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COMPARATIVE ASPECTS OF SPLENIC MICROCIRCULATORY PATHWAYS IN MAMMALS: THE REGION BORDERING THE WHITE PULP

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Abstract

Splenic microcorrosion casts prepared using minimal volumes of material show that most of the flow passes through the region bordering the white pulp. However, the nature of these microcirculatory pathways has received little attention. We have studied these in dog, cat, rat, mouse, and normal versus diseased human spleens. In all 5 species, a marginal sinus (MS) of anastomosing vascular spaces 5-10 μm thick lies between the white pulp and marginal zone (MZ). The morphology differs between species and the MS is absent in immune thrombocytopenia. The MS fills by circumferential flow before blood passes outward to the MZ. Many capillaries supply the MS and MZ, their arrangement and degree of branching differing among species. Capillaries never terminate within the reticulum of the white pulp. In immune thrombocytopenia, marked vascular hyperplasia occurs within white pulp and MZ. The perimarginal cavernous sinus plexus (PMCS), found in human, dog and rat, comprises large flattened spaces up to 300 μm $\times$ 1000 μm in area and 30-100 μm thick. It lies between the MZ and red pulp or directly adjacent to white pulp, and receives flow principally via the MZ. In **sinusal** spleens, the MS, MZ and PMCS are drained by open-ended venous sinuses. In **non-sinusal** spleens, the MS and MZ are drained by pulp venules. Approximately 90% of the splenic inflow passes through the region bordering the white pulp, bypassing the filtration beds of the red pulp. This suggests that immunologic functions of the spleen take precedence over the filtration of blood cellular elements in the red pulp.

Key Words: Spleen, microcirculation, corrosion casts, white pulp, marginal sinus, marginal zone, perimarginal cavernous sinus plexus, open-ended venous sinuses, human, dog, cat, rat, mouse, immune thrombocytopenia.

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Introduction

The microcirculation of the spleen is perhaps the most complex of all the organs and tissues of the body, and as such it has been the subject of much controversy. The issue that has received most attention is whether the microcirculation is "open" or "closed", i.e., whether the arterial blood flows first into the reticular meshwork of the red pulp or directly into the venous channels (via vessels with endothelial continuity). However, another important area of uncertainty exists which has remained largely unnoticed. This concerns the nature of the microcirculatory pathways bordering the white pulp. Although it is now becoming more widely recognized that the marginal zone (MZ) between the white pulp and red pulp is a distinct region, in fact the largest B-lymphocyte compartment within the spleen in some species (rat: Kumararatne *et al.*, 1981), the routes by which blood supplies and drains the MZ are poorly understood. What further complicates this issue is the fact that species differences exist.

Most standard medical histology texts describe the main features of splenic structure and blood circulation but do not include any reference to microcirculatory pathways bordering the white pulp. (An exception to this is the extensive chapter on spleen written by Weiss in his histology text (1988), in which some aspects of the microcirculation in the MZ are described). The view that is commonly presented is illustrated schematically in Figure 1. This shows follicular capillaries terminating within the lymphatic nodule, and all other vessels passing out substantial distances into the red pulp. The white pulp is shown as bordering directly on red pulp, and the venous drainage begins some distance away in the red pulp.

Using a modified microcorrosion casting technique, we have studied spleens of five mammalian species, including normal and diseased human spleens (Schmidt *et al.*, 1983a,b; 1985a,b; 1988; 1991). In this paper, we review the comparative aspects of microcirculatory pathways bordering the white pulp, drawing together our previous findings for individual species and new unpublished results. We show that the marginal sinus (MS) is a distinct vascular space, lying between the

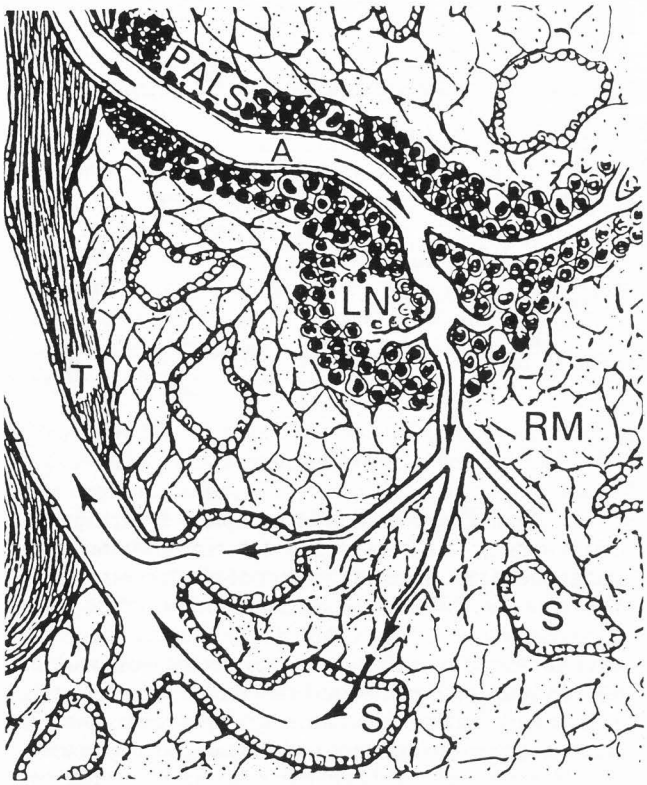

Figure 1. Schematic diagram showing structure of sinusal spleen. [(Reproduced with permission from Bellanti (1979), condensed to focus on region bordering white pulp]. A: Central artery; PALS: periarterial lymphatic sheath; LN: lymphatic nodule; RM: reticular meshwork of red pulp; S: venous sinus; T: trabecula.

Abbreviations in Text

MS	marginal sinus
MZ	marginal zone
PALS	periarterial lymphatic sheath
PMCS	perimarginal cavernous sinus
ITP	immune thrombocytopenia
SEM	scanning electron microscopy

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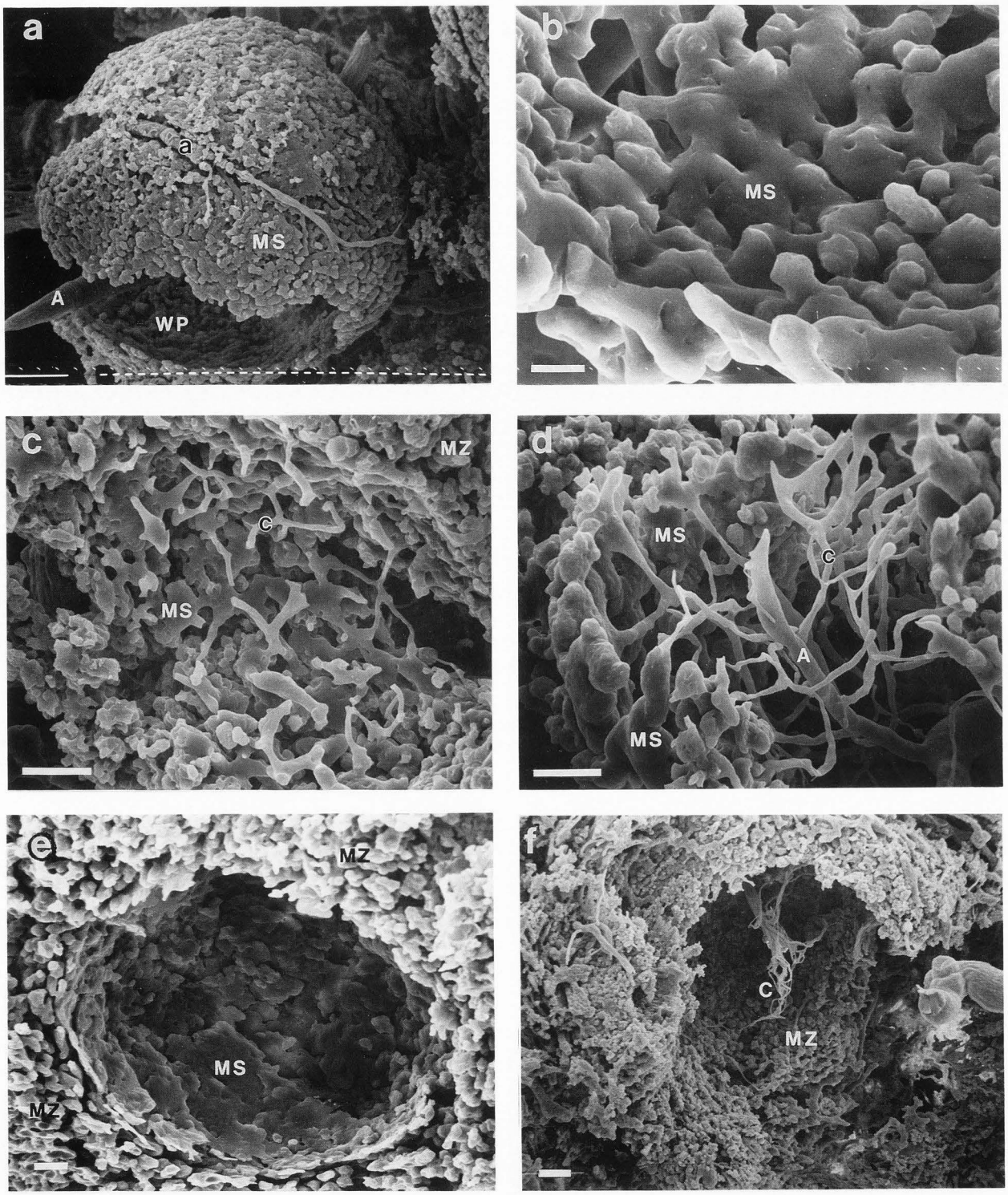
white pulp and MZ, in all five species studied. The 3-dimensional arrangement of the capillaries supplying the MS and MZ, which differs among species and changes with disease, is demonstrated. The perimarginal cavernous sinus (PMCS), so far reported only in humans (Yamamoto *et al.*, 1979; Schmidt *et al.*, 1988), has now been found in dog and rat also. Finally, we present evidence that a large proportion of the splenic venous drainage occurs directly from the MS and MZ, bypassing the filtration beds of the red pulp.

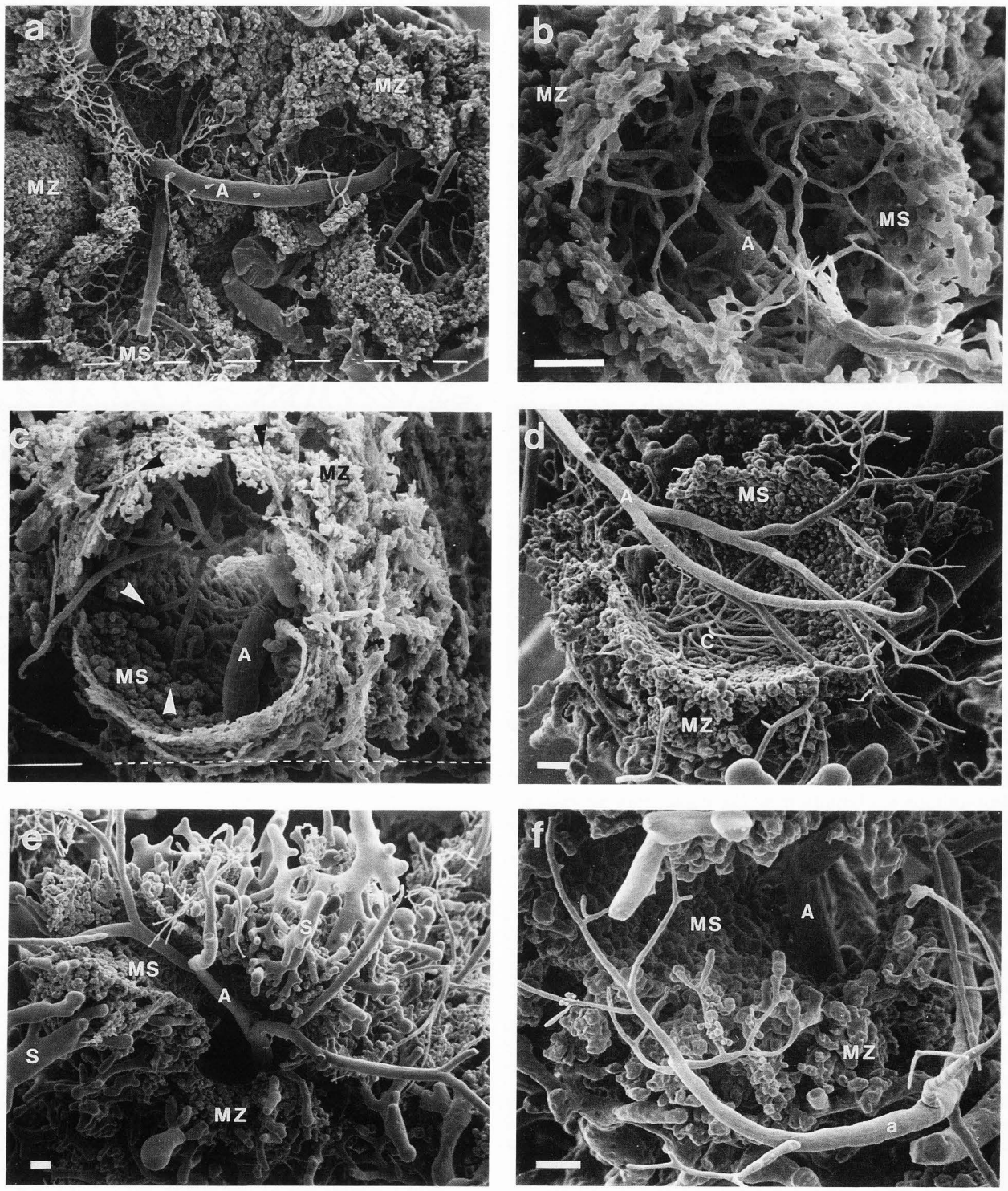
Materials and Methods

For full details of the procedures used for studying spleens of dog, cat, rat, mouse and human (normal and diseased) see Schmidt *et al.*, (1982; 1983a,b; 1985a, 1985b; 1988; 1991). In brief, the experimental animals were anesthetized with sodium pentobarbital (60 mg/kg ip). The spleen was removed and perfused via the splenic artery or a branch thereof, with phosphate-buffered Ringer solution which had been filtered and equilibrated at 37°C with 5% CO_2 in O_2. Perfusion was carried out at a pressure of 95 cm H_2O with the venous outflow unobstructed. When the tissue had lost its reddish color and the perfusate flowing from the splenic vein had cleared, a modified low-viscosity mixture of Batson's corrosion casting compound (Nopanitaya *et al.*, 1979) was injected via the arterial cannula. Usually, only a minimal amount was injected, until the material just reached the venous cannula. This left the reticular meshwork of the red pulp largely unfilled, which allowed the faster microcirculatory pathways to be traced over considerable distances in the cast. The actual volume injected (generally 0.3-2 ml) varied depending on the amount of tissue perfused. Following the injection the cannulae were clamped and the organ left undisturbed for 2 hours, in order for polymerization to occur. Several pieces of tissue approximately 1 cm^2 were cut from the filled segment (where necessary), and the tissue was then corroded away from these samples with 40% KOH at 60°C for 3-4 days. The vascular casts were rinsed in distilled water, left in 10% HCl for several hours, rinsed again with distilled water, air-dried, mounted on stubs and sputtered with gold for examination under the scanning electron microscope (SEM).

Figure 2 (facing page). Microcorrosion casts prepared using the minimal injection technique (as in all subsequent figures), which compare the morphology of the marginal sinus (MS) in spleens of different species. (**a**) Cat spleen. MS surrounds lymphatic nodule (white pulp, WP, corroded away). Arteriole (a) ramifies over convex surface of MS and terminates there via capillaries. Marginal zone and red pulp are unfilled due to minimal injection of material. A: central artery. Short bars = 10 μm. (**b**) Dog spleen. Aspect of MS facing a lymphatic nodule. Note flattened, almost continuous nature of MS. Bar = 20 μm. (**c**) Rat spleen. Interior of lymphatic nodule is seen, where follicular capillaries (c) are scarce due to breakage of casts. MS appears as discontinuous spaces interconnected by short vessels. MZ: marginal zone filling. Bar = 50 μm. (**d**) Mouse spleen. Central artery (A), within periarterial lymphatic sheath, gives rise to many capillaries (c) terminating in the MS. The discontinuous spaces of the MS are larger than those of rat spleen. Bar = 50 μm. (**e**) Normal human spleen. At the interior of a lymphatic nodule the MS appears as thin, flattened, almost continuous anastomosing spaces. MZ: marginal zone. Bar = 50 μm. (**f**) Spleen from patient with chronic immune thrombocytopenia. MS is absent and marginal zone (MZ) borders lymphatic nodule. c: follicular capillaries. Bar = 100 μm. [Fig. 1c: Reproduced with permission from Schmidt *et al.* (1985a)].



Eight normal human spleens from transplant donors (ages 2-62 years) were made available to us by the Multi-Organ Transplant Service, University Hospital, London. The spleens were maintained in sterile saline solution and kept on ice until use not more than an hour after removal from heparinized donors. Surgically removed spleens from seven patients (ages 20-76) with chronic immune thrombocytopenia, two with hypersplenism (ages 25 and 33), and one with chronic lymphocytic leukemia (age 58) were obtained through the Surgical Pathology Unit, University Hospital, London, immediately after removal. Splenic specimens for the



pathologist were quickly taken and a major arterial branch (at the hilus, distant from any incision) was cannulated. Both normal and diseased spleens were perfused with Ringer solution and casts prepared as described above.

In spleens from both humans and experimental animals, a limited region of the organ was usually perfused from the arterial branch cannulated. This produced in the cast a "natural dissection" at the boundary between the filled segment and the adjacent unperfused tissue. No cutting of the cast was needed on this face and, thereby, fragmentation of the replicas of fine

Figure 3 (facing page). Casts comparing capillary supply to marginal sinus (MS) and marginal zone (MZ) in animal spleens. (**a**) Mouse spleen. Central artery (A) runs through several adjacent lymphatic nodules, and gives rise to many capillaries, most of which terminate in the MS (on its white pulp side: see also Fig. 2d). MZ filling is seen around periarterial lymphatic sheaths and nodules. Bars = 100 μm. (**b**) Rat spleen. Central artery (A) within periarterial lymphatic sheath gives off many capillaries, most of which end in the MS on its white pulp side. Some MZ filling has begun. Bar = 50 μm. (**c**) Cat spleen. Vessels supplying MS and MZ consist of relatively few arterioles and capillaries deriving from central artery (A), with endings on both inner and outer aspect of MS (→). Capillaries are seen running circumferentially along the outer aspect of the MS and terminating there. Bars = 10 μm. (**d**) Dog spleen. Central artery (A) bifurcates repeatedly within lymphatic nodule, giving off many follicular capillaries (c) which terminate on either the inner or outer aspect of MS, or in the MZ. Some vessels pass further out to the red pulp (casts with 'blind' ends due to incomplete filling). Bar = 100 μm. (**e**) Dog spleen. Central artery (A) passes down the axis of periarterial lymphatic sheath. Arterioles extend out to MZ and red pulp, but capillaries are virtually absent within the sheath. Many venous sinuses (S) originating in the MS/MZ have begun to fill. Bar = 100 μm. (**f**) Dog spleen. Arteriole (a) branches from central artery (A) within lymphatic nodule, passes out through MS and MZ and curves circumferentially around nodule, giving off numerous capillaries that terminate in MS or MZ. Bar = 50 μm. [Fig. 3f: Reproduced with permission from Goresky and Groom (1984)].

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vascular paths was avoided. At this "natural" boundary, vascular pathways in the interior of the spleen could be followed without interruption. In small spleens from rats and mice, a similar result was obtained by ligating branches of the splenic artery at one end of the organ, just before injection of the casting material.

Results

The marginal sinus

The splenic white pulp generally includes cylindrical sheaths of lymphoid tissue (periarterial lymphatic sheath: PALS) surrounding central arteries after they leave trabeculae, with thickenings in places to form lymphatic nodules (Fig. 1). However, the relative proportions of these two components differ depending on the species, and in the cat the white pulp consists predominantly of lymphatic nodules. When only a small amount of corrosion casting material has been injected in cat spleen, the nodules are surrounded by a thin spherical layer (< 10 μm) of material outlining the limits of the nodule but not enclosing it completely (Fig. 2a: see **Discussion**). This structure is the marginal sinus (MS) which consists of a series of anastomosing blood spaces lying between the white pulp (corroded away in the casts) and the marginal zone (MZ). The MS was filled by circumferential spreading of the injected material.

In other casts where greater volumes of compound were injected, filling of the MZ occurred as well, by spreading of material radially outward from fenestrations in the outer wall of the MS. In Figure 2a, this has just begun to occur at the right of the micrograph. Although on its outward side, the MZ merges with the reticular meshwork of the red pulp, almost no filling of the red pulp has occurred in this figure.

Species in which both lymphatic sheaths (PALS) and nodules are plentiful include dog, rat and mouse. In all of these species, a marginal sinus surrounds both PALS and lymphatic nodules. In dog spleen the MS consists of a series of flattened anastomosing spaces, somewhat larger than in cat spleen but with a similar overall geometry. In Figure 2b, the side of the MS facing the white pulp is shown. On the opposite aspect of the MS, outward spread of material into the MZ occurred when larger quantities were injected, as reported above for cat. In contrast to the relatively continuous nature of the MS in casts from cat and dog, in rat spleen the MS consists of a series of discontinuous flattened spaces interconnected by short vessels of capillary dimensions. This may be seen in Figure 2c (a view from the white pulp side of a nodule), which also shows some filling of the MZ in the background. In mouse spleen, the MS is discontinuous, as in rat, but the individual anastomosing spaces are up to six times as large in area; moreover, these spaces merge together rather than being connected by capillary-sized vessels. This is shown in Figure 2d in a cast of the MS surrounding a region of PALS.

In human spleen, PALS is relatively sparse and the white pulp consists largely of lymphatic nodules. In Figure 2e, the white pulp side of the MS surrounding a nodule is seen. The MS comprises a flattened, almost continuous system of anastomosing spaces, similar to that in dog spleen. The sheet-like appearance of the MS cast contrasts markedly with the knobbly configuration of casts of the surrounding MZ meshwork (Fig. 2e). The casts showed that the MS was present very consistently in normal human spleens: in 213 out of 224 lymphatic nodules (95%) from 8 spleens. In contrast, in casts of spleens from patients with immune thrombocytopenia (ITP), the MS was seldom found: in only 20 out of 191 nodules (11%) from 7 spleens. The cast of the region bordering a lymphatic nodule shown in Figure 2f is typical of ITP spleens. The MS is totally absent and the cast shows the knobbly appearance characteristic of MZ filling. Even in the few cases where evidence for an MS was present in ITP, only small isolated patches of filling were found. Similar findings were obtained in splenic casts from two patients with hypersplenism and one with chronic lymphocytic leukemia.

Capillary supply to the marginal sinus and marginal zone

The arrangement of the capillaries supplying the region bordering the white pulp differs among species.

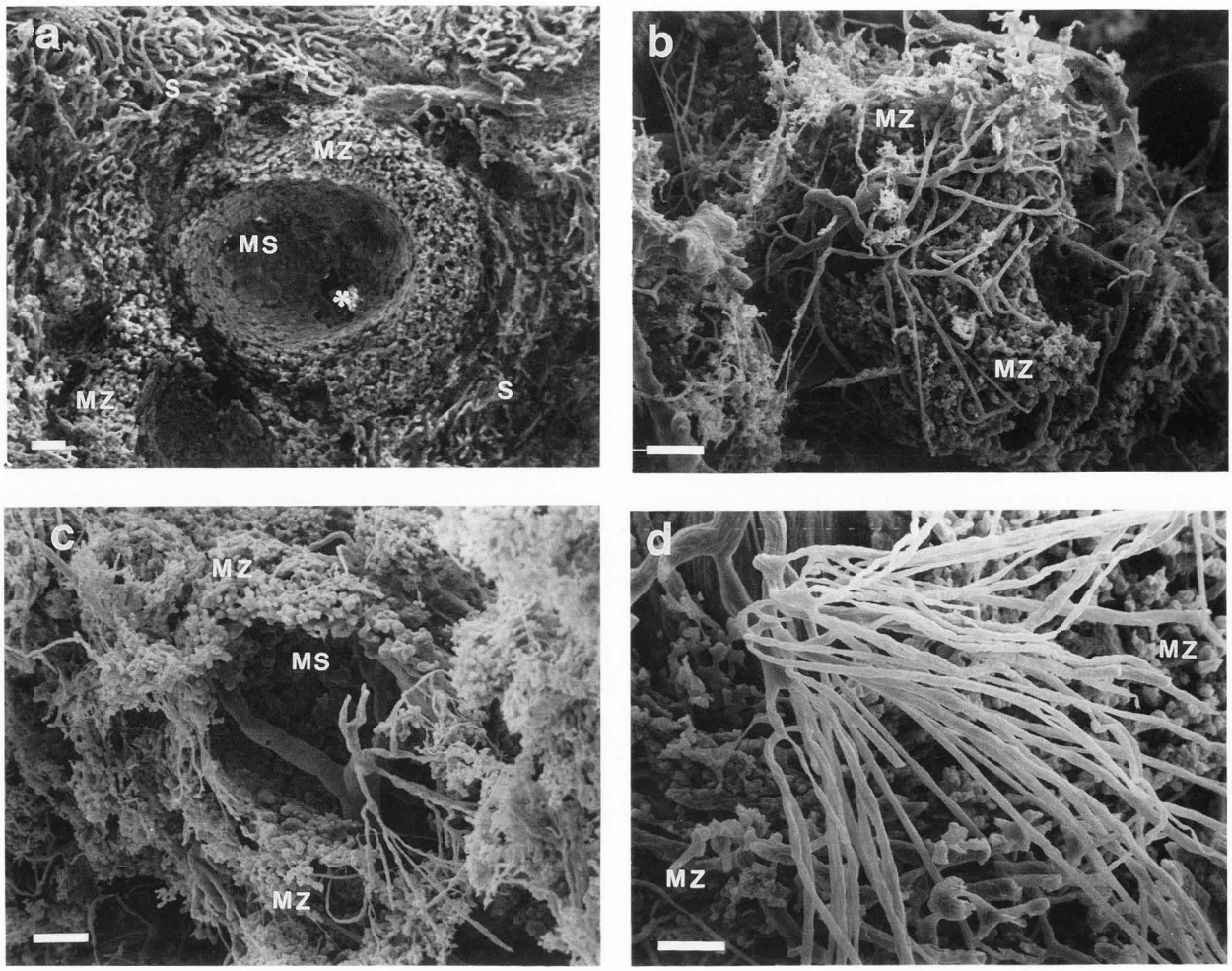
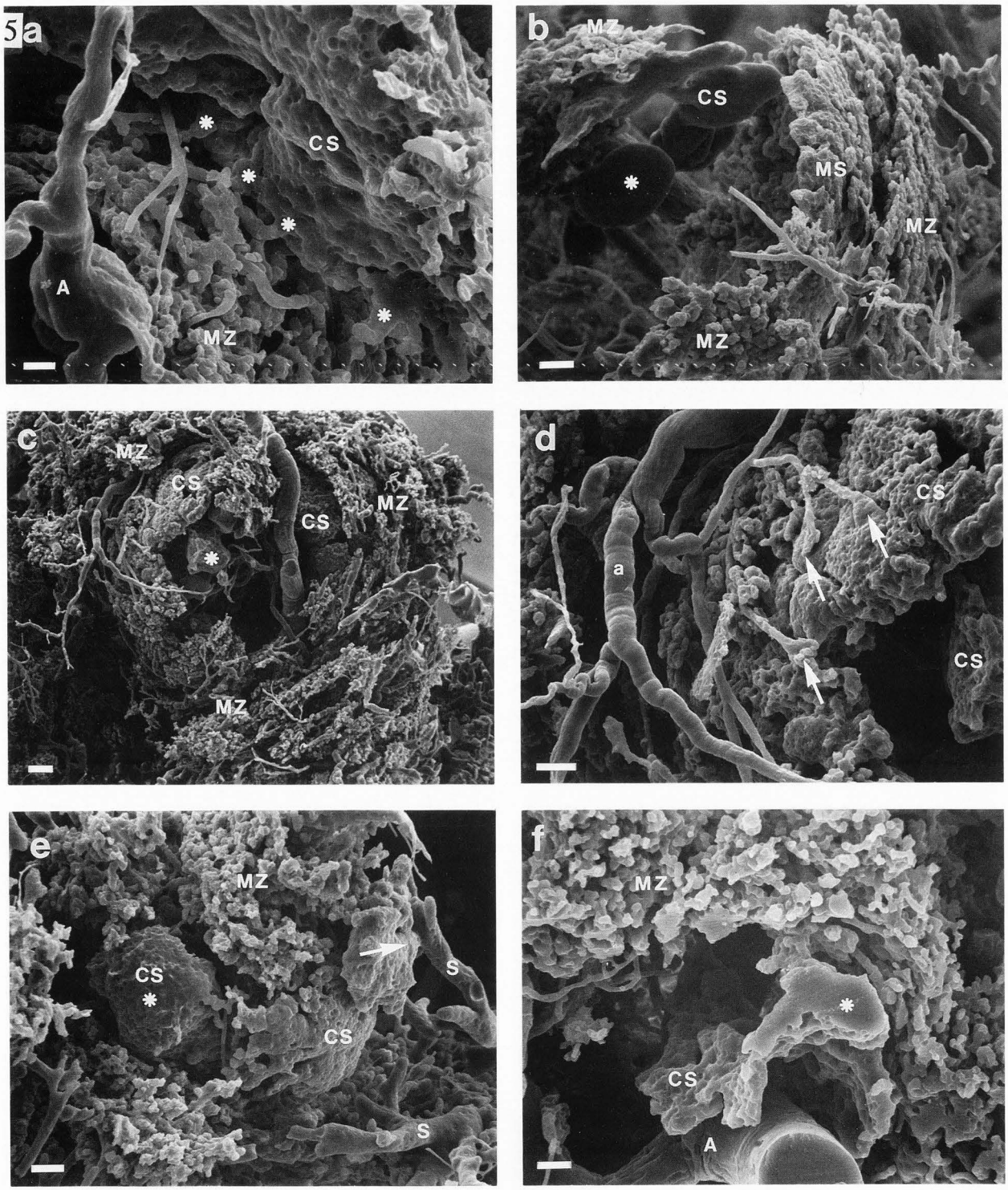
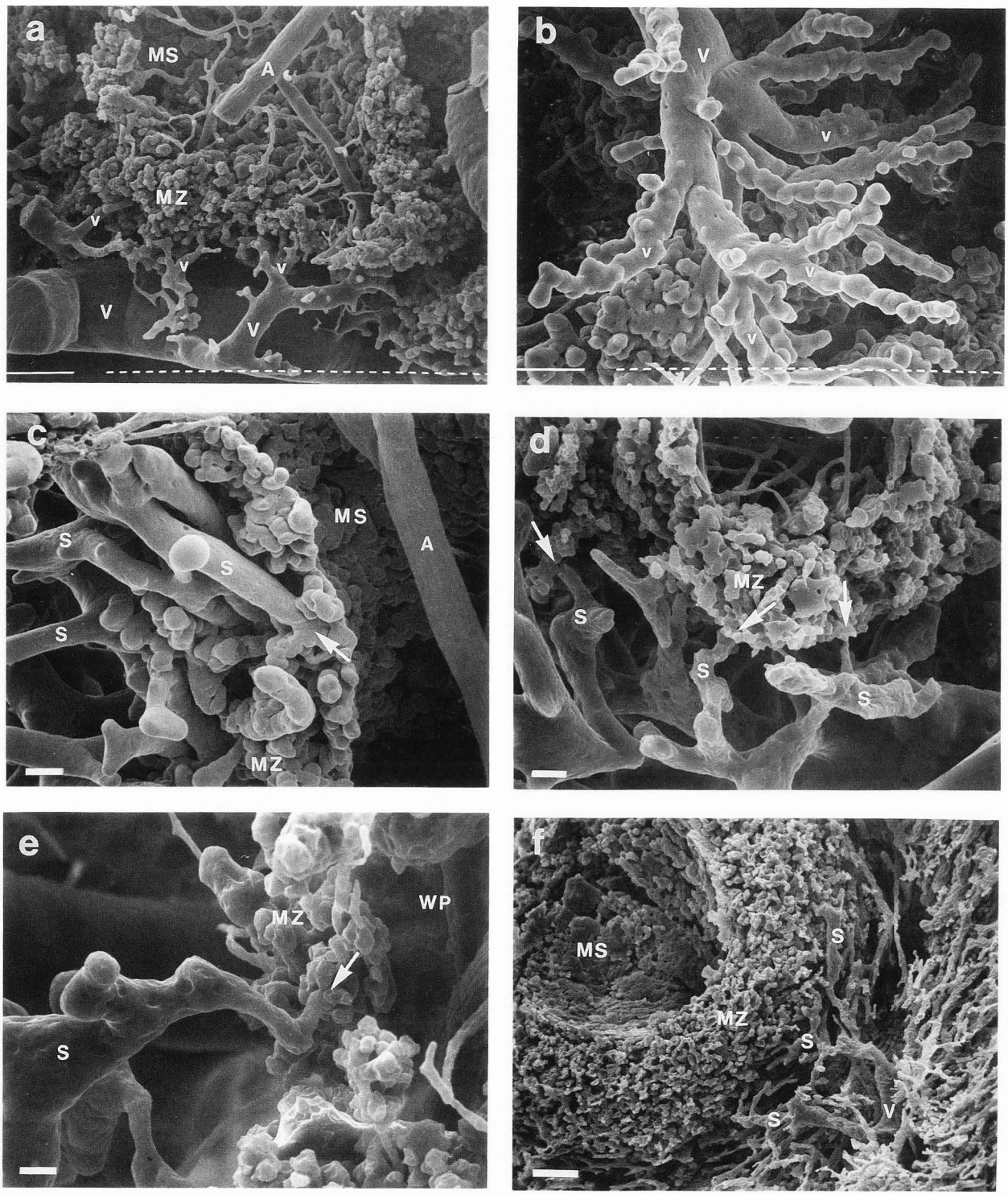


Figure 4 (above). Casts showing capillary supply to marginal sinus (MS) and marginal zone (MZ) in human spleens, normal versus diseased. **(a)** Normal human spleen, showing relationship between lymphatic nodules, MS, MZ, and network of venous sinuses (S) in red pulp. Opening in MS (*) is site where central artery (cast accidentally broken off) entered nodule. Bar = 100 μm. **(b)** Spleen from patient with chronic immune thrombocytopenia (ITP). Branches of central artery pass out from lymphatic nodule to the surrounding MZ, and there give rise to numerous circumferentially directed arterioles and capillaries, terminating in MZ or outer aspect of the MS. In normal spleens, the pattern of branching is similar but fewer vessels are found. Bar = 100 μm. **(c)** Normal human spleen, showing sparcity of vessels within lymphatic nodule. Bar = 100 μm. **(d)** Spleen from patient with ITP. Great proliferation of small arterioles and capillaries is found within lymphatic nodule and MZ. Bar = 100 μm. [Fig. 4a: Reproduced with permission from Schmidt *et al.* (1988)].

Figure 5 (on the facing page). Casts showing morphology of perimarginal cavernous sinus plexus (CS) in spleens of different species. **(a)** Normal human spleen. Large flattened mass of material, representing CS, is situated between MZ and red pulp. Central artery (A) within periarterial lymphatic sheath is seen at lower left. CS receives flow via numerous points of continuity with MZ (*). Bar = 25 μm. **(b)** Normal human spleen. CS is situated directly adjacent to lymphatic nodule and a part of the plexus extends into the white pulp (*). Note different morphology of CS, MS and MZ. Bar = 50 μm. **(c)** Dog spleen. Extensive CS surrounds lymphatic nodule (where MS is usually located) and extends into white pulp (*). MZ filling is seen external to CS, supplied by numerous vessels curving circumferentially within MZ. Bar = 100 μm. **(d)** Dog spleen. Close-up view of region from Fig. 5(c) showing circumferential arteriole (a) giving off several capillaries which terminate directly in CS (→). Bar = 50 μm. **(e)** Rat spleen. Regions of CS are situated directly adjacent to lymphatic nodule (*) or between MZ and red pulp. CS is drained (→) by open-ended venous sinuses (S). Bar = 50 μm. **(f)** Rat spleen. Region of CS extends into lymphatic nodule, close to central artery (A). Cross-sectioned areas of cast reveal thickness of CS (*), compared to much smaller spaces of MS or MZ. Bar = 25 μm. [Fig. 5c: Reproduced with permission from Schmidt *et al.* (1983a)].



Corrosion casts show that such capillaries terminate in the MS, from either the white pulp side or the MZ side (or both), and in the MZ. Capillaries were never seen terminating within the white pulp itself. In mouse and rat spleens, there is plentiful branching of vessels within the white pulp, and most capillaries terminate on the white pulp side of the MS. This is shown for mouse spleen in Figure 3a. The cast shows filling of the MZ around PALS and several lymphatic nodules, but virtually no filling of the red pulp. The narrowness of the gaps between adjacent MZ regions shows how small a volume is occupied by the red pulp. Within both PALS and



lymphatic nodules, very few capillaries pass into the MZ and terminate there or on the MZ side of the MS; the vast majority end in the MS where it faces the white pulp (Figs. 2d, 3a). The same is true of rat spleen, both for PALS (Fig. 3b) and lymphatic nodules (Fig. 2c).

In cat and dog spleens, capillaries within lymphatic nodules terminate in the MS on both the white pulp and MZ sides, as well as within the MZ itself. A view into a lymphatic nodule from cat spleen (Fig. 3c) shows a much lower number of vessels than in rat and mouse spleens; a few capillaries terminate on the inner aspect of the MS while most, after passing through, run in an arc along the outer aspect of the MS and terminate there or in the MZ. In contrast, capillaries are abundant

Figure 6 (facing page). Casts comparing venous drainage from marginal sinus (MS) and marginal zone (MZ) in spleens of different species. **(a)** Mouse spleen (non-sinusal). Photo shows entire pathway from central artery (A) to capillaries to MS to MZ to pulp venules (v) to collecting veins (V). Pulp venules appear as short, non-anastomosing vessels arranged as lateral branches of collecting veins. Bars = 10 μm. **(b)** Cat spleen (non-sinusal). Root-like system of non-anastomosing pulp venules (v) drains into collecting vein (V). Knobbly appearance results from emergence of material via fenestrations in venular walls and via open ends (retrograde filling). Bars = 10 μm. **(c)** Dog spleen (sinusal). Many venous sinuses (S) originate in MS or MZ as open-ended tubes (→). Extensive filling of interconnected system of venous sinuses has occurred in this way, while surrounding reticular meshwork remains unfilled. A: central artery of periarterial lymphatic sheath. Bar = 50 μm. **(d)** Rat spleen (sinusal). Venous sinuses (S) originate as open-ended tubes (→) in MZ bordering lymphatic nodule. Bar = 25 μm. **(e)** Human spleen (sinusal) from patient with ITP. Open-ended origin (→) of venous sinus (S) in MZ. WP: white pulp of lymphatic nodule corroded away. Bar = 25 μm. **(f)** Normal human spleen. Many venous sinuses (S) originate via open ends in MZ. Anastomosing system of venous sinuses drains into collecting veins (V). Reticular meshwork of red pulp is mostly unfilled. Bar = 100 μm. [Figs. 6b, 6c, and 6e: Reproduced with permission from Schmidt *et al.* (1983b, 1983a, and 1988) respectively].

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within lymphatic nodules of dog spleen (Fig. 3d) but virtually absent within PALS (Fig. 3e). The capillaries nestled within the nodule shown in Figure 3d terminate on both sides of the MS and in the MZ; in addition, there are arterioles that pass out through the MS and either curve around within the MZ or pass further out into the red pulp. In Figure 3f, a major arteriole arising from the central artery within the nodule passes intact through the MS and MZ. After curving circumferentially around the nodule, the arteriole branches to form an array of capillaries that terminate via ampullary dilatations in the outer aspect of the MS or in the MZ. The vascular supply to the MS and MZ around PALS (Fig. 3e) is achieved almost exclusively in this way.

An appreciation of the spatial relationship between the MS and MZ, including their relative thicknesses, may be obtained when a large amount of casting material is injected and a natural boundary between adjacent perfused and unperfused areas of spleen is studied (see **Methods**). Such a view is shown for normal human spleen in Figure 4a. Whereas the MS is generally < 10 μm in thickness (see also Fig. 2e) the MZ can be up to 200 μm thick, although it becomes a narrow band in regions where two nodules are in close approximation (Fig. 4a). The capillaries supplying the MS and MZ in human spleen are located almost exclusively around the outside of the nodules in the MZ. Typically, the central artery gives rise to only 2 or 3 branches, each of which passes out into the MZ and then branches repeatedly, forming numerous circumferentially directed capillaries that end in the MZ and the outer aspect of the MS (Fig. 4b). A complementary view showing the interior of a nodule (Fig. 4c) demonstrates the sparsity of vessels within the white pulp in normal human spleen. In contrast, in spleens of patients with ITP, a profusion of arterioles and capillaries is present both within the nodules and in the MZ (Fig. 4d). Such vascular hyperplasia was found very consistently in ITP spleens: in 176 out of 191 lymphatic nodules (92%) from 7 spleens. (Vascular hyperplasia was also found in casts from two patients with hypersplenism and one with chronic lymphocytic leukemia). However, in normal spleens, extensive vascularization was seldom found: in only 2 out of 224 nodules (1%) from 8 spleens.

The perimarginal cavernous sinus

In splenic casts of some species (human, dog, rat) the perimarginal cavernous sinus plexus (PMCS) may be clearly seen. (This structure was never found in spleens of cat or mouse). Its appearance is quite different from that of any other structure. In human spleen, large flattened masses of casting material up to 300 μm × 1,000 μm in area and 30-100 μm in thickness are found. These may be situated either between the MZ and the red pulp, or directly adjacent to the white pulp and often extending into it. In Figure 5a, a large area of PMCS surrounding the marginal zone around a periarterial lymphatic sheath is seen in human spleen. The surface of the PMCS cast is fairly smooth except for small "pock marks" and shallow impressions. Many points of flow continuity between the MZ and the PMCS are seen. In Figure 5b, the PMCS is situated directly adjacent to a lymphatic nodule in an area devoid of marginal sinus, and extends into the white pulp itself. The plexus-like nature of the PMCS is demonstrated in this cast.

Other casts from human spleen revealed that a considerable volume of material may reach the PMCS even when minimal filling of adjacent vascular spaces has occurred. Flow of material into the PMCS takes place not only via the MZ (Fig. 5a) but by several additional routes (not shown): from arterial capillaries connecting directly to the PMCS and from ellipsoid sheaths immediately adjacent to the PMCS. The above findings were obtained in normal human spleens but the PMCS was also present in spleens from patients with immune thrombocytopenia.

In dog spleen, the examples of PMCS we have found were located directly adjacent to or within the white pulp. This is shown in Figure 5c where the PMCS partly surrounds a lymphatic nodule in the way the MS usually does, and a portion of PMCS extends into the white pulp. In this example, the MZ surrounds the PMCS, and numerous arterioles curve circumferentially within the MZ. Some arterial capillaries ramify over the surface and terminate in the PMCS, as may be seen in the close-up view (Fig. 5d). In rat spleen, the PMCS is found surrounding the MZ (Fig. 5e), directly adjacent to or within the white pulp (Figs. 5e, 5f).

Venous drainage from the marginal sinus, marginal zone, and perimarginal cavernous sinus

Two quite different types of venous circulation are found among mammalian species. The smallest venous vessels begin either as venous sinuses or as pulp venules (for details about the structural differences of these venous channels see **Discussion**). Species with sinusal spleens include dog, rat and human, while those with non-sinusal spleens include mouse and cat. The nature of the pathways for flow in non-sinusal spleens, from MZ to pulp venules, is shown in Figures 6a and 6b. Pulp venules are short, non-anastomosing vessels 6-10 μm in diameter in mouse (Fig. 6a) and slightly larger in cat (Fig. 6b). In both species, they are arranged either as lateral branches of collecting veins (Fig. 6a) or as a 'root system' with many fine rootlets merging into one trunk (Fig. 6b). Flow into pulp venules occurs via their open ends and via fenestrations in their walls. Figure 6b illustrates the latter route in a cast from cat spleen, where the knobbly appearance of pulp venules results from the emergence of casting material from these fenestrations, due to retrograde filling of the collecting vein.

Venous sinuses can be distinguished from pulp venules by their larger size, greater abundance, and arrangement into a richly anastomosing system (for other distinctive features which cannot be determined from corrosion casts, see **Discussion**). This may be seen in Figure 6c for dog spleen, where many interconnecting venous sinuses drain the marginal sinus and marginal zone around the periarterial lymphatic sheath. These venous sinuses do not all originate as blind-ended channels, as is commonly thought, but receive flow freely through their "open" ends. Note that the region of continuity between MS and venous sinus is approximately 25 μm in diameter and that extensive filling of the sinuses has occurred, even though the surrounding reticular meshwork remains unfilled. Such open-ended venous sinuses are present in rat spleen also, and Figure 6d shows several sinuses draining the MZ around a lymphatic nodule. In human spleen, the venous sinuses also originate at the MZ via "open" ends (Fig. 6e). In the absence of filling of the red pulp reticular meshwork in these casts, the abundance of such open-ended origins and the interconnected nature of the venous sinuses may be seen clearly (Fig. 6f). The perimarginal cavernous sinus, which was found only in sinusal spleens, was also drained by open-ended venous sinuses. This is shown for rat spleen in Figure 5e, but the same pattern was found in human spleen also.

Discussion

The involvement of the splenic red pulp in blood filtration has long been recognized. However, the importance of the region bordering the white pulp for filtration and immunologic functions has not been so widely appreciated. Recently there has been an emphasis on immunologic aspects of the spleen, including the migration pathways of recirculating lymphocytes (van Ewijk and Nieuwenhuis, 1985; Pabst, 1988; Pellas and Weiss, 1990). However, the very basic issue of the nature of microcirculatory routes within and out of the region bordering the white pulp still remains unclear. Microcorrosion casts, produced by injection of minimal amounts of material, offer a unique opportunity to study these flow pathways.

The marginal sinus

This structure was first described in rat spleen by Andrew (1946), who referred to it as a "sinus-like cleft", and by Altschul and Hummason (1947) and Snook (1950) who assigned to it the name of "the perifollicular space". Subsequently, the term "marginal sinusoid" was used by Baillif (1953). The currently used term, marginal sinus, was adopted by Snook (1964) and by subsequent investigators (e.g., Sasou *et al.*, 1976; Schmidt *et al.*, 1983a,b; Pellas and Weiss, 1990). The original reports of this structure described it as a series of anastomosing vascular spaces lying between the white pulp and the MZ. Serial sections indicated that the MS is not a vessel in the usual sense, since it does not have a longitudinal axis, but is a cleft-like space surrounding the white pulp. The above authors found that many white pulp capillaries terminate in the MS, their endothelial walls continuous with the endothelial cells lining the MS. This endothelial lining has been shown particularly well by Sasou *et al.* (1982). Red blood cells within the MS have direct access to the MZ via discontinuities in the outer wall of the MS (Snook, 1964; Sasou *et al.*, 1982). By means of histological sections or SEM of tissue, it is difficult to gain an appreciation of the morphology of the MS. Many studies of splenic morphology have omitted all mention of this structure, and the existence of the marginal sinus in mammalian spleens is not yet generally recognized. It has been claimed that the MS is absent in human spleen (Van Krieken *et al.*, 1985; Sasou *et al.*, 1986) or that it is a "poorly delimited" component of the MZ (Barnhart and Lusher, 1979).

Our microcorrosion casts have shown the three-dimensional morphology of the MS and demonstrated that it is a distinct vascular space in all five species studied (Schmidt *et al.*, 1983a,b; 1985a,b; 1988). However, its morphology differs among species, ranging from a continuous (cat, dog, normal human) to a discontinuous (rat, mouse) system of anastomosing spaces. The individual spaces may be small in area (cat, rat) or large (dog, mouse, normal human). Casts prepared by the minimal injection technique show that the MS is filled by circumferential spreading of material, before radial spreading occurs outwardly into the MZ through fenestrations in the outer surface of the MS. This indicates that a large proportion of the inflowing blood is distributed over the surface of the white pulp, before passing outward into the marginal zone and on to the red pulp. (In minimal-injection casts lymphatic nodules appear to be incompletely surrounded by MS. We suspect that this incomplete enclosure may be artefactual. One cannot be certain of this, however, for when larger amounts of casting compound are injected the view of the MS is

obscured by the cast of the surrounding MZ, which does enclose the white pulp entirely: see Schmidt *et al.*, 1985b, Fig. 4).

It is interesting that in immune thrombocytopenia (and some other pathological conditions) the MS is consistently absent in casts from human spleens (Schmidt *et al.*, 1991). Since many studies of the human spleen have been based on pathological material, this may partly explain why the MS has escaped detection. In casts of normal spleens from organ transplant donors, we found that the MS was present with great consistency, in 95% of 224 lymphatic nodules examined from eight spleens. In marked contrast to this, the MS was absent in 89% of 191 nodules examined from casts of seven spleens of patients with immune thrombocytopenia. Thus, absence of the marginal sinus from human spleen is not a species characteristic as suggested by Van Krieken *et al.* (1985) and Sasou *et al.* (1986), but appears to be a consequence of a disease state such as immune thrombocytopenia. There remains the possible objection that an absence of MS filling in the casts may merely reflect a failure to perfuse a structure which is actually present. The consistency of our findings makes this possibility extremely unlikely. Moreover, any part of the microvasculature deprived of blood perfusion for long periods of time will eventually disappear.

What functions could the MS serve? The fact that by means of the MS the incoming blood is spread widely over the surface of the white pulp and then evenly distributed out to the MZ suggests that immunological interactions may be facilitated thereby, as well as filtration functions in the MZ. There is evidence in the literature that lymphocytes and macrophages can migrate from the MS into lymphatic nodules. This is based on views from tissue sections and SEM of tissue, showing cells caught in transit between MS and nodule (Moore *et al.*, 1964; Goldschneider and McGregor, 1968; Veerman and van Ewijk, 1975; Sasou *et al.*, 1980). Although such static views do not indicate the direction of cell movement, other investigations of lymphocyte migration based on autoradiography or immunocytochemistry have demonstrated the movement of B cells from the MS into lymphatic nodules, and possibly in the reverse direction as well (Goldschneider and McGregor, 1968; Mitchell, 1973; Brelinska and Pilgrim, 1982; van Ewijk and Nieuwenhuis, 1985; Willführ *et al.*, 1990; Pellas and Weiss, 1990). The issue of migration pathways of recirculating lymphocytes is complex and not yet resolved, and this route from the MS into the nodule is by no means the only one involved. Nevertheless, the fact that the MS was present in spleens of all five mammalian species studied suggests that it may provide an important part of the microenvironment for promoting immune responses.

Routes for lymphocyte migration via channels bridging the MZ (and, presumably, the MS also) have been reported (Mitchell, 1973; Sasou and Sugai, 1992). The absence of red cells from these channels (Mitchell, 1973) supports the view that these are not channels for blood flow and, thus, one would not expect them to fill with corrosion casting material. In our casts, we have never seen filling of such bridging channels.

In immune thrombocytopenia, we speculate that the absence of the MS could have significant consequences. Blood flow through the MZ could become less uniformly distributed, giving rise to areas of slow flow; therefore, platelets and other blood cells would spend increased time in the proximity of splenic macrophages. This change, along with the presence of high concentrations of antiplatelet antibody, could lead to the accelerated destruction of platelets characteristic of immune thrombocytopenia.

Capillary supply to the marginal sinus and marginal zone

The major conclusion from our studies regarding this topic is that in all five species examined a plentiful supply of capillaries is distributed to the MS and MZ. Capillaries were never seen terminating in an 'open' fashion in the reticulum of the white pulp itself (Fig. 1). It could be argued that capillary terminations in the white pulp are in fact present, but that such routes have not been filled in the casts. However, in our studies several hundred lymphatic nodules were examined in spleens of each species, including patients with ITP, and the consistent absence of casting material flowing out into the white pulp from capillary endings strongly suggests that capillaries rarely (if at all) terminate there. It is probable that during the proliferation of capillaries within nodules, as in a secondary immune response or in ITP, many capillary sprouts form. From histological sections, these might be interpreted as capillary terminations but in corrosion casts such vessels would not fill, since no blood flows through them at this stage of their development. Moreover, in histological sections direct connections of capillaries to regions of PMCS lying within the white pulp could also be misinterpreted as terminations within the reticulum of the white pulp. These two possibilities may explain the discrepancy between our observations and previous reports (e.g., Snook, 1950; Weiss, 1988).

Species differences exist in the degree of branching of the arterial tree, e.g., much more extensive branching in human than in cat spleen. Nevertheless, in all species examined a large proportion of the inflowing blood empties into the MS/MZ, as indicated by microcorrosion casts prepared using the minimal injection technique. Weiss (1988) has described the MZ as the "major receiving depot or vestibule of the spleen". Histological studies have shown that the MZ is a distinct region in which the reticular meshwork is more finely meshed than in the red pulp, and which contains a large population of lymphocytes, macrophages, and other blood cells (Blue and Weiss, 1981b). In particular, the MZ contains a unique population of macrophages not found elsewhere in the body (Dijkstra *et al.*, 1985). The MZ, which occupies 28% of the total splenic volume in rats (Willführ *et al.*, 1990), appears to provide an ideal microenvironment for cell-cell interactions subserving

immunologic and filtration functions of the spleen. Even though the actual arrangement of the capillary supply to the MS/MZ differs among species (directed towards the white pulp and/or marginal zone side of the MS), in all species studied the MS/MZ receives a large blood supply commensurate with its important functional roles.

A remarkable proliferation of capillary supply to the MS/MZ was found in spleens of patients with immune thrombocytopenia (and some other pathological conditions). In immune thrombocytopenia, this may be related to the increased activity associated with antiplatelet antibody production and phagocytosis of platelets. Support for this view comes from the finding that there is an increase in capillary density in peripheral lymph nodes undergoing immunologic reactions (Herman *et al.*, 1972), and that activated macrophages induce vascular proliferation (Polverini *et al.*, 1977).

The perimarginal cavernous sinus

The PMCS was first reported in 1979, when Yamamoto *et al.* presented evidence of its existence in human spleen and named it the "perimarginal cavernous sinus plexus". Using histological sections and SEM of tissue, these investigators showed that (a) the PMCS is located either between the MZ and the red pulp, or borders the white pulp directly in areas where the MZ is not well developed; (b) thin endothelial cells with flattened nuclei line the PMCS, in contrast to the long spindle-shaped endothelial cells lining venous sinuses; (c) individual sinuses of the PMCS plexus communicate with each other through narrow canals; (d) many thin endothelial cells and their processes bridge opposing walls of the PMCS; (e) the PMCS communicates with the reticular spaces of the MZ. Vascular connections between the PMCS and other structures were not clear in that study (Yamamoto *et al.*, 1979).

By means of microcorrosion casts, we obtained the first three-dimensional views of the PMCS in human spleens, and were also able to elucidate its vascular connections (Schmidt *et al.*, 1988). In the present paper, we have shown that the PMCS exists in dog and rat spleens also. In all three species, our casts have confirmed points a, c and e above, and provided evidence consistent with points b and d. Moreover, we have demonstrated that the PMCS often extends right into the white pulp, especially in dog and rat spleens.

Blood flow into the PMCS occurs via three routes: capillaries connected directly to the PMCS (human, dog, rat), connections with ellipsoid sheaths (human, dog), and connections with the MZ (human, dog, rat). Drainage from the PMCS occurs directly into venous sinuses (human, dog, rat) via "open" ends 15-20 μm in diameter, not via interendothelial slits in sinus walls. Tissue spaces in the PALS, which may represent deep lymphatic vessels, have been reported in rat spleen (Sasou and Sugai, 1992). However, the dimensions of the PMCS are much larger than these lymphatic spaces. Moreover, the nature of the routes for inflow and drainage of blood for the PMCS strongly argue against the possibility that the casts we have identified as PMCS are actually lymphatic spaces. We have never seen lymphatic vessels filled with casting material in casts produced by the minimal injection technique.

The function of the PMCS is not yet clear, but Yamamoto *et al.* (1979) suggested that it provides a route for lymphocyte migration into the white pulp. Their SEM micrographs of splenic tissue showed a great number of lymphocytes, together with some macrophages, adhering to the inner wall of the PMCS; also, some lymphocytes were seen in the process of passing through the wall into the white pulp (see their Figs. 7 and 9). The extension of PMCS into the white pulp, found in our casts, would presumably enhance this process. The large volume of the individual sinuses comprising the PMCS plexus will lead to diminished blood flow velocities and wall shear rates, producing conditions more favorable for cellular adhesion to the walls and migration into the white pulp.

Venous drainage from the marginal sinus and marginal zone

The fact that mammalian spleens must be classified as sinusal or non-sinusal, depending on the nature of their venous origins in the reticular meshwork, was first pointed out by Snook (1950). Only about 50% of the 15 species he examined had venous sinuses. Venous sinuses and pulp venules can be distinguished by their gross morphologic differences (e.g., size, abundance, degree of anastomosis) and by the characteristic structural differences of their walls. The former are best appreciated from examination of corrosion casts, while the latter obviously requires examination of tissue.

The differences in wall structure of venous sinuses and pulp venules have been shown beautifully by Fujita (1974), Blue and Weiss (1981a,c), and Hataba *et al.* (1981). The walls of venous sinuses consist of long spindle-shaped endothelial cells aligned parallel to the axis of the vessel, their nuclear regions bulging into the lumen. These cells are held in position by processes of reticular cells, which fit into transverse grooves in the endothelial cells and thus form 'hoops' around the abluminal surface of the sinus. Narrow, slit-like gaps exist between endothelial cells, through which blood cells from the surrounding reticular meshwork must squeeze in order to enter the venous sinuses. The kinetics of entry of red blood cells in rat spleen has been studied by intravital videomicroscopy (MacDonald *et al.*, 1987). In contrast, the walls of pulp venules lack the characteristic spindle-shaped endothelial cells aligned with the axis of the vessel. Instead, the venules are lined with smooth, flattened, irregularly-shaped endothelial cells, and have relatively few and irregularly-distributed openings ('fenestrations') in their walls. The sizes of the fenestrations are mostly large enough to allow unimpeded entry of red cells into the venule. In non-sinusal spleens, trapping of immature and abnormal red cells occurs by adhesion to the fine structures of the reticular meshwork. In sinusal spleens, the reticular meshwork must, presumably, serve a similar function but the interendothelial slits provide an important second mechanism,

based on size restriction, for trapping red cells.

It has been generally believed that venous sinuses begin in the red pulp or MZ as blind-ended sacs, and that entry of blood occurs exclusively via interendothelial slits in sinus walls (Chen and Weiss, 1973; Leblond, 1973; Cho and DeBruyn, 1975; Fujita *et al.*, 1985). However, our corrosion casts have shown that many venous sinuses begin as open-ended tubes continuous with the MS or MZ, allowing free entry of blood into the venous system and bypassing both the reticular meshwork of the red pulp and the interendothelial slits at sinus walls. It is obvious from casts produced by the minimal injection technique that a large volume of material has entered the venous system without passing through the reticular meshwork. Thus, the route provided by open-ended venous sinuses at the MS/MZ must be one of very low resistance to flow.

Studies of the washout kinetics of red cells and plasma from isolated perfused spleens (cat: Levesque and Groom, 1976; rat: Cilento *et al.*, 1980) have shown that at least 90% of the inflowing blood travels through the organ via a 'fast' pathway. Our corrosion casts demonstrate that this fast pathway corresponds almost entirely to flow through the MS/MZ into open-ended venous sinuses (or pulp venules, in non-sinusal spleens). This means that approximately 90% of the total flow is distributed to the region bordering the white pulp, presumably to subserve immunologic functions, and then passes directly to the venous outflow. The remaining 10% of the flow passes into the reticular meshwork of the red pulp, where filtration of cellular elements of the blood occurs. These proportions imply that the primary function of the spleen is immunologic, and that the filtration of blood cellular elements in the red pulp represents a secondary function.

Conclusions

The schematic diagram presented at the start of this paper (Fig. 1) can now be modified, incorporating the new insights obtained regarding microcirculatory pathways bordering the white pulp of mammalian spleen. It is not possible to depict in a single diagram the microcirculatory pathways for all five species examined, due to the species differences that exist. Even within the groupings of sinusal and non-sinusal spleens, species differences exist. In light of this, we have chosen to present in Figure 7 an updated schematic diagram which shows the microcirculatory pathways bordering the white pulp in the normal human spleen. Below are indicated the major conclusions for human spleen, and a brief summary of the differences found in other mammalian species.

In the normal human spleen, a marginal sinus 5-10 μm thick borders the white pulp directly. It consists of a series of cleft-like, anastomosing vascular spaces between the white pulp and the surrounding marginal zone, and receives a plentiful blood supply via vessels which terminate mostly on its outer aspect. These vessels are derived from branches of the central artery which curve circumferentially within the marginal zone. Microcorrosion casts show that the marginal sinus fills preferentially, before filling of the marginal zone and surrounding red pulp occurs. A uniform distribution of blood from marginal sinus to marginal zone is provided through discontinuities in the outer wall of the marginal sinus. In addition, the marginal zone receives a substantial blood supply from capillaries that terminate within its reticular meshwork. In the white pulp itself relatively few capillaries are usually found; these originate either from the central artery or from vessels in the marginal zone (for details see Schmidt *et al.*, 1988). Capillaries do not terminate in an open fashion within the reticulum of the white pulp.

The perimarginal cavernous sinus of human spleen is a very large blood space situated either between the marginal zone and red pulp or directly adjacent to the white pulp itself. It receives blood from direct connections with arterial capillaries, through ellipsoid sheaths, and via points of continuity with the marginal zone. The venous drainage from the perimarginal cavernous sinus, as well as the marginal sinus and marginal zone occurs via open-ended venous sinuses. These allow free entry of blood into the venous system, bypassing both the reticular meshwork of the red pulp and interendothelial slits in venous sinus walls. In addition, some blood from the marginal zone percolates out into the reticular meshwork of the red pulp and eventually gains access to venous sinuses through interendothelial slits in their walls.

The marginal sinus is also present in the other species we studied (dog, cat, rat, mouse), although its morphology differs somewhat among species. However, the marginal sinus is absent in humans with immune thrombocytopenia. There are differences between species in the degree of branching of vessels within the white pulp, as well as in the arrangement of the capillary supply to the marginal sinus and marginal zone. In mouse and rat spleens, a profusion of capillaries terminates on the inner aspect of the marginal sinus, whereas in cat and dog spleens the capillaries terminate on both the inner and outer aspects. It is noteworthy that dramatic vascular hyperplasia occurs in the white pulp and marginal zone of humans with immune thrombocytopenia.

The perimarginal cavernous sinus is also present in spleens of dog and rat. The vascular supply and drainage are similar to those in human spleen, except that ellipsoid sheaths are absent in rat spleen. No evidence of a perimarginal cavernous sinus was found in spleens of cat and mouse. Venous drainage from the marginal sinus and marginal zone in spleens of dog and rat occurs via open-ended venous sinuses, as described above for human spleen. In the non-sinusal spleens of cat and mouse, pulp venules drain the region bordering the white pulp via open ends and fenestrated walls near the vessel origins.

The scope of this paper has been limited to microcirculatory pathways bordering the white pulp, and the

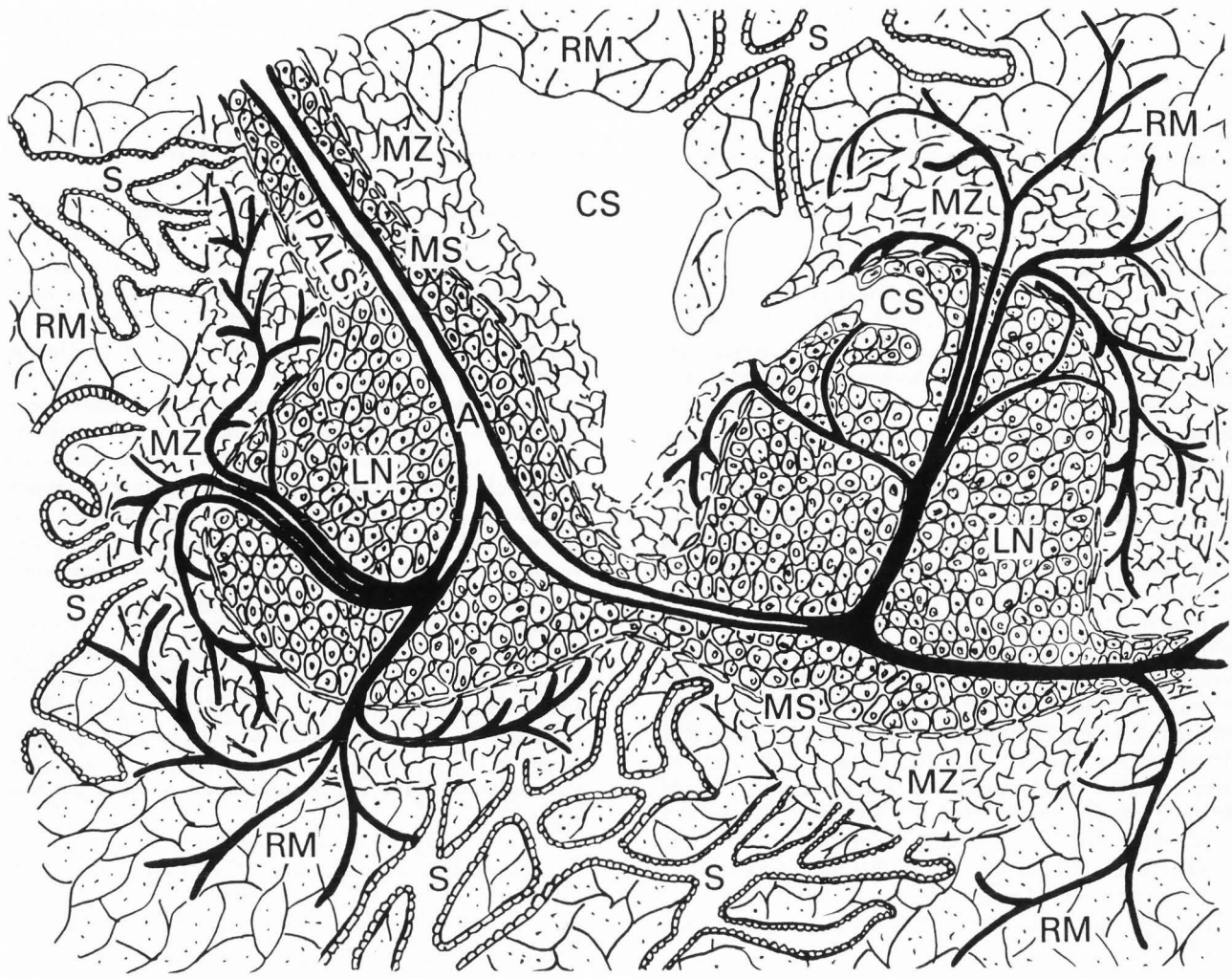

Figure 7. Schematic diagram of microcirculatory pathways in and around the white pulp of normal human spleen, summarizing the findings of the present study. The central artery (A) passes through the periarterial lymphatic sheath (PALS) and lymphatic nodules (LN), giving rise to numerous arterioles and capillaries (shown in black). Directly bordering the white pulp is the marginal sinus (MS), consisting of a series of thin anastomosing vascular spaces. Surrounding the MS lies the marginal zone (MZ), in which the reticulum is more finely meshed than that of the reticular meshwork (RM) in the red pulp beyond. Abundant venous drainage is provided by anastomosing venous sinuses (S), most of which originate as open-ended vessels at the boundary between the MZ and RM. Some venous sinuses originate within the MZ itself or at the MS. (For simplicity, only a few representative regions of venous sinuses are shown, and capillary terminations in the RM are illustrated in separate areas of the Figure. In reality, both the system of venous sinuses and the capillary terminations completely surround the MZ). The perimarginal cavernous sinus (CS), a plexus of large blood spaces, lies either between the MZ and RM or else adjacent to and extending into the white pulp. The CS receives flow principally from the MZ and is drained by open-ended venous sinuses. After leaving the white pulp, numerous arterial capillaries curve circumferentially and terminate at the outer aspect of the MS or within the MZ (or occasionally at the CS). Other capillaries extend out into the RM and terminate in the meshwork itself; rarely, direct connections to venous sinuses are present. Many capillaries in the MZ and RM are surrounded by ellipsoid sheaths (not shown) before their terminations.

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updated schematic diagram (Fig. 7) does not consider the pathways through the red pulp (manuscript in preparation). It is noteworthy that only ~10% of the arterial inflow to the spleen passes through the reticular meshwork of the red pulp (the 'slow' pathway), whereas 90% is distributed to the region bordering the white pulp (the 'fast' pathway: Levesque and Groom, 1976; Groom and Schmidt, 1990). Thus, it seems appropriate to shift focus away from the much belabored issue of 'open' versus 'closed' circulations in the red pulp, toward the nature and functions of microcirculatory pathways bordering the white pulp.

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Discussion with Reviewers

S. Aharinejad: The partial or segmental injection method is certainly a good technique to avoid the extensive filling of vascular structures in the spleen, which could cover insight into the splenic microvascular bed. However, does not a possibility of misinterpretation exist due to incomplete filling of some structures, e.g., capillaries?

Authors: Because this possibility does indeed exist, one should not draw conclusions, for example, from "blind-ended" capillary casts. Interpretations regarding other structures (e.g., the MS) must not be based upon anecdotal information but on the integration of information from many casts obtained using different degrees of filling.

S. Sasou: In the human spleen, the marginal sinus observed by the authors may be a pooling of the material for the corrosion cast in the MZ along the flat reticular cells demarcating the white pulp and the MZ, because it is difficult to observe the vascular meshwork around the white pulp such as in the rat spleen by the light, electron and scanning microscope. Do you have any comments?

Authors: Corrosion casts reveal that the MS in normal human spleen is a very distinct entity, filling preferentially before filling of the MZ and surrounding red pulp occurs. The sheet-like appearance of the MS casts contrasts markedly with the knobbly configuration of casts of the surrounding MZ meshwork (Schmidt *et al.*, 1988). In species such as rat, examination of splenic tissue by light and electron microscopy has clearly demonstrated the existence of the MS (Snook, 1964; Sasou *et al.*, 1976). However, in human spleen such a demonstration based on tissue samples has not yet been reported. This may be because **normal** human splenic tissue is difficult to obtain and the MS may indeed be absent in some disease states (Schmidt *et al.*, 1991). Confusion in the literature may have arisen because of extrapolation to the normal from pathological material.

S. Aharinejad: You describe vascular hyperplasia for ITP and CLL patients and refer to possible immunologic hyperreaction and consequent increased phagocytosis. The same phenomenon, you say, was observed in patients with "hypersplenism". Splenic enlargement might be due to portal vein obstruction and portal hypertension. How could one understand the genesis of higher vascular density in such cases?

Authors: Corrosion casts were prepared from spleens of two patients with a clinical diagnosis of hypersplenism. In one case, the hypersplenism was secondary to chronic hepatitis, an autoimmune disorder in which additional phagocytic activity would be expected [Toghill PJ (1990). The syndromes of splenic dysfunction: A clinical overview. In: The Spleen: Structure, Function and Clinical Significance. Bowdler AJ (ed.). Chapman and Hall, London, pp. 209-232]. In the other case, the hypersplenism was secondary to portal hypertension, and the question of such spleens demonstrating excessive immunological activity has not yet been resolved (Toghill, 1990: see above).