

The spleen of the one humped camel (*Camelus dromedarius*) has a unique histological structure

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ABSTRACT

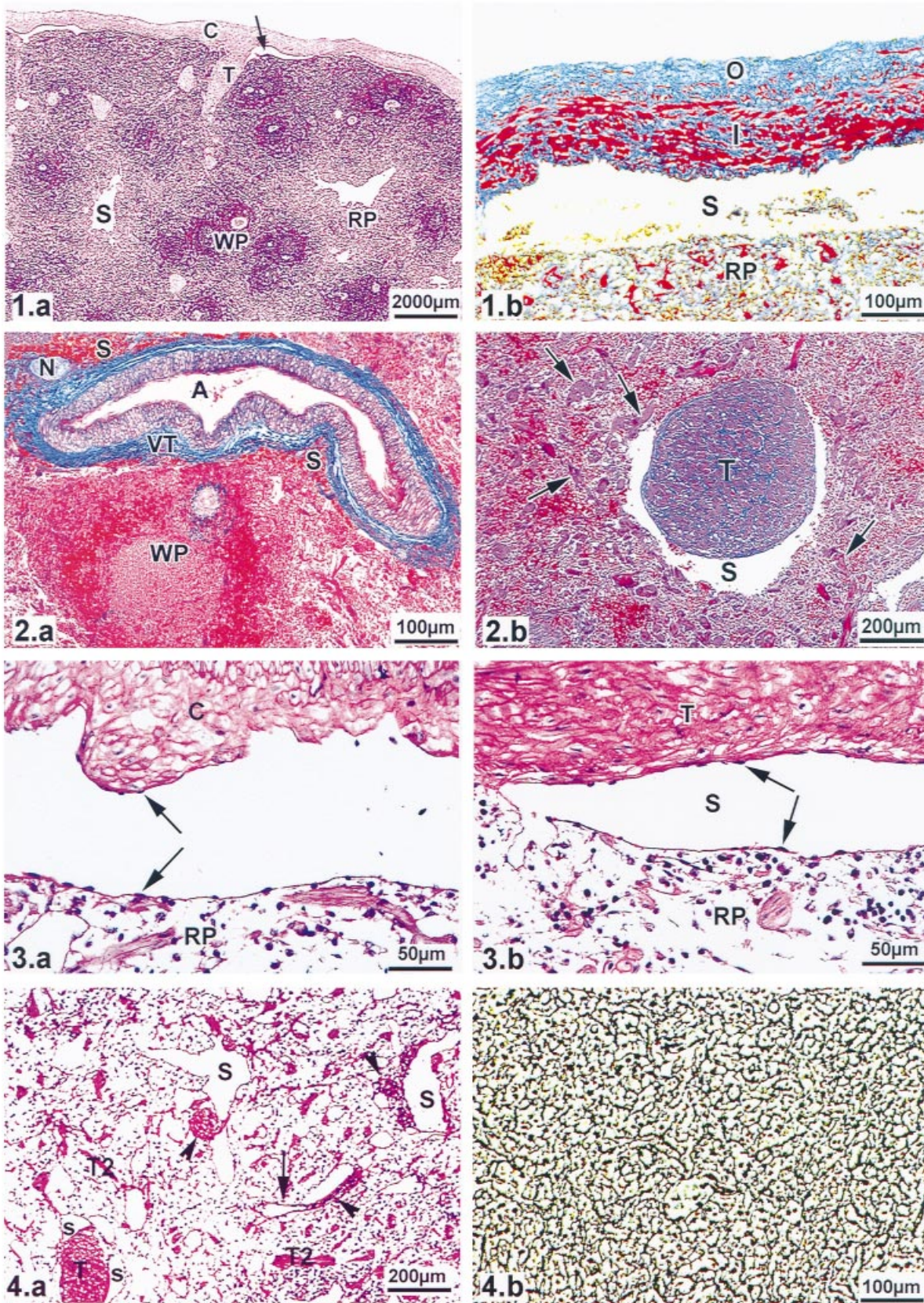
The histology and structure of 38 spleens of the dromedary (aged 0.5–15 y) were studied in relation to age. The spleen was found to have a thick capsule (292 ± 106 mm) divided into an outer layer (113 ± 39 mm) composed mainly of connective tissue and an inner layer (180 ± 81 mm) consisting mainly of smooth muscle cells. Vascular and avascular trabeculae extend from the capsule, the former containing arteries and nerves but no trabecular veins, the latter being divided structurally into primary and secondary trabeculae. Subcapsular and peritrabecular blood sinuses around primary and vascular trabeculae are unique to the camel spleen. The central artery emerges from the periarterial lymphatic sheath and branches into up to 4 penicilli which extend as sheathed arterioles (42 ± 8 μ m). These are found near or surrounded by blood sinusoids of the red pulp. A wide marginal zone surrounds the white pulp and contains sheathed arteries but no marginal sinuses. The red pulp is characteristically divided into cords by secondary trabeculae and contains venous sinusoids of different sizes. The camel spleen is of a sinusal type that can store blood. The thick muscular capsule and trabeculae pump the stored blood according to the body's need. Both closed and open circulations are found. The venous return is unique as the blood flow is from the venous sinusoids of the red pulp to the peritrabecular sinuses to the subcapsular sinuses to the splenic vein. No significant structural differences related to age were found.

Key words: White pulp; red pulp; marginal zone; periarterial macrophage sheath.

INTRODUCTION

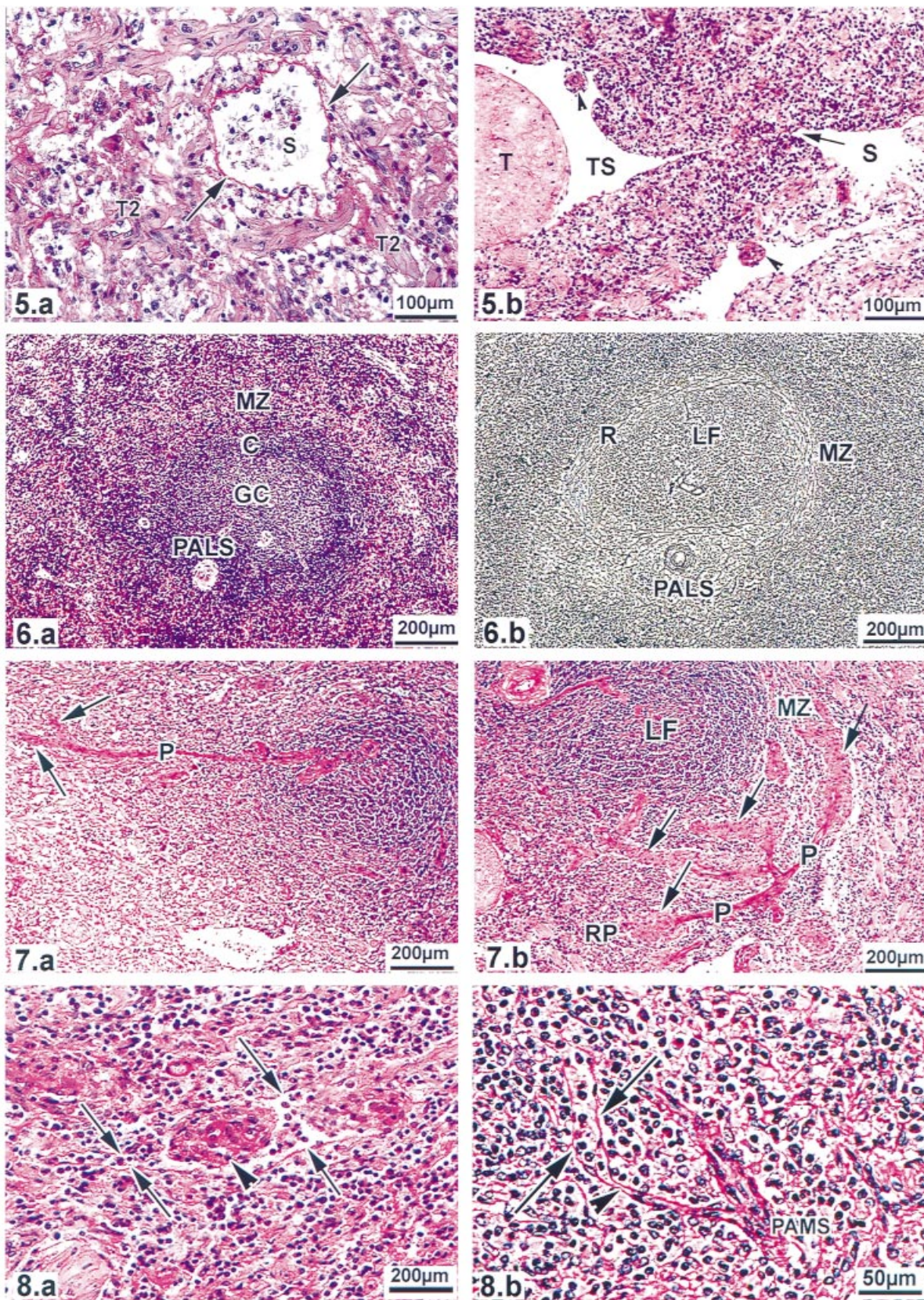
The camel is an important source of meat, milk and hide in several countries and there is growing interest in its meat and milk products (Hamers & Muyldermans, 1998). The most important disease to affect the camel is the blood parasite, *Trypanosoma evansi* (Ngeranwa et al. 1993). The blood parasites are removed and phagocytosed in the spleen (Schnizer et al. 1972; Chen & Weiss, 1973). In general, it is the largest lymphoid organ and the most important organ of immunological defence for the blood (Pabst, 1993). The white pulp of the mammalian spleen is composed of the periarterial lymphatic sheath (PALS) and lymph follicles (Raviola, 1994). Lymph follicles predominate in the human spleen whereas the PALS

predominates in the rat (Steiniger et al. 1997). The reticular framework of the white pulp is divided into 2 parts in mice (Tanaka et al. 1996) and man (Satoh, 1997), one part surrounding the PALS and the other surrounding the lymph follicles. The marginal zone is the site of heavy blood traffic and filtration. Venous sinusoids are found near or within it (More et al. 1964; Weiss, 1964; Burk & Simon, 1970). It envelops the white pulp in rodents and rabbits (Snook, 1950) but surrounds only the lymph follicles in humans and contains no marginal or perimarginal sinuses. It is separated from the red pulp by a perifollicular zone (Steiniger et al. 1997). The red pulp of several species is composed of splenic cords separated by blood sinusoids (Dellmann & Brown, 1976). The periarterial macrophage sheath (PAMS) is composed of macro-



Figs 1-4. For legends see p. 429.

Fig. 1. (a) Overview of the camel spleen showing the capsule (C), trabecula (T), subcapsular sinus (arrow), white pulp (WP) and red pulp (RP). H&E. (b) The splenic capsule is divided into an outer layer (O) composed mainly of connective tissue and an inner layer (I) composed mainly of smooth muscle cells. S, subcapsular blood sinus; RP, red pulp. Trichrome.



Figs 5–8. For legends see p. 429.

phages supported by a reticular network (Raviola, 1994). Mice, rats and rabbits lack sheathed arteries (Snook, 1950). The diameter of the PAMS has been determined in several animals (Seki & Abe, 1985). The splenic circulation may be open (Thomas, 1967; Fukuta et al. 1976; Irino et al. 1977; Blue & Weiss, 1981*a*), closed (Simon, 1970*a*; Murkami et al. 1973) or a combination of both (Barnhart et al. 1976; Chen, 1978). There are sinusal type spleens, e.g. in horses, dogs and pigs (Brown & Dellmann, 1976), man, rats and rabbits (Blue & Weiss, 1981*b*) and nonsinusal types, e.g. in cats, ruminants (Brown & Dellmann, 1976) and mice (Blue & Weiss, 1981*a*). The age-structure relationship of the human spleen has been studied (Timens et al. 1989) and the relationship between the regeneration of the splenic transplant and the age of the host and the donor has been investigated in rats (Westermann et al. 1988). In spite of the immunological and haematological importance of the spleen and the advantages of knowledge of its cell physiology, very little is known about the spleen of the camel (Hegazi, 1953; Bareedy et al. 1982; Abd El Aal, 1994; Radmehr, 1997). It has been shown that the white pulp is composed of lymph follicles around blood vessels. The marginal zone is poorly delimited from the white and the red pulp and composed of diffuse lymphatic tissue (Bareedy et al. 1982; Abd El Aal, 1994). There are numerous ellipsoids in the red pulp (Hegazi, 1953) and the marginal zone (Abd El Aal, 1994). Radmehr (1997) described the branching of the splenic artery. The following structural characteristics found in other species have not been described in the camel: the PALS, corona and the reticular framework of the white pulp, splenic circulation, marginal sinuses and morphometry. The aim of this study was therefore to examine the different splenic compartments and age-structure relationships using morphometry and to describe the splenic circulation in the camel.

MATERIALS AND METHODS

The study was carried out on 38 spleens obtained from clinically healthy camels of both sexes with a mean age 9.5 y (range 0.5–15 y). Fresh specimens were obtained from 35 spleens directly after slaughter and were fixed in 10% buffered formalin. As these spleens probably contracted during bleeding to an unphysiologically small size, to show the splenic compartments of a normal spleen 3 spleens were perfused with 0.9% saline through the splenic artery to remove the blood from the blood vessels and avoid blood clots. The splenic vein was then ligated and each

spleen infused with 10 l of 10% buffered formalin through the splenic artery, after which the splenic artery was tied off. The infused spleens were immersed in the same fixative for 24 h before specimen collection. All specimens were prepared for paraffin sections and stained with haematoxylin and eosin, (H&E) Crossman trichrome, Gomori's reticulin, Orcein, PAS (Böck, 1989) and Giemsa (Burck, 1988). The thickness of the capsule and the diameter of the PAMS of all samples were measured using a micrometer eye piece (Carl Zeiss, Germany) and compared at different ages. The results are given as mean \pm standard deviation.

RESULTS

The camel spleen was composed of a thick capsule surrounding the splenic parenchyma (Fig. 1*a*). The capsule was 292 ± 106 μ m thick and was divided into clearly demarcated outer and inner layers (Fig. 1*b*). The outer layer (113 ± 39 μ m) consisted mainly of connective tissue including collagen, elastic and reticular fibres with few smooth muscle cells. The inner layer (180 ± 81 μ m) was composed predominantly of smooth muscle cells supported by reticular, collagen and elastic fibres. Vascular and avascular trabeculae extended from the capsule into the splenic parenchyma. The former contained trabecular arteries and nerves but no veins and were composed mainly of connective tissue made up of collagen, reticular and elastic fibres and a few laterally located smooth muscle cells parallel to the longitudinal axis of the trabeculae (Fig. 2*a*). The latter were divided into primary and secondary trabeculae. The primary trabeculae originated from the capsule and had a similar structure to that of the inner layer of the capsule, being composed mainly of smooth muscle cells lying parallel to the longitudinal axis of the trabeculae and supported by reticular, collagen and elastic fibres (Fig. 2*b*). The secondary trabeculae were composed mainly of parallel smooth muscle cells with reticular fibres among them. Subcapsular blood sinuses extended under the capsule and connected to peritrabecular sinuses which surrounded both the primary and the vascular trabeculae (Fig. 3*a, b*). The red pulp was divided into small compartments (cords) by the secondary trabeculae (Fig. 4*a*), each cord being composed of a reticular network containing the different blood cells (Fig. 4*b*). Blood sinusoids with a continuous basement membrane were irregularly distributed in the red pulp (Figs 4*a, 5a*) and connected to the peritrabecular sinuses (Fig. 5*b*). The white pulp was demarcated by circumferential reticular fibres

that clearly divided the compartments of the white pulp into PALS and lymphoid follicle (Fig. 6*a, b*). The lymphoid follicles were spherical in shape and sometimes indented at the site of the PALS. Secondary follicles had germinal centres surrounded by a corona (Fig. 6*a*). After emerging from the PALS, the central artery branched into a maximum of 4 straight branches (penicillary arteries), which were able to branch further (Fig. 7*a, b*). The extensions of the penicillary arteries were ensheathed by cylindrical PAMS ($42 \pm 8 \mu\text{m}$) forming the sheathed arteries which were possibly surrounded by or associated with blood sinusoids (Fig. 8*a*). The arterial capillaries extended beyond the edge of the PAMS into the red pulp to open directly into the sinusoids (Fig. 8*b*) or into splenic cords. The white pulp was separated from the red pulp by a marginal zone that was clearly separated from the white pulp by reticular fibres, but there was no clear line of demarcation from the red pulp (Fig. 6*a, b*). It contained PAMS but no marginal sinuses or secondary trabeculae (Fig. 7*b*). There was no significant difference in the morphometric measurements of the capsule or the PAMS at different ages. A schematic overview of the different compartments and the circulation of the camel spleen is depicted in Figure 9.

DISCUSSION

The present study has shown that the capsule of the camel spleen is characteristically thick and divided into outer connective tissue and inner smooth muscle layers constituting about 1/3 and 2/3 of the capsule thickness, respectively. Abd El Aal (1994) described

these 2 layers only in passing without quantitative measurements and reported that the outer layer was thicker than the inner. Ruminants have only 2 thin layers of smooth muscle cells. In the horse, the capsule consists of an outer thick connective tissue and an inner thinner smooth muscle layer, in the pig the capsule is formed mainly from smooth muscle, while in the dog and cat smooth muscle makes up about 2/3 of the capsule thickness (Brown & Dellmann, 1976). The capsule of the human spleen is composed of connective tissue with little smooth muscle (Weiss, 1983). As in other species (Brown & Dellmann, 1976), the splenic trabeculae extend from the capsule into the parenchyma, but in the camel they were uniquely divided into vascular and avascular trabeculae. The vascular trabeculae contained arteries and nerves but no veins. The avascular outnumbered the vascular trabeculae and were divided into primary and secondary trabeculae. The primary trabeculae have a similar structure to the inner layer of the capsule, while the secondary trabeculae are composed mainly of smooth muscle and extend through the red pulp as in the pig (Ueda et al. 1991).

The current work revealed a unique structure which has not previously been observed in other species in that the trabecular and capsular veins were replaced by peritrabecular and subcapsular sinuses respectively. The peritrabecular sinuses were noted by Bareedy et al. (1982) and collecting veins similar to large sinuses extending under the capsule were recorded by Abd El Aal (1994). The peritrabecular and the subcapsular sinuses play the role of the trabecular and capsular veins in collecting the venous blood from the spleen to the splenic vein. Bareedy et

Fig. 2. (a) A vascular trabecula (VT) composed mainly of connective tissue with a few laterally located smooth muscle cells and containing a trabecular artery (A) and nerve (N) but no vein. S, peritrabecular blood sinus; WP, white pulp. Trichrome. (b) An avascular trabecula divided into a primary trabecula (T) which is composed of parallel smooth muscle cells and connective tissue, and secondary trabeculae (arrows) composed of parallel smooth muscles and reticular fibres. S, peritrabecular blood sinus. Trichrome.

Fig. 3. Photomicrograph of an infused fixed spleen showing (a) a subcapsular sinus lined with flat endothelial cells (arrows) lying on a PAS positive basement membrane. C, capsule; RP, red pulp. PAS (b) A peritrabecular sinus (S) lined with flat endothelial cells (arrows) resting on a PAS-positive basement membrane. T, trabecula; RP, red pulp. PAS.

Fig. 4. (a) Photomicrograph of an infused fixed spleen showing red pulp composed of splenic cords separated by secondary trabeculae (T2) and containing blood sinusoids (S) and PAMS (arrowheads) which may open into blood sinusoids (arrow). T, primary trabecula; S, peritrabecular sinus. PAS. (b) Red pulp supported by a reticular connective tissue network. Gomori's reticulin.

Fig. 5. (a) Red pulp composed of splenic cords separated by secondary trabeculae (T2) and containing blood sinusoids (S) with a continuous basement membrane (arrow). PAS. (b) A red pulp sinusoid (S) connected to a peritrabecular sinus (TS). The arrow shows the site of connection. The PAMS (arrowheads) is located inside the blood sinusoid. T, primary trabecula. PAS.

Fig. 6. (a) White pulp composed of PALS and a lymph follicle which possesses a germinal centre (GC) and corona (C). MZ, marginal zone. Giemsa. (b) White pulp divided by reticular fibres into clearly demarcated PALS and a lymph follicle (LF), separated from the marginal zone (MZ) by circumferential reticular fibres (R). Gomori's reticulin.

Fig. 7. (a) Central artery continuing as a penicillary artery (P) which divides into 2 sheathed arteries (arrows). PAS. (b) Central artery dividing into 4 penicillary arteries (P) which extend as sheathed arteries surrounded by cylindrical PAMS (arrows) located in the marginal zone (MZ) or in the red pulp (RP). LF, lymph follicle. PAS.

Fig. 8. (a) PAMS (arrowhead) surrounded by a blood sinusoid (arrows) in the red pulp. (b) A sheathed artery extends beyond the edge of the PAMS as an arterial capillary (arrowhead) and opens into a blood sinusoid which is surrounded by basement membrane (arrows). PAS.

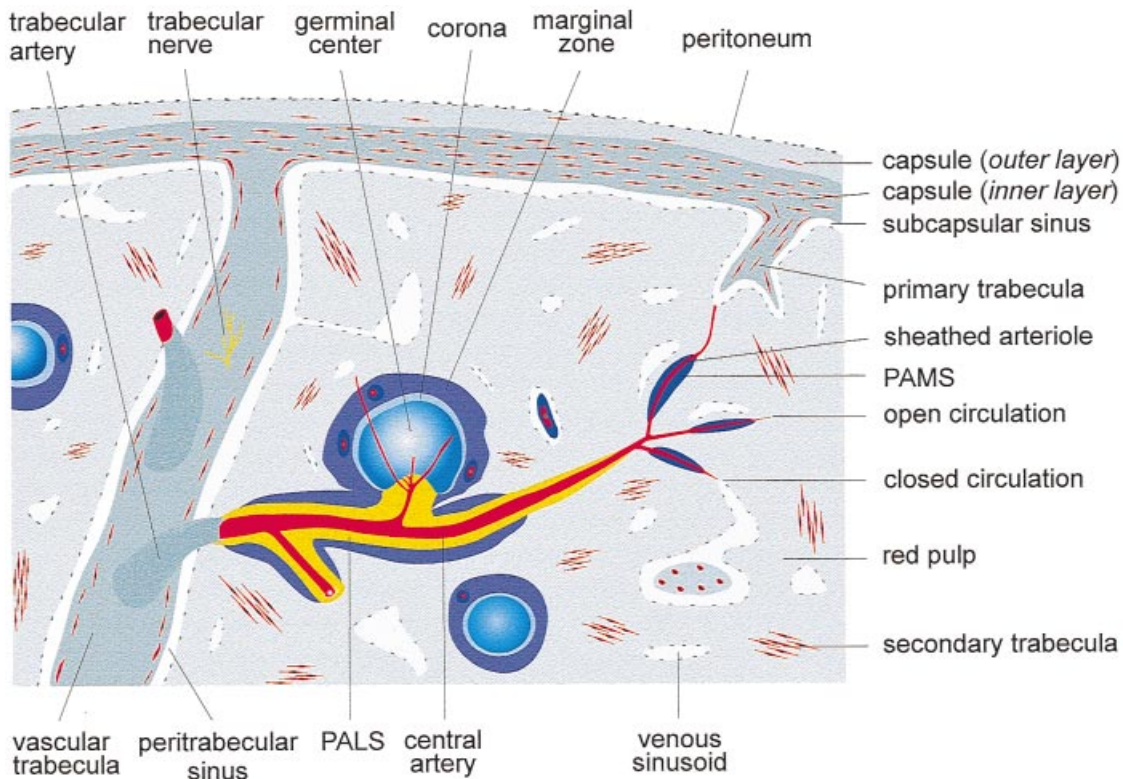


Fig. 9. Schematic diagram showing the different compartments and the circulation of the camel spleen.

al. (1982) and Abd El Aal (1994) found that the red pulp was divided into cords by blood sinusoids. The present study has revealed that the cords are separated by secondary trabeculae and the sinusoids are distributed irregularly in the red pulp. The massive distribution of the secondary trabeculae among the splenic cords and sinusoids facilitates quick outflow of blood during splenic contraction.

Due to the presence of blood sinusoids in the red pulp of the camel spleen it can be classified, in agreement with Bareedy et al. (1982), as sinusal in type. The increase in the diameter of the blood sinusoids in the infused spleen further supports its blood storage function. The volume of the stored blood may exceed 10 l as although we infused 10 l of fixative the spleen appeared to be able to accommodate more. The type of spleen found in the camel differs from that in other ruminants which are of nonsinusal type (Brown & Dellmann, 1976). The camel spleen can store blood like other sinusoidal type spleens of the pig, horse and dog (Brown & Dellmann, 1976) and human, rat and rabbit (Blue & Weiss, 1981*b*). The present finding indicates that the spleen of the one humped camel with its muscular capsule and trabeculae represents a highly contractile bag that can force the stored blood out to the circulation according to the needs of the body, e.g. on extreme

physical activity or in haemorrhagic conditions. The white pulp was demarcated by circumferential reticular fibres that divided it into 2 compartments, one surrounding the lymph follicles and the other forming a network at the PALS. This is in agreement with the finding of Tanaka et al. (1996) in mice and Sataho et al. (1997) in man. They concluded that this reticular framework plays an essential role in lymphocyte homing and compartmentalisation, which may also be true for the camel white pulp. The marginal zone surrounds all the white pulp as in other species (Brown & Dellmann, 1976), except in man where it surrounds only the lymphoid follicles (Steiniger et al. 1997).

The PAMS was cylindrical in shape as in man (Raviola, 1994). This finding is in contrast to the finding of Bareedy et al. (1982) and Abd El Aal (1994) who described an ellipsoid, rounded or oval shaped PAMS in the camel. It is ellipsoid shape in the dog (Jacobsen, 1971). The PAMS of the camel ($42 \pm 8 \mu\text{m}$) was smaller than that of the pig (60–90 μm), similar to that of the horse (40–60 μm) and larger than that of the dog, cat (30–40 μm) and cow (< 30 μm) (Seki & Abe, 1985) and also man (20–30 μm) (Raviola, 1994). There is no PAMS in rats, rabbits or mice (Snook, 1950). The sheathed arteries were clearly demarcated from the red pulp. Blood sinusoids extended adjacent

to the sheathed arteries as in dogs (Jacobsen, 1971), or—uniquely in the camel—surrounded them. The current study showed that the arterial capillary may extend over the end of the PAMS in the red pulp. Similar results were recorded by Seki & Abe (1985) in the cow, horse, pig, dog and rat, but not in the cat. This arterial capillary may open directly into sinusoids or into splenic cords. Therefore both closed and open circulations were observed in the camel spleen. This agrees with the finding of Barnhard et al. (1976) in man and of Seki & Abe (1985) in the dog and rat.

CONCLUSIONS

The camel spleen is of sinusal type that can store blood. The thick muscular capsule and trabeculae pump the stored blood according to the body's need. Both closed and open circulations are found. The venous return is unique as the blood flows from the venous sinusoids of the red pulp to the peritrabecular sinuses to the subcapsular sinuses to the splenic vein. The absence of a marginal sinus and the presence of a closed circulation in the camel spleen may reduce the role of the marginal zone in blood filtration. This may explain why blood parasites are the main health problem in the camel.

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