Hystrix It. J. Mamm. (n.s.) 17 (2) (2006): 99-116

EVOLUTION OF SINUSAL AND NON-SINUSAL SPLEENS OF MAMMALS

ION UDROIU

Department of Animal and Human Biology, University of Rome "La Sapienza", Via A. Borelli 50, 00161 Rome, Italy; e-mail: ion.udroiu@uniroma1.it

Received 24 March 2006; accepted 25 October 2006

ABSTRACT - Mammals present different types of spleen. In the past, attempts have been made to define characteristics for a classification of this organ, but the evolutionary process that has led to the splenic types of modern mammals has been poorly investigated. This review categorizes all mammalian spleens studied so far, synthesizing several earlier classification principles and updating them in the light of recent research. The main qualitative features are the type of circulation (closed, open or both) and the nature of the venous vessels in the splenic cords (venules or sinuses). The main quantitative feature is the proportion between red and white pulp. Moreover, a phylogeny of this organ is proposed: from a 'primitive type' with closed circulation (characteristic of monotremes, insectivores and tree shrews), the spleen has evolved into an 'archetypal type' with open circulation (peculiar to mice, gerbils, bats and marsupials). It has subsequently diversified into different models: blood-storing spleens (ungulates and carnivores) and defensive spleens (Old World monkeys and the majority of rodents).

Key words: spleen, red pulp, blood storage, sinus, mammals, phylogeny

RIASSUNTO - ***Evoluzione delle milze sinusale e non-sinusale dei mammiferi.*** I mammiferi presentano diversi tipi di milza. Nel passato, sono stati fatti dei tentativi per definire le caratteristiche utili alla classificazione di quest'organo, ma il processo evolutivo che ha portato ai tipi di milza dei mammiferi moderni è stato poco studiato. Questa *review* classifica tutte le milze dei mammiferi studiate finora, facendo una sintesi dei criteri di classificazione precedenti ed aggiornandoli alla luce delle più recenti ricerche. I principali caratteri qualitativi sono il tipo di circolazione (chiusa, aperta o entrambe) e la natura dei vasi venosi nei cordoni splenici (venule o seni). Il principale carattere quantitativo è la proporzione tra la polpa rossa e quella bianca. Inoltre, viene proposta una filogenesi di quest'organo: da un tipo 'primitivo' con circolazione chiusa (caratteristico di monotremi, insettivori e scandenti), la milza si è evoluta in un modello 'archetipico' con circolazione aperta (proprio di topi, gerbilli, chirotteri e marsupiali), diversificandosi, successivamente, in diversi modelli, vale a dire milze accumulatrici di sangue (ungulati e carnivori) e milze difensive (scimmie del vecchio mondo e la maggior parte dei roditori).

Parole chiave: milza, polpa rossa, accumulo ematico, seno, mammiferi, filogenesi

INTRODUCTION

The spleen is a somewhat unusual organ, long mysterious despite numerous efforts to produce a thorough record of its morphology and physiology. Despite pioneering authors such as Stuckley, who, in 1723, hypothesized the spleen as an organ with blood-storing properties (quoted in Wilkins, 2002), or Mollier (1911), who described the lattice nature of the sinus endothelium, a number of basic characteristics remained unclear for many years. Notably, the nature of intermediate circulation in the red pulp (i.e. of an open or closed type) has been the subject of controversy for over 50 years: this stems from the lack of one single type of spleen. In fact, different species present different types of intermediate circulation and among mammals, in particular, many species present both open and closed circulation. Moreover, different morphological-functional principles for classifying the mammalian spleen have been proposed. Von Herrath (1935) catalogued different species according to the splenic content of trabeculae and lymphatic tissue. Spleens with a predominance of trabeculae were defined 'storage spleens' (Speicher-milz), while the ones with more lymphatic tissue were termed 'defensive' (Abwehrmilz). Thus, the author catalogued several species from horses (extreme storage type), through cats, pigs, cows, sheep, dogs, men, to rabbits (extreme defensive type). Several authors (Hoepke, 1951; Zwillenberg, 1958; Hartwig and Hartwig, 1985) criticised this categorization, concluding that von Herrath's criterion relied mainly on quantitative characteristics, while omitting qualitative ones. Snook (1950) divided mammalian spleens according to the nature of their venous vessels in the reticular meshwork of the red pulp. Those with venous sinuses were classified as 'sinusal', while the others with smaller, non-anastomosing "primordial veins" were termed 'non-sinusal'. Although some species were eventually classified incorrectly by Snook (Seki and Abe, 1985), this standard is still most useful, especially for understanding inter-specific physiological differences. Finally, Hartwig and Hartwig (1985) studied a large number of mammalian species and divided the spleen into four types, depending on the abundance of muscle cells and on the nature of the external capsule. However, only one parameter is taken into consideration (i.e. the degree of muscularization) and such widely-differing spleens as the murine and human are classed together. On the basis of these contributions and research with the most recent techniques, it is now possible to make a synthesis and suggest an overview of the evolution of the mammalian spleen.

NON-MAMMALIAN SPLEENS

The complexity of splenic phylogeny may lie in the mesenchymal origin of this organ, the presence of other lymphoid organs (Pitchappan, 1980) and its peculiar insertion in the hematic (rather than lymphatic) circulation. The evolutionary trend towards the division of labour allows for the coexistence of different functions in the spleen: immunological, hemopoietical, hemoclastic and blood-storing. The predominance of one/some of these functions over others determines the morphology of the organ.

The spleen is not present in Acrania, which also lack erythrocytes, and first appears in cyclostomes, although not as a proper organ. This "pre-spleen" is contained by the gut-wall and functionally corresponds to the spleen and bone marrow of higher vertebrates (Tanaka, 1998). An internal lymphoid component and an external myeloid one can be distinguished, but the division is not sharp. Subsequently, in fish, the spleen appears as a separate organ delimited by a capsule (although lungfishes still have an intestinal spleen), with sharply segregated white and red pulp; the former is composed of PeriArterial Lymphoid Sheath (PALS), while the latter coheres to form cords (Murata, 1959). Erythropoiesis is still present, but not indispensable. The aim of this paper is not to compare non-mammalian spleens in depth (for detailed reviews, see Pitchappan, 1980, Tischendorf, 1985 and Tanaka, 1998), although it should be pointed out that in birds erythropoiesis is confined in the bone marrow and the white pulp reaches the highest level of evolution among non-mammals, with the appearance of secondary nodules, i.e. germinal centers (Murata, 1959).

THE PRIMITIVE SPLEEN

Among mammals, Monotremata (Basir, 1932; Tanaka, 1990; Connolly *et al.*, 1999), Insectivora (Tanaka, 1990), Scandentia (Bamroongwong *et al.*, 1991) and Sirenia (Blessing *et al.*, 1972) show a primitive spleen (Fig. 1). Besides the presence of a capsule (composed of collagen, elastic fibers and little smooth muscle), the general morphology recalls the intestinal, three-layered spleen of lungfishes (a white pulp, a red pulp and an interposed sinusoid), which might well represent a predecessor of the mammalian spleen. In the white pulp, the arteries are surrounded by the PALS (rich in T-lymphocytes), which may contain lymphatic nodules (rich in B-lymphocytes). The white pulp is surrounded by an 'Intermediate Zone' (IZ), which is encircled by the red pulp. The IZ consists of an inner domain of lymphoreticular tissue (almost absent in the mole) and an outer domain of venous capillaries; it is separated from the white pulp by an arterial net. The red pulp consists of venous vessels, trabeculae and splenic cords (Billroth's cords). The trabeculae, which depart inward from the capsule, consist of a dense fibrous connective tissue, composed of numerous collagen fibers and fibroblasts. The cords can be considered cavernous vascular spaces, since they receive arterial blood and convey it to venous vessels, and act as a three-dimensional filter. They consist of a spongy mass of cells, mainly macrophages, held in by a reticular meshwork. The meshwork presents an enormous contact surface and its great section determines a low shear rate. Hence, the opposite of the Fahraeus effect (erythrocytes travelling faster than plasma) takes place (Groom *et al.*, 1991). Moreover, the plasmatic component is separated from the cellular component by sympathetic-stimulated contraction of the splenic

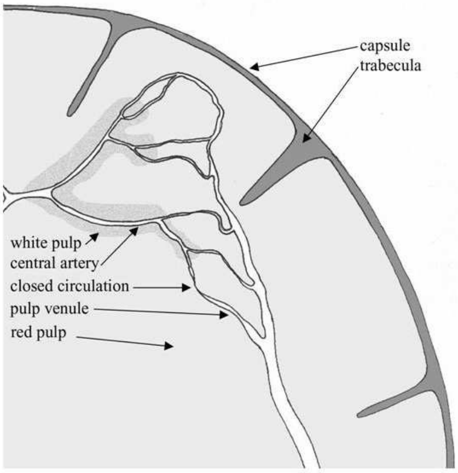

Figure 1 - Primitive spleen (not in scale): the trabecular system is poorly developed, the capsule is thin, splenic contraction and storage of blood are almost absent; the white pulp is abundant and envelopes the central artery; arterioles departing from the central artery run into the red pulp and communicate directly with the venules (closed circulation).

vein: the plasma is pushed out from veins and cords and drained by the lymphatic vessels. This explains the high hematocrit values found in the spleen, magnifying the filtration (and blood-storing – see below) property of this organ (Weiss, 1985). Thus, the erythrocytes slowly passing through the pulp cords are brought into contact with a high number of macrophages before reaching the venous vessels and the filtration depends on the adhesion to the meshwork. This means that filtration is mainly based on surface properties of the membrane. Aged erythrocytes, in particular, lose their surface sialic acid, exposing galactose residues which macrophages recognize and bind to start phagocytosis. Moreover, the reticulocytes (immature erythrocytes), being bigger and less deformable, remain trapped in the meshwork and are "nursed" by the macrophages for one or two days until they reach maturity (Blue and Weiss, 1981a; Groom *et al.*, 1991).

Splenic circulation is still closed (like in non-mammals): the arterioles originating from the white pulp communicate directly with the venules both in the IZ and in the red pulp. Furthermore, erythropoiesis is still an important splenic function, alongside the immunological function, and in the platypus, *Ornithorhyncus,* it prevails over the medullar one. Finally, it is interesting to note that in *Tupaia glis* the splenic gross morphology resembles that of primates (Bamro-ongwong *et al.*, 1991), demonstrating its relationship with this Order.

In Cetacea this primitive type is remodelled along pathways similar to some non-mammalian spleens (Tanaka, 1994). The Intermediate Zone is more developed and wider, whereas the most external layer (PeriVenous Layer, homologous to the properly called red pulp of other mammals) shows clear signs of involution (Zwillenberg, 1958; Zwillenberg, 1959; Romano *et al.*, 1993; Tanaka, 1994). This has also led to the formation of capsular veins, absent in all other mammals.

THE ARCHETYPAL SPLEEN

In mice (Schmidt *et al.*, 1985a), gerbils (Hayes, 1973), bats (Snook, 1950; Kallen, 1977; Tanaka, 1988) and marsupials (Hayes, 1968a; Basden *et al.*, 1996; Cisternas and Armati, 1999;

Young *et al.*, 2003; Old *et al.*, 2003), the spleen has undergone a radical change (Fig. 2). The white pulp is enveloped by the Marginal Zone (MZ), topographically equivalent to the Intermediate Zone, and continuous with the intervascular space of the red pulp. This area is characterised by a fine reticular meshwork and contains B-lymphocytes, macrophages and migratory cells. Here a distinct vascular space is formed by the Marginal Sinus (MS), which is an important part of the microenvironment involved in the promotion of the immune response. It is probably an evolutionary modification of the arterial net between the white pulp and the IZ of the primitive spleen (Tanaka, 1990). Many arterioles run externally from the central arteries in the white pulp, most of which form terminal capillaries ending either in the MS or in the MZ, while others run into the red pulp. Here the blood passes in the reticular meshwork of the cords and is drained by an extensive system of non-anastomosing pulp venules ("primordial veins" according to Snook, 1950). The pulp venules, opened to the cords at their distal ends, consist of a trilaminar wall composed of endothelium, basement membrane and adventitial reticular cells. The endothelium is formed by smooth, flattened, irregular cells, defined by Seki and Abe (1985) as 'stellate-shaped'. Basally, it is covered by a diffuse feltwork of thin argyrophilic filaments. It has been suggested (Yadava *et al.*, 1996) that during pre-crisis in malaria, the spleen may switch to a closed circulation, returning to an open one during crisis.

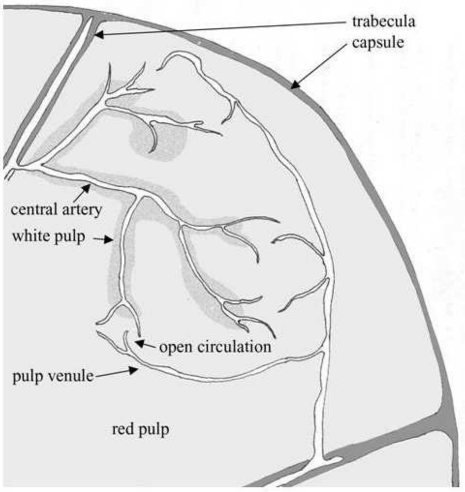

Figure 2 - Archetypal spleen (not in scale): the trabecular system is poorly developed, the capsule is thin, splenic contraction and storage of blood are almost absent; the white pulp is abundant and envelopes the central artery; arterioles departing from the central artery end in the pulp cords; the blood is drained by the pulp venules (open circulation).

Thus, the main innovation is the change from a closed to an open circulation in the red pulp, beside the specialization of the MZ and the further loss of importance of splenic erythropoiesis.

DIVERSIFICATION AND SPECIALIZATION OF MAMMALIAN SPLEENS

I. The storage type (non-sinusal)

From now on, mammalian spleens run along different evolutionary pathways. As bone marrow becomes the site for erythropoiesis in adult life, this function in the spleen is confined to fetal life, as an intermediate phase between vitelline and medullar hemopoiesis, ending at birth (or soon after in some species). Thus, the further phylogenetic development of the spleen determines the specialization of the remaining splenic activities and allows new functions to emerge.

In some species, increased spleen dimensions together with accentuated muscularization (capsule, trabeculae and red pulp rich in smooth muscle cells) and consequent contractility allow this organ to store (and release) blood. Within the red pulp, which now forms the clearly predominant part of the organ, the cords undergo a vast extension (Fig. 3). Here, a large amount of blood, and therefore erythrocytes, is retained. If necessary (haemorrhage, hypoxia, or intensive stress), blood is pumped into the circulation by the contraction of the spleen, i.e. of the muscular capsule and the trabeculae. These latter also determines the "canalisation" of the blood flow, which, after the arterial terminals, does not disperse in a wide, impeding space such as the dilated splenic cords. On the contrary, it runs through short, narrow channels and flows directly to the pulp venules, determining a functionally closed circulation. The structure of the pulp venules does not differ significantly from that of the mouse, except for the presence of larger fenestrations. These may facilitate blood passage during splenic contraction.

Actually, all spleens can store some blood. In particular, this organ acts as a reservoir of platelets in many species, but the quantity of erythrocytes that can be accumulated in the 'primitive' and 'archetypal' spleens is negligible. On the contrary, in the 'storage' spleen up to one half of the total blood volume can be retained in the red pulp. This is the case of the horse (Tablin and Weiss, 1983; Seki and Abe, 1985), cervids (Blumenthal, 1952; Hartwig and Hartwig, 1975), cat (Blue and Weiss, 1981a; Seki and Abe, 1985), lion (Tischendorf, 1956) and pinnipeds (Blessing *et al.*, 1972; Schumacher and Welsch, 1987; Cabanac *et al.*, 1999; Stewardson *et al.*, 1999; Thornton *et al.*, 2001). With regard to bats, the blood-storing property of their spleen (linked to hibernation and/or hemopoiesis) is controversial (Kallen, 1977).

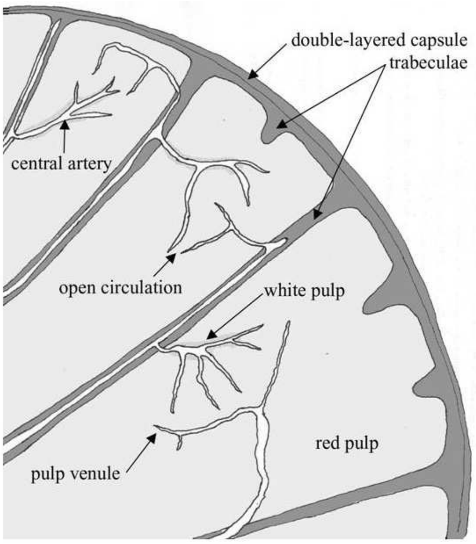

Figure 3 - Storage spleen (not in scale): the well developed trabecular system and the thick capsule are rich in muscle cells, allowing splenic contraction; a great quantity of blood can be stored in the large cords of the red pulp; the white pulp occupies a minor portion; the blood is drained by the pulp venules (open circulation).

II. The defensive type (sinusal)

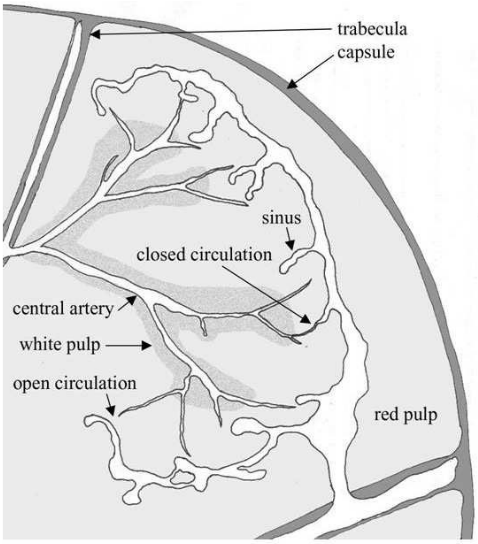

Figure 4 - Defensive spleen (not in scale): the trabecular system is poorly developed, the capsule is thin, splenic contraction and storage of blood are almost absent; the white pulp is abundant and envelopes the central artery; the blood is drained by the sinuses (open circulation), which also have few direct connections with the arterioles (closed circulation).

In man (Chen and Weiss, 1972; Schmidt *et al.*, 1988), Old World monkeys (Dustin, 1938; Eberl-Rothe, 1960; Matejka, 1977; Lowenstine, 2003), Lagomorpha (Roberts and Latta, 1964; Miyoshi *et al.*, 1970; Abe *et al.*, 1986), guinea pig (Tischendorf, 1969), sciurids (Watzka, 1937; Hayes and Eglitis, 1967), meadow and prairie voles (Steven, 1981), rat (Schmidt *et al.*, 1985b; Seki and Abe, 1985) and probably also in mole rat (Jankovic and Paunovic, 1973) and degu (Donnelly and Quimby, 2002), the red pulp is not predominant; on the contrary, the white pulp occupies a larger portion (Fig.4). Beside this quantitative difference, the red pulp itself undergoes a significant change. The few, non-anastomosing pulp venules are no longer present. Instead, the venous drainage of the red pulp is carried out by sinuses, a specific splenic vessel, never observed in non-mammalian spleens (Murata, 1959). Table 1 illustrates the species studied so far, discriminating between the sinusal and non-sinusal nature of their spleens. The sinusal wall is made up of rod-shaped endothelial cells aligned parallel to the vessel axis, with the nuclear region bulging into the lumen, and is covered by characteristic argyrophilic annular fibers. In contrast to pulp venules, characterised by wide, irregularly distributed fenestrations, the sinus wall is perforated by narrow, regularly distributed interendothelial slits (IES), through which the erythrocytes have to squeeze. While normal erythrocytes can easily do this, abnormal cells without the ability to deform cannot pass. Moreover, erythrocytes with a rigid inclusion (Howell-Jolly bodies, Heinz bodies or parasites) can pass through the slits, but while passing the inclusion is "pitted out" (Chen and Weiss, 1973; Chotivanich *et al.*, 2002). The calibre of the IES can change, determining a variation in the speed of the blood flow (Groom *et al.*, 1991). On the other hand, the open terminals of the venous sinuses represent a pathway of low resistance for blood flow (Schmidt *et al.*, 1993), together with the few direct connections between arterial capillaries and venous sinuses (Su *et al.*, 1991; Schmidt *et al.*, 1993).

Table 1 - Sinusal and non-sinusal spleens. Systematics according to Wilson and Reeder (1993); see text for references.

Order	Family	Species	Spleen
Monotremata	Ornithorhynchidae	*Ornithorhynchus anatinus*	non-sinusal
	Tachyglossidae	*Tachyglossus aculeatus*	non-sinusal
Didelphimorphia	Didelphidae	*Didelphis virginiana*	non-sinusal
Peramelemorphia	Peramelidae	*Isoodon macrourus*	non-sinusal
Diprotodontia	Macropodidae	*Macropus eugenii*	non-sinusal
		Lagorchestes hirsutus	non-sinusal
Dasyuromorphia	Dasyuridae	*Sminthopsis macroura*	non-sinusal
Xenarthra	Dasypodidae	*Dasypus novemcinctus*	sinusal
		Dasypus hybridus	non-sinusal
		Zaëdyus pichiy	non-sinusal
		Chaetophractus villosus	semi-sinusal
		Euphractus sexcinctus	semi-sinusal
		Cabassous unicinctus	non-sinusal
	Myrmecophagidae	*Myrmecophaga tridactyla*	non-sinusal
		Tamandua tetradactyla	non-sinusal
	Megalonychidae	*Choloepus hoffmanni*	non-sinusal
Insectivora	Soricidae	*Suncus murinus*	non-sinusal
	Erinaceidi	*Erinaceus europaeus*	non-sinusal
	Talpidae	*Talpa wogura*	non-sinusal
Rodentia	Muridae	*Microtus pennsylvanicus*	sinusal
		Microtus ochrogaster	sinusal
		Mus domesticus	non-sinusal
		Rattus norvegicus	sinusal
		Nannospalax leucodon	sinusal?
		Meriones unguiculatus	non-sinusal
		Mesocricetus auratus	semi-sinusal
	Sciuridae	*Sciurus vulgaris*	sinusal
		Spermophilus citellus	sinusal
		Marmota monax	sinusal
	Octodontidae	*Octodon degus*	sinusal?
	Cavidae	*Cavia porcellus*	sinusal
Lagomorpha	Ochotonidae	*Ochotona rufescens*	sinusal
	Leporidae	*Oryctolagus cuniculus*	sinusal
Scandentia	Tupaidae	*Tupaia glis*	non-sinusal
Primates	Callitrichidae	*Callithrix jacchus*	non-sinusal
	Cebidae	*Aotus lemurinus griseimembra*	non-sinusal?
		Aotus trivirgatus	non-sinusal
	Galagonidae	*Galago senegalensis*	sinusal?
	Cercopithecidae	*Theropithecus gelada*	sinusal
		Cercopithecus aethiops	sinusal
		Macaca mulatta	sinusal
		Papio hamadryas	sinusal
		Cercocebus torquatus	sinusal
	Hominidae	*Pongo pygmaeus*	sinusal
		Homo sapiens	sinusal

Table 1 - continues

Chiroptera	Vespertilionidae	*Vespertilio superans*	non-sinusal
		Myotis lucifugus	non-sinusal
Carnivora	Otariidae	*Zalophus californianus*	non-sinusal
		Arctocephalus gazella	non-sinusal
		Arctocephalus pusillus	non-sinusal
	Phocidae	*Phoca vitulina*	non-sinusal
		Leptonychotes weddellii	non-sinusal
		Lobodon carcinophagus	non-sinusal
		Mirounga angustirostris	non-sinusal
		Mirounga leonina	non-sinusal
		Cystophora cristata	non-sinusal
	Ursidae	*Ursus thibetanus*	sinusal
	Canidae	*Canis lupus*	sinusal
	Procionidae	*Procyon lotor*	sinusal
	Mustelidae	*Mephitis mephitis*	sinusal
		Mustela vison	sinusal
		Mustela putorius furo	semi-sinusal
		Mustela nivalis	semi-sinusal
		Mustela erminea	semi-sinusal?
		Mustela lutreola	semi-sinusal?
	Felidae	*Felis silvestris*	non-sinusal
		Panthera leo	non-sinusal
Cetacea	Monodontidae	*Delphinapterus leucas*	non-sinusal
	Delphinidae	*Globicephala macrorhynchus*	non-sinusal
		Tursiops truncatus	non-sinusal
	Balaenopteridae	*Balaenoptera musculus*	non-sinusal
		Balaenoptera physalus	non-sinusal
		Balaenoptera borealis	non-sinusal
	Phocoenidae	*Phocaena phocaena*	non-sinusal
Artiodactyla	Camelidae	*Camelus dromedarius*	sinusal
		Camelus bactrianus	sinusal
	Bovidae	*Bos taurus*	sinusal
		Ovis aries	sinusal
		Capra hircus	sinusal
	Hippopotamidae	*Hippopotamus amphibius*	sinusal
	Suidae	*Sus scrofa*	semi-sinusal
	Cervidae	*Cervus elaphus*	non-sinusal
		Capreolus capreolus	non-sinusal
		Alces alces	non-sinusal
		Rangifer tarandus	non-sinusal?
Perissodactyla	Equidae	*Equus caballus*	non-sinusal
Proboscidea	Elephantidae	*Elephas maximus*	sinusal
Sirenia	Trichechidae	*Trichechus inunguis*	non-sinusal

III. Another storage type (sinusal)

Snook's distinction (1950) between sinusal and non-sinusal spleens led von Herrath (1958) to identify the former with defensive spleens and the latter with storage ones. This concept held for over 20 years, but in reality it is not completely correct. The two canons cannot be superposed, as dogs demonstrate having a sinusal spleen that can store a large volume of blood (Blue and Weiss, 1981b; Seki and Abe, 1985; Alexandre-Pires *et al.*, 2003). The same is true for cows (Seki and Abe, 1985), sheep (Mollier, 1911; Lewis, 1957), goats (Ohta, 1957; Saigal *et al.*, 1977), camels (Abe *et al.*, 1999; Zidan *et al.*, 2000), hippopotamuses (Tischendorf, 1958), elephants (Tischendorf, 1953), bears (Zhang *et al.*, 1997), raccoons (Hayes and Eglitis, 1967), skunks (Snook, 1950) and minks (Abe *et al.*, 1989).

EVOLUTIONARY INTERMEDIATES

In some species, an interesting arrangement of the venous vessels in the red pulp has been found. These species present some zones, in the same vessel, characterised by rod-shaped endothelial cells (therefore sinusal) and others by the stellate-shaped type (thus non-sinusal). We suggest considering these spleens as 'semi-sinusal'. They may represent evolutionary intermediate types between the archetypal spleen and the sinusal. Hamsters (Hataba and Suzuki, 1986), pigs (Seki and Abe, 1985), weasels and ferrets (Hataba and Suzuki, 1989) belong to this category. The latter was earlier classified as non-sinusal and its spleen was described as identical to that of the ermine and European mink (Watzka, 1937; Tischendorf, 1969). Therefore we presume these carnivores also belong to the semi-sinusal category. One might be surprised by the difference, within the same genus, between the American mink (*Mustela vison*) and the European mink (*Mustela lutreola*). However, this can be explained by the fact that, despite the common name, the European mink is phylogenetically more closely related to the ferret and the weasel (Fig. 5) than to the American mink (Sato *et al.*, 2003).

CONCLUSIONS

This review suggests an interpretation of the phylogeny of the mammalian spleen on the basis of available literature dealing with a total of 82 species. Many others have not been considered, either because of a total lack of published works on the subject (Dermoptera and Pholidota, for example) or because the existing literature deals only with specific aspects: Hartwig and Hartwig (1985) put the peccary, the lama and the chamois in the storage category, but give no indication about the sinusal or non-sinusal nature of their spleens; Mishra and Verma (2004) describe the spleen of the buffalo in the same manner (although it is reasonable to expect it to be like that of the cow); Dustin (1938) reports that the sinuses are much more developed in the Cynomorpha (Cercopithecidae) than in *Galago senegalensis*, making us wonder whether it belongs to the

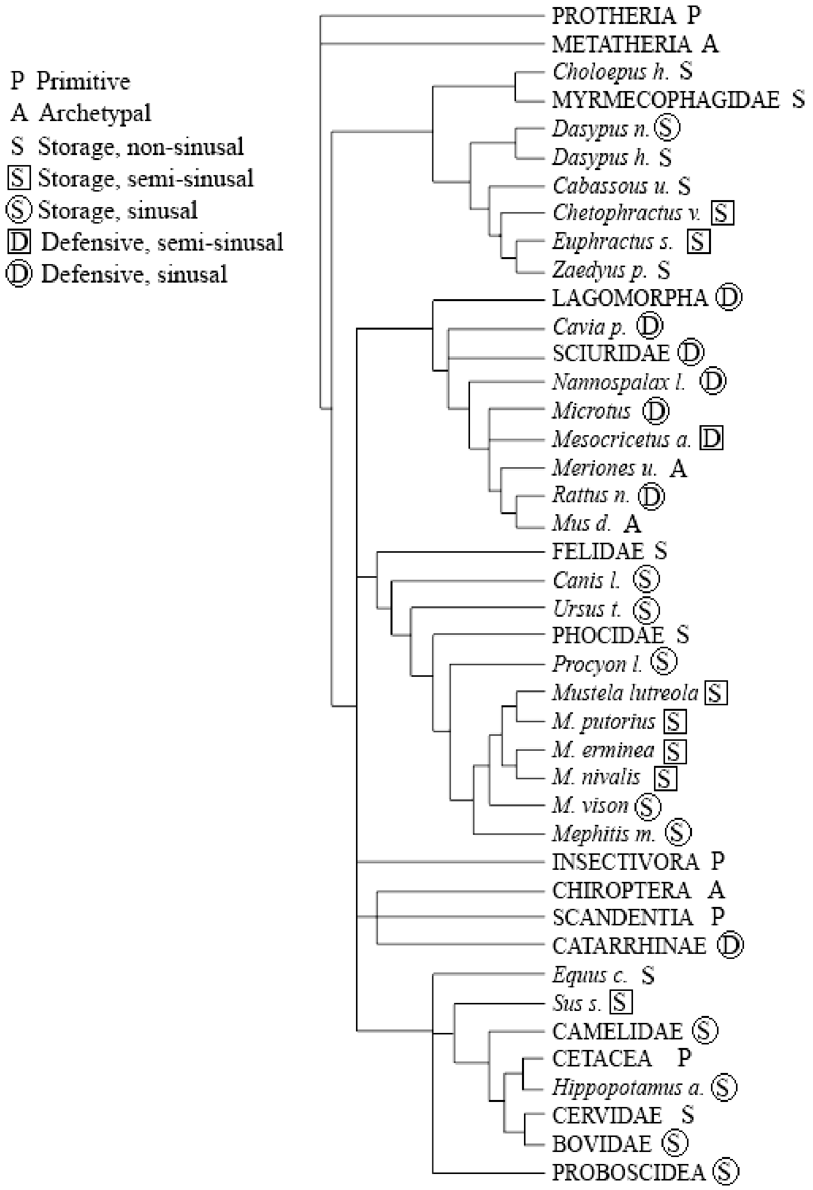

Figure 5 - Phylogenetic tree showing the evolutionary relationships between the *taxa* and their splenic types.

sinusal or non-sinusal type; Baitchman and Kollias (2000) report that otters have a very large spleen (in line with other carnivores and with their predator and diving habit), but the vascular architecture is unknown. On the other hand, despite the lack of literature, we can deduce the splenic characteristics of some species in another way. As explained above, the sinus wall (and not the wall of the 'primordial veins' or pulp venules) "pits out" intra-erythrocytic inclusions such as Howell-Jolly bodies and Heinz bodies. Therefore, if the absence of these inclusions is not proof of the presence of sinuses, the presence of Howell-Jolly and/or Heinz bodies is clear evidence of the absence of splenic sinuses. This is confirmed by the fact that the species commonly known in hematology for presenting Howell-Jolly bodies in their blood, such as cat, horse, mouse (Feldman *et al.*, 2000), and marsupials (Canfield, 1998) possess nonsinusal spleens (see above). In this way, we can presume that reindeer, which present Howell-Jolly bodies in the circulating erythrocytes (Feldman *et al.*, 2000), possess a non-sinusal spleen like the other cervids described here, and probably one of the extreme storage type. The same is true for the owl monkey (Mrema *et al.*, 1987).

However, if one agrees with the model suggested here, it is likely that the spleens to be studied in future research will fall into one of the types illustrated in this article. Originating from a single, more simple model, the spleen runs along different evolutionary pathways. In some mammals the spleen developed more the filtering and immunological functions, in others the blood-storing and in others more both. Moreover, in the organ of some species, we can find primitive characteristics alongside highly evolved features. One example is the lobed form of some spleens. This plesiomorph character, common in many fish, is present in monotremes, but also in marsupials and several Xenarthra, which have developed more advanced characteristics such as the open splenic circulation and the Marginal Sinus (in Xenarthra also blood storage and/or sinusation).

The spleen first appears, both phylogenetically and ontogenetically, as a lympho-myeloid organ. With the appearance of blood-forming bone marrow, splenic erythropoiesis is no longer indispensable. As a consequence, the spleen has become more involved in immunological processes. Confirmation comes from the fact that 90% of blood passes through the Marginal Sinus (where the humoral, immune response takes place), and only 10% passes through the "slow path" in the cords of the red pulp (Schmidt *et al.*, 1993). This functional shift, together with the presence of other lymphatic organs and consequent division of labour, has determined the high specialization of the white pulp, a character already present in Monotremes. These, in fact, present a lymphatic system that is, regarding its most important features, common to all mammals. Therefore, we can suppose that the terrestrial ancestors of mammals (therians) had developed a spleen similar to the here defined 'primitive type'. Following the appearance of an open circulation, the further diversification of the spleen was probably due to adaptative advantages related to the evolution of mammals. Changes leading to the storage or defensive types seem to be mainly quantitative. The only new qualitative character that may appear is the presence of sinuses in the red pulp (these cannot appear in non-mammals, as their nucleated erythrocytes would not be able to pass through the interendothelial slits). In some carnivores (Fig. 5), the trend seems to lead towards a blood-storing and sinusal spleen, with ferret and weasel representing an evolutionary intermediate (semi-sinusal). An important distinction, among carnivores, is that between these sinusal and semi-sinusal spleens and the non-sinusal type of the cat (and lion). Here the development of the blood-storing ability, with obvious advantages for such a predatory species, has not been accompanied by the development of sinuses. This pattern may be related to the higher susceptibility of cats to Heinz bodies formation, due to their high number (eight) of reactive sulfhydril groups per hemoglobin molecule (Christopher *et al.*, 1989; Weiss *et al.*, 1990). Also seals did not develop a sinusal spleen probably as a consequence of their aquatic environment (to the author's knowledge, marine mammals are not infected by intraerythrocytic parasites, thus the appearance of sinuses would have brought no evolutionary advantage). On the other hand, their diving habit is allowed by the great quantity of blood which the spleen can store. A non-sinusal red pulp is possibly more suitable in this case.

Living into an aquatic environment has also led to significant changes in the splenic morphology of cetaceans. Beside the vascular architecture, the size of the spleen represents another striking difference between seals and cetaceans. It is definitely small, both in relative and absolute terms, and clearly does not possess blood-storing properties. According to Hartwig and Hartwig (1985) the absence on natural predators could explain cetaceans small spleen. However, it should be noted that in this Order the *Retia Mirabilia* perform the blood-storing function. On the contrary, in terrestrial ungulates the development of a blood-storing spleen provides exactly the advantage of avoiding predation.

Among rodents, the evolutionary trend seems to lead towards the defensive type. However, the difference between mouse and rat, both members of the same subfamily, is striking. Future research on comparative hemopoiesis and, above all, on murine malaria will possibly solve this apparent contradiction.

Among primates, the spleen of Old World monkeys (and man) differs from that of New World monkeys: the first is of the sinusal, extreme defensive type, while the second is non-sinusal.

Finally, Xenarthra present pathways for the specialization of the spleen as in the other placentals (constituting the Magnorder of Epitheria), from which they diverged early: anteaters, two-toed sloths, southern naked-tailed armadillos (Claussen, 1969), pichi (Claussen, 1969; Galindez *et al.*, 2003) and southern long-nosed armadillos (Galindez *et al.*, 2000) belong to the non-sinusal, storage type; large hairy armadillos and six-banded armadillos to the semi-sinusal, storage type (Claussen, 1969); nine-banded armadillos to the sinusal, storage type (Hayes, 1968b), although all of them show an archaic characteristic such as the lobed shape.

In conclusion, the classification proposed here could be helpful, together with other morphological and biomolecular parameters, to expand and clarify the phylogenetic relationships among mammalian *taxa* and could also be useful in understanding inter-specific differences in physiology, hematology and veterinary pathology.

REFERENCES

Abe M., Sugimura M., Suzuki Y. and Atoji Y. 1986. Morphology and microvascular pathways in the spleen of afghan pikas (*Ochotona rufescens rufescens*). *Jpn. J. Vet. Sci.*, 48(4): 655-665.

Abe M., Takehana K., Iwasa K. and Hiraga T. 1989. Scanning electron microscopic studies on the red pulp of the mink spleen. *Jpn. J. Vet. Sci.*, 51(4): 775-781.

Abe M., Eerdunchaolu, Kobayashi A. and Takehana K. 1999. Fine structure of the Bactrian Camel (*Camelus Bactrianus*) spleen. *J. Rakuno Gakuen Univ.*, 24(1): 45-53.

Alexandre-Pires G., Pais D. and Esperança Pina J.A. 2003. Intermediary spleen microvasculature in *Canis familiaris* – Morphological evidences of a closed and open type. *Anat. Histol. Embryol.*, 32:263-270.

Baitchman E.J. and Kollias G.V. 2000. Clinical anatomy of the North American river otter (*Lontra canadensis*). *J. Zoo Wildl. Med.*, 31(4): 473-483.

Bamroongwong S., Somana R., Rojananeungnit S., Chunhabundit P. and Rattanachaikunsopon P. 1991. Scanning electron microscopic study of the splenic vascular casts in common tree shrew (*Tupaia glis*). *Anat. Embryol.*, 184(3): 301-304.

Basden K., Cooper D.W. and Deane E.M. 1996. Development of the blood-forming tissues of the tammar wallaby *Macropus eugenii. Reprod. Fertil. Dev.*, 8(6): 989-994.

Basir M.A. 1932. The histology of the spleen and suprarenals of Echidna. *J. Anat.*, 66: 628-649.

Blessing M., Ligensa K. and Winner R. 1972. Zur Morphologie der Milz einiger im Wasser lebender Säugetiere. *Zeitsch. Wiss. Zool.*, 184(1-2): 164-204.

Blue J. and Weiss L. 1981a. Vascular pathways in nonsinusal red pulp-An electron microscope study of the cat spleen. *Am. J. Anat.*, 161: 135-168.

Blue J. and Weiss L. 1981b. Electron microscopy of the red pulp of the dog spleen including vascular arrangements, periarterial macrophage sheaths (ellipsoids) and the contractile innervated reticular meshwork. *Am. J. Anat.*, 161: 189-218.

Blumenthal I. 1952. Die Milz des Elches (*Alces alces* L.). *Z. Mikrosk. Anat. Forsch.*, 58(2): 230-255.

Cabanac A.J., Messelt E.B., Folkow L.P. and Blix A.S. 1999. The structure and blood-storing function of the spleen of the hooded seal (*Cystophora cristata*). *J. Zool.*, 248 (1): 75-81.

Canfield P.J. 1998. Comparative cell morphology in the peripheral blood film from exotic and native animals. *Aust. Vet. J.*, 76 (12): 793-800.

Chen L. and Weiss L. 1972. Electron microscopy of the red pulp of human spleen. *Am. J. Anat.*, 134: 425-458.

Chen L. and Weiss L. 1973. The role of the sinus wall in the passage of the erythrocytes through the spleen. *Blood*, 41: 529-537.

Chotivanich K., Udomsangpetch R., McGready R., Proux S., Newton P., Pukrittayakamee S., Looareesuwan S. and White N.J. 2002. Central role of the spleen in malaria parasite clearance. *J. Infect. Dis.*, 185(10): 1538-1541.

Christopher M.M., Perman V. and Eaton J.W. 1989. Contribution of propylene glycol-induced Heinz body formation to anemia in cats. *J. Am. Vet. Med. Assoc.,* 194(8): 1045-1056.

Cisternas P.A. and Armati P.J. 1999. Development of the thymus, spleen, lymph nodes and liver in the marsupial, *Isoodon macrourus* (Northern brown bandicoot, Peramelidae). *Anat. Embryol.*, 200: 433–443.

Claussen C.P. 1969. Vergleichende Untersuchungen zur makroskopischen und mikroskopischen Anatomie der Milzen einiger Edentata. *Zeitschr. Wiss. Zool.*, 179: 333-424.

Connolly J.H., Canfield P.J., McClure S.J. and Whittington R.J. 1999. Histological and immunohistological investigation of lymphoid tissue in the platypus (*Ornithorhynchus anatinus*). *J. Anat.*, 195(2): 161-171.

Donnelly T.M. and Quimby F.W. 2002. Biology and diseases in other rodents. In: Fox J.G., Anderson L.C., Loew F.M. and Quimby F.W. (eds) Laboratory Animal Medicine. 2nd ed. Academic Press, San Diego.

Dustin P. 1938. Les housses splenique de Schweigger-Seidel. Étude d'histologie et d'histophysiologie compareés. *Arch. de Biol.*, 49: 1-99.

Eberl-Rothe G. 1960. Die mikroskopische Anatomie der Primatenmilz. *Primatologia*, 3(2): 71-86.

Feldman B.F., Zinkl J.G. and Jain N.C. 2000. Schalm's Veterinary Hematology. Lippincott Williams & Wilkins, Philadelphia.

Galindez E.J., Codon S.M. and Casanave E.B. 2000. Spleen of *Dasypus hybridus* (Mammalia, Dasypodidae): a light and electron microscopic study. *Anat. Rec.*, 258: 286-291.

Galindez E.J., Estecondo S. and Casanave E.B. 2003. The spleen of *Zaedyus pichiy*, (Mammalia, Dasypodidae): a light and electron microscopic study. *Anat. Histol. Embryol.*, 32: 194-199.

Groom A.C., Schmidt E.E. and MacDonald I.C. 1991. Microcirculatory pathways and blood flow in spleen: new insights from washout kinetics, corrosion casts, and quantitative intravital video-microscopy. *Scanning Microscopy*, 5(1): 159-174.

Hartwig H. and Hartwig H.G. 1975. Über die Milz der Cervidae (Gray, 1821). Quantitativ-morphologische Untersuchungen an Milzen des Rothirsches (*Cervus elaphus*, L.1785) und des Rehes (*Capreolus capreolus*, L. 1758). *Gegenbaurs Morphol. Jahrb.*, 121(6): 669-697.

Hartwig H. and Hartwig H.G. 1985. Structural characteristics of the mammalian spleen indicating storage and release of red blood cells. Aspects of evolutionary and environmental demands. *Experientia*, 41: 159-163.

Hataba Y. and Suzuki T. 1986. In : Proceedings of the 11th International Congress of Electron Microscopy. Vol IV: p 2855.

Hataba Y. and Suzuki T. 1989. Scanning electron microscopic study of the red pulp of ferret spleen. *J. Electron. Microsc.*, 38(3): 190-200.

Hayes T.G. and Eglitis J.A. 1967. The microscopic structure of the adult Raccoon (*Procyon lotor*) and Woodchuck (*Marmota monax*) spleens. *J. Morphol.*, 121(1): 47-54.

Hayes T.G. 1968a. Studies of a primitive mammalian spleen, the opossum (*Didelphis virginiana*). *J. Morpho.*, 124(4): 445-450.

Hayes T.G. 1968b. Electron and light microscopic observations of the armadillo (*Dasypus novemcinctus mexicanus*) spleen. *Anat. Rec.*, 160: 473.

Hayes T.G. 1973. The marginal zone and marginal sinus in the spleen of the gerbil. A light and electron microscopy study. *J. Morphol.,* 141(2): 205-215.

von Herrath E. 1935. Bau und Funktion der Milz. Z Zellforsch 23: 375-430.

von Herrath E. 1958. Bau und Funktion der normalen Milz. W. de Gruyter & Co, Berlin.

Hoepke H. 1951. Die Leistungen der Milz. *Anat. Anz.*, 98(1-2): 7-12.

Jankovic B.D. and Paunovic V.R. 1973. Immunological responses in the mole rat (*Spalax leucodon*). I. Antibody production, delayed hypersensitivity and lymphatic tissue. *Ann. Immunol.*, 124(1): 133-152.

Kallen F.C. 1977. The cardiovascular systems of bats: structure and function. In: Wimsatt W.A. (ed) Biology of bat. Academic Press, New York.

Lewis OJ (1957) The blood vessels of the adult mammalian spleen. *J. Anat.*, 91(2): 245-250.

Lowenstine L.J. 2003. A primer of primate pathology: lesions and nonlesions. *Toxicologic Pathology*, 31: 92-103.

Matejka M. 1977. An electron microscopic study of the perfusion-fixed spleen. II. Fine structure of the sinus lining cells with respect to filamentous structure in monkey spleen. *Anat. Anz.*, 141: 331-339.

Mishra B.K. and Verma P.K. 2004. Capsule and trabeculae of the spleen: a comparative histology. *J. Anat. Soc. India*, 53(1): 59-60.

Miyoshi M., Fujita T. and Tokunaga J. 1970. The red pulp of the rabbit spleen studied under the scanning electron microscope. *Arch. Histol. Jpn.*, 32(3): 289-306.

Mollier S. 1911. Über den Bau der Kapillaren Milzvenen (Milzsinus). *Arch. Mikrosk. Anatomie*, 76: 608-657.

Mrema J.E.K., Caldwell C.W., Stogsdill P.L., Kelley S.T. and Green T.J. 1987. Erythrocytes and erythrocyte morphologies of healthy and colony-born owl monkeys (*Aotus lemurinus griseimembra*). *J. Med. Primat.*, 16: 13-25.

Murata H. 1959. Comparative studies of the spleen in submammalian vertebrates. II. Minute structure of the spleen, with special reference to the periarterial lymphoid sheath. Bull. *Yamaguchi Med. School*, 6: 83-105.

Ohta Y. 1957. Cubical anatomy of several ducts and vessels by injection method of acrylic resin. III. On the vascular system of the spleen in some mammals. *Okajimas Folia Anat. Jpn.*, 30(1): 13-41.

Old J.M., Selwood L. and Deane E.M. 2003. A histological investigation of the lymphoid and immuno-haematopoietic tissues of the adult stripe-faced dunnart (*Sminthopsis macroura*). *Cells Tissues Organs*, 173:115-121.

Pitchappan R.M. 1980. Review on the phylogeny of splenic structure and function. *Dev. Comp. Immunol.*, 4(3): 395-416.

Roberts D.K. and Latta J.S. 1964. Electron microscopic studies on the red pulp of the rabbit spleen. *Anat. Rec.*, 148: 81-101.

Romano T.A., Felten S.Y., Olschowka J.A. and Felten D.L. 1993. A microscopic investigation of the lymphoid organs of the beluga, *Delphinapterus leucas. J. Morphol.*, 215(3): 261-287.

Saigal R.P., Nanda B.S., Roy K.S. and Nagpal S.K. 1977. Histomorphological and morphometric changes in the spleen of ageing goats. *Anat. Anz.*, 141(3): 292-307.

Sato J.J., Hosoda T., Wolsan M., Tsuchiya K., Yamamoto M. and Suzuki H. 2003. Phylogenetic relationships and divergence times among mustelids (Mammalia: Carnivora) based on nucleotide sequences of the nuclear interphotoreceptor retinoid binding protein and mitochondrial cytochrome b genes. *Zoolog. Sci.*, 20(2): 243-264.

Seki A. and Abe M. 1985. Scanning electron microscopic studies on the microvascular system of the spleen in the rat, cat, dog, pig, horse and cow. *Jpn. J. Vet. Sci.*, 47(2): 237-249.

Schmidt E.E., MacDonald I.C. and Groom A.C. 1985a. Microcirculation in mouse spleen (nonsinusal) studied by means of corrosion casts. *J. Morphol.*, 186: 17-29.

Schmidt E.E., MacDonald I.C. and Groom A.C. 1985b. Microcirculation in rat spleen (sinusal), studied by means of corrosion casts, with particular reference to intermediate pathways. *J. Morphol.*, 186: 1-16.

Schmidt E.E., MacDonald I.C. and Groom A.C. 1988. Microcirculatory pathways in normal human spleen, demonstrated by scanning electron microscopy of corrosion casts. *Am. J. Anat.*, 181: 253-266.

Schmidt E.E., MacDonald I.C. and Groom A.C. 1993. Comparative aspects of splenic microcirculatory pathways in mammals: the region bordering the white pulp. *Scanning Microscopy*, 7(2): 613-628.

Schumacher U. and Welsch U. 1987. Histological, histochemical, and fine structural observations on the spleen of seals. *Am. J. Anat.*, 179(4): 356-368.

Snook T. 1950. A comparative study of the vascular arrangements in mammalian spleen. *Am. J. Anat.*, 87: 31-78.

Steven W.M. 1981. The relationship of macrophages and erythrocytes receptors to vascular patterns in vole spleen. *Acta Anat.*, 109: 197-208.

Stewardson C.L., Hemsley S., Meyer M.A., Canfield P.J. and Maindonald J.H. 1999. Gross and microscopic visceral anatomy of the male Cape fur seal, *Arctocephalus pusillus pusillus* (Pinnipedia: Otariidae), with reference to organ size and growth. *J. Anat.*, 195(2): 235-255.

Su C.Y., Tsai A.I., Liu H.P., Chen L.T. and Chien S. 1991. Ultrastructural studies on splenic microcirculation of the rat. *Chin. J. Physiol.*, 34(2): 223-234.

Tablin F. and Weiss L. 1983. The equine spleen: an electron microscopic analysis. *Am. J. Ana.*, 166: 393-416.

Tanaka Y. 1988. A histo-anatomical study on the spleen of a bat, *Vespertilio orientalis. Acta Anat. Nippon.*, 63(6): 502-512.

Tanaka Y. 1990. “Intermediate Zone” of mammalian spleens: light and electron microscopic study of three primitive mammalian species (Platypus, Shrew, and Mole) with special reference to intrasplenic arteriovenous communication. *Am. J. Anat.*, 187: 313-337.

Tanaka Y. 1994. Microscopy of vascular architecture and arteriovenous communications in the spleen of two odontocetes. J. *Morphol.*, 221(2): 211-233.

Tanaka Y. 1998. Structure of the reptilian spleen. In: Gans C, Gaunt AS, editors. Biology of the Reptilia. Society for the Study of the Amphibians and Reptiles. Vol XIX (Morphology G), Ithaca.

Thornton S.J., Spielman D.M., Pelc N.J., Block W.F., Crocker D.E., Costa D.P., LeBoeuf B.J. and Hochachka P.W. 2001. Effects of forced diving on the spleen and hepatic sinus in northern elephant seal pups. *Proc. Natl. Acad. Sci.*, 98(16): 9413-9418.

Tischendorf F. 1953. Über die Elefantenmilz. *Z. Anat. Entwicklungsgesch*, 116: 577-590.

Tischendorf F. 1956. Milz. In: Helmcke J.G., v. Lengerken H. (eds) Handbuch der Zoologie. W. de Gruyter & Co., Berlin, Vol VIII, 5(2), pp 1-32.

Tischendorf F. 1958. Über die Hippopotamidenmilz. *Z. Mikrosk. Anat. Forsch.*, 64(2): 228-257.

Tischendorf F. 1969. Die Milz. In: Möllendorff W. and Bargmann W. (eds) Handbuch der mikroskopische Anatomie der Menschen. Springer-Verlag, Berlin. Vol VI (6).

Tischendorf F. 1985. On the evolution of the spleen. *Experientia*, 41: 145-152.

Watzka M. 1937. Zur Kenntnis der Milz der Säugetiere (Kapillarhülsen; Megakaryocyten, Sinusendothel und Plasmaabbau). *Z. Mikrosk. Anat. Forsch.*, 41: 498-524.

Weiss D.J., McClay C.B., Christopher M.M., Murphy M. and Perman V. 1990. Effects of propylene glycolcontaining diets on acetaminophen-induced methemoglobinemia in cats. *J. Am. Vet. Med. Assoc.*, 196(11): 1816-1819.

Weiss L. 1985. Milza. In: Weiss L. and Greep R.O. (eds) Istologia. Zanichelli, Bologna.

Wilkins B.S. 2002. The spleen. Br J Haematol 117(2): 265-274.

Wilson D.E. and Reeder D.M. 1993. Mammal Species of the World. Smithsonian Institution Press, Washington.

Yadava A., Kumar S., Dvorak J.A., Milon G. and Miller L.H. 1996. Trafficking of *Plasmodium chabaudi adami* infected erythrocytes within the mouse spleen. *Proc. Natl. Acad. Sci.*, 93: 4595-4599.

Young L.J., McFarlane R., Slender A.L. and Deane E.M. 2003. Histological and immunohistological investigation of the lymphoid tissue in normal and mycobacteria-affected specimens of the Rufous Hare-wallaby (*Lagorchestes hirsutus*). *J. Anat.*, 202(3):315-325.

Zhang Y., Zhang L. and Feng G. 1997. Light and electron microscopic observation in black bear spleen. *Chinese J. Zool.*, 32(5): 29-30.

Zidan M., Kassem A., Dougbag A., El. Ghazzawi E., Abd El Aziz M. and Pabst R. 2000. The spleen of the one humped camel (*Camelus dromedarius*) has a unique histological structure. *J. Anat.*, 196: 425-432.

Zwillenberg H.H. 1958. Die mikroskopische Anatomie der Milz der Furchenwale. *Acta Anat.*, 32(1-2): 24-39.

Zwillenberg H.H. 1959. Über die Milz des Braunfisches (*Phocaena phocaena*, L.). *Z. Anat. Entwicklungsgesch*, 121 (1): 9-18