Structural characteristics of the mammalian spleen indicating storage and release of red blood cells. Aspects of evolutionary and environmental demands

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Different morphological and functional compartments can be distinguished within the spleen of mammals (fig. 1). Reticular cells form a meshwork exhibiting regional specializations (white and red pulp). Defense functions seem to be bound to the white pulp of the spleen. Metabolic functions including accumulation of various substance (e.g. ferritin) can be primarily located within the red pulp. An important storage of intact red blood cells and the rapid release of erythrocytes are correlated with the development of a capsule and trabeculae containing numerous smooth muscle cells. In the following contribution attention is focused on the evolution of capsule and trabeculae of the spleen in correlation with storage and rapid release of considerable amounts of intact red blood cells.

According to Björkman[2], as early as 1723 Stuckeley 'regarded the spleen as a storing place for a surplus of blood, which could, in case of a need, be used'. This statement could be verified in several experiments. In 1925, Barcroft[1] detected in cats which had been bled to death a considerable decrease of the spleen weight (from approximately 25 g to 6–7 g). A similar reduction of spleen weight was observed following rapid movements[1]. In the same decade Scheunert and Krzywanek[23,24] reported that forced exercise in horses and dogs was followed by an increase of red blood cell counts and of hemoglobin in blood samples drawn from the jugular vein. This phenomenon could not be observed in splenectomized dogs or in a horse suffering from a tuberculosis of the spleen. In animals adapted to exercise the increase is higher than in nontrained animals of the same species[16,17]; e.g. following complete exhaustion in 17 race horses, initial values of packed cell volume (hematocrit) were raised by 47%, hemoglobin values increased by 49% and O_2-saturation by 10%. In dogs the hematocrit values were elevated for only about 30%[23], whereas the spleen of man may store only 30–40 ml of blood[3]. In splenectomized sheep neither stress (disturbance) nor adrenaline injections seems to induce an increase of hematocrit values observed in intact specimens[6]. Finally, storage and release of red blood cells has been convincingly demonstrated using reinjected radioactively labeled erythrocytes[22,26].

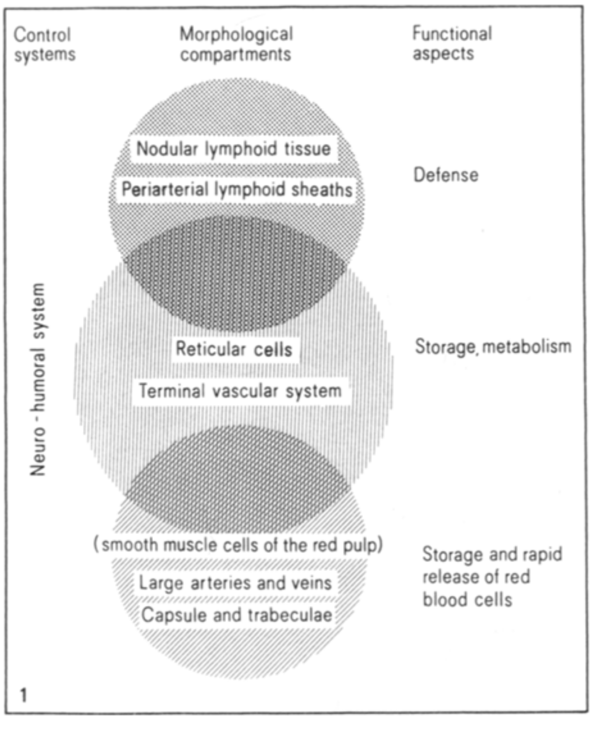

Figure 1. Diagram showing overlapping of morphological and functional compartments of the mammalian spleen.

Von Herrath developed the concept, as a working hypothesis, that various types of spleens observed in different species of mammals can be described as a graded series between two extreme types; on the one hand the storage type and on the other the defense type. In a comparative morphological approach von Herrath[9,10] investigated structural characteristics of the spleen in seven domesticated mammals (rabbit, cat, dog, pig, sheep, cow and horse). In addition, he studied the spleen of man using quantitative techniques. Von Herrath characterized the spleen of horses, animals known to be able to store and release considerable amounts of red blood cells (see above), by a high value of relative weight (spleen weight divided by b.wt), a high amount of trabeculae rich in smooth muscle cells, and a small amount of white pulp. The spleen of rabbits, on the other hand, has a small relative weight, is low in numbers of trabeculae and smooth muscle cells and rich in lymphatic tissues (for detailed review, see von Herrath[11]). The spleen of dogs exhibits an intermediate position between that of the horse and that of the rabbit (cf. fig. 2). Between the extreme types there exist transitional types. They may be scaled according to their relative weight, content of trabeculae and smooth muscle cells, as well as content of red and white pulp. Von Herrath showed a close correlation between the amount of smooth muscle cells in the trabeculae of the spleen and the effectiveness of release of red blood cells[12]. Other investigators completed the scaling list of spleen types using the quantitative morphological techniques proposed by von Herrath. According to the criteria suggested by von Herrath the spleen of the elk seems to possess the highest capacity to store and release red blood cells[11].

Figures 3 and 4 show randomly selected microscopical visual fields of a spleen of man and a spleen of chamois redrawn from original histological sections. The camera lucida representation is chosen to demonstrate the technique applied by von Herrath in evaluating the relative amount of trabeculae, white and red pulp (planimetry of areas occupied by white and red pulp, capsule and trabeculae). According to von Herrath's criteria, man seems to be a mammal with a poor capacity to store red blood cells, whereas chamois should show a high capacity of storing and releasing red blood cells. The investigated individuals were of roughly the same b.wt; thus, the likelihood of problems of allometry was quite small.

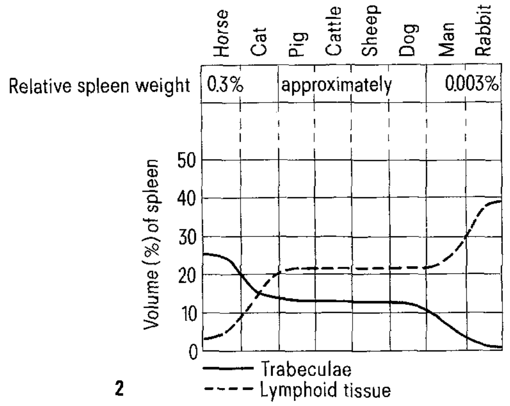

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Figure 2. Diagram showing relative spleen weight and splenic content of trabecular and lymphoid tissue in seven domesticated species of mammals and in man. To the left the extreme 'storage' type (storage and release of red blood cells) is found whereas to the right the extreme 'defense' type of spleen is represented (after von Herrath[11]).

In addition to the difference in the area occupied by capsule and trabeculae as well as by white pulp it has to be noted that the capsule and trabeculae of man are extremely poor in smooth muscle cells whereas these structures in chamois as well as in other Artiodactyla contain numerous smooth muscle cells (cf. figs 5 and 6). However, the scaling procedure developed by von Herrath has to be criticized in a number of different respects. A few of these will be briefly discussed here.

1) Most important is the influence of the sacrificing procedure. Fright and stress are well-known factors inducing rapid and maximal contractions of the spleen (e.g. maximal decrease of spleen thickness in sheep within 30 sec following disturbance, cf. Dooley et al.[6]; for further details observed in wild animals, see Dooley[8]).

2) When wild animals are compared with their domesticated varieties, it has to be considered that only some domesticated species have been selectively bred for exhaustive exercise. Furthermore, relative spleen weights of domesticated and wild species may belong to different lines of allometry since domestication results in a change of proportions of different organs (for details and review, see Herre and Röhrs[13]).

3) The estimated contents of spleens in capsular and trabecular tissues as well as in white and red pulp depends on the measuring technique applied. Von Herrath included the capsule in every fourth visual field evaluated. However, the relative amount of capsule is low in spleens with absolute high volume and high in spleens with absolute low volume (Hartwig and Hartwig[8]). Fur-

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Figure 3. Camera lucida drawing showing trabeculae (T) and monolayered capsule as well as lymphoid tissue (L) in a histological section of a human spleen. Please note that trabeculae and capsule contain mainly connective tissue.

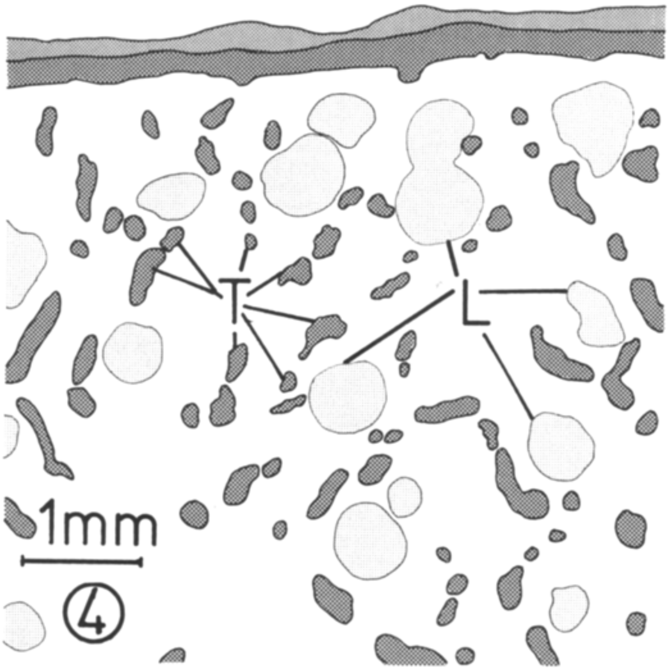

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Figure 4. Camera lucida drawing showing trabeculae (T) and bilayered capsule as well as lymphoid tissue (L) in a histological section of a spleen of chamois. Please note that trabeculae and capsule are rich in smooth muscle cells.

thermore, in our own unpublished investigations extremely high values of variance between different randomly selected visual fields have been observed. In addition it has to be mentioned that trabeculae may exhibit preferential orientations. This results in great variations of measured values if different section angles are investigated. Finally, relative values of volumes of red pulp, trabeculae and white pulp vary considerably if contracted or dilated spleens are investigated (for further and detailed discussions, see Tischendorf[28]).

In consequence the quantitative morphometric parameters reported in the literature have to be interpreted with a considerable amount of criticism. Therefore, we propose to evaluate exclusively qualitative parameters such as occurrence and distribution of smooth muscle cells as well as existence of a mono- or bilayered capsule. Regarding these structural characteristics, four different types of spleens can be distinguished in mammals: Spleens with 1) monolayered capsule and trabeculae composed mainly of connective tissue, 2) monolayered capsule and trabeculae rich in smooth muscle cells, 3) bilayered capsule and trabeculae rich in smooth muscle cells, and 4) bilayered capsule and trabeculae rich in smooth muscle cells accompanied by additional smooth muscle cells in the red pulp. Spleens characterized by the presence of a bilayered capsule have been claimed to be most efficient in storing red blood cells. The rich network of veins and lymphatic vessels in the subserosal part of the bilayered capsule (fig. 5) apparently serves to transport the fluid compartment of the blood which has been separated from erythrocytes (for review, see Tischendorf[28]). In principle, the scaling according to these criteria is in agreement with the results of von Herrath (compare fig. 2 with the table). However, since in most species listed in the table only morphology and not storage capacity has been investigated a complete morpho-functional scaling following the fruitful ideas of von Herrath (storage type versus defense type of spleen) cannot be performed. In contrast to the results of von Herrath the present listing does not show the spleen of the horse to be an extreme type among those of domesticated animals. However, we think that the scaling proposed in the table reflects both aspects of evolution and adaptation (especially to environmental demands, see below) and therefore might complete the scaling proposed by von Herrath.

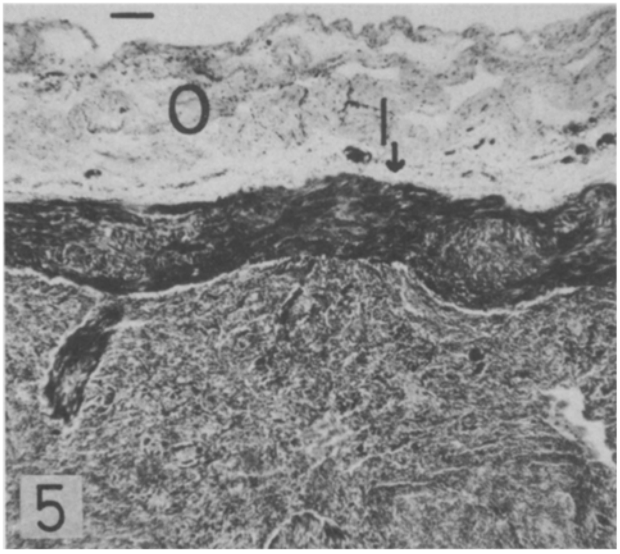

Figure 5. Histological section showing bilayered spleen capsule in *Cervus elaphus*. O: outer or subserosal layer of the capsule. Please note numerous profiles of veins. I: inner layer of capsule rich in darkly stained smooth muscle cells. Scale marker: 100 µm.

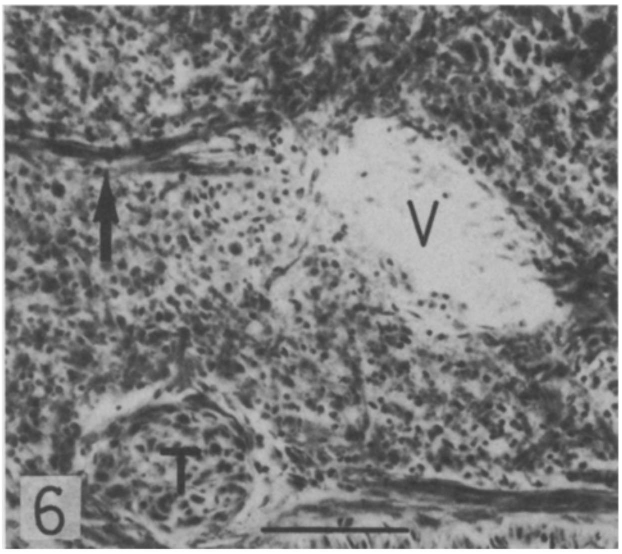

Figure 6. Smooth muscle cells (arrow) of the red pulp in the spleen of chamois. Please note arrangement of smooth muscle cells perpendicularly to the wall of a pulp vein (V). T: cross-sectioned trabecule rich in darkly stained smooth muscle cells. Scale marker: 100 µm.

Aspects of evolutionary and environmental demands interacting with the phylogenetic development of the spleen are most probably closely related to the biology of this enigmatic organ. When one discusses the biology of the spleen, ideas developed by Meyer[20], who dealt with functional biology and evolutionary biology, as well as concepts put forward by Koenig[14] should be acknowledged. In an attempt to combine ideas based on natural sciences with those arising from humanities, Koenig[14] distinguished four ethological determinants of final causes, allowing the comparison of functional capabilities of different species 1) phylogenetic position and level of evolution; 2) ecological and behavioral position such as e.g. feeding insectivorous, carnivorous, herbivorous; 3) general ecological situation such as living in narrow and subdivided territories as opposed to occupying wide nonstructured regions; 4) social structure such as family group in contrast to social herds.

For the present analysis, and also with view to future research, the evolution of the spleen may be discussed within an ethological framework. Apparently only those mammals evolving rather late in phylogeny possess spleens with a bilayered capsule and trabeculae rich in smooth muscle cells (type 3 in the table). It has been claimed that the order of Artiodactyla is probably the youngest order in the phylogeny of mamals[27]. In addition to the above-mentioned structural criteria of our spleen of type 3, Artiodactyla developed a meshwork of smooth muscle cells in the red pulp (spleen of type 4, table, fig. 6). The latter has been considered to be most efficient in the rapid release of stored red blood cells (for review, see Tischendorf[28]). In this context it is interesting to note that possible ancestral forms of Artiodactyla have been presumed to exist no earlier than the Lower Eocene period[4]. During this period apparently a new type of environment (grassland and prarie) developed[19]. Thus, environmental demands may have influenced the evolution of spleen biology and spleen morphology in context with the evolution of ethological determinants[14]. This suggestion is further supported by characteristic patterns of evolution of other organ systems, e.g. the gastrointestinal tract, especially the stomach (for details, see Langer[18]). Unfortunately conditions of paleoecology are not known in detail. However, it has been shown that at least the phylogenetically older Perissodactyla evolved as leaf eaters (cf. Thenius[27]) whereas Artiodactyla might have fed on grass.

Considering these aspects, diversities of spleen morphology may serve as a model elucidating at least partly the modification of structures under the pressure of evolution. Apparently acquirement of new structures, e.g. bilayered capsule and smooth muscle cells of the red pulp seem to be under the pressure of evolutionary demands (see table, and also Popper in this context: 'It is much more likely that a new feeding habit leads ... to new anatomical adaptations, than that accidental anatomical changes enforce new feeding habits'[21]). Newly developed structural elements can be observed in all members of the same phylogenetic branch independently of the present environmental demands and the present ecological and behavioral position (see above; cf. table). The spleen of Cetacea belongs to type 3 of the classification presented in the table. The very small relative weight of the spleen of Cetacea was interpreted by Zwillenberg[29] as an example for the limited value of the classification of different types of spleens proposed by von Herrath. However, Zwillenberg[29] apparently did not regard the biology of the spleen in Cetacea. Cetacea occupy an environment without natural enemies and most probably have no need to store and release red blood cells in emergency situations induced by enemies. Apparently only quantitative values (e.g. relative weight, volume occupied by capsule and trabeculae, etc.) of the different splenic compartments are under influence of the environment whereas the presence or absence of a certain structural element, e.g. smooth muscle cells of the red pulp, seems to be under the influence of evolution. This assumption is further supported by findings obtained in kangaroos. Kangaroos possess spleens characterized by the presence of smooth muscle cells in the red pulp, and in some species a bilayered capsule is observed[15]. Although ethological determinants[14] of kangaroos and Artiodactyla are very similar there is a considerable difference. Both Artiodactyla and kangaroos engage in long lasting flight activities. However, the relative weight of the spleen is high in Artiodactyla and low in kangaroos. This apparently paradoxical observation may be easily explained since kangeroos at high hopping speeds have lower energy costs in comparison to other quadrupedal herbivores at the same speed (bouncing ball phenomenon; cf. Dawson and Taylor[5]). In a discussion of the above-mentioned problem, the structure of the spleen of Hyracoidea is of special interest since Hyracoidea apparently represent the most ancestral order of Ungulata[27]. The spleen of Procavia (Hartwig and Hartwig, unpublished results) belongs to type 2 of the classification presented in the table. Thus, Hyracoidea might have been separated from the branch of developing Ungulata before pressure of evolutionary or environmental demands resulted in the evolution of a bilayered spleen capsule.

In summary, this short and deliberately subjective review indicates that structural characteristics shown in the table evolved in mammals along the following line: 1) monolayered capsule and trabeculae poor in smooth muscle cells to 2) development of smooth muscle cells within the capsule and trabeculae and 3) via the evolu-

Selected structural details characterizing four different morphological types of spleens observed in different orders of mammals. To keep the list of references manageable predominantly the detailed review presented by Tischendorf[28] has been cited. (+) refers to unpublished observations of Hartwig and Hartwig or Hartwig, Tischendorf and Hartwig

Type of spleen	Orders and species
1) Monolayered capsule and trabeculae poor in smooth muscle cells	Primates Man[9]
	Lagomorpha *Lepus europaeus* (+) *Oryctolagus cuniculus* (+) Rabbit[9]
	Rodentia *Marmota monax*[28] *Rattus norvegicus*[25] *Mus musculus*[28] Laboratory rat[25] Guinea pig[28] Golden hamster[28]
2) Monolayered capsule and trabeculae rich in smooth muscle cells	Carnivora *Procyon lotor*[28] *Mustela putorius* (+) *Vulpes vulpes* (+) *Panthera leo*[28] Dog[9] Cat[9]
3) Bilayered capsule and trabeculae rich in smooth muscle cells	Perissodactyla Horse[9] Donkey[29]
4) Bilayered capsule and trabeculae rich in smooth muscle cells, network of smooth muscle cells in the red pulp	Proboscidea *Elephas maximus*[28]
	Artiodactyla *Hippopotamus amphibius*[28] *Tayassu tajacu* (+) Pig[9] Camel (+) Lama (+) *Alces alces*[28] *Cervus elaphus*[8] *Capreolus capreolus*[8] Reindeer (+) *Rupicapra rupicapra* (+) Cattle[9,28] Sheep[9] Goat[28]

tion of a bilayered capsule to 4) finally the development of a network of smooth muscle cells in the red pulp. According to this line of evolution spleens of type 4 (table) should possess the highest capacity to store and release red blood cells. This assumption is further supported by the observation that this latter type of spleen receives the highest density of sympathetic innervation as revealed by the histochemical visualization (formaldehyde-induced fluorescence technique) of catecholamines (unpublished comparative investigations in rabbit, horse and cattle by Piontkowski and Hartwig). Physiological experiments conducted in nondomesticated representatives of Perissodactyla and Artiodactyla are urgently required to test the presented hypothesis. Moreover, only these experiments can elucidate the functional role of the meshwork of smooth muscle cells observed in the red pulp of Artiodactyla. At present a controlled active contraction of reticular cells in Perissodactyla and other mammals cannot be excluded since contractile filaments have been located in a number of different reticular cells using immunocytochemical techniques[7].

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