by stellate reticular cells. The reticular cells were having numerous branching processes supported by reticular fibres. The membranous processes of reticular cells tend to form the channel like structures that may function to conduct blood toward the inter-endothelial slits in the sinus wall. Some reticular cells may actually represented fixed macrophages of monocytic origin. The macrophages were large rounded or irregularly shaped cells, with a vesicular nucleus and abundant cytoplasm. They often contained engulfed erythrocytes.

neutrophils and platelets. The number of the lymphocytes and leucocytic cells was drastically reduced, however, at places small clumps were also present (Fig. 35). Small groups of smooth muscle fibres were oriented in varying directions (Fig. 35). In the marginal zone the reticular fibres of the splenic cords formed a closely knit concentric network and the meshes of the cords had a greater content of small lymphocytes and plasma cells than the rest of the red pulp. The collagenous fibres of the trabeculae continued directly into the reticular fibres of the red pulp. The capillaries opened into the mesh work and occasionally, nerve endings were observed in the red pulp. The splenic sinuses were elongated, irregularly contoured slit like vascular channels. These were fairly distributed within the red pulp and mainly present along the longitudinal axis of the trabeculae (Fig. 36, 37). The sinuses permeated the entire red pulp and were numerous around the white pulp. At places the sinuses opened into the red pulp where they branched irregularly (Fig. 12). The sinusoids displayed a unique arrangement of endothelium and basal lamina. The sinuses were lined by the elongated reticuloendothelial cells. These cells were thick in the central nuclear region with tapered ends and had longitudinally oriented nucleus. The occurrence of intercellular gaps was a common feature. The endothelial cells were oriented parallel to the long axis of the sinuses. These cells were active phagocytes and were noticed to have engulfed foreign bodies. The lining cells rested upon the fenestrated basal lamina and were supported by the fine layers of reticular fibres. The cellular elements of circulating blood can easily migrate through the sinus wall by traversing the

interendothelial clefts and the fenestrations of the basal lamina. The basal lamina of venous sinuses was moderately PAS positive. The walls of sinuses lacked the muscular coat but at places the smooth muscle cells were present in the vicinity of these sinuses. The lumen of sinusoids had clusters of RBCs and other leucocytic cells. However, the sinuses in the red pulp were of comparatively lesser dimensions. Each arteriole was surrounded by connective lamellae of reticular tissue with lymphocytes. The reticular cells had the same cytologic appearance as those in white pulp, marginal zone and cords but were different in having an elongated, tapered form and not a branched one. The penicillar arteries or the arteries of pulp pursued a radiating course (Fig. 38). The periarterial tissue was drastically reduced. The endothelial cells were tall being oriented along the longitudinal axis of the blood vessels. The single layer of smooth muscle cells constituted the tunica media. The elastica interna was absent, however, thin tunica adventitia was formed by reticular, collagen and elastic fibres but they lacked the elastica externa. The penicillar artery further divided in the red pulp and led to the formation of sheathed capillaries which were ellipsoidal or spherical in shape (Fig. 38). These capillaries had characteristic thickening of their walls called as Schweigger-Seidel sheath. These capillaries were generally associated with the single sheath, however, at places two to four capillaries were ensheathed by a single layer or two to three sheaths arranged in a series (Fig. 38, 39, 40). The cells were rounded toward the lumen and stellate towards the periphery. The RBCs formed a regular constituent and were generally present among the cells of the sheath. The lumen of these

capillaries was not visible when these were cut obliquely. The endothelium was formed by tall fungiform cells which were connected by the intercellular junctions, and rested on a thin basal lamina. The iron and hemosiderin pigment were irregularly distributed in the form of small clumps or aggregations in the splenic parenchyma (Fig. 41, 42).

A maximum portion of splenic parenchyma was constituted by the red pulp. The red pulp formed maximum proportion at the level 2nd ( $80.13 \pm 0.87\%$ ). The values recorded in groups 1st, 8th, 7th, 6th and 4th although were in decreasing order but were statistically significant with each other (Table 12, 13). A similar type of significance was exhibited among the levels 4th, 5th and 3rd. The minimum red pulp portion of  $73.13 \pm 0.76$  per cent was noticed at the level 3rd. The overall mean value of all the regions was  $75.74 \pm 0.28$  per cent. The per cent red pulp area had a highly significant negative correlation with the capsule and trabecular thickness, mean diameter of Malpighian follicle, diameter of largest follicle and different per cent areas of splenic parenchyma (Table 11).

**Table 1:** Statistical analysis showing multiple range test for capsule and trabecular thickness at eight different regions of the spleen

Parameters	Calculated "F' values values from the Analysis of variance	Region	I	II	III	IV	V	VI	VII	VIII
Capsule thickness(μm)	57.22	Mean	279.70	236.39	469.73	475.41	196.14	274.51	300.25	248.37
		SD	27.24	40.46	99.76	162.97	46.31	16.01	43.11	56.37
		SE	4.97	7.38	18.21	29.75	8.45	2.92	7.87	10.29
Trabecular thickness(μm)	29.08	Mean	254.10	212.30	314.53	377.71	267.00	254.90	281.79	242.33
		SD	18.63	35.72	72.57	65.64	64.52	65.05	32.29	25.74
		SE	3.40	6.52	13.25	11.98	11.78	11.87	5.89	4.70

SD - Standard deviation; SE - Standard error

**Table 2: Statistical analysis showing Duncan's multiple range test for capsule and trabecular thickness**

Duncan grouping			Capsule thickness		Duncan grouping			Trabecular thickness	
			Mean ( $\mu\text{m}$ )	Region				Mean ( $\mu\text{m}$ )	Region
	A		475.41	4		A		377.71	4
	A								
	A		469.73	3		B		314.53	3
	B		300.25	7		C		281.79	7
	B								
C	B		279.70	1	D	C		267.00	5
C	B								
C	B	D	274.51	6	D	C		254.90	6
C		D							
C		D	248.37	8	D	C		254.10	1
		D							
		D	236.39	2	D			242.23	8
	E		196.14	5		E		212.31	2

Means with the same letter are not significantly different at  $P < 0.05$ .

**Table 3: Statistical analysis showing correlation coefficients between different parameters at the first region**

	CT	TT	MDF	DSF	DLF	NF	PWP	PRP	PT
CT	1.00000**	0.53658**	0.26122	0.05891	-0.00288	-0.40786*	0.56242**	-0.58306**	0.55980**
TT	0.53658**	1.00000**	0.07567	-0.11172	-0.13551	-0.47142**	0.45562**	-0.46000**	0.32339
MDF	0.26122	0.07567	1.00000**	-0.06903	0.07505	-0.17942	0.11648	-0.08341	0.19470
DSF	0.05891	-0.11172	-0.06903	1.00000**	0.13839	-0.12785	-0.03768	-0.01341	0.32671
DLF	-0.00288	-0.13551	0.07505	0.13839	1.00000**	0.26672	0.11269	-0.12991	0.03074
NF	-0.40786*	-0.47142**	-0.17942	-0.12785	0.26672	1.00000**	-0.62101**	0.54619**	-0.56809**
PWP	0.56242**	0.45562**	0.11648	-0.03768	0.11269	-0.62101**	1.00000**	-0.90738**	0.78462**
PRP	-0.58306**	-0.46000**	-0.08341	-0.01341	-0.12991	0.54619**	-0.90738**	1.00000**	-0.81141**
PT	0.55980**	0.32339	0.19470	0.32671	0.03074	-0.56809**	0.78462**	-0.81141**	1.00000**

\* Significant at 5% level of significance.

\*\*Highly significant at 5% level of significance.

CT: Capsule thickness; TT: Trabecular thickness; MDF: Mean diameter of follicle;

DSF: Diameter of smallest follicle; DLF: Diameter of largest follicle;

NF: Number of follicle; PWP: Per cent white pulp; PRP: Per cent red pulp;

PT: Per cent trabecular tissue

**Table 4: Statistical analysis showing correlation coefficients between different parameters at the second region**

	CT	TT	MDF	DSF	DLF	NF	PWP	PRP	PT
CT	1.00000**	0.62201**	-0.71899**	0.63137**	-0.13266	-0.60081**	-0.14298	0.39905*	-0.37058*
TT	0.62201**	1.00000**	-0.50060**	0.46013**	-0.09023	-0.37554*	-0.38860*	0.61983**	-0.53193**
MDF	-0.71899**	-0.50060**	1.00000**	-0.51869**	0.21866	0.61926**	0.10030	-0.29830	0.26228
DSF	0.63137**	0.46013**	-0.51869**	1.00000**	-0.09164	-0.35292*	-0.14909	0.29813	-0.21353
DLF	-0.13266	-0.09023	0.21866	-0.09164	1.00000**	0.22285	0.11117	-0.22401	0.24562
NF	-0.60081**	-0.37554*	0.61926**	-0.35292*	0.22285	1.00000**	-0.00102	-0.41210*	0.33296
PWP	-0.14298	-0.38860*	0.10030	-0.14909	0.11117	-0.00102	1.00000**	-0.65966**	0.42191*
PRP	0.39905*	0.61983**	-0.29830	0.29813	-0.22401	-0.41210*	-0.65966**	1.00000**	-0.76246**
PT	-0.37058*	-0.53193**	0.26228	-0.21353	0.24562	0.33296	0.42191*	-0.76246**	1.00000**

\* Significant at 5% level of significance.

\*\*Highly significant at 5% level of significance.

CT: Capsule thickness; TT: Trabecular thickness; MDF: Mean diameter of follicle;

DSF: Diameter of smallest follicle; DLF: Diameter of largest follicle;

NF: Number of follicle; PWP: Per cent white pulp; PRP: Per cent red pulp;

PT: Per cent trabecular tissue



**Table 5: Statistical analysis showing correlation coefficients between different parameters at the third region**

	CT	TT	MDF	DSF	DLF	NF	PWP	PRP	PT
CT	1.00000**	0.71765**	-0.22836	0.03883	-0.04177	-0.49841**	0.56795**	-0.73410**	0.63719**
TT	0.71765**	1.00000**	-0.50452**	0.05786	0.07224	-0.53651**	0.41175*	-0.67423**	0.69294**
MDF	-0.22836	-0.50452**	1.00000**	-0.08179	0.09895	0.27312	-0.11524	0.31662	-0.44067**
DSF	0.03883	0.05786	-0.08179	1.00000**	0.10478	-0.06902	-0.17540	-0.04823	0.24008
DLF	-0.04177	0.07224	0.09895	0.10478	1.00000**	-0.33335	-0.15092	-0.07331	-0.00427
NF	-0.49841**	-0.53651**	0.27312	-0.06902	-0.33335	1.00000**	-0.21117	0.33167	-0.39198*
PWP	0.56795**	0.41175*	-0.11524	-0.17540	-0.15092	-0.21117	1.00000**	-0.67347**	0.31664
PRP	-0.73410**	-0.67423**	0.31662	-0.04823	-0.07331	0.33167	-0.67347**	1.00000**	0.77712**
PT	0.63719**	0.69294**	-0.44067**	0.24008	-0.00427	-0.39198*	0.31664	-0.77712**	1.00000**

\* Significant at 5% level of significance.

\*\*Highly significant at 5% level of significance.

CT: Capsule thickness; TT: Trabecular thickness; MDF: Mean diameter of follicle;

DSF: Diameter of smallest follicle; DLF: Diameter of largest follicle;

NF: Number of follicle; PWP: Per cent white pulp; PRP: Per cent red pulp;

PT: Per cent trabecular tissue

**Table 6: Statistical analysis showing correlation coefficients between different parameters at the fourth region**

	CT	TT	MDF	DSF	DLF	NF	PWP	PRP	PT
CT	1.00000**	-0.05863	-0.25994	-0.48132**	-0.13415	0.13987	-0.65795**	0.60470**	-0.29411
TT	-0.05863	1.00000**	-0.10973	0.00704	0.01802	0.07875	0.02452	-0.01809	0.00568
MDF	-0.25994	-0.10973	1.00000**	-0.02207	0.15354	0.08595	0.01039	-0.06833	-0.01103
DSF	-0.48132**	0.00704	-0.02207	1.00000**	-0.10094	0.01418	0.33682	-0.40734*	0.40132*
DLF	-0.13415	0.01802	0.15354	-0.10094	1.00000**	-0.32607	0.11255	0.03678	-0.25094
NF	0.13987	0.07875	0.08595	0.01418	-0.32607	1.00000**	-0.19247	0.24566	-0.21906
PWP	-0.65795**	0.02452	0.01039	0.33682	0.11255	-0.19247	1.00000**	-0.85971**	0.34914*
PRP	0.60470**	-0.01809	-0.06833	-0.40734*	0.03678	0.24566	-0.85971**	1.00000**	-0.76004**
PT	-0.29411	0.00568	-0.01103	0.40132*	-0.25094	-0.21906	0.34914*	-0.76004**	1.00000**

\* Significant at 5% level of significance.

\*\*Highly significant at 5% level of significance.

CT: Capsule thickness; TT: Trabecular thickness; MDF: Mean diameter of follicle;

DSF: Diameter of smallest follicle; DLF: Diameter of largest follicle;

NF: Number of follicle; PWP: Per cent white pulp; PRP: Per cent red pulp;

PT: Per cent trabecular tissue

**Table 7: Statistical analysis showing correlation coefficients between different parameters at the fifth region**

	CT	TT	MDF	DSF	DLF	NF	PWP	PRP	PT
CT	1.00000**	0.86057**	0.90377**	0.81582**	-0.19728	-0.49787**	-0.07336	0.15881	-0.16936
TT	0.86057**	1.00000**	0.73162**	0.63347**	-0.11756	-0.59083**	0.11088	-0.04181	-0.02510
MDF	0.90377**	0.73162**	1.00000**	0.75008**	-0.21150	-0.26631	-0.02797	0.16952	-0.13342
DSF	0.81582**	0.63347**	0.75008**	1.00000**	-0.19516	-0.26302	-0.12380	0.30895	-0.24724
DLF	0.19728	-0.11756	-0.21150	-0.19516	1.00000**	0.09499	0.29476	-0.11676	-0.26004
NF	-0.49787**	-0.59083**	-0.26631	-0.26302	0.09499	1.00000**	0.08855	0.04818	-0.01645
PWP	-0.07336	0.11088	-0.02797	-0.12380	0.29476	0.08855	1.00000**	0.68603**	0.35624*
PRP	0.15881	-0.04181	0.16952	0.30895	-0.11676	0.04818	-0.68603**	1.00000**	-0.67883**
PT	-0.16936	-0.02510	-0.13342	-0.24724	-0.26004	-0.01645	0.35624*	-0.67883**	1.00000**

\* Significant at 5% level of significance.

\*\*Highly significant at 5% level of significance.

CT: Capsule thickness; TT: Trabecular thickness; MDF: Mean diameter of follicle;

DSF: Diameter of smallest follicle; DLF: Diameter of largest follicle;

NF: Number of follicle; PWP: Per cent white pulp; PRP: Per cent red pulp;

PT: Per cent trabecular tissue

**Table 8: Statistical analysis showing correlation coefficients between different parameters at the sixth region**

	CT	TT	MDF	DSF	DLF	NF	PWP	PRP	PT
CT	1.00000**	0.55434**	-0.59107**	0.19360	-0.13453	-0.39554*	-0.40236*	0.43217**	-0.30624
TT	0.55434**	1.00000**	0.01068	0.37876*	-0.22171	-0.30279	-0.39906*	0.52465**	-0.48020**
MDF	-0.59107**	0.01068	1.00000**	0.11860	-0.16590	0.15689	0.25905	-0.16475	-0.00378
DSF	0.19360	0.37876*	0.11860	1.00000**	-0.23616	-0.13989	-0.13356	0.12538	-0.12290
DLF	-0.13453	-0.22171	-0.16590	-0.23616	1.00000**	0.18107	0.13614	-0.13943	0.06187
NF	-0.39554*	-0.30279	0.15689	-0.13989	0.18107	1.00000**	0.32142	-0.35587*	0.26450
PWP	-0.40236*	-0.39906*	0.25905	-0.13356	0.13614	0.32142	1.00000**	-0.81633**	0.31195
PRP	0.43217**	0.52465**	-0.16475	0.12538	-0.13943	-0.35587*	-0.81633**	1.00000**	-0.78650**
PT	-0.30624	-0.48020**	-0.00378	-0.12290	0.06187	0.26450	0.31195	-0.78650**	1.00000**

\* Significant at 5% level of significance.

\*\*Highly significant at 5% level of significance.

CT: Capsule thickness; TT: Trabecular thickness; MDF: Mean diameter of follicle;

DSF: Diameter of smallest follicle; DLF: Diameter of largest follicle;

NF: Number of follicle; PWP: Per cent white pulp; PRP: Per cent red pulp;

PT: Per cent trabecular tissue

**Table 9: Statistical analysis showing correlation coefficients between different parameters at the seventh region**

	CT	TT	MDF	DSF	DLF	NF	PWP	PRP	PT
CT	1.00000**	0.82430**	0.48002**	-0.08925	0.35467*	0.27627	-0.13868	0.22710	-0.13926
TT	0.82430**	1.00000**	0.15029	-0.16022	-0.02950	-0.02748	-0.18395	0.22524	-0.10088
MDF	0.48002**	0.15029	1.00000**	0.12681	0.89895**	0.67912**	0.43745**	-0.00656	-0.34383
DSF	-0.08925	-0.16022	0.12681	1.00000**	0.13606	-0.10103	-0.14398	0.11580	-0.01222
DLF	0.35467*	-0.02950	0.89895**	0.13606	1.00000**	0.78225**	0.42216*	-0.01616	-0.32096
NF	0.27627	-0.02748	0.67912**	-0.10103	0.78225**	1.00000**	0.47952**	-0.14556	-0.22424
PWP	-0.13868	-0.18395	0.43745**	-0.14398	0.42216*	0.47952**	1.00000**	-0.48640**	-0.26589
PRP	0.22710	0.22524	-0.00656	0.11580	-0.01616	-0.14556	-0.48640**	1.00000**	-0.71296**
PT	-0.13926	-0.10088	-0.34383	-0.01222	-0.32096	-0.22424	-0.26589	-0.71296**	1.00000**

\* Significant at 5% level of significance.

\*\*Highly significant at 5% level of significance.

CT: Capsule thickness; TT: Trabecular thickness; MDF: Mean diameter of follicle;

DSF: Diameter of smallest follicle; DLF: Diameter of largest follicle;

NF: Number of follicle; PWP: Per cent white pulp; PRP: Per cent red pulp;

PT: Per cent trabecular tissue

**Table 10: Statistical analysis showing correlation coefficients between different parameters at the eighth region**

	CT	TT	MDF	DSF	DLF	NF	PWP	PRP	PT
CT	1.00000**	0.81172**	-0.59296**	0.79280**	-0.74659**	-0.66137**	-0.23755	0.36483*	-0.34842*
TT	0.81172**	1.00000**	-0.43921**	0.58925**	-0.57768**	-0.63255**	-0.28898	0.51441**	-0.52125**
MDF	-0.59296**	-0.43921**	1.00000**	-0.40908*	0.43611**	0.52461**	0.04804	-0.22809	0.31284
DSF	0.79280**	0.58925**	-0.40908*	1.00000**	-0.82135**	-0.60781**	-0.16875	0.30689	-0.31017
DLF	-0.74659**	-0.57768**	0.43611**	-0.82135**	1.00000**	0.50713**	0.27641	-0.34789*	0.28221
NF	-0.66137**	-0.63255**	0.52461**	-0.60781**	0.50713**	1.00000**	0.02105	-0.11750	0.17430
PWP	-0.23755	-0.28898	0.04804	-0.16875	0.27641	0.02105	1.00000**	-0.74255**	0.23736
PRP	0.36483*	0.51441**	-0.22809	0.30689	-0.34789*	-0.11750	-0.74255**	1.00000**	-0.82375**
PT	-0.34842*	-0.52125**	0.31284	-0.31017	0.28221	0.17430	0.23736	-0.82375**	1.00000**

\* Significant at 5% level of significance.

\*\*Highly significant at 5% level of significance.

CT: Capsule thickness; TT: Trabecular thickness; MDF: Mean diameter of follicle;

DSF: Diameter of smallest follicle; DLF: Diameter of largest follicle;

NF: Number of follicle; PWP: Per cent white pulp; PRP: Per cent red pulp;

PT: Per cent trabecular tissue

**Table 11: Statistical analysis showing overall correlation coefficient between different parameters**

	CT	TT	MDF	DSF	DLF	NF	PWP	PRP	PT
CT	1.00000**	0.62543**	0.22884**	0.03481	0.04776	-0.22651**	0.18414**	-0.12742*	0.03867
TT	0.62543**	1.00000**	0.34904**	0.18573**	0.08767	-0.30721**	0.24696**	-0.20081**	0.06422
MDF	0.22884**	0.34904**	1.00000**	0.12873*	0.27309**	0.07643	0.27096**	-0.21516**	0.04935
DSF	0.03481	0.18573**	0.12873*	1.00000**	-0.19485**	-0.27540**	-0.03438	0.10136	-0.06026
DLF	0.04776	0.08767	0.27309**	-0.19485**	1.00000**	0.21774**	0.23801**	-0.20850**	0.02408
NF	-0.22651**	-0.30721**	0.07643	-0.27540**	0.21774**	1.00000**	-0.08058	0.03579	-0.05485
PWP	0.18414**	0.24696**	0.27096**	-0.03438	0.23801**	-0.08058	1.00000**	-0.79144**	0.34496**
PRP	-0.12742*	-0.20081**	-0.21516**	0.10136	-0.20850**	0.03579	-0.79144**	1.00000**	-0.73206**
PT	0.03867	0.06422	0.04935	-0.06026	0.02408	-0.05485	0.34496**	-0.73206**	1.00000**

\* Significant at 5% level of significance.

\*\*Highly significant at 5% level of significance.

CT: Capsule thickness; TT: Trabecular thickness; MDF: Mean diameter of follicle;

DSF: Diameter of smallest follicle; DLF: Diameter of largest follicle;

NF: Number of follicle; PWP: Per cent white pulp; PRP: Per cent red pulp;

PT: Per cent trabecular tissue

**Table 12: Statistical analysis showing Duncan's multiple range test for per cent trabecular tissue, white pulp and red pulp**

Duncan grouping		% Trabecular tissue		Duncan grouping		% White pulp		Duncan grouping		% Red pulp			
		Mean (μm)	Region			Mean (μm)	Region			Mean (μm)	Region		
	A	12.500	7		A	14.667	3		A	80.133	2		
	A	12.467	3	B	A	13.767	4		B	76.433	1		
	A	12.333	5	B	A	13.700	5		B	76.433	8		
B	A	12.200	6	B		C	13.067	1		B	76.133	7	
B	A	11.733	8			C	12.433	6	C	B	75.367	6	
B	A	11.600	4	D			11.867	8	C	B	D	74.700	4
B		11.000	1	D			11.367	7	C		D	73.633	5
B		10.967	2			E	10.233	2		D	73.133	3	

Mean with the same letter are not significantly different at  $P < 0.05$



**Table 13:** Statistical analysis showing multiple range test for per cent trabecular tissue, white pulp and red pulp at eight different regions of the spleen

Parameters	Calculated "F" values values from the Analysis of variance	Region	I	II	III	IV	V	VI	VII	VIII
% Trabecular tissue	2.47	Mean	11.00	10.96	12.46	11.60	12.33	12.20	12.50	11.73
		SD	2.42	2.47	2.68	1.94	1.95	1.84	2.02	1.91
		SE	0.44	0.45	0.49	0.35	0.35	0.33	0.37	0.34
% White pulp	14.54	Mean	13.06	10.23	14.66	13.76	13.70	12.43	11.36	11.86
		SD	2.95	1.79	1.68	2.40	1.93	2.29	1.62	1.59
		SE	0.54	0.32	0.30	0.43	0.35	0.41	0.29	0.29
% Red pulp	9.16	Mean	76.43	80.13	73.13	74.70	73.63	75.36	76.13	76.43
		SD	5.73	4.77	4.17	3.82	3.28	3.44	2.23	2.75
		SE	1.04	0.87	0.76	0.69	0.59	0.62	0.40	0.50

SD - Standard deviation; SE - Standard error

**Table 14: Statistical analysis showing multiple range test for mean diameter, number of follicles, diameter of smallest and largest follicle at eight different regions of the spleen**

Parameters	Calculated "F" values values from the Analysis of variance	Region	I	II	III	IV	V	VI	VII	VIII
Mean diameter of follicle ( $\mu\text{m}$ )	13.92	Mean	281.59	277.25	329.72	339.12	336.43	294.52	311.72	295.43
		SD	18.68	27.49	30.78	19.40	67.58	28.86	36.07	35.73
		SE	3.41	5.02	5.62	3.54	12.33	5.27	6.58	6.52
Diameter of smallest follicle( $\mu\text{m}$ )	3.54	Mean	183.18	194.58	186.10	193.09	195.79	180.65	177.33	195.10
		SD	11.17	31.46	17.02	20.80	30.86	10.51	8.36	25.67
		SE	2.09	5.74	3.10	3.79	5.63	1.91	1.52	4.68
Diameter of largest follicle( $\mu\text{m}$ )	6.49	Mean	399.49	405.86	433.09	442.78	434.41	449.60	415.60	420.13
		SD	20.71	16.12	37.24	31.15	52.38	22.12	48.38	55.01
		SE	3.78	2.94	6.79	5.68	9.56	4.03	8.83	10.04
No. of follicles/ $\text{cm}^2$ area	3.85	Mean	128.41	115.72	118.10	114.14	124.44	144.26	133.96	131.58
		SD	31.59	29.77	23.76	21.99	34.02	27.88	31.52	27.74
		SE	5.76	5.43	4.33	4.01	6.21	5.09	5.75	5.06

SD - Standard deviation; SE - Standard error

**Table 15: Statistical analysis showing Duncan's multiple range test for mean diameter and number of follicles**

Duncan grouping		Mean diameter of follicles		Duncan Grouping		Number of follicle	
		Mean ( $\mu\text{m}$ )	Region			Mean	Region
	A	339.127	4		A	144.265	6
	A	336.432	5	B	A	133.961	7
B	A	329.724	3	B	A	131.583	8
B	C	311.721	7	B D	A C	128.412	1
D	C	295.439	8	B D	C	124.449	5
D	C	294.521	6	B D	C	118.107	3
D		281.593	1	D	C	115.729	2
D		277.551	2	D		114.144	4

Means with the same letter are not significantly different at  $P < 0.05$ .

**Table 16: Statistical analysis showing Duncan's multiple range test for diameter of smallest and largest follicle**

Duncan grouping			Smallest follicle		Duncan grouping			Largest follicle	
			Mean ( $\mu\text{m}$ )	Region				Mean ( $\mu\text{m}$ )	Region
A			195.793	5	A			449.608	6
B	A		195.105	8	A			442.785	4
B	A		194.589	2	B	A		434.415	5
B	A		193.099	4	B	A		433.096	3
B	A	C	186.104	3	B	C		420.139	8
B		C	183.180	1	B	C		415.609	7
		C	180.657	6		C		405.863	2
		C	177.332	7		C		399.499	1

Means with the same letter are not significantly different at  $P < 0.05$ .

Fig. 1. Photograph showing crescent shaped spleen (S) in relation to kidney (K), colon (C) and rumen (R). Note its extent from lumbar vertebrae 3rd to 7th

Fig. 2. Photograph showing crescent shaped spleen (S) in relation to kidney (K), colon (C) and rumen (R). Note its extent from lumbar vertebrae 3rd to 7th





Fig. 3. Arteriogram showing splenic artery (A) entering the hilus. Note its primary (P), secondary (S) and tertiary (T) branches

Fig. 4. Arteriogram showing splenic artery (A) entering the hilus. Note its primary (P), secondary (S) and tertiary (T) branches

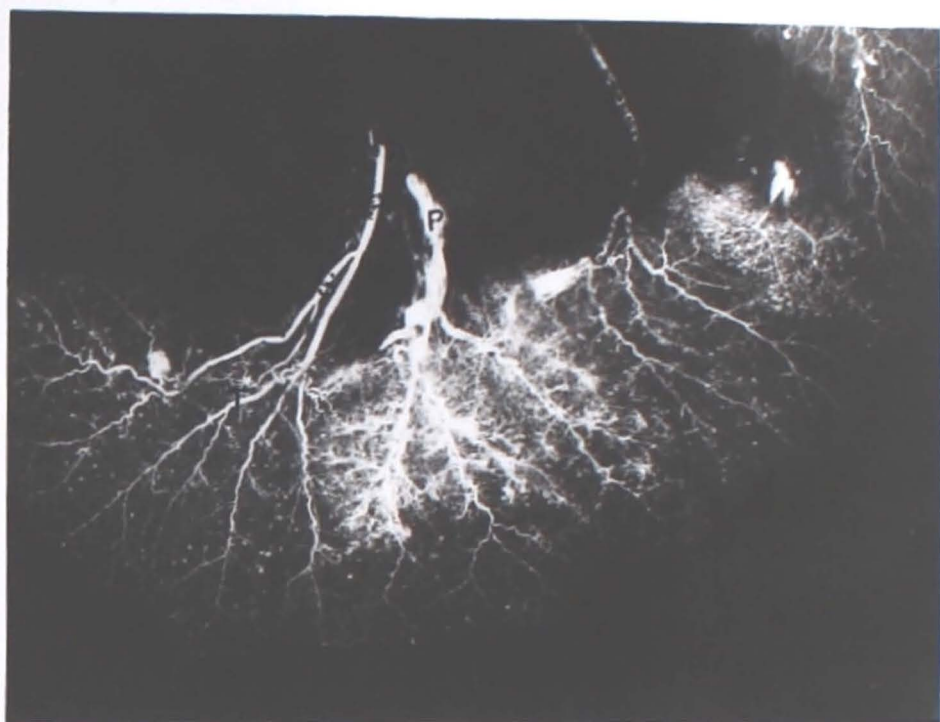




Fig. 5. Arteriogram showing splenic artery entering the hilus. Note its primary (P), secondary (S) and tertiary (T) branches

Fig. 6. Photomicrograph showing capsule (C) of the spleen along with the flattened mesothelium (m) and longitudinally oriented smooth muscle (M) layers. Note nerve fibres (N) in the upper layer of the capsule

H. & E. x 50

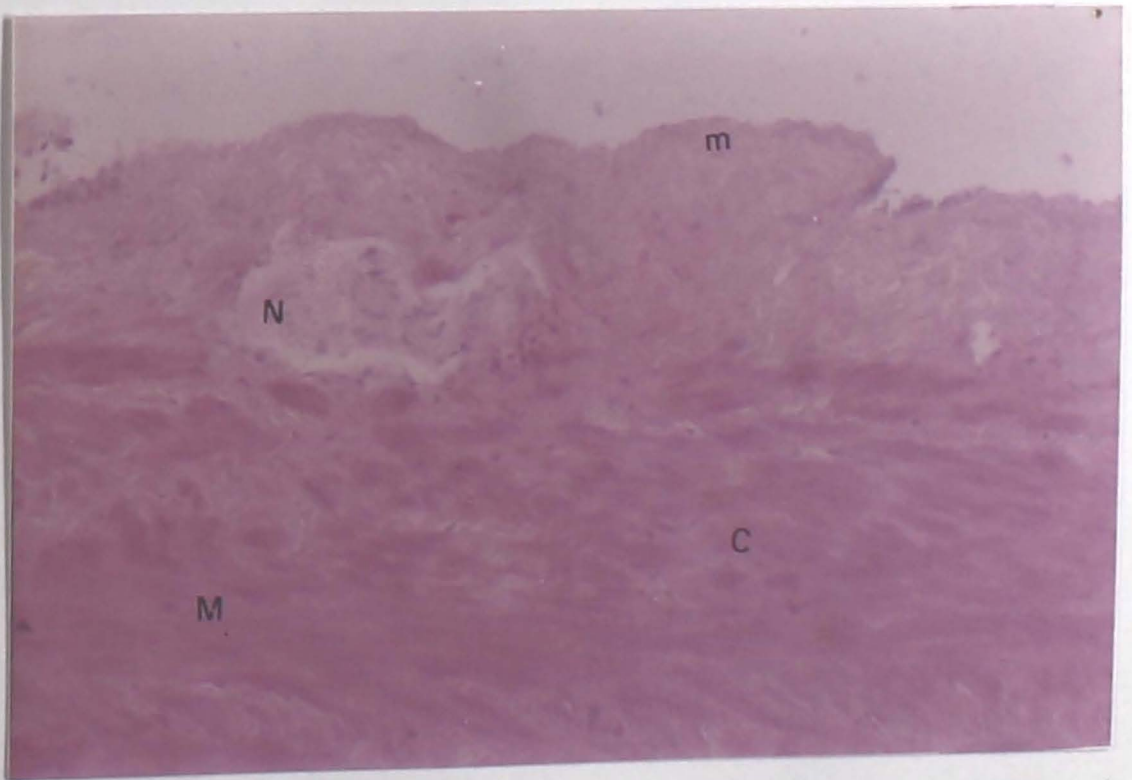
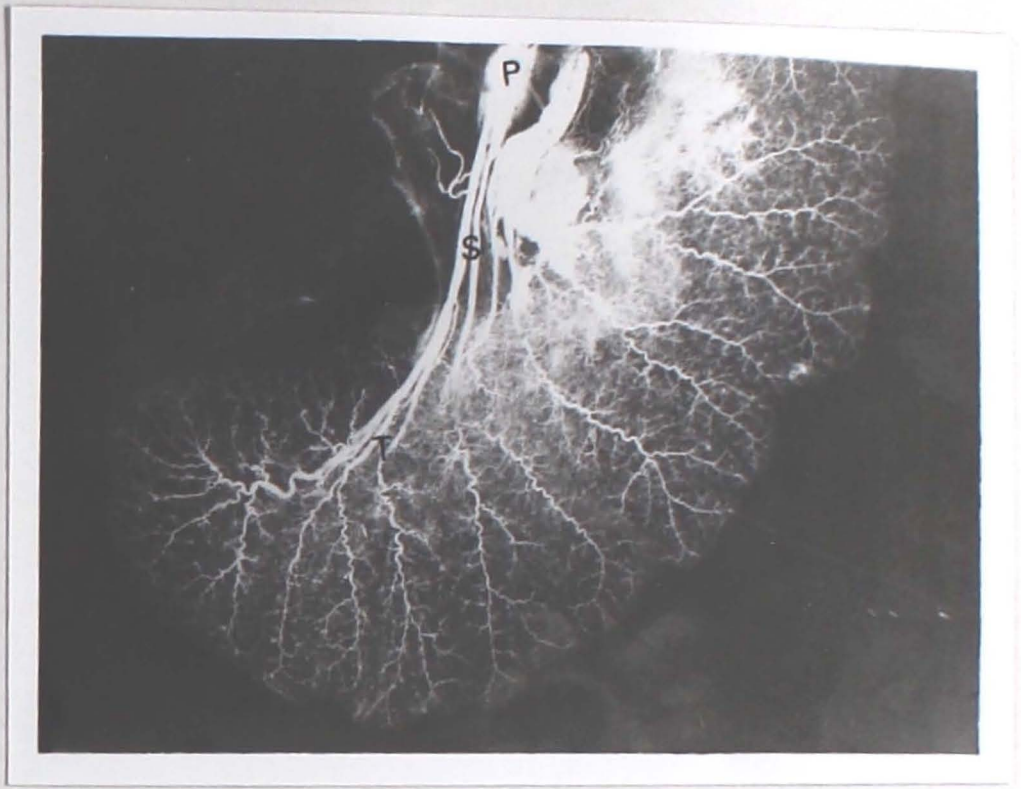




Fig. 7. Photomicrograph showing distribution of reticular fibres (r) in the capsule. Note the vertical orientation of the fibre in the lower part of the capsule from where the trabeculae (T) emanates

Gomori's method x 50

Fig. 8. Photomicrograph showing distribution of collagen fibres (blue colour) in the capsule (C) and trabeculae (T)

Crossmann-trichrome method x 50

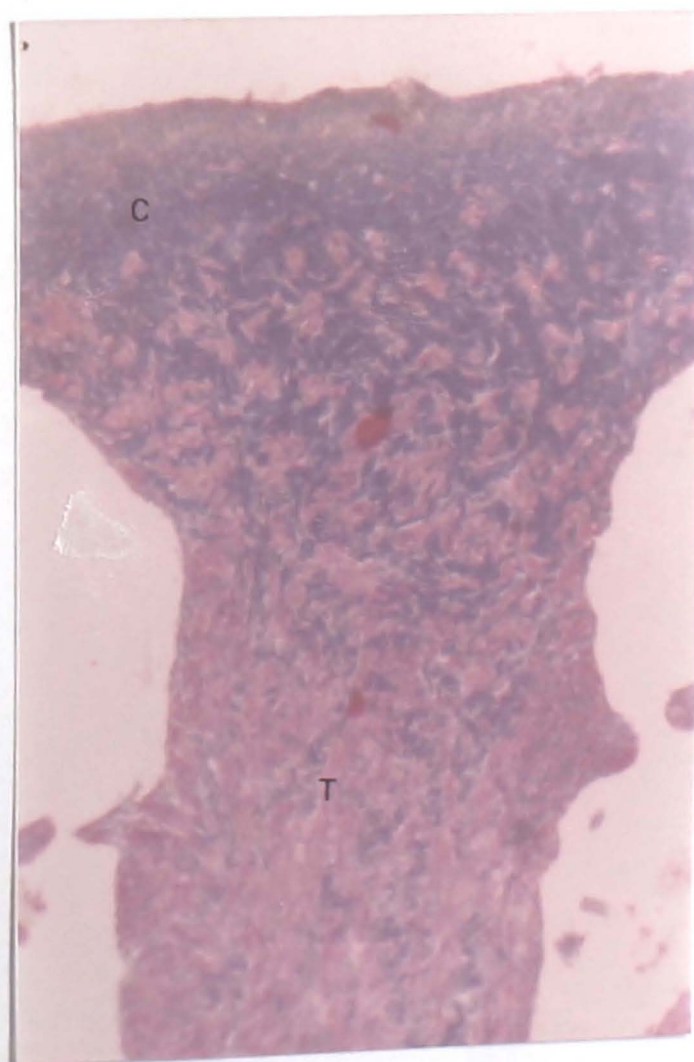
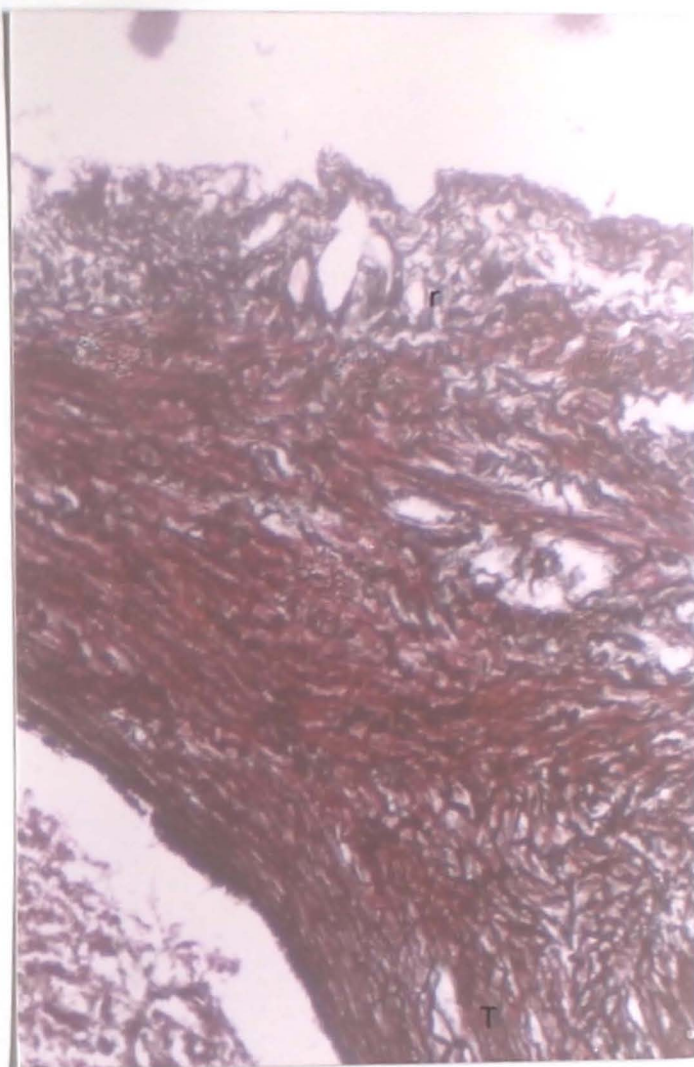


Fig. 9. Photomicrograph showing distribution of elastic fibres (e)  
in the different layers of capsule (c)  
Weigert's method x 50

Fig. 10. Photomicrograph showing distribution of elastic fibres (e)  
in the different layers of capsule (c) and trabeculae (T)  
Weigert's method x 50



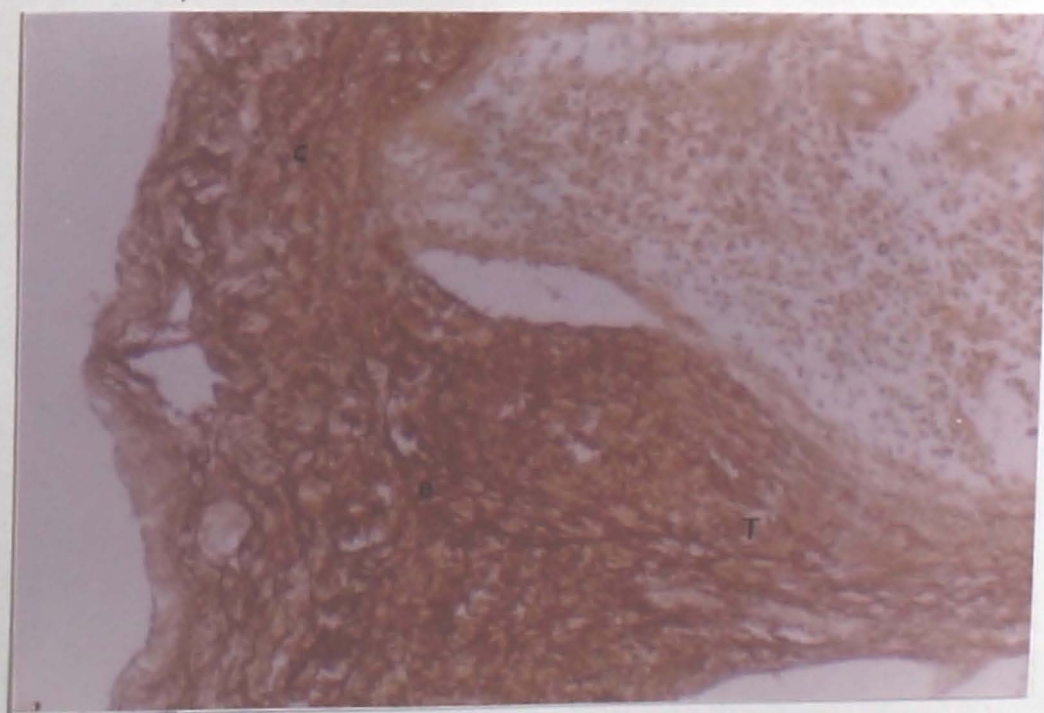


Fig. 11. Photomicrograph showing the branching pattern of the trabeculae (T), white pulp (W) and red pulp (R)  
H. & E. x 26.8

Fig. 12. Photomicrograph showing smooth muscles (M) arrangement in trabeculae (T). Note the presence of venous sinuses (V) along the longitudinal axis of the trabeculae (T)  
H. & E. x 50



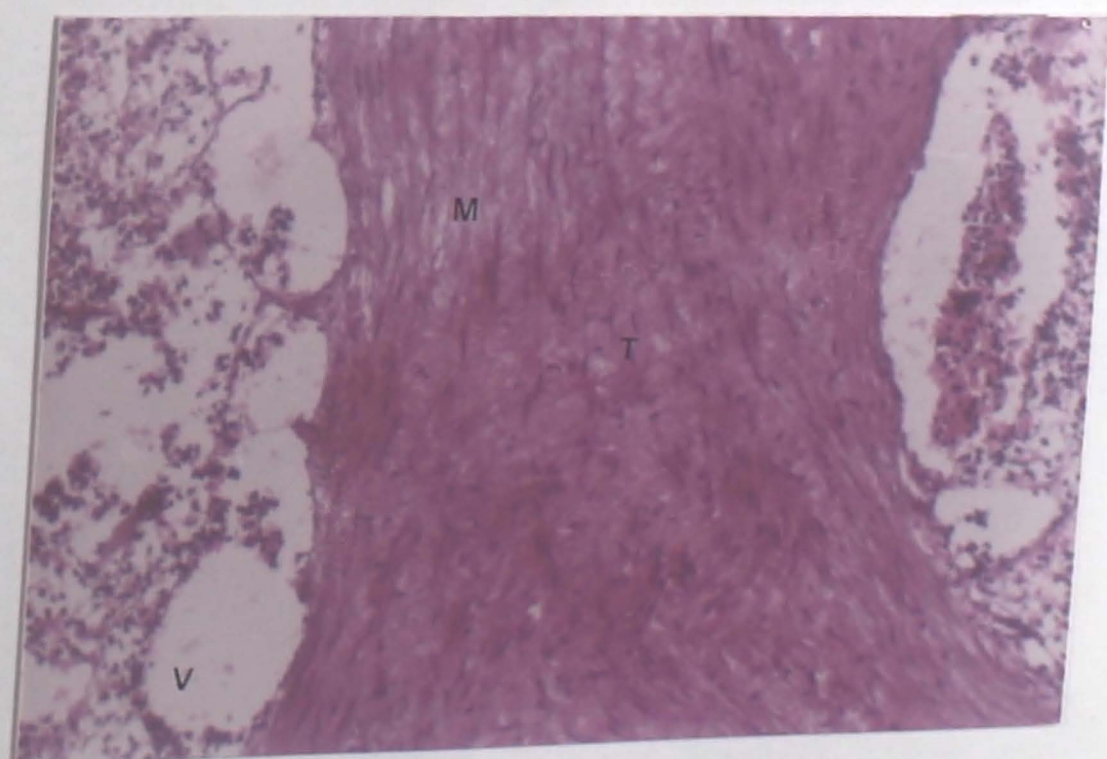
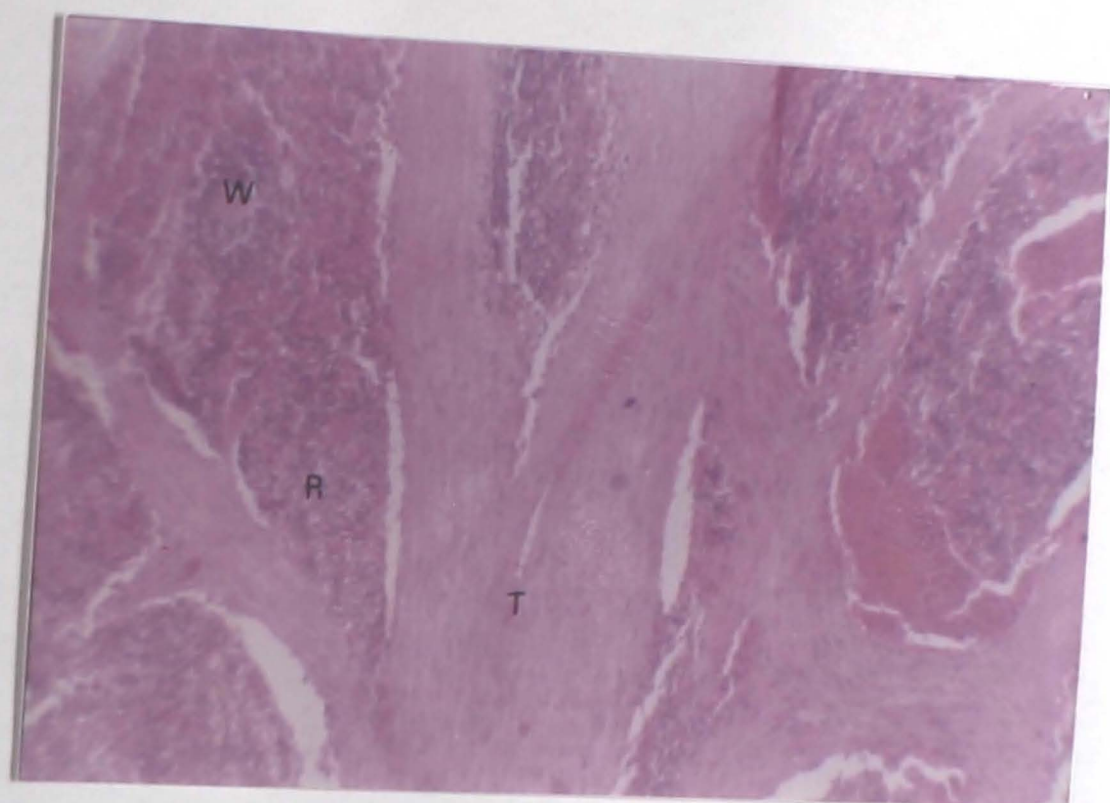




Fig. 13. Photomicrograph showing distribution of elastic fibres (E)  
in the trabeculae (T)

Weigert's method x 50

Fig. 14. Photomicrograph showing distribution of collagen fibres  
(blue colour) in the trabeculae (T)

Crossmann-trichrome method x 50

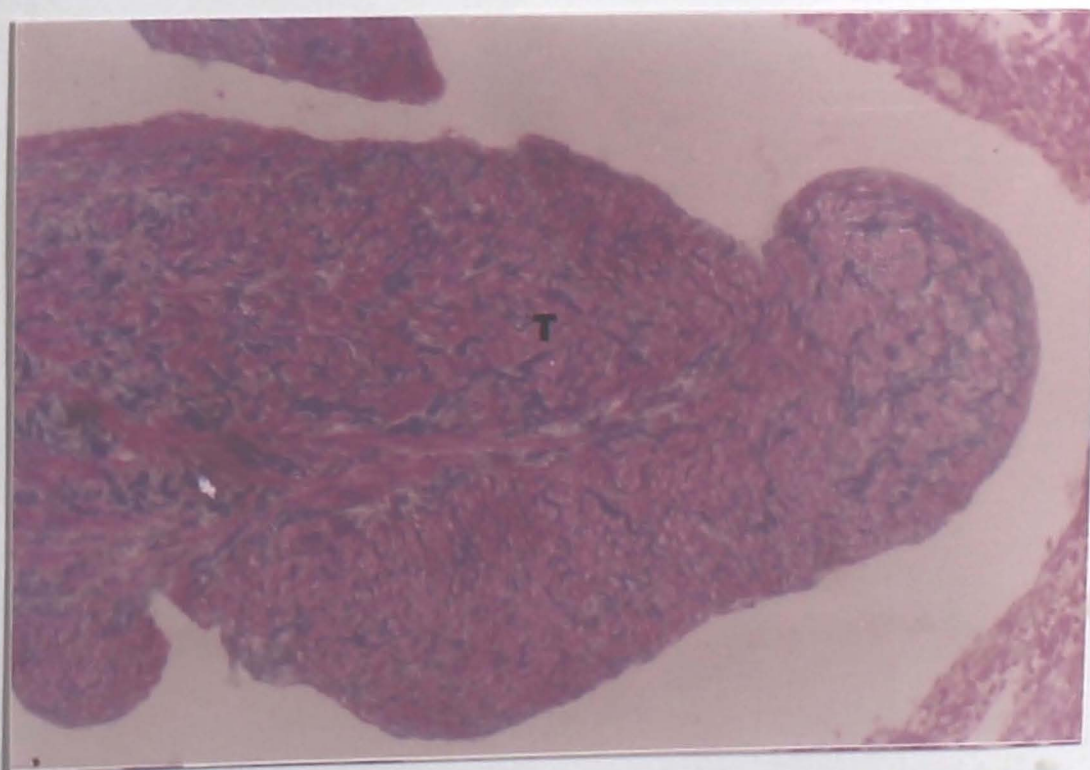
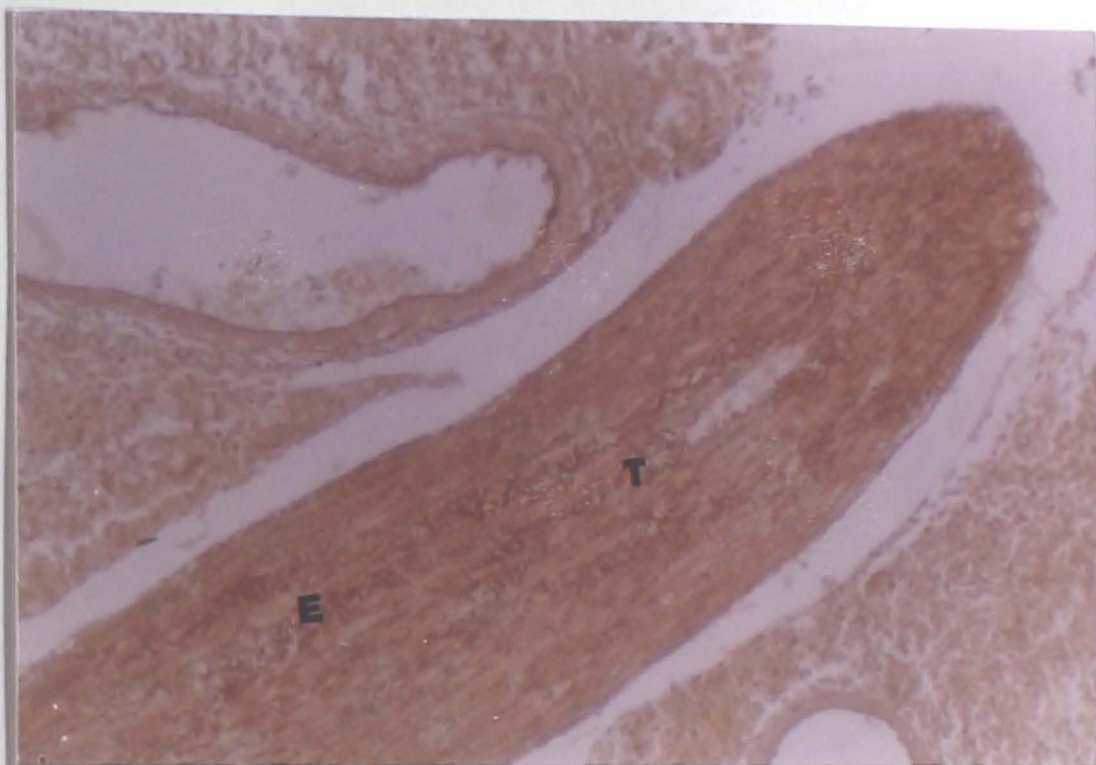


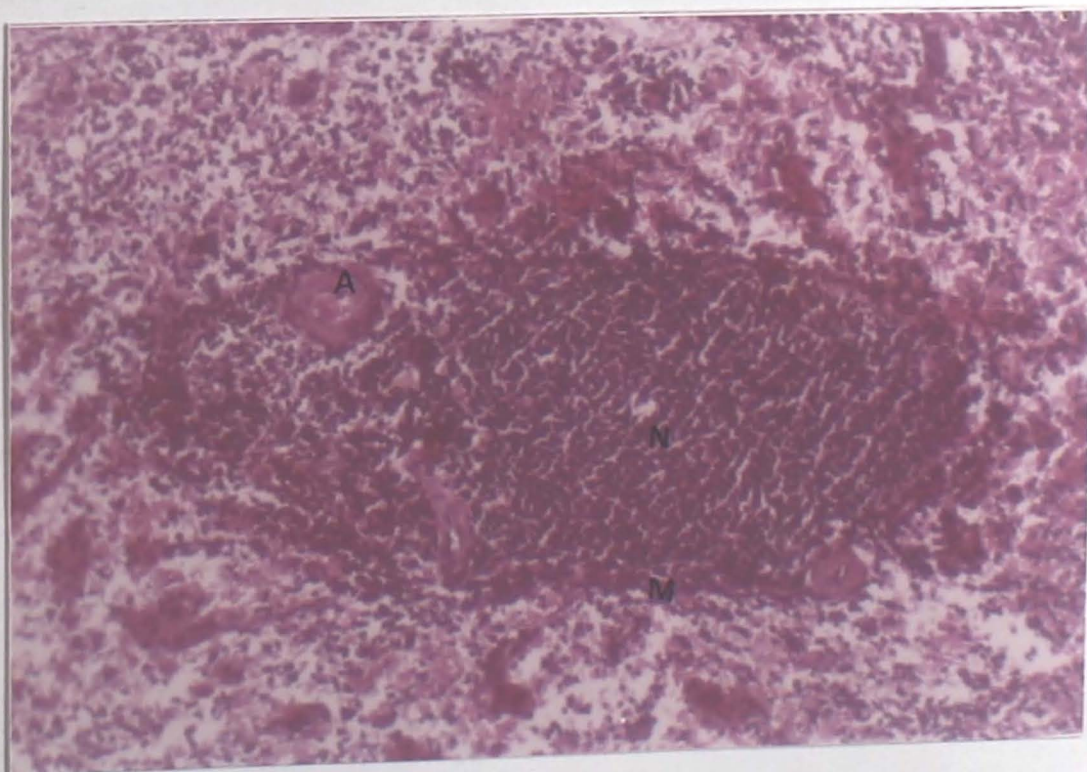
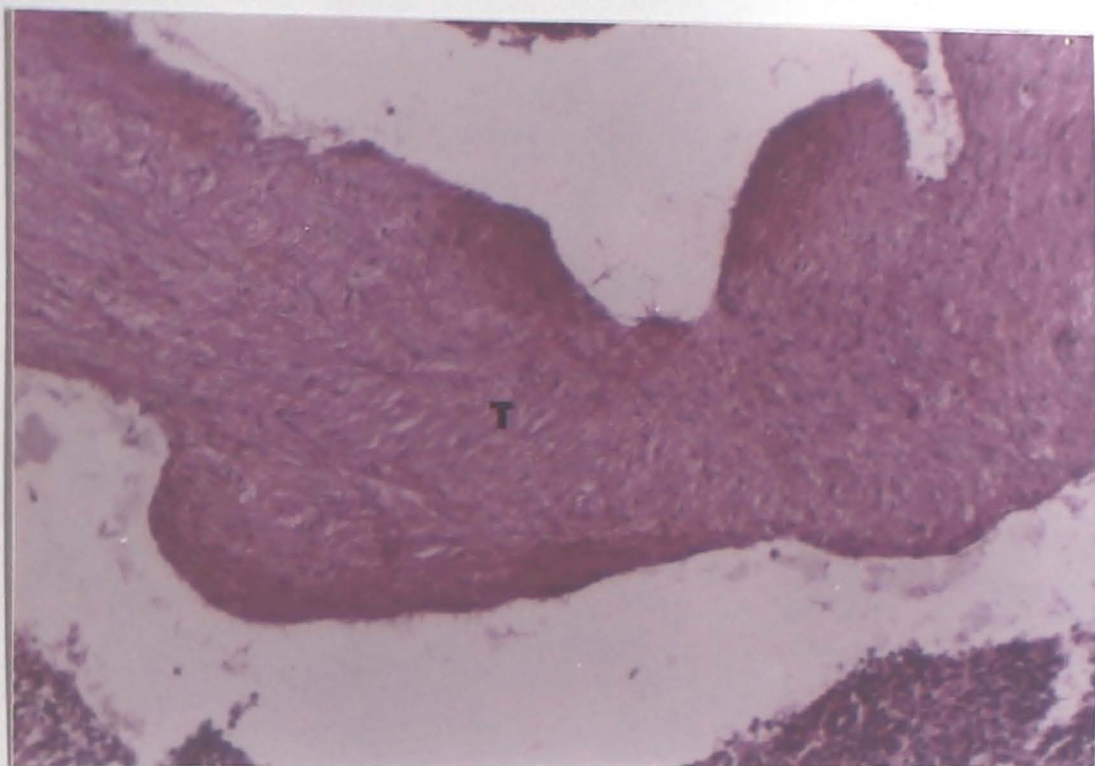
Fig. 15. Photomicrograph showing mild to moderate PAS activity in the trabeculae (T)

McManus PAS method x 50

Fig. 16. Photomicrograph showing splenic nodule (N), nodular artery (A), marginal zone (M) and smooth muscle fibres (m)

H. & E. x 50





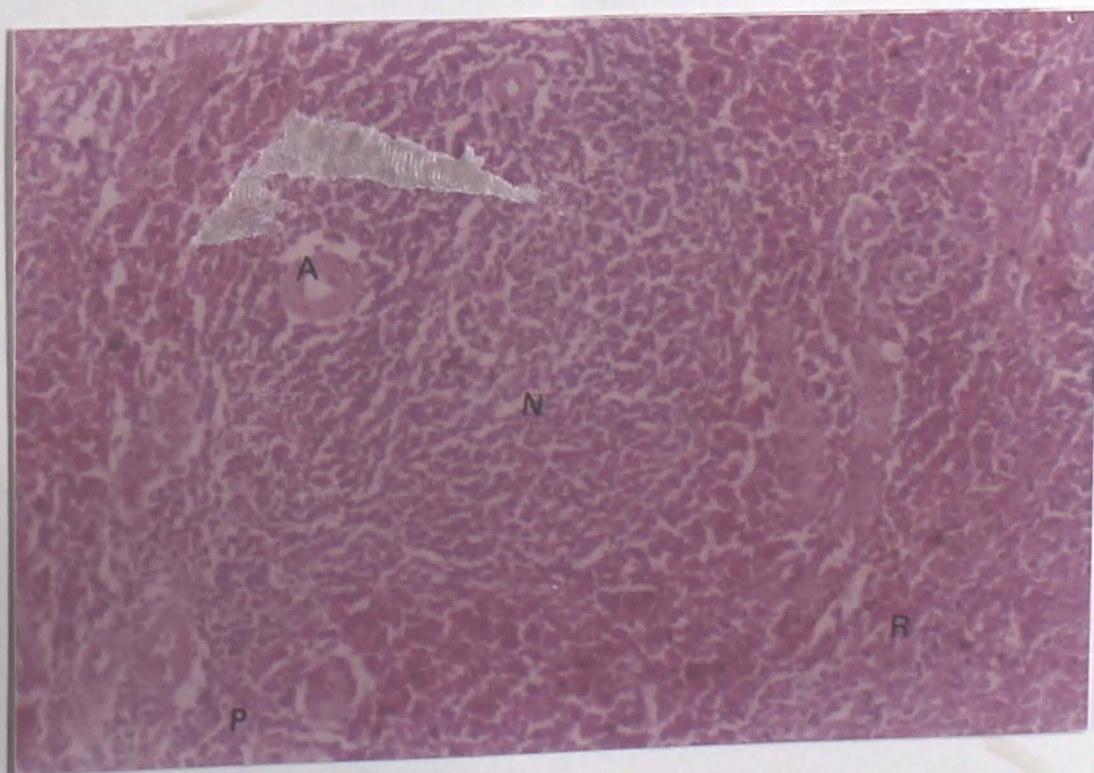
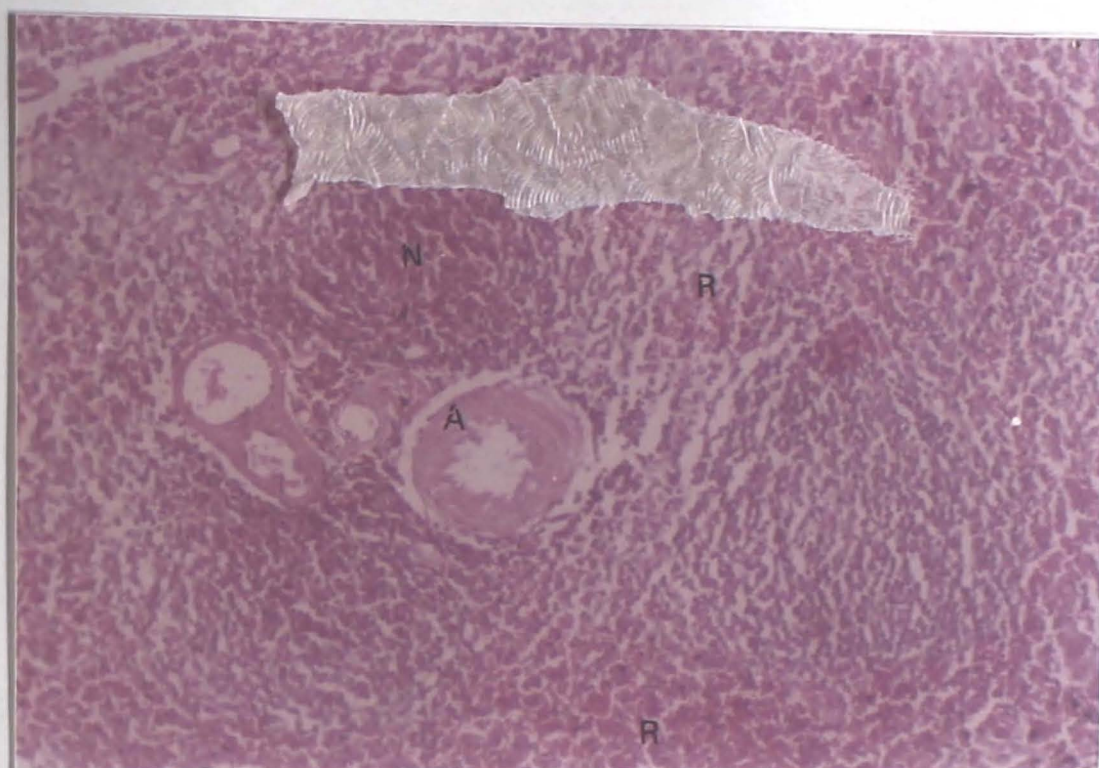
Photomicrograph showing splenic nodule (N), nodular artery (A). Note extension of the red pulp (R) in between the two nodules (N)

H. & E. x 50

Photomicrograph showing splenic nodule (N), nodular artery (A), periarterial lymphatic sheath (P) and the red pulp (R)

H. & E. x 50



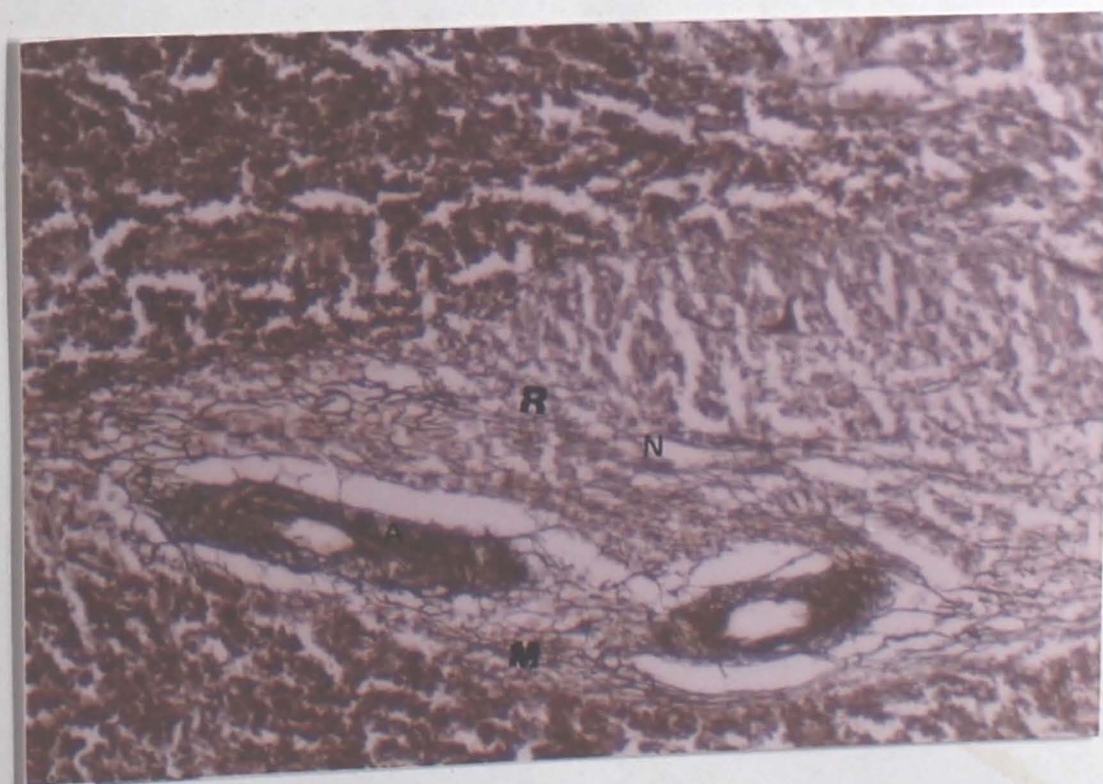
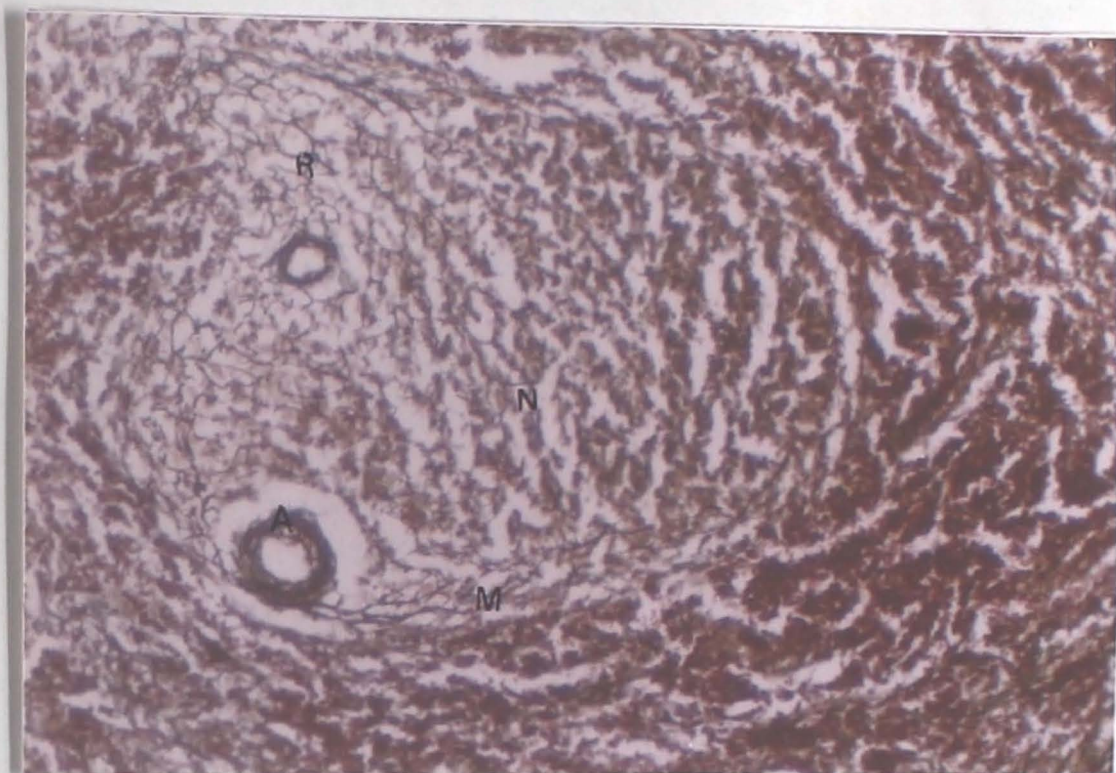


Photomicrograph showing periarterial lymphatic sheath (P)  
H. & E. x 100

Photomicrograph showing splenic nodule (N), marginal zone (M). Note the course of the elipsoidal capillaries (C) in the red pulp (R)

H. & E. x 200





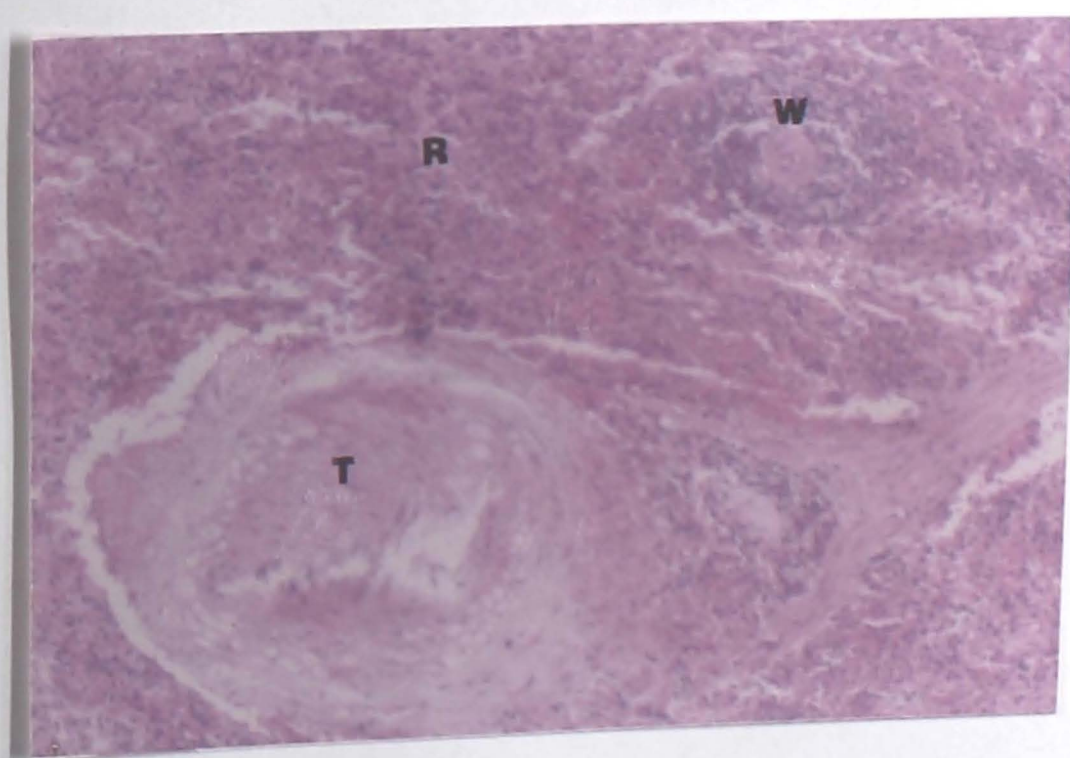
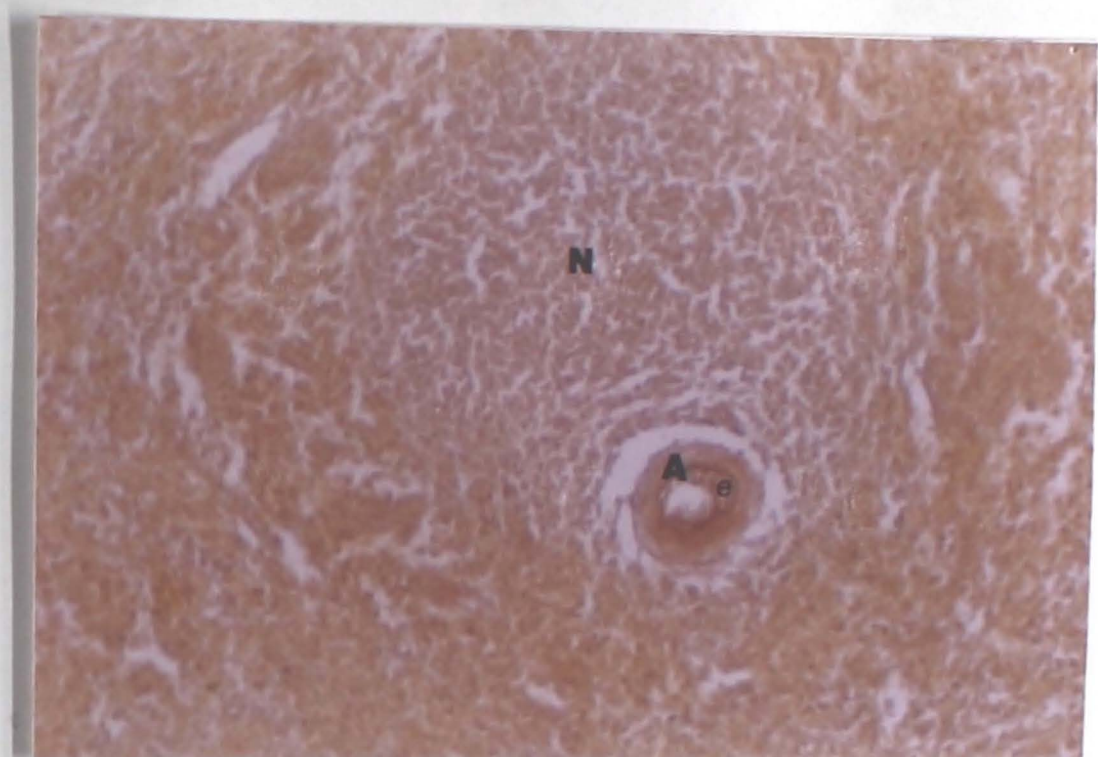


Photomicrograph showing presence of elastic fibres (e) in the tunica intima of the nodular artery (A). Note the absence of the fibres in the splenic nodule (N)

Weigert's method x 50

Photomicrograph showing the branching of trabecular artery (T), white pulp (W) and red pulp (R)

H. & E. x 50



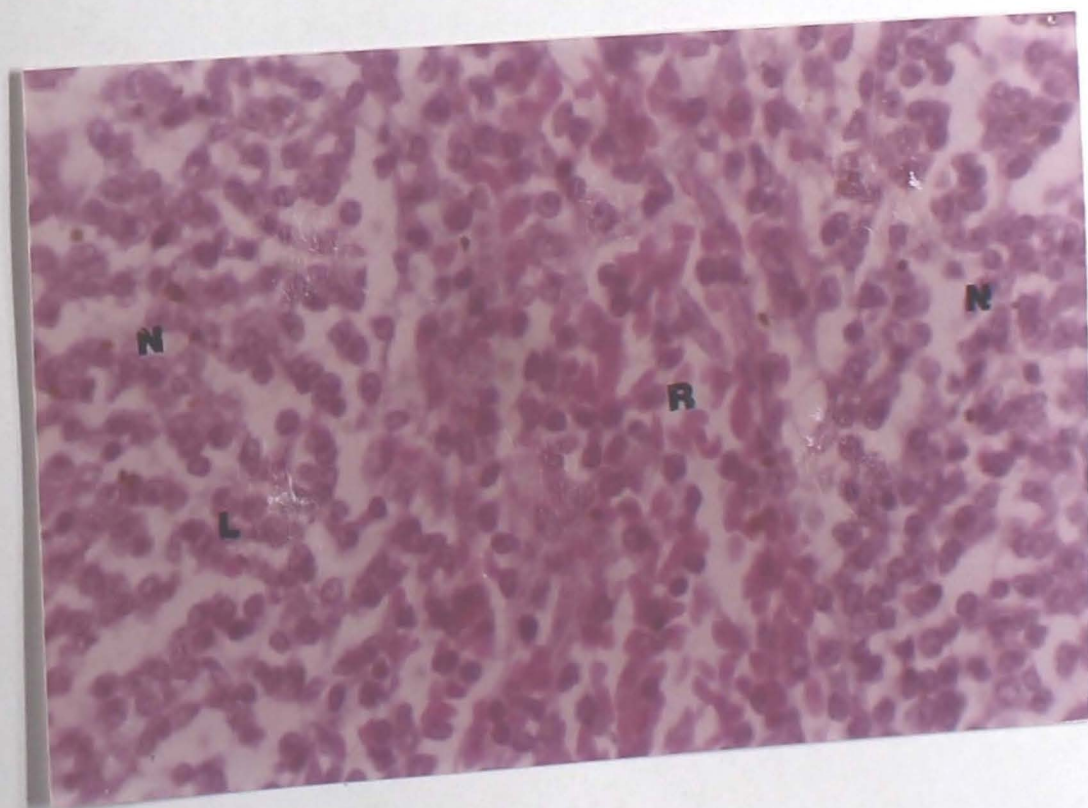
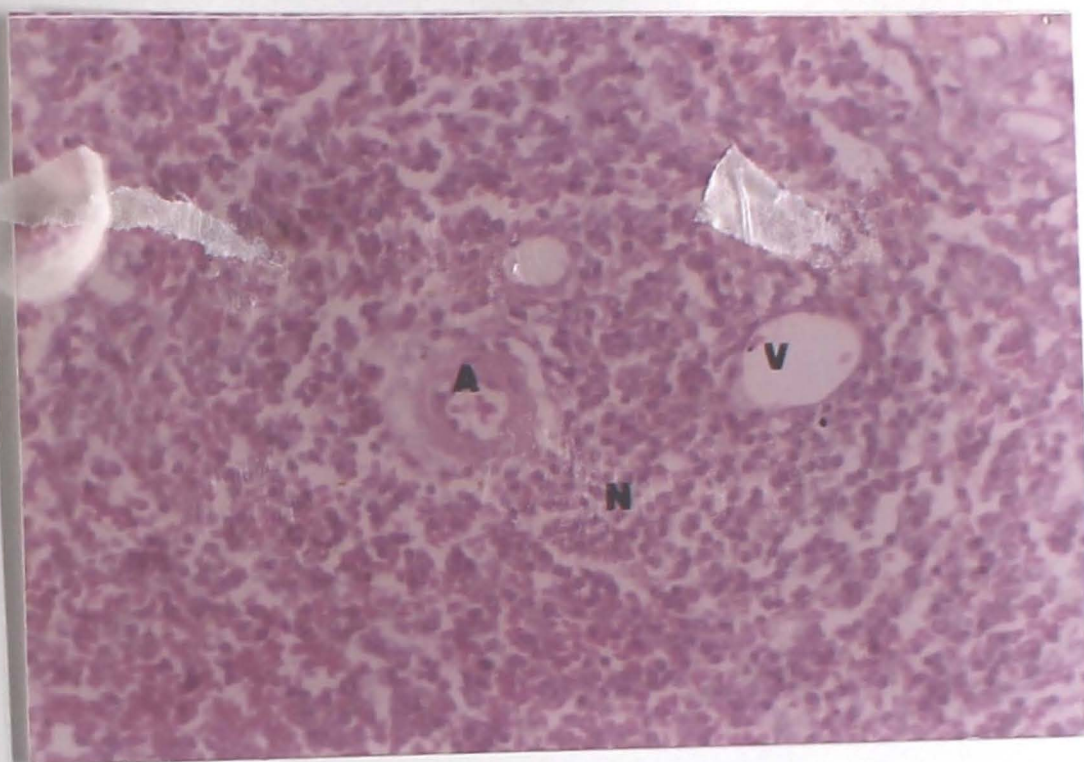
Photomicrograph showing splenic nodule (N), nodular artery (A) and pulp vein (V)

H. & E. x 100

Photomicrograph showing distribution of lymphocyte (L) in the splenic nodule (N). Note the red pulp (R) penetrating the two splenic nodules

H. & E. x 200





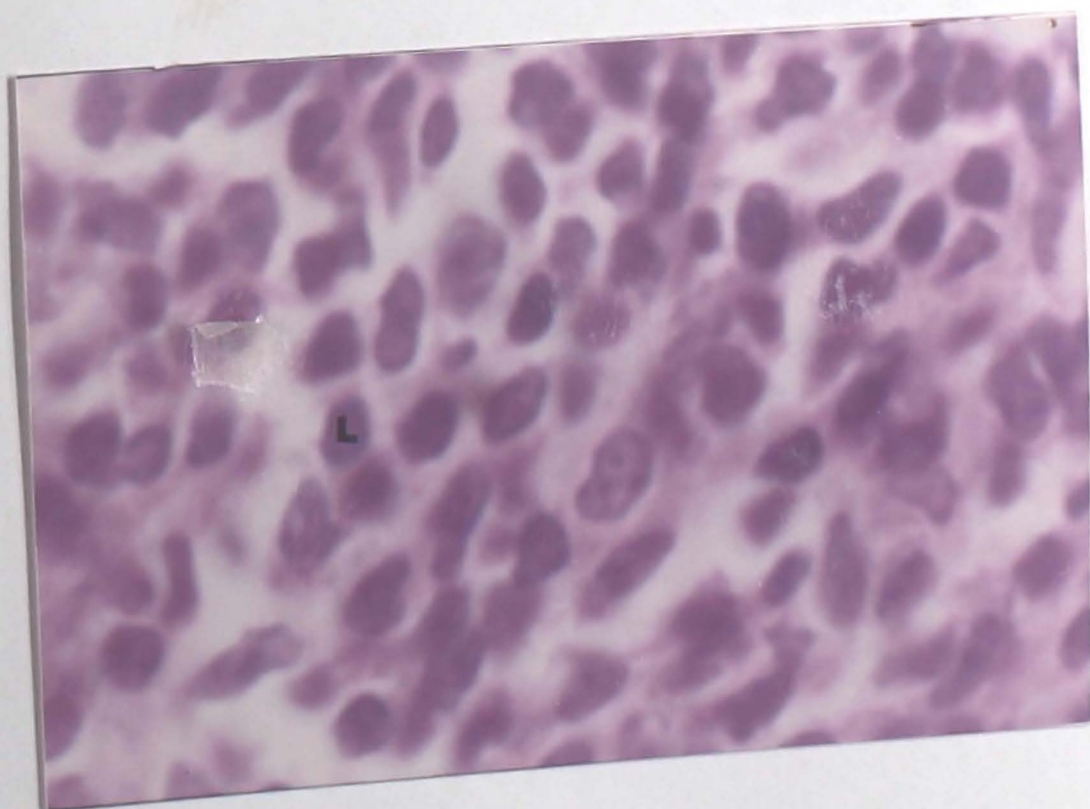
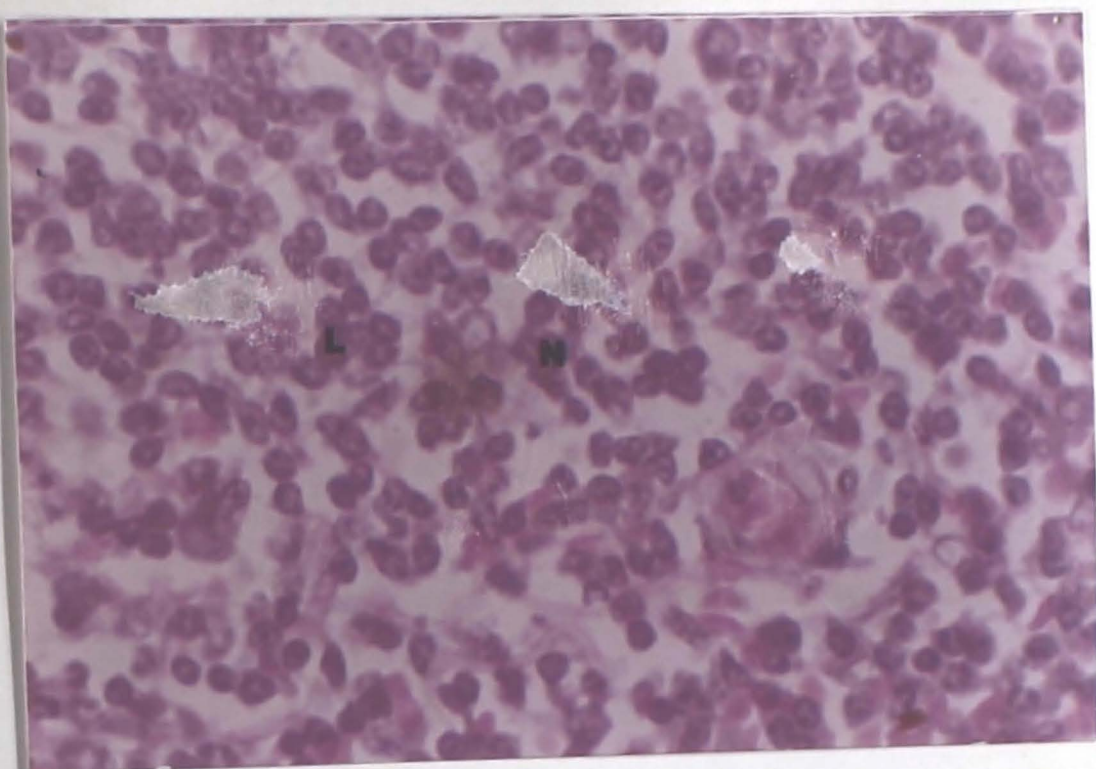
Photomicrograph showing distribution of lymphocytes (L)  
of different types in the splenic nodules (N)

H. & E. x 268

Photomicrograph showing distribution of lymphocytes (L)

H. & E. x 670





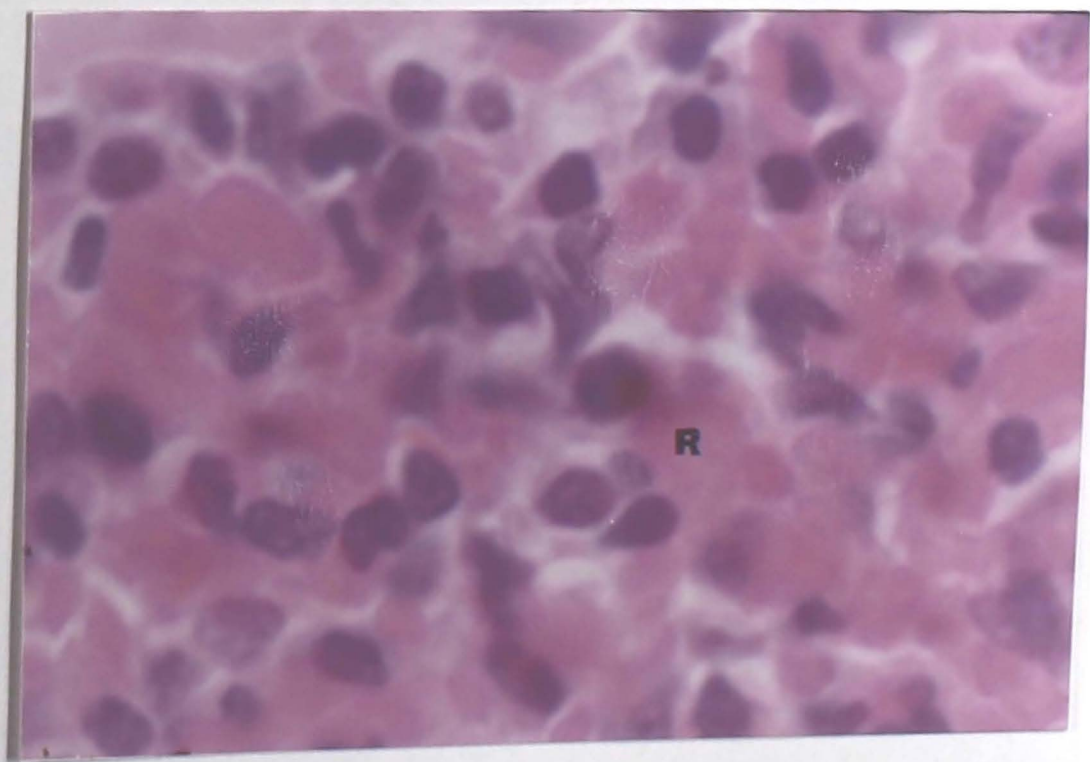
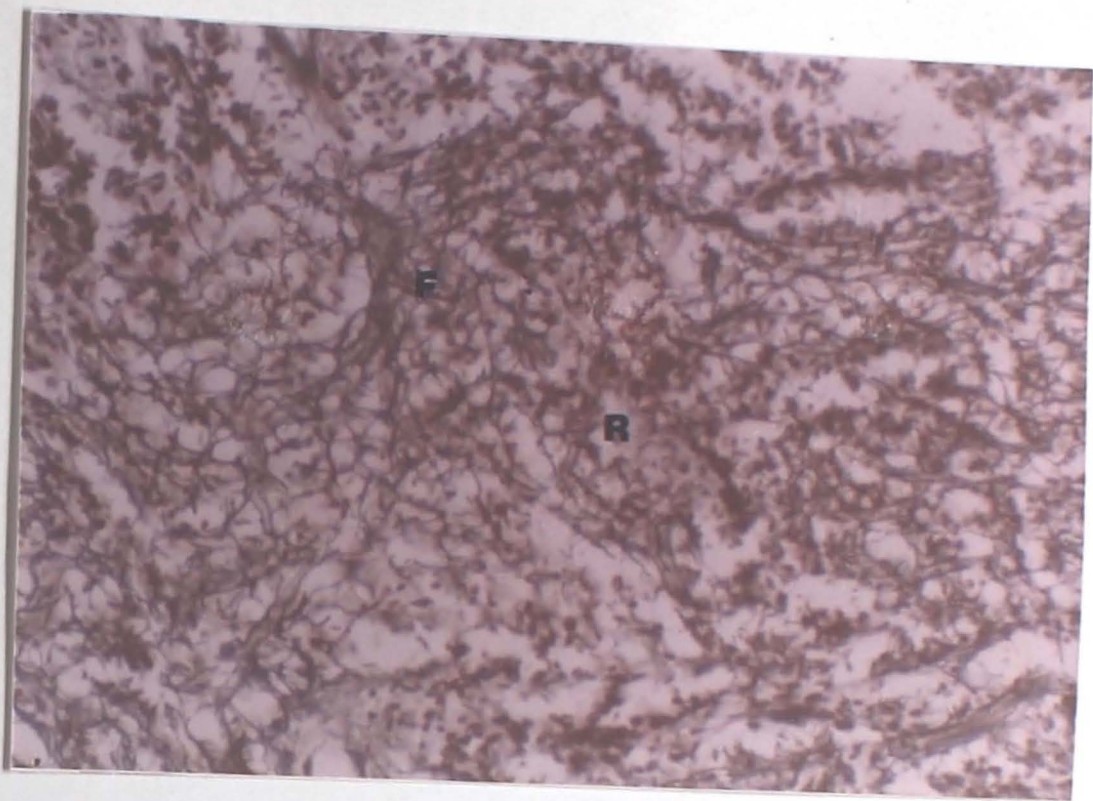
Photomicrograph showing distribution of iron (I) in the white pulp (W).

Perl's method x 200

Photomicrograph showing presence of collagen fibres (blue colour) in the marginal zone (M) and around the nodular artery (A).

Crossmann-trichrome method x 100





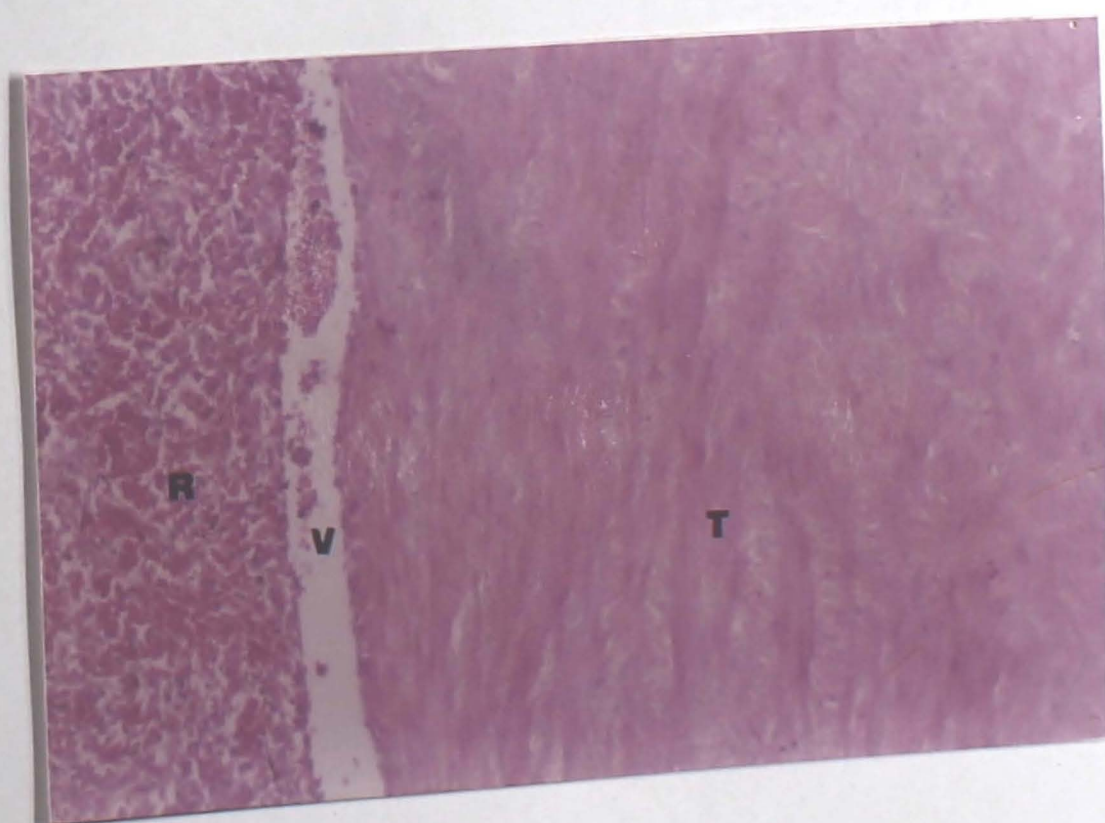
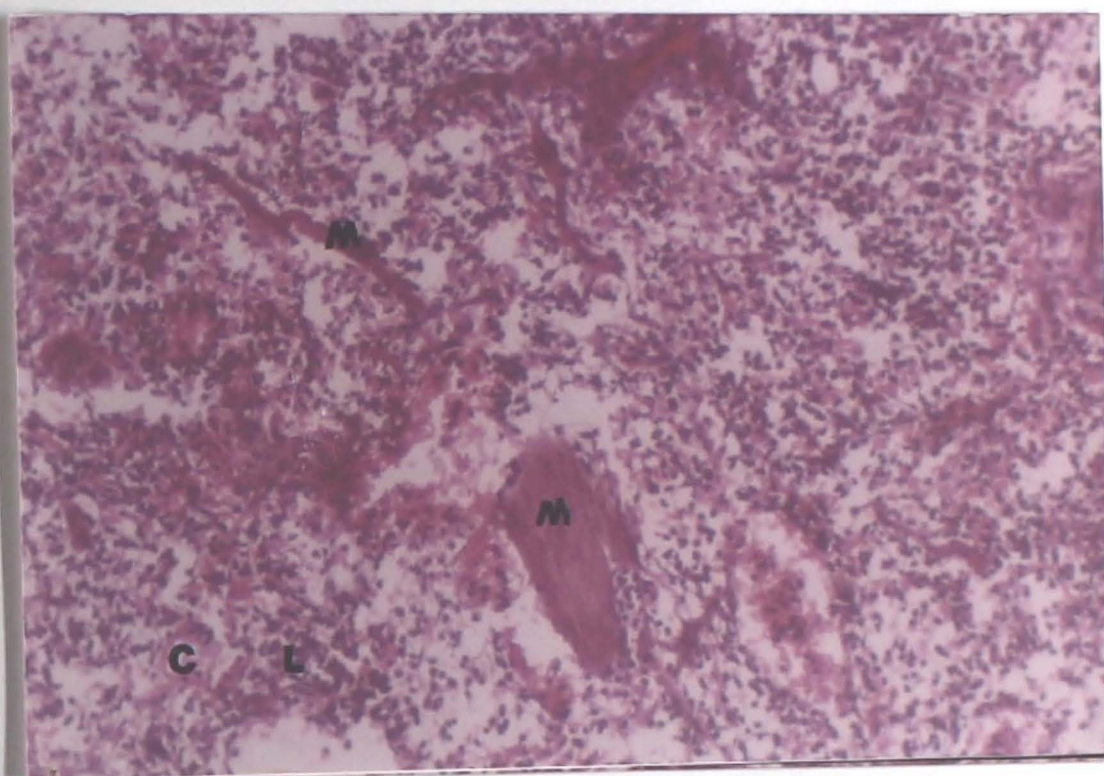


Photomicrograph showing smooth muscle fibres (M), fine blood capillaries (C) and small aggregation of lymphocytes (L)

H. & E. x 50

Photomicrograph showing trabeculae (T), red pulp (R) and venous sinuses (V) along the longitudinal axis of the trabeculae

H. & E. x 50



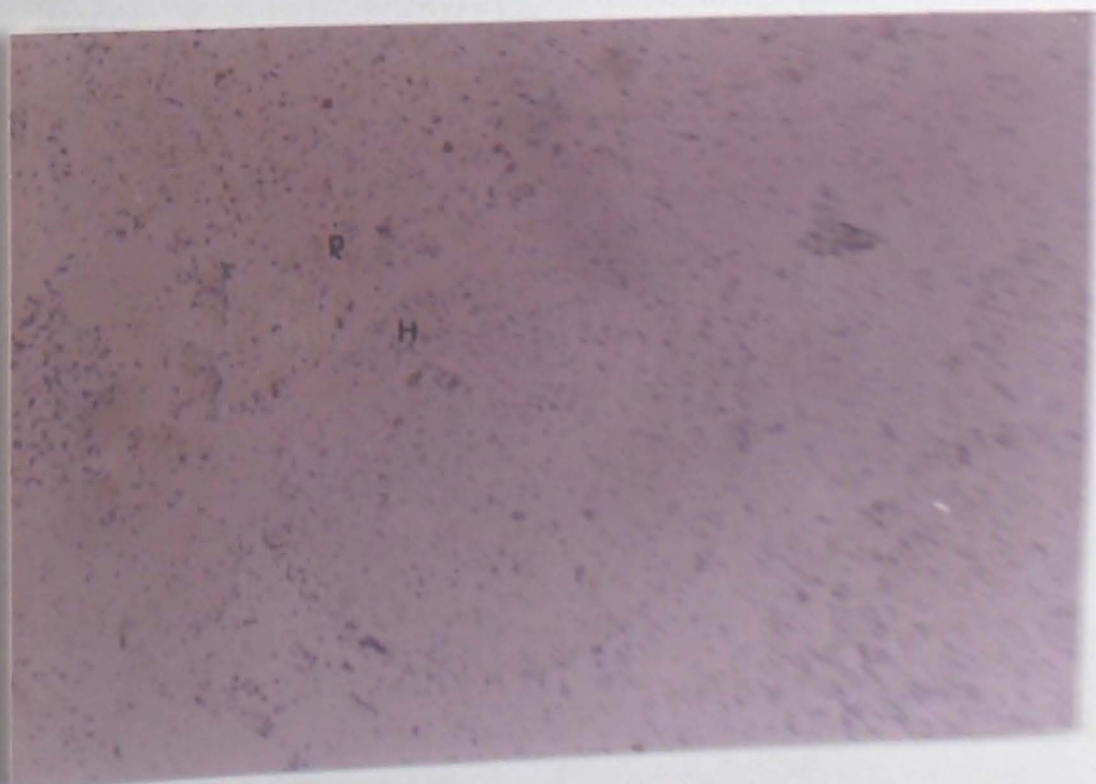
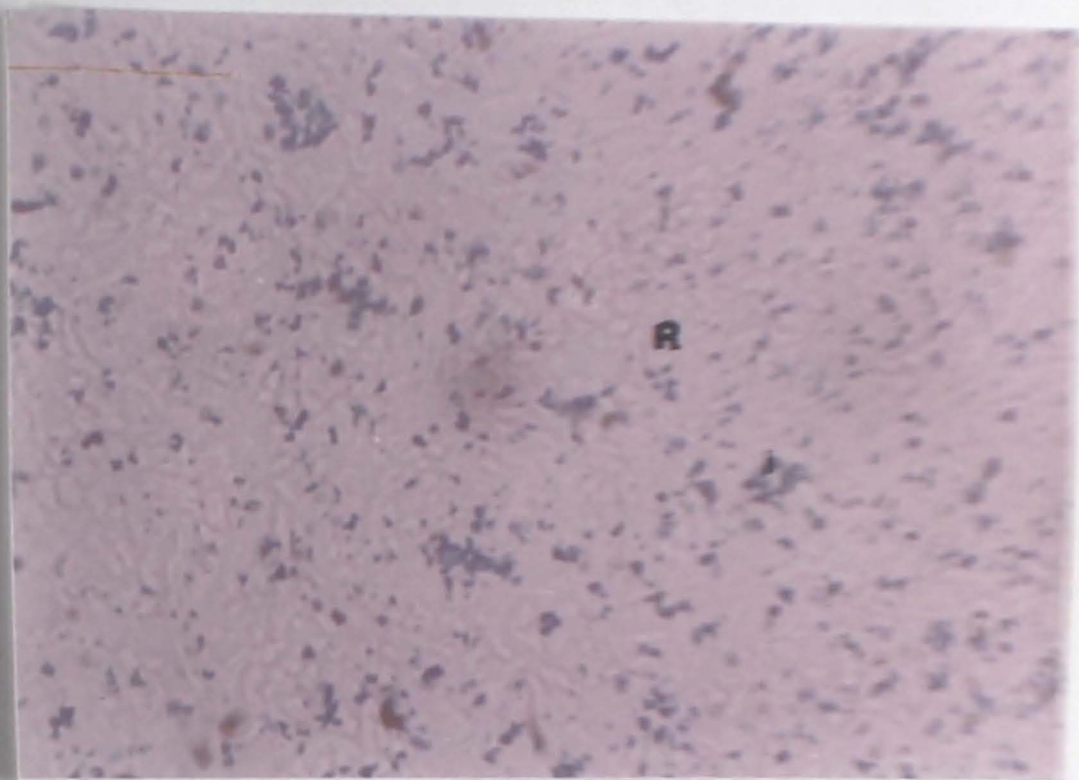


Photomicrograph showing the presence of venous sinuses (V) and smooth muscle fibres (M) in the red pulp (R)

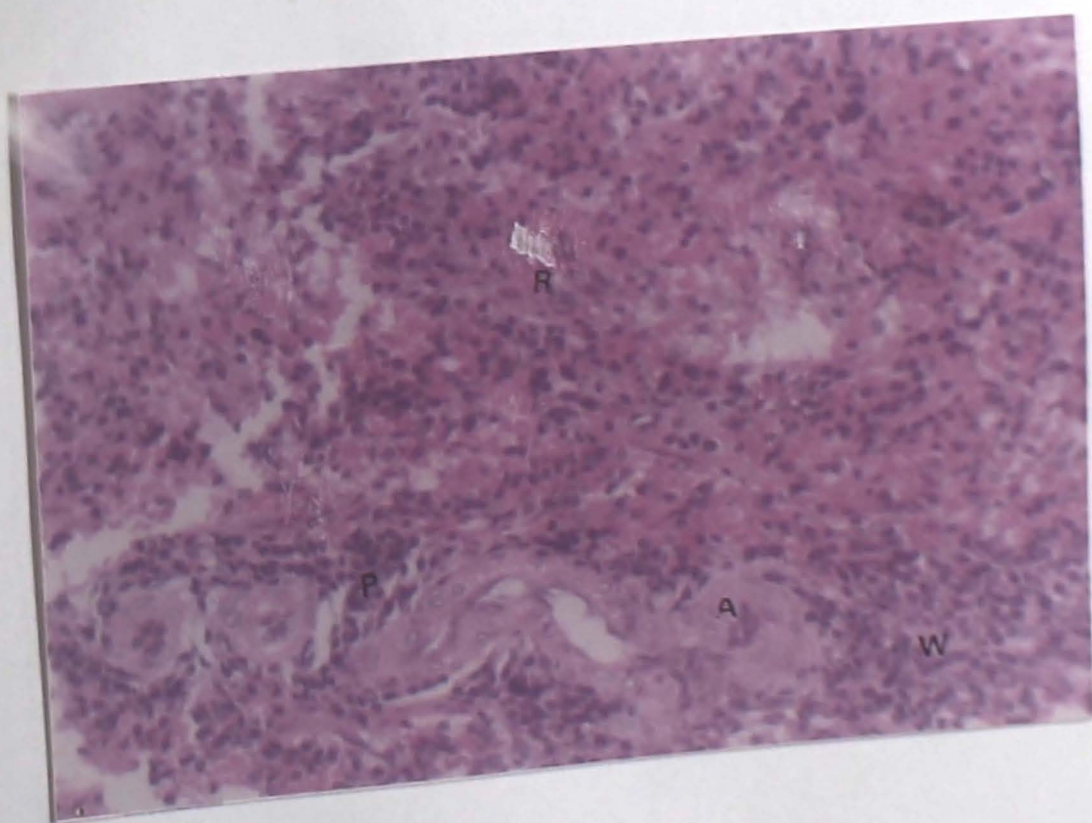
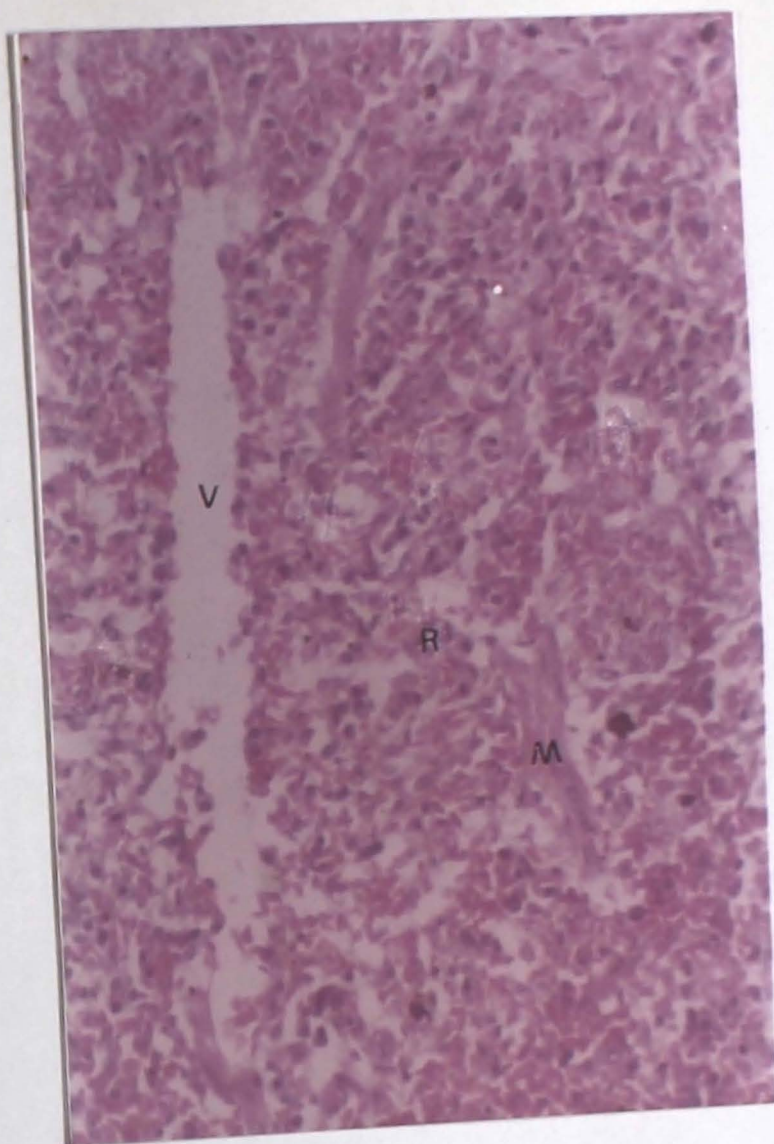
H. & E. x 100

Photomicrograph showing the course of the nodular artery (A) in the white pulp (W), periarterial lymphatic sheath (P) and the red pulp (R)

H. & E. x 200







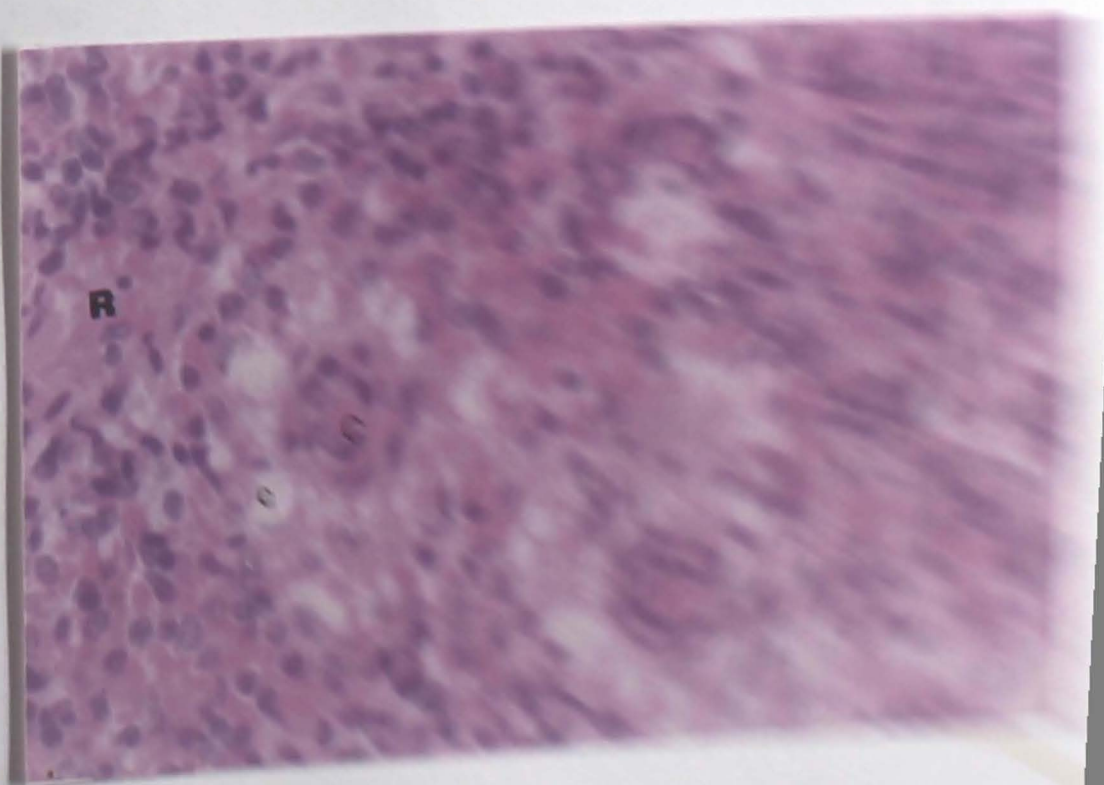
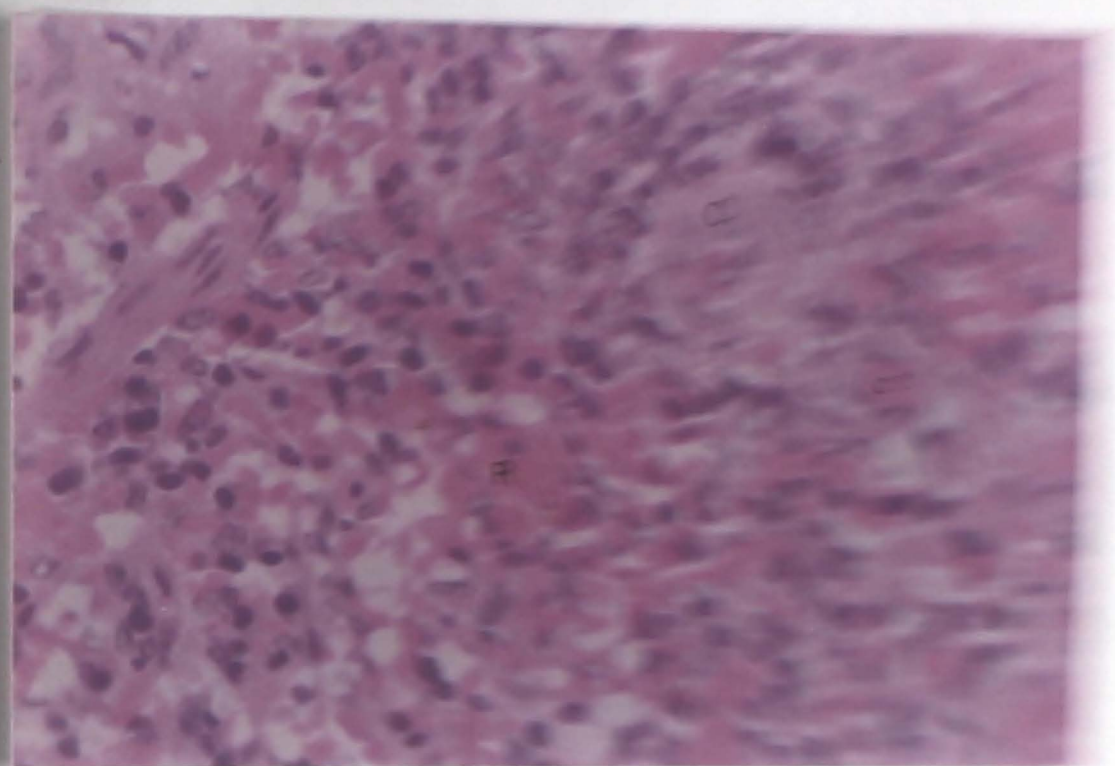
Photomicrograph showing sheathed capillaries (C), fine capillaries (c) in the red pulp (R)

H. & E. x 200

Photomicrograph showing sheathed capillaries (C), fine capillaries (c) in the red pulp (R)

H. & E. x 200







Photomicrograph showing distribution of iron (I) in the red pulp (R)

Perl's method x 200

Photomicrograph showing distribution of hemosiderin (H) in the red pulp (R)

Turnbull blue method x 200

## DISCUSSION

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The spleen of the camel differed greatly in structure and position from that of other domesticated animals as it was much smaller in proportion to the body weight and size. The crescent shaped spleen of camel presented a head, a body and a tail as reported by Hegazi (1953) and Smuts and Bezuidenhout (1987). Whereas, Purohit and Rathor (1958) mentioned the sickle shaped spleen in camel. The spleen was elongated and elliptical in ox (McLeod *et al.*, 1964; Getty, 1975; Nickel *et al.*, 1979 and Awal *et al.*, 1991a), tongue or falciform shaped in dog (Miller, 1965 and Getty, 1975), triangular in sheep and goat (May, 1970; Getty, 1975; Nickel *et al.*, 1979 and Awal *et al.*, 1991b) and long and narrow in pig (Getty, 1975). The spleen of the horse was coma shaped with its wide dorsal extremity and a pointed ventral extremity (Nickel *et al.*, 1979). Smallwood (1992) reported that the spleen of ox was tongue shaped whereas, that of the sheep and the goat were oval shaped.

The average weight, volume, length and width of spleen of camel was about 365 gm, 470 ml, 45 cm and 14 cm, respectively. The width decreased gradually toward both extremities. Similarly, Hegazi (1953) mentioned that the spleen of camel weighed about 450 gm in adult camel with its average length 40 cm, and a width of 9 cm in the middle part. However, Purohit and Rathor (1958) have reported that the average weight,

length and width of camel spleen was about 900 gm, 52.5 cm and 20 cm. respectively. Smuts and Bezuidenhout (1987) reported the weight of spleen was approximately 1000 gm in the adult camel. Getty (1975) and Nickel *et al.* (1979) reported that the average weight, length and width of spleen was about 900 gm, 50 cm and 15 cm, respectively, in bovine; 100 gm, 15 cm and 10 cm, respectively in sheep, 350 gm, 60 cm and 10 cm in pig and 1000 gm, 50 cm and 25 cm in horse. Awal *et al.* (1991a) observed that the mean length, breadth and weight of the spleen of indigenous cattle were 34.86 cm, 8.85 cm and 355 gm, respectively, whereas, these parameters in sheep were 9.75 cm, 6.91 cm and 50.72 gm (Awal *et al.*, 1991b).

In the fresh state the spleen of camel was soft and elastic but not yielding and presented a dark greyish colour as reported by Purohit and Rathor (1958). It was hard in consistency indicating the large amount of interlobular connective tissue as earlier described by Hegazi (1953). However, a bluish red or somewhat purple colour has been reported in horse (Getty, 1975), cattle (McLeod *et al.*, 1964; Getty, 1975 and Awal *et al.*, 1991a) and in sheep (Awal *et al.*, 1991b).

The spleen of camel was situated in the left dorsal part of the abdominal cavity just above the dorsocaudal aspect of the rumen, extending obliquely from the root of the third lumbar transverse process to the tip of the seventh one. These findings were similar to the observations as noticed by Hegazi (1953) and Purohit and Rathor (1958) in camel. The spleen of bovine extended downward and forward from the upper part of the last two ribs to the level of sixth rib (McLeod *et al.*, 1964; Getty, 1975; Nickel

*et al.*, 1979 and Awal *et al.*, 1991a). The long axis of spleen of sheep was oblique and corresponded to the line drawn from ventral end of last rib to the middle of tenth intercostal space (Getty, 1975). The long axis of the spleen in pig was dorsoventral, curved and conformed to the left part of greater curvature of stomach under the vertebral end of last three ribs. Whereas, the dog spleen was situated ventral to the vertebral ends of last rib and first lumbar transverse process (Getty, 1975).

The spleen of camel was firmly attached to the dorsocaudal aspect of rumen by a small triangular area through the peritoneum as observed by Purohit and Rathor (1958). The dorsal part of the spleen was visible from the anterior of fourth lumbar vertebra and was related to the left kidney. The spleen of camel presented two surfaces, two borders, two extremities and a hilus. The parietal surface was convex and related to the obliquus abdominis internus muscle and to the left sublumbar region. The visceral surface was concave and related to the left dorsocaudal part of rumen. This confirmed the earlier findings of Hegazi (1953) and Purohit and Rathor (1958) in camel. The spleen of bovine, sheep, horse and black Bengal goat also had two surfaces, two borders, two extremities, a base and an apex (McLeod *et al.*, 1964; Getty, 1975; Nickel *et al.*, 1979; Ahmed *et al.*, 1982; Awal *et al.*, 1991a and Awal *et al.*, 1991b). The parietal surface was slightly convex and was related to the diaphragm. It presented the costal impressions of the last three ribs. The visceral surface was concave and was intimately attached to the parietal surface of dorsal sac of rumen. The similar findings were also observed in bovine, sheep and horse (McLeod

*et al.*, 1964; Getty, 1975 and Nickel *et al.*, 1979), in black Bengal goat (Ahmed *et al.* 1982), cattle (Awal *et al.*, 1991a) and sheep (Awal *et al.*, 1991b).

The lateral border of camel spleen was thin, convex, free and generally irregular in outline due to marked indentations throughout its length. A prominent notch was present anteriorly while posteriorly it terminated into a pointed end as reported by Purohit and Rathor (1958). Whereas, Hegazi (1953) found this border as insinuated inbetween the rumen and the diaphragm. The medial border was folded towards the parietal surface at the hilus on which the left kidney rested and marked a renal impression. Whereas, this border has been reported concave, thick and rounded up to the hilus, where it became thin up to the level of posterior extremity (Purohit and Rathor, 1958). The splenic artery entered the organ at the posterior third of this border. Both the borders of the spleen were thin and straight in dog (Miller, 1965), bovine (McLeod *et al.*, 1964), sheep (Getty, 1975) and cattle (Awal *et al.*, 1991a). Whereas, Hegazi (1953) observed that caudal border encircled the lateral convex border of the left kidney. The anterior extremity of camel spleen was thick and rounded with constricted neck whereas. the posterior extremity was thinner flattened and tapered at the end.

The hilus in the form of a wide depression was present in the lower third of the anterior border where the blood vessels were placed apart from each other as observed by Purohit and Rathor (1958). However, it was a small area located on the dorsal third of the visceral surface where

the artery and the vein were very close to each other in bovine (McLeod *et al.*, 1964; Getty, 1975 and Awal *et al.*, 1991a). Whereas, the hilus was close to the cranial border of the slightly concave visceral surface and extended to the entire length of the organ in horse (Nickel *et al.*, 1979). However, in sheep, it was close to the cranial angle as a round depression (Getty, 1975). It was like a long furrow dividing the visceral surface of the dog spleen longitudinally in two almost the equal halves (Miller, 1965).

The splenic branch of the hepatic artery ran along the visceral surface to enter the hilus about the middle of its medial border and supplied the middle and distal parts of the camel spleen as earlier described by Purohit and Rathor (1958) and Smuts and Bezuidenhout (1987). Whereas, the splenic artery, branch of right ruminal artery entered the anterior extremity and supplied that particular area only. However, in ox and sheep the splenic artery was a branch of right ruminal artery and passed cranially and to the left across the dorsal curvature of the rumen to enter the hilus of the spleen (McLeod *et al.*, 1964 and May, 1970). The splenic artery bifurcated into a cranial and a caudal primary branches which gave off several secondary branches. These further divided into tertiary branches leading into several fine branches. Both the primary branches entered the hilus independently and supplied a particular segment of the spleen without any anastomosis. Similar type of observations have been reported in man (Gupta *et al.*, 1976), dog (Gupta *et al.*, 1978a), goat (Gupta *et al.*, 1978b and Bajpai and Chandra, 1995a), sheep (Gupta *et al.*, 1979a and Jain and Singh, 1986), buffalo (Jain and Singh, 1988), cattle (Awal *et al.*, 1989) and camel (Radmehr, 1997).



## Capsule

A thick fibromuscular capsule covered the spleen of camel as already reported by Hegazi (1953), bovine (McLeod *et al.*, 1964 and Awal *et al.*, 1992) and domestic animals (Dellmann and Brown, 1987 and Dellmann, 1993). The capsule was constituted by dense connective tissue and smooth muscle fibres as described in domestic animals (Dellmann, 1971), horse (Getty, 1975) and goat (Bajpai and Chandra, 1995b). In the present study, the capsule thickness varied from 196 to 475  $\mu\text{m}$  at different levels of the spleen. A capsule thickness of 128  $\mu\text{m}$  to 232  $\mu\text{m}$  has been reported in goat (Bajpai and Chandra, 1995b). The capsule was covered by a serous peritoneal coat of squamous to cuboidal mesothelial cells as reported in other domestic animals (Dellmann, 1971; Bloom and Fawcett, 1975; Dellmann and Brown, 1987, Dellmann, 1993 and Awal *et al.*, 1992). McLeod *et al.* (1964) in bovine reported an incomplete serous coat covering the posteroventral part of visceral surface and parietal surface except for a narrow area near the anterior border. Whereas, the peritoneum was closely adhered to the capsule by cuboidal mesothelium in human beings (Greep, 1965) and horse (Getty, 1975).

A thin layer of loosely arranged connective tissue along with sparsely distributed connective tissue cells was present below the mesothelial cell layer as reported in domestic animals (Dellmann, 1971 and Bloom and Fawcett, 1975) and goat (Bajpai and Chandra, 1995b). In the present study, the capsule was rich in smooth muscle fibres which were arranged in three layers. An outer and inner layers of smooth muscle

fibres were oriented longitudinally and the middle layer was obliquely placed. The inner layer was of variable thickness and densely populated with smooth muscle cells. However, these smooth muscle fibres arranged in two layers were oriented at right angle to each other in horse and ruminants (Dellmann and Brown, 1987 and Dellmann, 1993). In general, the capsule was richly populated with smooth muscle fibres in camel (Hegazi, 1953), dog (Miller, 1965), pig (Dellmann, 1971), horse and ruminant (Bloom and Fawcett, 1975).

The present observations in camel were similar to the findings of Awal *et al.* (1992) in cattle and Bajpai and Chandra (1995b) in goat that a mixed population of reticular, collagenous and elastic fibres supported the framework of the capsule. The collagen fibres were densely arranged just above the outer layer of smooth muscle fibres. The smooth muscle fibres interwoven with collagen and elastic fibres have been reported in the outer layer of capsule in ruminant spleen (Dellmann and Brown, 1987 and Dellmann, 1993). The inner most zone of capsule was rich in elastic fibres in cattle (Awal *et al.*, 1992) and laboratory animals (Bloom and Fawcett, 1975) and goat (Bajpai and Chandra, 1995b). However, the connective tissue just below the mesothelium was devoid of elastic fibres.

The concentration of fine reticular fibres in the form of meshwork was maximum in the inner layer of capsule in camel as observed in cattle (Awal *et al.*, 1992) and goat (Bajpai and Chandra, 1995b). The reticular fibres were arranged longitudinally to form a uniform layer in the inner most part of the capsule. However, these fibres continued to the framework of the trabeculae where their orientation was vertical as compared to the

fibres present in capsule. The capsule was slightly thicker at the point where the trabeculae were emitted from its inner most surface.

During present study small blood capillaries and nerve fibres of varying number were irregularly distributed in the capsule as reported in dog (Miller, 1965) and other domestic animals (Dellmann and Brown, 1987 and Dellmann, 1993). A varying PAS reaction has been observed in the different components of the capsule. A highest and lowest capsule thickness of  $475.41 \pm 29.75 \mu\text{m}$  and  $196.14 \pm 8.45 \mu\text{m}$ , respectively, was recorded in the camel as compared to  $227.1 \pm 34.19 \mu\text{m}$  and  $135 \mu\text{m}$  in ageing goat, respectively (Saigal *et al.*, 1977).

The branching and anastomosing trabeculae were given off from the deeper face of the capsule which subdivided the spleen into smaller compartments. Similar type of findings have been reported in camel (Hegazi, 1953), bovine (McLeod *et al.*, 1964), horse (Getty, 1975), ox (Raghavan, 1964), goat (Bajpai and Chandra, 1995b), dog (Miller, 1965) and cattle (Awal *et al.*, 1992). Whereas, Greep (1965) in human beings reported a rich arborizing network of trabeculae subdividing the organ into communicating compartments.

The trabeculae of varying thickness reached to a variable depth and some of them were uniform with a free pointed end. Two to three branches of trabeculae were encountered as observed in goat (Bajpai and Chandra, 1995b). The smooth muscle fibres, elastic, reticular and collagen fibres constituted the framework of the trabeculae along its longitudinal axis which was vertical to their orientation in the capsule. The smooth muscles

in the capsule assisted the smooth muscles of the trabeculae. The elastic and smooth muscle fibre in the capsule and trabeculae probably help in the volume changes possible in domestic animals (Dellmann, 1971, 1993; Bajpai and Chandra, 1995b). Greep (1965) reported that the capsule in human beings contained little muscle and was, therefore, incapable of the profound contraction exhibited by the muscular capsule of the spleen in dog and cat.

The thin reticular fibres along with few collagen fibres formed a wide mesh work in camel. The reticular fibres became progressively thinner in the terminal branches of the trabeculae as mentioned in goat (Bajpai and Chandra, 1995b). The elastic fibres of varying concentrations were oriented in different planes in the trabeculae. At places the large clumps were present particularly in the deeper part of the free end of trabeculae. In goat, Bajpai and Chandra (1995b) observed that the elastic fibres were extended from the capsule into the trabeculae. The trabeculae contained a large number of elastic fibres than the capsule as reported by Bloom and Fawcett (1975) in domestic animals. Only few collagen fibres were observed in the trabeculae. In dog, Miller (1965) reported that the collagenous fibres of the trabeculae continued directly in to the reticular fibres of the splenic pulp. The connective tissue fibres and smooth muscle fibres of the trabeculae were moderately PAS positive.

The large trabeculae were occupied by the arteries, veins and nerve bundles with a peripheral layer of smooth muscle fibres like that of goat (Bajpai and Chandra, 1995b). According to Greep (1965) in human beings,

Dellmann (1971, 1993) and Bloom and Fawcett (1975) in domestic animals, the trabeculae contained arteries, veins, lymph vessels and nerves. In dog, Miller (1965) reported that the large intrasplenic arteries were present mainly in the trabeculae. The trabeculae originated from the hilus usually contained major vessels but were poor in smooth muscle fibres in cattle (Awal *et al.*, 1992). A few nerve fibres and fine blood capillaries were irregularly distributed in the trabeculae. The highest trabecular thickness was  $377.71 \pm 11.98 \mu\text{m}$  and the least was  $212.30 \pm 6.52 \mu\text{m}$ . Whereas, a maximum per cent trabecular tissue of  $12.50 \pm 0.37$  and minimum  $10.96 \pm 0.45$  per cent was observed in camel. But a maximum 9.13 per cent and minimum 6.05 per cent trabecular tissue was observed by Saigal *et al.* (1977) in ageing goat.

### **White pulp**

The splenic white pulp was a lymphoreticular tissue composed of lymphocytes, plasma cells macrophages and other free cells lying in a reticular meshwork. Whereas, in domestic animals Dellmann and Brown (1987) reported that the reticular fibres and reticular cells formed a three dimensional stroma containing sequestered lymphocytes, macrophages and other accessory cells similar to those seen in lymph nodes. The white pulp was distributed throughout the spleen as lymphatic nodules and as periarterial lymphatic sheaths (PALS) already reported in domestic animals (Dellmann, 1971, 1993 and Dellmann and Brown, 1987) The well developed PALS coaxially surrounded the central artery. Whereas, Miller (1965) reported that the diffuse lymphatic tissue was elaborated along the



arteries in the dog spleen. The PALS as a cylindrical sheath of lymphoreticular tissue was present around a central artery in domestic animals (Dellmann and Brown, 1987 and Dellmann, 1993).

Greep (1965) and Bloom and Fawcett (1975) further reported that the PALS were present until the vessels became small arterioles. In the present study, the PALS at its periphery merged with the marginal zone of the splenic nodules. The sheath had a loose, irregular framework of reticular fibres and the reticular cells. About the periphery of lymphatic sheath, these fibres became circumferentially arranged where flattened reticular cells formed the concentric layers and thereby delimiting the lymphoid tissue from the surrounding red pulp as reported in domestic animals (Bloom and Fawcett, 1975). Similarly, Greep (1965) stated that the reticular elements followed a circumferential pathway and formed a rim to the white pulp in domestic animals. Whereas, Dellmann (1971, 1993) reported that the lymphoreticular tissue was concentrically arranged in the periphery of the sheath in domestic animals. The reticular framework was occupied by lymphocytes of various dimensions predominantly belonging to the small and medium sized variety like in domestic animals (Bloom and Fawcett, 1975) and cattle (Awal *et al.*, 1992).

A few elastic fibres were interspersed among the reticular fibres near the nodular artery. However, the concentration of lymphocytes was variable at different places and generally it constituted a major portion of splenic nodule. The plasma cells and macrophages were only occasionally found but their number increased towards the periphery of the sheath.

The erythrocytes were present at the junction of white and red pulp as reported in domestic animals (Bloom and Fawcett, 1975).

Small rounded bodies were scattered throughout the red pulp called Malpighian corpuscles or splenic nodule in camel as also reported by Hegazi (1953). These nodules composed of dense lymphoid tissue along with reticular fibres surrounded the small arteries. Similarly, the splenic nodules of different dimensions were observed as ovoid mass in domestic animals (Getty, 1975; Nickel *et al.*, 1979; Raghavan, 1964; Awal *et al.*, 1992 and Bajpai, 1992). The splenic nodules were randomly distributed in the splenic parenchyma as observed in goat (Bajpai, 1992). The splenic nodules were often observed at bifurcation of nodular artery. The lymphatic tissue occurred mainly as splenic nodules and occasionally as PALS in goat (Bajpai, 1992). Whereas in cat and dog the lymphatic follicles were large and the PALS were small (Greep, 1965)

The nodules generally occurred singly and occasionally aggregations of two to three nodules were also observed. The union between the different nodules was differentiated by the presence of marginal zone and part of red pulp in between the two. The nodules had a fine meshwork of reticular connective tissue containing mainly lymphocytes of various sizes as observed in cattle by Awal *et al.* (1992). Only few reticular fibres were interspersed in the central part of white pulp, however, these were coarse and concentrically arranged in the marginal zone as observed in cattle (Awal *et al.*, 1992). Similarly, Greep (1965) reported that the cellular component of splenic nodules was mainly contributed by the lymphocytes, plasma cells, macrophages and few RBCs and hemosiderin pigments.

The lymphocytes were mainly occupied by the round to oval shaped lightly stained nuclei. The nucleolus was mostly centric in position and strongly basophilic. The scanty lightly eosinophilic cytoplasm occupied the periphery of the cell. The lymphocytes were aggregated into small irregular clumps and small cords. In goat, Bajpai (1992) also noticed that the lymphocyte, presented the mitotic figures indicating the active proliferative stage.

The small vacuolated areas were observed in between the aggregations of the lymphocytes being occupied by RBCs and hemosiderin pigment. The macrophages were irregularly distributed inbetween the lymphocytes having less basophilic nuclei with uniform distribution of fine chromatin material. Few plasma cells were also observed in the nodule. Few collagenous fibres were interspersed inbetween the reticular fibres of the marginal zone particularly around the blood vessels.

In domestic animals, Dellmann (1971, 1993) reported that the lymphoreticular tissue was concentrically arranged at the periphery of the splenic nodules. The marginal zone was sandwiched in between the Malpighian follicle and red pulp being constituted by several concentrically arranged reticular cells, macrophages, lymphocytes, plasma cells and the reticular network where the blood capillaries of white and red pulp were also observed. However, a transitional region in between the lymphoid tissue and red pulp has been reported in domestic animals (Dellmann, 1971, 1993 and Bloom and Fawcett, 1975). The constituents of marginal zone closely resembled the red pulp as described in domestic animals

(Dellmann, 1971), however, it was clearly distinguished by the presence of a thin zone of reticular and collagen fibres encircling the splenic nodule. Greep (1965) in human beings, reported that the cords of the red pulp were directly continuous with the marginal zone and were of similar structure.

In the marginal zone the reticular fibres of cords formed a closely knit concentric network which contained greater amount of small lymphocytes and plasma cells than the rest of the red pulp as reported in domestic animals (Bloom and Fawcett, 1975). The concentration of lymphocytes was drastically reduced in the marginal zone as compared to the nodule. The branched reticular cells were concentrically arranged in the marginal zone like in domestic animals (Dellmann, 1971, 1993). The reticular cells nuclei were elongated and cylindrical with less basophilic chromatin material. The number of blood capillaries and red blood cells was increased. The small venous sinuses were circumferentially oriented around the white pulp like in domestic animals (Bloom and Fawcett, 1975). The presence of marginal zone in each splenic nodule was not a constant feature in the camel.

The two or more branches of nodular arteries were observed in a single nodule. These arteries were *eccentrically oriented* in the nodules thus the terminology "central artery" given in some text books was not true in the camel like in goats (Bajpai, 1992). The loose tunica adventitia was surrounded by reticular fibres and a sheath of lymphoid tissue. The tunica media was constituted by few layers of concentrically arranged

smooth muscle fibres. The nodular artery and its branches were having cuboidal or even columnar endothelium which may be so high as to completely efface the lumen.

The capillaries observed in the marginal zone lacked the tunica intima and smooth muscle fibres and were surrounded by few reticular cells and a less concentration of the lymphocytes, in the camel. Whereas, in domestic animals, Bloom and Fawcett (1975) reported that the nodular artery was a muscular artery with tall endothelial cells and one or two layers of smooth muscle cells. Throughout its course within the white pulp, the artery gave off numerous collateral capillaries which supplied the lymphoid tissue of the sheath. Initially, the capillary wall had tall endothelial cells, basal lamina and an investment of pericytes; further on, the endothelium became low and the pericytes disappeared. The reticular fibres formed a meshwork around the blood capillaries where a few elastic fibres were also observed.

The density and arrangement of reticular framework varied greatly in different layers of the blood vessels as previously noticed by Awal *et al.* (1992) in cattle. The large reticular fibres were comparatively more numerous and densely arranged in the different layers of nodular artery whereas, these fibres formed irregular meshwork in the small branches of the arteries in the camel. In the present study, the thick elastic fibres were observed in the tunica intima whereas, thin fibres surrounded the periphery of the nodular artery. The collagen fibres were noticed only in the peripheral layers of the nodular artery. Similar, observations have been



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which constituted the splenic pulp and most of the pulp was red. Whereas, in domestic animals Dellmann (1971, 1993) reported that the pulp between the capsule and the trabeculae was subdivided in the red and white pulp. However, Dellmann and Brown (1987) and Dellmann (1993) reported that the capsule, trabeculae and reticular fibres supported the splenic parenchyma composed of red and white pulp. The red pulp was composed of pulp arterioles, sheathed and terminal capillaries, splenic sinuses or sinusoids and splenic cords in camel like other domestic animals (Dellmann, 1971, 1993; Bloom and Fawcett, 1975 and Dellmann and Brown, 1987). Whereas, Hegazi (1953) in camel observed the numerous ellipsoids, arterioles and sinusoids. The meshwork of fine reticular fibres and processes of reticular cells contained all the blood elements of the blood as already reported in camel (Hegazi, 1953) and cattle (Awal *et al.*, 1992).

The splenic cords of variable thickness were present between splenic sinuses. The loosely arranged reticular fibres network contained numerous free erythrocytes, reticular cells, plasma cells, macrophages, lymphocytes, other leucocytes and phagocytic cells as reported in domestic animals (Greep, 1965; Dellmann, 1971, 1993; Bloom and Fawcett, 1975 and Dellmann and Brown, 1987). The splenic cords were completely invested by stellate reticular cells. The reticular cells were having numerous branching processes supported by reticular fibres which divided the cordal spaces into freely communicating compartments (Greep, 1965) The membranous processes of reticular cells tended to form the channel-like

structure that may function to conduct blood toward the interendothelial slits in the sinus wall as described by Dellmann and Brown (1987) and Dellmann (1993) in domestic animals. Some reticular cells may actually represented fixed macrophages of monocytic origin. The macrophages were large rounded or irregularly shaped cells, with a vesicular nucleus and abundant cytoplasm. They often contained engulfed erythrocytes, neutrophils, etc.

The number of the lymphocytes and leucocytic cells was drastically reduced, however, at places small clumps were also present. The small groups of smooth muscle fibres were oriented in varying directions. However, numerous smooth muscle cells have been reported in red pulp of ruminant and pig (Dellmann and Brown, 1987 and Dellmann, 1993). In the marginal zone the reticular fibres of the splenic cords formed a closely knit concentric network and the meshes of the cords had a greater content of small lymphocytes and plasma cells than the rest of the red pulp. The collagenous fibres of the trabeculae continued directly into the red pulp as described in dog (Miller, 1965) and domestic animals (Bloom and Fawcett, 1975). The large number of phagocytic cells of wandering and fixed types were found to have been engorged with the wornout blood cells, presence of iron pigments in the meshes of red pulp. Similar type of observations have also been described by Trautman and Fiebiger (1957) in horse and ruminants and Ahmed *et al.* (1987) in black Bengal goats.

The capillaries opened into the meshwork and occasionally, encapsulated nerve endings were observed in the red pulp. The splenic

sinuses in camel were elongated, irregularly contoured slit like vascular channels, whereas, Dellmann and Brown (1987) and Dellmann (1993) reported wide vascular channels being lined with elongated, longitudinally oriented endothelial cells in domestic animals.

The splenic sinuses were fairly distributed within the red pulp as observed by Awal *et al.* (1992) in cattle. The sinuses permeated the entire red pulp and were numerous around the white pulp and displayed a unique arrangement of endothelium and basal lamina. The sinuses were lined by the elongated reticuloendothelial cells. These cells were thick in the central nuclear region with tapered ends and had longitudinally oriented nucleus as reported in domestic animals (Bloom and Fawcett, 1975). These endothelial cells were active phagocytes and were noticed to have engulfed foreign bodies in cattle (Awal *et al.*, 1992). The lining cells rest upon the fenestrated basal lamina and were supported by a layer of fine reticular fibres. The intercellular gaps were commonly observed like in domestic animals (Dellmann, 1971, 1993). Whereas, Greep (1965) in human beings reported that reticular cells were without desmosomes or without interposition of intercellular cement and thus these were easily separated from each another. Gaps might appeared between the lining cells of the sinuses where the blood cells were found traversing the wall of the sinuses. In dog, Thomas (1967) observed that the numerous apertures through the sinus wall connected cord spaces and sinuses, thus the blood cells appeared to be in transit through the sinus wall. The cellular elements of circulating blood can easily migrate through the sinus wall by traversing the

vessels. The single layer of smooth muscle cells constituted the tunica media whereas, the reticular, collagen and elastic fibre constituted thin tunica adventitia. The elastica interna and externa were absent. The penicillar artery further divided in the red pulp leading to the formation of sheathed capillaries which were elipsoidal or spherical in shape. These capillaries had characteristic thickening of their walls called a Schweigger-Seidel sheath. These capillaries were generally associated with the single sheath, however, at places two to four capillaries were ensheathed by a single layer or two to three sheaths arranged in a series. The cells were rounded toward the lumen and stellate towards the periphery. The erythrocytes formed a regular constituent and were generally present among the cells of the sheath. The lumen of obliquely cut capillaries was not visible. The endothelium was formed by tall fungi-form cells which were connected by the intercellular junctions and rest on a thin basal lamina. This arrangement was remarkably similar to that described by Bloom and Fawcett (1975) in domestic animals.

In domestic animals, Dellmann (1971) and Nickel *et al.* (1979) reported that the blood reached the pulp through the pulp arteries which break up and formed little brushes (Penicilli). The arteries of the pulp then became the sheathed arteries and gave rise to capillaries supplying to the reticular meshwork of the red pulp. Whereas, Dellmann and Brown (1987) and Dellmann (1993) reported that the pulp artery formed the penicillus (brush like tuft), composed of two to six straight branches, each with three segments. The initial part, the pulp arteriole was the longest



interendothelial clefts and the fenestrations of the basal lamina in domestic animals (Bloom and Fawcett, 1975). The basal lamina of venous sinuses was moderately PAS positive in camel.

The walls of sinuses lacked the muscular coat, however, the smooth muscle cells were observed in the vicinity of these sinuses in camel. The clusters of erythrocytes and leucocytes were present in the lumen of sinusoids. Numerous ellipsoids and sinusoids were scattered in the red pulp. The ellipsoids were formed of the arterioles of the penicilli which were the branches of smaller arteries. Each arteriole was surrounded by connective lamellae of reticular tissue with lymphocytes as earlier reported in camel (Hegazi, 1953).

According to Lewis (1957) the splenic ellipsoids in dog were highly developed where they ensheathed the arterial capillaries a short distance prior to their opening into the pulp and had two or more sinusoids in close relation. They contained a closely packed aggregation of cells identical to the reticular cells of the pulp. However, these ellipsoids were poorly developed in sheep and contained small aggregations of reticular cells surrounding the arterial capillaries. Jacobsen (1971) described that the ellipsoid sheaths of Schweigger Seidel (sheathed capillaries) in dog were cellular structures encircling terminal branches of penicillar arteries. A very thin layer of red pulp reticulum separated the ellipsoid from sinus.

The penicillar arteries or the arteries of the red pulp pursued a radiating course. The periarterial tissue was drastically reduced. The tall endothelial cells were oriented along the longitudinal axis of the blood

vessels. The single layer of smooth muscle cells constituted the tunica media whereas, the reticular, collagen and elastic fibre constituted thin tunica adventitia. The elastica interna and externa were absent. The penicillar artery further divided in the red pulp leading to the formation of sheathed capillaries which were elipsoidal or spherical in shape. These capillaries had characteristic thickening of their walls called a Schweigger-Seidel sheath. These capillaries were generally associated with the single sheath, however, at places two to four capillaries were ensheathed by a single layer or two to three sheaths arranged in a series. The cells were rounded toward the lumen and stellate towards the periphery. The erythrocytes formed a regular constituent and were generally present among the cells of the sheath. The lumen of obliquely cut capillaries was not visible. The endothelium was formed by tall fungi-form cells which were connected by the intercellular junctions and rest on a thin basal lamina. This arrangement was remarkably similar to that described by Bloom and Fawcett (1975) in domestic animals.

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and continued into peculiar structures called sheathed capillaries or ellipsoids.

The maximum portion of splenic parenchyma was constituted by the red pulp. A maximum and minimum per cent red pulp of  $80.13 \pm 0.87$  and  $73.13 \pm 0.76$  was observed, respectively, in camel as compared to 87.66 and 78.05 per cent observed in ageing goats (Saigal *et al.*, 1977).

## SUMMARY

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The present study was planned on 8 adult healthy camels to record gross anatomy, histomorphology and micrometry of various components of the spleen. The spleen of camel was situated in the left dorsal part of the abdominal cavity extending obliquely from the posterior border of third lumbar transverse process to anterior border of the seventh lumbar transverse process. The anterior most part of spleen was firmly attached to dorsocaudal part of rumen by a small triangular area through gastrosplenic ligament. The crescent shaped spleen presented a head, a body and a tail with an average weight and volume of 365 gm and 470 ml, respectively. However, the average length and width were 45 cm and 14 cm at the widest part of the spleen.

It was dark greyish coloured and hard in consistency indicating the large amount of interlobular connective tissue. The hilus was a depression, where the blood vessels were placed quite well apart from each other. The splenic branch of the hepatic artery ran along the visceral surface to enter the hilus of the organ about the middle of its medial border. The splenic artery before entering the hilus bifurcated into cranial and caudal primary branches. Both the primary branches were supplying the caudal two-third part of the spleen. The cranial part was supplied by the splenic branch of right ruminal artery. The several secondary branches emerged from the

primary branches, which led to tertiary and several finer branches. Both of the primary branches supplied a particular segment of the spleen without any anastomosis.

The capsule of the spleen was lined with an outer layer of mesothelial cells. The smooth muscle fibres were arranged in three layers along with collagen, reticular and elastic fibres. These fibres were oriented along the longitudinal axis of capsule and later these constituted the trabeculae. The white pulp of the spleen was a lymphoreticular tissue consisting of lymphocytes, plasma cells and macrophages surrounding the major arterial blood vessels. The lymphatic tissue was either arranged as splenic nodules or periarterial lymphatic sheath. The isolated nodules were mainly scattered singly in the splenic parenchyma having a fine meshwork of reticular connective tissue containing mainly lymphocytes of various sizes. These fibres were concentrically arranged in the marginal zone with a sparse distribution in the central part of white pulp. Small vacuolated areas were observed inbetween the aggregations of lymphocytes being occupied by erythrocytes and hemosiderin pigment. Eccentrically located nodular arteries were generally divided into two to three branches. The different layers of nodular artery were moderately PAS positive. The statistical values for mean diameter of splenic nodule, mean diameter of smallest and largest follicles were 308.26, 188.23 and 420.13  $\mu\text{m}$ , respectively.

A maximum portion of splenic parenchyma was constituted by the red pulp (73.13 to 80.13%) having pulp arterioles, sheathed capillaries,



terminal capillaries, splenic sinuses and splenic cords. A meshwork of reticular fibres was observed throughout the red pulp. The splenic cords of varying thickness contained numerous erythrocytes, reticular cells, plasma cells, macrophages, lymphocytes, other leucocytic and phagocytic cells. Small groups of isolated smooth muscle fibres were oriented in varying directions. The splenic sinuses in the form of elongated, slit like vascular channels were mainly present along the longitudinal axis of the trabeculae just adjacent to red pulp. However, their dimensions were reduced in the red pulp. The sinuses were lined with elongated reticuloendothelial cells. The penicillar artery divided in the red pulp and formed the sheathed capillaries with the characteristic thickening of their walls. There was a significant correlation between the capsule and trabecular thickness, mean diameter of Malpighian follicle, diameter of smallest and largest follicle, number of Malpighian follicle per  $\text{cm}^2$  area and different per cent areas of splenic parenchyma.

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