Preface to the Second Edition

The human spleen is an organ of very special and in some respects unique clinicopathological significance, and has emerged from a centuries-old scientific and medical obscurity only within living memory. Understanding of the biological functions and structure of the spleen has progressed through several phases: classical speculation, comparative studies confounded by profound differences between species, empirical studies of the disordered, and usually enlarged, spleen, and the outcome of splenectomy.

Each of these sources has left lingering fingerprints, often as widely accepted and persistent concepts at the clinical level, including those of (1) hypersplenism as a process or mechanism, (2) that there is no exclusive function of the spleen that cannot be performed by elements of the immune system everywhere, and (3) that the spleen acts as a useful reservoir of blood cells. The inherent errors of these concepts have proved limiting to the development of the rational interpretation of splenic disorder.

In the clinical context, the spleen long appeared as a silent, almost anonymous organ, presenting for notice principally when enlarged, or when peripheral blood cytopenias suggested the possibility of a splenic disorder. The discrete structural entity of the organ permitted surgical removal of the spleen (splenectomy) as a therapeutic intervention, which was long believed to carry no long-term penalties.

The first edition of this book, published in 1990 by Chapman and Hall Medical of London under the title *The Spleen: Structure, Function, and Clinical Significance* principally addressed the wealth of new information on the microscopic structure of the spleen, its immune functions, the mechanisms of related cytopenias, and the clinical sequelae of splenic disorders.

In the interval since that time there has been increasing recognition of the adverse consequences of absent or impaired splenic function, not only following splenectomy, but in a surprisingly wide range of diseases and disorders. This has led to a broad range of new surgical techniques designed to preserve sufficient splenic tissue to maintain the protective function of the organ. Related to this has been an increasing clinical interest, especially with respect to the investigation of the spleen radiologically, that has greatly improved the recognition of splenomegaly, atrophy, and intrasplenic pathology. In addition there has been a significant improvement in the sensitivity of techniques providing quantitative estimates of the various functions that are impaired in hyposplenism.

The changes appearing in this edition have therefore increased the clinical emphasis of the work, although some significant revisions and additions that focus on the supporting basic sciences related to the organ are also widely distributed throughout the chapters.

It is with great regret that I record the untimely passing of three distinguished contributors to the first edition. First, Professor Aage Videbaek of the Gentofte University Hospital, Copenhagen, Denmark, whose rigorous and insightful contributions to hematology ably represented the discipline in Scandinavia. He was the editor of the *Scandinavian Journal of Haematology,* and established the standards for critical research and clinical application for which the journal and its successor, the *European Journal,* are well known. He will be greatly missed. Second, Dr. Jack Chamberlain, a former student of Professor Leon Weiss, and a man whose hematological research in the field of scanning electron microscopy at the Universities of Rochester and East Carolina contributed greatly to an understanding of the structure of the spleen. I am grateful to Mrs. Chamberlain for her permission to consolidate elements of his first edition chapter into Chapter 2 of this edition. Last, I regretfully record the passing of Eric Schmidt who taught in the Department of Medical Biophysics at the University of Western Ontario. In the words of his colleague and chief, Professor Alan Groom, "Eric was a gifted experimentalist and electron microscopist, a shrewd observer, whose scientific observations have been of enormous value." Fortunately, one of his last collaborative contributions to the science of the spleen is incorporated into this edition.

I am grateful to those former authors who, while unable to complete revisions of their former work, nevertheless provided the framework for the contributions in this Edition. I also wish to thank Mr. Thomas Lanigan of the Humana Press for his interest and encouragement in the preparation of *The Complete Spleen: Structure, Function, and Clinical Disorders*, and also to Chapman and Hall of London, who were most helpful in making the transition to a new publisher practicable. I am also indebted to Ms. Lisa Watts and Ms. Sherry Puckett of the Department of Medicine at Marshall University, WV, and Ms. Katherine Carolan of the Department of Surgery at the University of Iowa, for their secretarial expertise and dedication. I am also grateful to the Huntington Clinical Foundation for support with respect to the editorial resources required, to Dr. F. G. Renshaw of Michigan State University and Dr. N. C. Bowdler of the University of Iowa for invaluable assistance in the preparation of this edition, to Mr. William Arnold for his valuable expertise with the illustrations, and to Mr. Jonathan Bowdler, whose communication skills were used to great advantage.

Anthony J. Bowdler, ***MD, PhD***

2 The Microanatomy of the Mammalian Spleen

Mechanisms of Splenic Clearance

FERN TABLIN, VMD, PhD, JACK K. CHAMBERLAIN, MD, FACP, AND LEON WEISS, MD

2.1. INTRODUCTION

The spleen is a uniquely adapted lymphoid organ that is dedicated to the clearance of blood cells, microorganisms, and other particles from the blood. This chapter deals with the microanatomy of the spleen, its highly specialized extracellular matrix components, distinctive vascular endothelial cell receptors, and the extraordinary organization of the venous vasculature. We also address the cellular mechanisms of splenic clearance, which are typified by the vascular organization of the spleen; mechanisms and regulation of clearance, and the development of a unique component; specialized barrier cells, which may be essential to the spleen's clearance functions in stress.

2.2. ANATOMICAL ORGANIZATION OF THE SPLEEN

The mammalian spleen consists of an encapsulated, trabeculated pulp, made up of stroma and vasculature supporting a large population of circulating, migrating, and differentiating blood and hematopoietic cells (Figs. 1–6). The vascular layout of the spleen is as follows: arteries enter the capsule and move into the splenic parenchyma within the trabeculae. From there, they enter the white pulp (WP), where they are surrounded by lymphocytes. The white pulp selectively clears lymphocytes and their accessory cells from the blood. They equip the spleen to engage in immunological reactions. Arteries continue into the adjacent marginal zones (MZs), which consist of shells of tissue surrounding the white pulp, and are interposed between white pulp centrally and red pulp (RP) peripherally. Marginal zones are heavily trafficked, receiving blood from many arterial terminals, and selectively distribute its components to other parts of the spleen. The marginal zone also stores erythrocytes, platelets, and monocyte-macrophages, and initiates their processing. The red pulp is that large part of the pulp that extends outward from the marginal zone. It too receives arterial terminals, clears, tests, and stores erythrocytes, and is primed for erythroclasia and erythropoiesis. Blood deposited in the marginal zone and red pulp moves through the filtration beds, and is drained by a system of venous vessels in both the marginal zone and red pulp.

From: *The Complete Spleen: A Handbook of Structure, Function, and Clinical Disorders* Edited by: A. J. Bowdler © Humana Press Inc., Totowa, NJ

2.2.1. CAPSULE AND TRABECULAE The human spleen weighs approx 150 g, in adults, and is enclosed by a capsule composed of dense connective tissue, with little smooth muscle (Faller, 1985; Weiss, 1983, 1985). This arrangement reflects the minimal contractile role of the capsule and trabeculae in altering the blood volume of the human spleen, under normal circumstances. The capsule measures 1.1–1.5 mm thick, and is covered by a serosa, except at the hilus, where blood vessels, nerves, and lymphatics enter the organ. There are two layers of the capsule: This can be determined by the orientation of collagen fibers (Faller, 1985), which are moderately thick and uniform, but which become finer in the deeper regions, where the transition to pulp fibers occurs. There are also elastic fibers present in the capsule. The capsule is continuous on its inner surface, with a richly ramified system of trabeculae, which penetrates and supports the pulp.

In most mammalian spleens, the capsule is sympathetically innervated, and there is a species-dependent blend of smooth muscle and collagenous tissue. In certain species, the capsule and trabeculae are rich in smooth muscle. These spleens, of which the equine and feline are examples, are termed "storage spleens." Sympathetic stimulation results in the contraction of the capsule and trabeculae, causing delivery of large reserves of blood into the circulation. The horse has a huge spleen, and its outstanding athletic prowess is dependent on the spleen's capacity to increase the hematocrit, and, thus, the oxygen-carrying capacity of the circulation (Persson et al., 1973a,b). In fact, splenic reserves of mature erythrocytes are so large and readily mobilized that the reticulocytes are rarely present in the circulation, although they are produced in the bone marrow, which holds them in reserve and releases them only under conditions of severe chronic anemia. Thus, the splenic store of mature erythrocytes can be used to compensate for all but the most persistent, long-term blood loss. In contrast, in the spleen of humans, rabbits, dogs, and mice, erythrocyte reserves are quite small. These spleens do retain some significant storage capacity, however, holding large numbers of platelets in ready reserve. Because these less-contractile spleens have been thought to have greater immunological and other antimicrobial capacity, they have been termed "defense spleens."

2.2.2. SPLENIC PARENCHYMA The splenic parenchyma, or pulp, consists of white pulp, the intermediate marginal zone, and

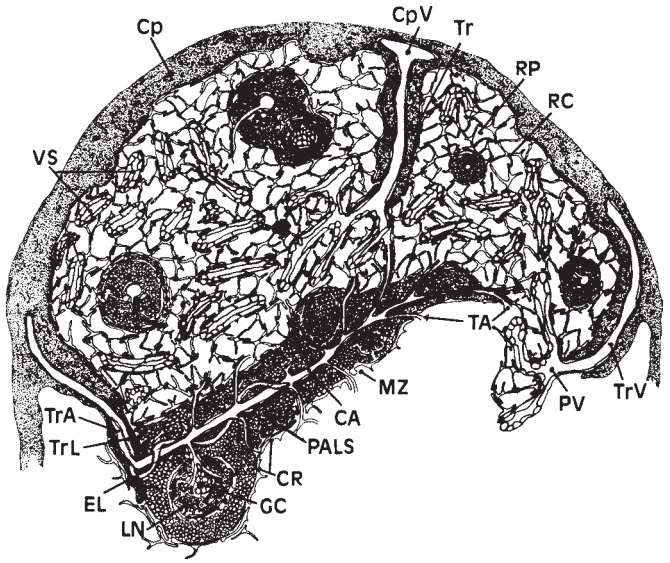

Fig. 2.1. Schematic drawing of a sinusal spleen. The spleen is enclosed by a capsule (Cp) and, from its internal surface, trabeculae (Tr) branch to compartmentalize the pulp. The splenic artery pierces the capsule and branches into trabecular arteries (TrA). The trabecular artery enters the pulp as the central artery (CA), which runs in the central axis of the periarteriolar lymphatic sheath (PALS). This is the component of the white pulp (WP) in which T-cells are concentrated. The second major component of the white pulp consists of the lymphatic nodules (LNs), which occur as nodules within the PALS, where their presence forces the central artery into an eccentric position. LNs are sites of concentration of B-cells, and may contain germinal centers (GCs), when there is a high level of antibody formation. Branches of the central artery supply LNs, and pass laterally through the white pulp to terminate in the marginal zone (MZ), which closely surrounds the WP. In addition to its arterial vessels, efferent lymphatic vessels (EL) drain the white pulp, entering the trabeculae as trabecular lymphatics (TrL). In this schema, the lymphocytes of the WP are shown, and, with the vasculature, dominate the picture. Note, however, that there is a circumferential reticulum (CR) limiting the periphery of WP. The MZ and red pulp (RP), which occupy the bulk of the spleen, are schematized, and show no free cells. The red pulp consists of terminating arterial vessels (TA), a meshwork, or filtration bed, consisting of reticular cells (RCs) and associated reticular fibers, and a system of venous vessels. The proximal venous vessel, which receives blood from the filtration beds, is the venous sinus (VS). These distinctive vessels end blindly, anastomosing richly, deeply penetrating the filtration beds of RP, supported by the reticular network. VSs receive blood through their interendothelial slits, which then drains into pulp veins (PVs), trabecular veins (TrVs), and capsular veins (CpVs).

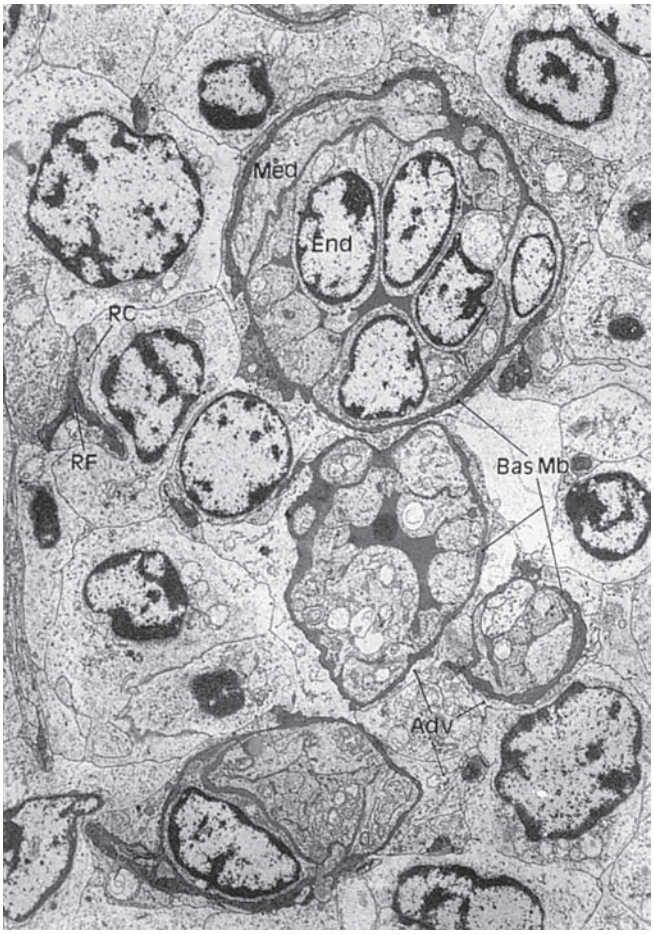

Fig. 2.2. White pulp, human spleen. Four cross-sections of an arteriole and its smaller branches lie in white pulp. Each of the vessels may possess an endothelium (End), basement membrane (Bas Mb), media (Med), and adventitia (Adv). Especially in the smaller vessels, the media and adventitial layers are rather incomplete. Lymphocytes occupy much of the rest of this field, held in a meshwork of reticular cells (RCs) and reticular fibers (RFs) (×6250).

the red pulp (Fig. 7; Kashimura and Fujita, 1987; Sasou et al., 1986; van Krieken and te Velde, 1988).

2.2.2.1. White Pulp The white pulp consists predominantly of lymphocytes, antigen-presenting cells, and macrophages, lying on a specialized reticular meshwork composed of concentric layers of stromal cells, now recognized to be specialized fibroblasts (Borrello and Phipps, 1996; Van Vliet et al., 1986; Fujita et al., 1982, 1985). The reticular meshwork is most dense in association with the periarterial lymphatic sheath (PALS) and marginal zone. Matrix proteins produced by these fibroblasts include: type III collagen, laminin, fibronectin, vitronectin, and tenascin (Liakka et al., 1995). These proteins may play an important role in the migration of lymphocytes during fetal development of lymphatic tissue, as well as

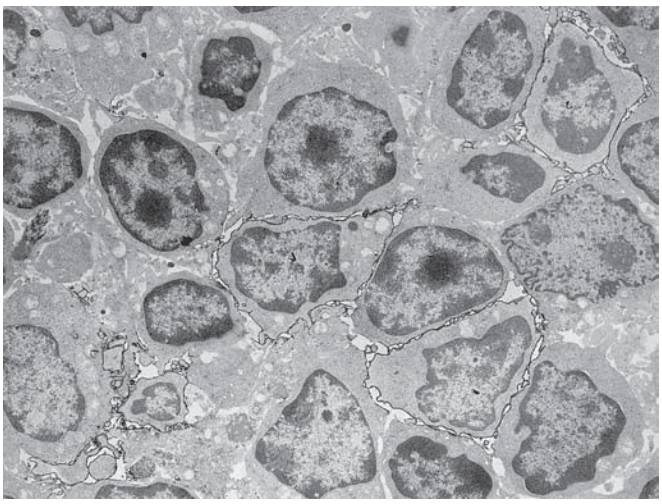

Fig. 2.3. Normal murine white pulp stained for T-cells. The field consists mostly of T-cells, the surfaces of some of which are stained by immunocytochemistry for the T-cell marker, Thy 1.2. Note the short cell processes of many of the lymphocytes, establishing transient junctional complexes with vicinal cells (×2500).

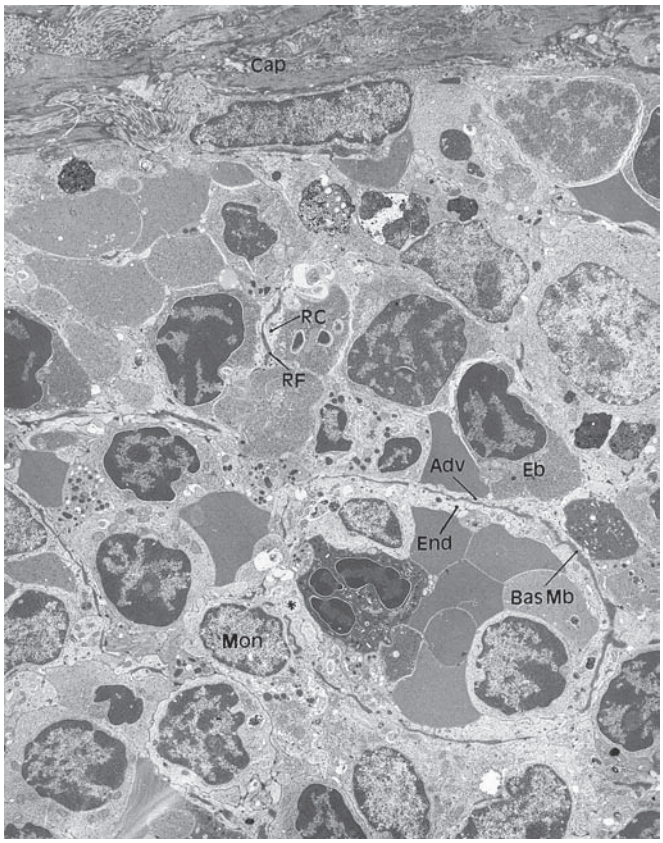

Fig. 2.4. Subcapsular red pulp mouse spleen. The capsule is rich in muscle and collagen. The subjacent red pulp contains a bifurcating pulp vein. The right limb has endothelium (End), basement membrane (Bas Mb), and adventitia labeled. A mononuclear cell (Mon) sends delicate cytoplasmic processes into the vascular wall, penetrating adventitia and basement membrane (*). The perivascular red pulp is pervaded by a reticular meshwork consisting of reticular cells (RCs), which branch from the adventitial layer of pulp veins, and reticular fibers (RFs). The reticular meshwork of mouse red pulp is, however, typically scanty. The red pulp contains many reticulocytes and some erythroblasts (Eb) (×5000).

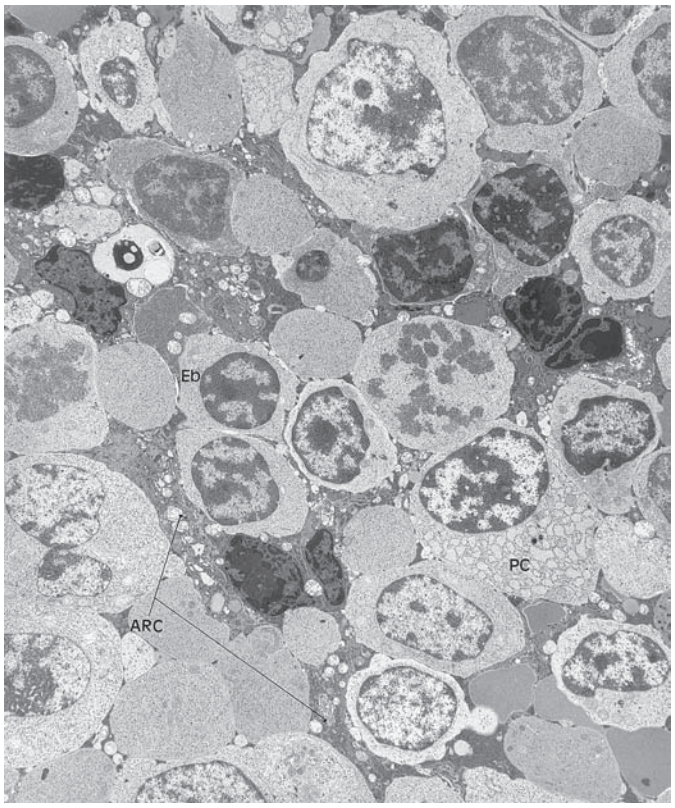

Fig. 2.5. Red pulp, murine spleen in malaria. This field of red pulp, in precrisis *Plasmodium yoelii* malaria, consists of many erythroblasts (Eb), some in mitosis, and a few plasma cells (PCs). Note the dark, syncytially fused stromal cells, forming a complex, extensive membrane, which constitutes a barrier surrounding many free cells. These stromal cells are barrier cells (ARC) (×5000).

during the normal adult immune response. Fibroblasts in the white pulp and marginal zone may express the lymphoid marker, Thy-1 (Borrello and Phipps, 1996), thus forming a distinct microenvironment for T-cell interaction (Van Vliet et al., 1986).

The organization of the white pulp is closely associated with its arterial supply (Fig. 2). Those lymphocytes immediately adjacent to the central arteries constitute the PALS. The lymphocytes in the PALS are predominantly T-cells; B-cells are concentrated in the lymphatic nodules, most often situated at the periphery, or at points of arterial branching (Fig. 3).

The central artery supplies radial branches to the white pulp, marginal zone, and red pulp, and terminates in an attenuated vessel of variable structure supplying the red pulp (Fig. 8). The distal portion of the vessel may be surrounded by a loose macrophage arrangement known as the "periarterial macrophage sheath" (PAMS), which is usually not prominent in humans (Fig. 9; Blue and Weiss, 1981; Biussens et al., 1984; Weiss, 1983; Weiss et al., 1985). It is, however, more obvious in younger subjects. PAMS are also found in mouse and rabbit spleens; however, in canine, feline, herbivore, and avian spleens, the macrophages are organized in a tight cuff or sheath, also referred to as an "ellipsoid."

Deep efferent lymphatic vessels are also present in white pulp, where they are entwined with arterial vessels. These lymphatics run from the white pulp into the trabeculae, then leave the spleen at the hilus. The splenic lymphatic vessels are mostly well-developed, but inconspicuous, because, running in lymphocyte-crowded beds and possessing a lymphocyte-crowded lumen surrounded by the thinnest of vascular walls, they are difficult to discern from their background. These lymphatic vessels carry lymph countercurrent to the flow of blood in their adjacent arterial vessels. They provide splenic lymphocytes with a major efferent pathway for the migration of immunologically competent lymphocytes of the recirculating lymphocyte pool. Venous vessels are notably absent from the white pulp, and are discussed further in the Subheading 2.2.3.

2.2.2.2. Marginal Zone The marginal zone, as its name implies, lies at the periphery of the white pulp and its outer surface blends with the structure of the red pulp. In the human spleen, the reticular meshwork is fine; and the zone is the site of termination of many arterioles, which frequently bifurcate just before their termination (Fig. 10). The marginal zone receives a disproportionately large number of terminal arterial vessels. Blood entering the marginal zone is directed selectively to other arterial beds: Lymphocytes and their accessory cells pass to the white pulp (van Ewij and Nieuwenhuis, 1985); platelets and erythrocytes pass into the red pulp. Studies on the kinetics of splenic cell migration have shown that 25% of the cells that transit through the spleen stay in the marginal

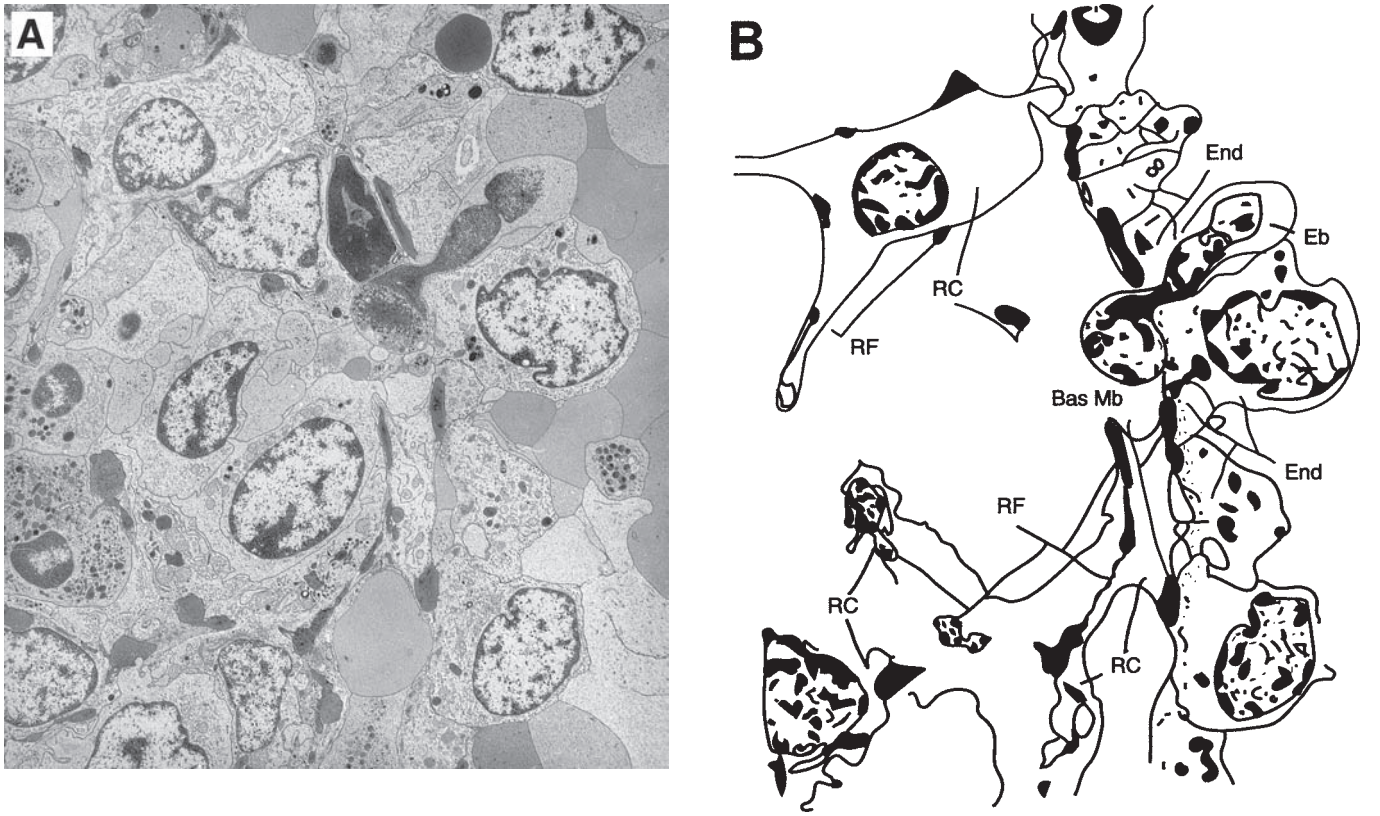

Fig. 2.6. (**A**) Transmission micrograph; (**B**) key to part (**A**). Red pulp, human spleen, thalassemia. Human spleen is a sinusal spleen, and this field contains a venous sinus. Its wall runs vertically, its luminal surface to the right lined by cross-sections of the rod-shaped endothelial cells. Nuclei are present in three of these endothelial cells. The basal portion of the endothelial cell is rich in longitudinally running filaments, which stipple the cell, and, when interwoven, present as dense plaques. The fenestrated basement membrane (Bas Mb) appears in short segments at the base of the endothelium. Red blood cells in thalassemia vary considerably in appearance, and are floppy, tending to fold flexibly on one another. Many reticulocytes are present, and erythroblasts (Ebs) circulate. An Eb, with its nucleus deeply constricted in two places, is passing through the endothelium in an interendothelial slit (the erythroblast itself is deeply constricted in the interendothelial slit at the lower nuclear constriction). The cord on the left is fully packed with leukocytes and reticular cells (RCs). The latter serve as the fibroblastic stroma of the cord, and ensheathe reticular fibers (RFs) (×8000).

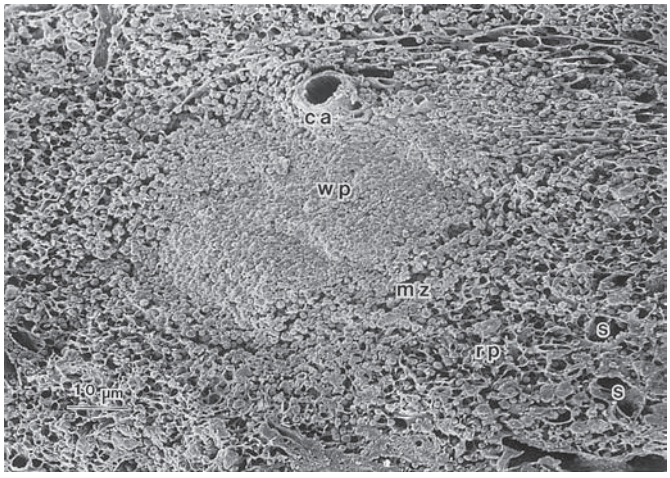

Fig. 2.7. Scanning electron micrograph of the human spleen at low power. Central artery (CA), white pulp (WP), marginal zone (MZ), red pulp (RP) and sinuses (S) in a freeze-cracked surface of the splenic pulp. (Reproduced with permission from Kashimura, M. and Fujita, T. [1987]).

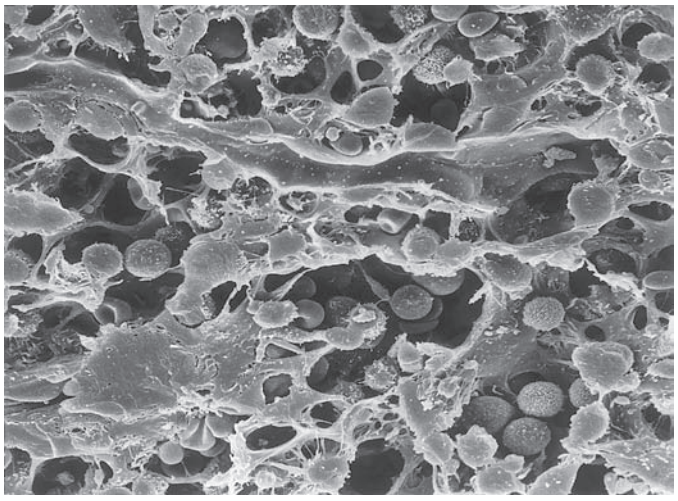

Fig. 2.8. Scanning electron micrograph of the human spleen. A longitudinally fractured arterial capillary terminates in the cordal spaces of the red pulp. The arterial capillary fans out to the right-hand side, where fenestrations provide openings to the cordal spaces (×1350). (Adapted with permission from Fujita, T., et al. [1985]).

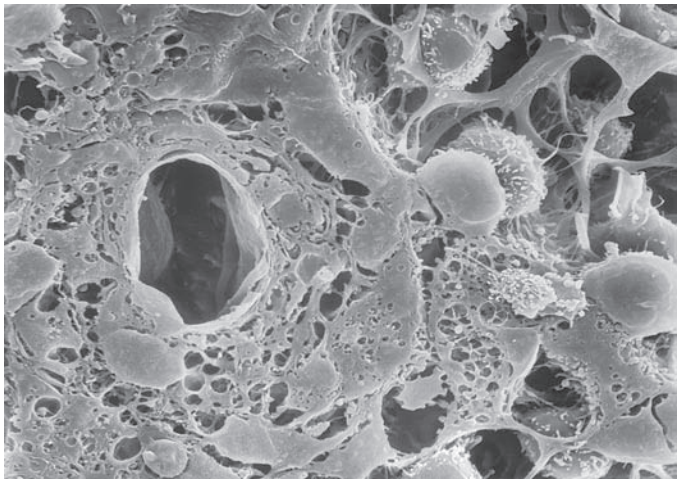

Fig. 2.9. Scanning electron micrograph of the human spleen, to show a sheathed artery enveloped by macrophages and reticular cells. The specimen is taken from the spleen of a patient with immune thrombocytopenia: The honeycombing of the cytoplasm of the macrophages results from the phagocytosis of platelets (×4000). (Reproduced with permission from Fujita, T., et al. [1985]).

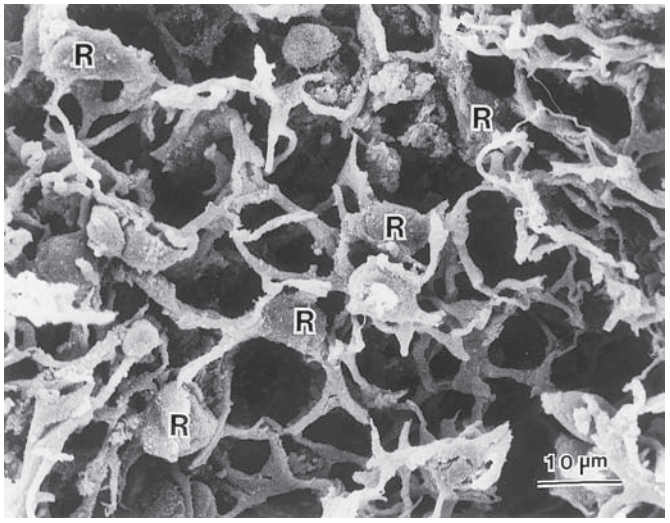

Fig. 2.11. Scanning electron micrograph of the red pulp of the human spleen. The meshwork is formed by reticular cells (RCs), their processes and reticular fibers. Critical point drying has produced cell retraction, so that the cells shrink against the reticular fibers they surround (×1500).

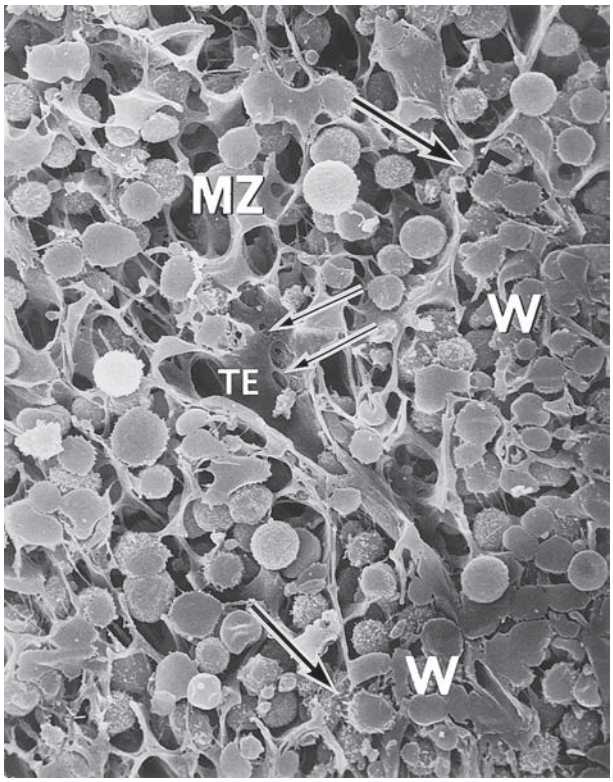

Fig. 2.10. Scanning electron micrograph of the human spleen, showing the terminal end (TE) of a follicular artery in the marginal zone (MZ). The thin arrows show openings in the flat reticular cells, which demarcate the MZ from the white pulp (W). (Reproduced with permission from Sasou, S., et al. [1986]).

zone for approx 50 min; 10% of the lymphocytes will migrate to white pulp, and stay an average of 4–5 h. The majority of the cells rapidly pass through the spleen via the venous vasculature of the red pulp (Hammond, 1975; Ford, 1968; Pabst, 1988; *see also* Chapter 7). An enormous number of lymphocytes migrate through the spleen at any given time, and it has been calculated to surpass the combined traffic of all lymph nodes in the body (Ford, 1969). Numerous studies clearly demonstrate that entrance and retention of T- and B-cells into white pulp is not a random process, but requires a selective interaction between lymphocytes and endothelial cells. This interaction may be mediated by the mucosal adressin adhesion molecule, MAdCAM-1, which has previously been shown to be involved in lymphocyte homing to mucosal sites, and is expressed on the high endothelial venules of Peyer's patches and mesenteric lymph nodes. MAdCAM-1 has been shown to be present on endothelial cells of marginal zone terminal arterioles closest to the white pulp of the mouse spleen (Kraal et al., 1995), and may serve to regulate lymphocyte traffic to the white pulp. Additionally, marginal zone macrophages have been suggested to play a similar role in the migration of lymphocytes to white pulp (Buckley et al., 1987; Lyons and Parish, 1995). In the alymphoplastic *aly* mutant mouse, which is affected by a spontaneous autosomal-recessive mutation, there is a deficiency in systemic lymph nodes, Peyer's patches, and the splenic marginal zone. This phenotype can be rescued by bone marrow transplantation, and provides further evidence for the role of the marginal zone in lymphatic development and its relationship to sites of lymphocyte-mucosal homing (Koike et al., 1996).

2.2.2.3. Red Pulp Three-fourths of the volume of the human spleen consists of the red pulp, which comprises four vascular structures in sequence: slender nonanastomosing arterial vessels (penicilli), the splenic cords, or cords of Bilroth; the venous sinuses; and the pulp veins. All of these vessels are supported by a reticular meshwork (Fig. 11), provided by fibroblasts and their various extracellular matrix proteins, similar to those noted for the white pulp: fibronectin, laminin, vitronectin, tenascin, type III collagen, as well as type IV collagen (Liakka et al., 1995). Macrophages are also found in the splenic cords, both as single cells associated with the reticular fibroblasts and as constituents of the PAMS associated with arterioles of the red pulp.

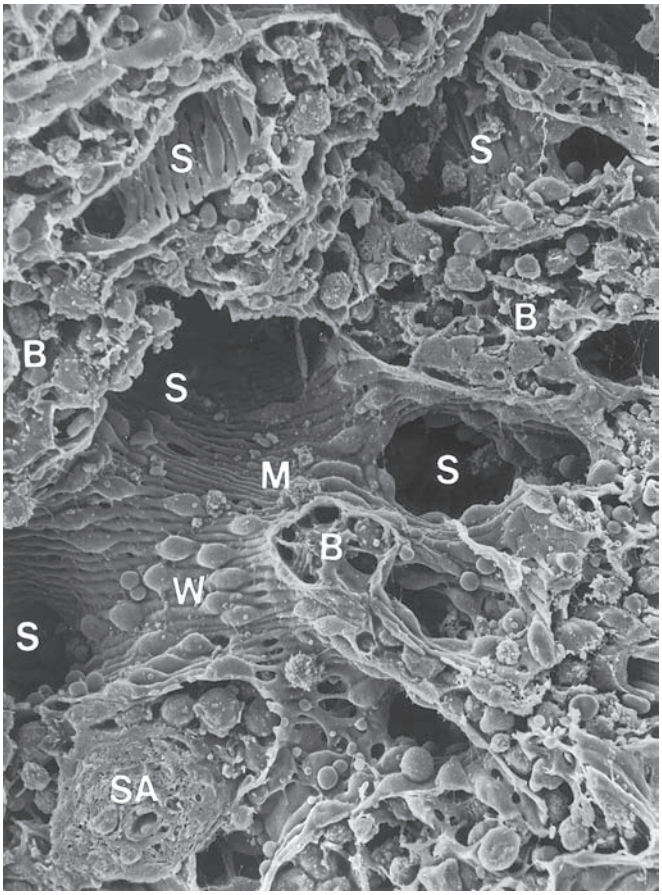

Fig. 2.12. Scanning electron micrograph of the red pulp of the human spleen at low power. The splenic cords (B) are fractured, and the sinuses (S) are seen mostly from the surface. The endothelial cells (or "rod cells of Weidenreich," W) are arranged in parallel, and show enlarged nuclear portions, which project into the lumen of the sinus. Red blood cells can be seen protruding into the sinuses through the junctions of the endothelial cells. MP, macrophages. SA, sheathed artery (×875). (Reproduced, with modification, with permission from Fujita, T. [1974]).

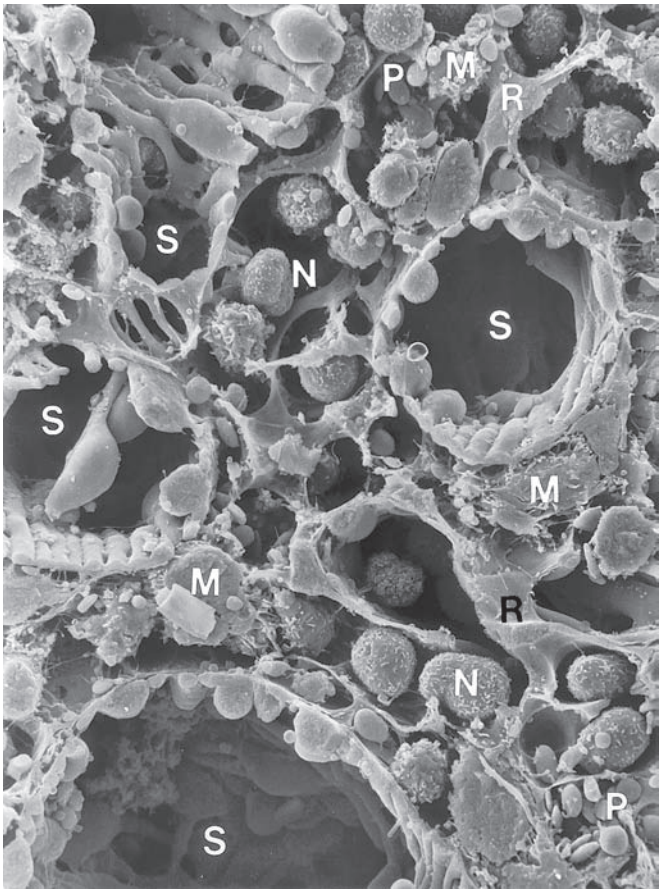

Fig. 2.13. Scanning electron micrograph of the red pulp of the human spleen, at higher power than in Fig. 12. The splenic cords are seen to be supported by reticulum cells (RCs), and the cord meshwork contains macrophages, leukocytes, mainly neutrophils (N), and blood platelets (P). The endothelial pattern of the sinuses (S) is demonstrated; the sinuses in the upper part of the micrograph suggest a perforated structure (×1800). (Reproduced by permission from Fujita, T. [1974]).

The human, rat, and dog spleens are of the sinusal type. In the human spleen, the splenic sinuses comprise approx one-third of the volume of the red pulp (van Krieken and te Velde, 1988); they consist of long anastomosing vascular channels, which ultimately drain into the pulp veins. The sinuses have a unique endothelium, in which the cells are arranged longitudinally, like the staves of a barrel, and run parallel to the long axis of the sinus. Tight junctional complexes are present at regular intervals along their lateral and basolateral surfaces (Uehara and Miyoshi, 1997), and, in the rat spleen, macula occludens are also present at irregular intervals.

Sinusal endothelial cells have two sets of cytoplasmic filaments: The intermediate filaments (vimentin) are loosely arranged; thin filaments are tightly organized into dense actin bands in the basal cytoplasm (Drenckhahn and Wagner, 1986). These stress fibers arch between attachments to circumferential components of the basement membrane, and contain nonmuscle myosin, and probably contract, to vary the tension in the endothelial cell. Endothelial cell-signaling, via adherens junctions, can result in contraction of adjacent endothelial cells resulting in the production of inter-endothelial slits. Potential slit-like spaces, which can be penetrated by cells flowing from the pulp spaces (Fig. 12–16), are a critical point in the flow pathway of particulates through the spleen, and represent an important regulator of selective particulate flow (Fig. 6).

A fenestrated basement membrane is present on the abluminal surface of the endothelial cells; its transverse ring-like component reinforces the sinus structure, like the hoops of a barrel (Fig. 15; Groom, 1987; Weiss, 1983). Immunoelectron microscopic studies have shown that these ring-like fibers are predominantly composed of type IV collagen and laminin, with sparser components of type III collagen and tenascin (Liakka et al., 1995), produced both by the adventitial reticular cells and by endothelial cells which probably associate with this matrix through the β-1 integrins on their basolateral surfaces.

In the human spleen, the basement membrane of the venous sinuses has well-marked circumferential components and a lesser longitudinal component. It is continuous with the reticular meshwork of the surrounding cords, and is overlaid with fibroblasts. The junction of the venous sinuses with the pulp veins is obvious, because there is a transition from rod-like endothelial cells with a fenestrated basement membrane, to the flattened endothelium of the pulp veins and a continuous basement membrane. This transition is most easily seen as the pulp veins enter the trabeculae, and

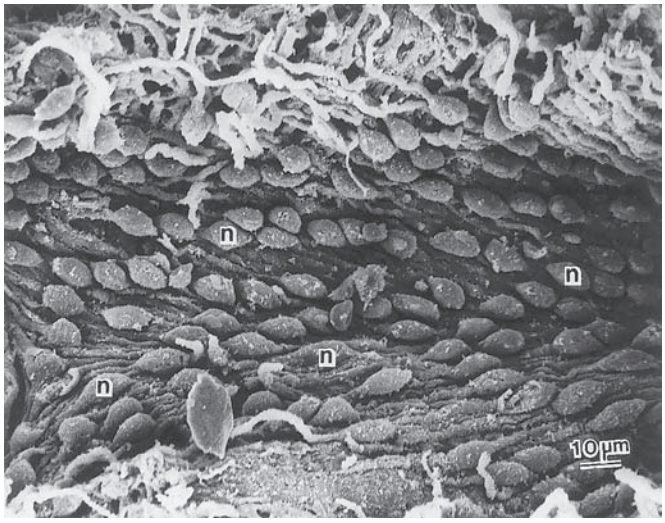

Fig. 2,14. Scanning electron micrograph of the human spleen, showing the luminal surface of a vascular sinus. The endothelial cells are arranged in parallel rows. The portions of the cells containing nuclei (n) bulge into the lumen; there are no visible gaps between the endothelial cells (×3000).

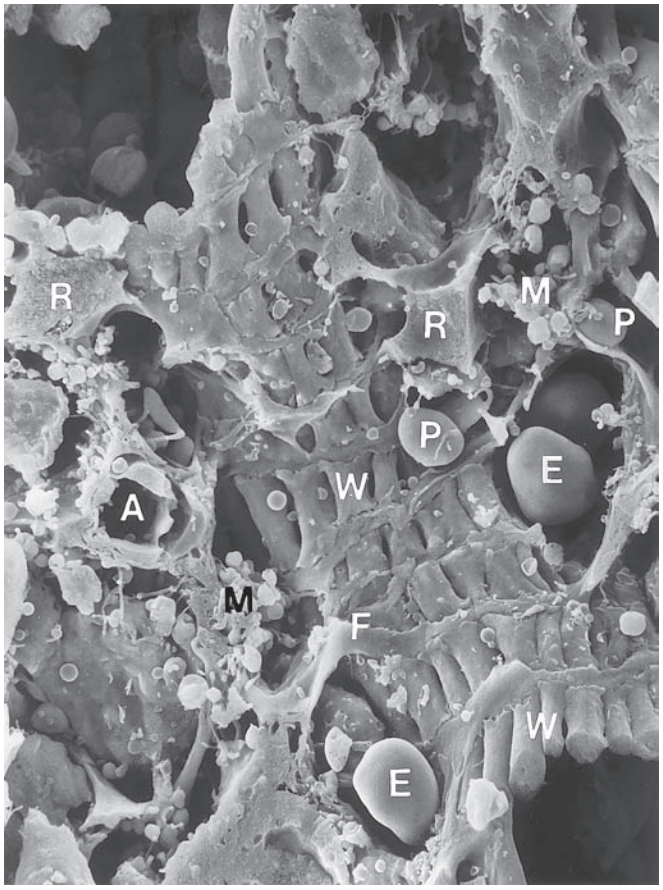

Fig. 2.15. Scanning electron micrograph of the human spleen, showing the abluminal surface of a vascular sinus. The rod-like endothelial cells are crossed by the encircling hoops of basement membrane. Sinus endothelial cells (W). Reticular cell foot processes (F). Artery (A). Erythrocytes (E). Macrophage (MP). Platelets (P). Reticulum cells (Rs) (×3700). (Reproduced by permission from Fujita, T. [1974]).

can be abrupt and without an intermediate structural organization (Weiss, 1983).

Nonsinusal spleens lack venous sinuses, and efferent blood is received initially by pulp veins. In the mouse, cat, and horse, pulp veins are thin-walled, large-lumened vessels with squamous endothelium, and a thin, intermittent basement membrane (Fig. 4). They typically display transmural apertures, which may be large. Pulp veins often lie close to trabeculae, and enter them, becoming trabecular veins. The walls of pulp veins, unlike venous sinuses, offer little impedance to the passage of blood cells, because of their large transmural apertures.

2.3. PATHWAYS OF BLOOD FLOW

The afferent arterial vessels course through the white pulp as central arteries, and their radial branches supply the marginal zone or red pulp. The attenuated main stem of a central artery drains, in most instances, into the reticular meshwork of the red pulp. In humans, the majority of the arterial terminations have no endothelial continuity with venous structures, and the circulation is predominantly "open" in form, with the pathway of blood flow crossing a connective tissue space. This does not mean that, in normal circumstances, there is a random process of flow in the intermediate circulation, since the orientation of the arterial terminations to the splenic sinus walls, and of the reticular cell stroma intervening between arterial termination and sinus wall, effectively produces an unimpeded pathway. As a consequence, the greater part of the blood flow through the human spleen passes through a functional "fast pathway," in contrast to the small fraction of flow that traverses the slow pathway (Groom, 1987). However, the volume of blood is greater in the "slow pathway," because of its slow turnover; this occupies part of the pulp cords, where they are both physiologically and structurally open. Groom et al. and Levesque and Groom (1981) have demonstrated vascular pathways in the marginal zone, which have the potential for bypassing the red pulp; these are discussed in more detail in Chapter 3.

The regulation of volume and distribution of blood flow is critical to the effective functioning of the spleen. A high proportion of a bolus of abnormal red blood cells entering the spleen is retained on initial passage through the organ, indicating that the filtration process is dependent on the structures of the afferent circulation, and does not require adaptive changes and prolonged flow (Groom, 1987). The arterial structures appear to direct plasma to the marginal zone, marginated cells to the white pulp, and marginal zone; the axial flow (especially erythrocytes and platelets) is directed to the red pulp. The marginal zone receives an overly large number of terminal arterial vessels, and blood entering this area is selectively channeled to either the white or red pulp.

The arteries of the human spleen appear to have sympathetic innervation, but parasympathetic innervation remains to be demonstrated (Reilly, 1985). Plasma skimming appears to require an intact nerve supply; pooling and concentration of red blood cells are inhibited by sympathomimetic drugs.

The reticular stroma of the human marginal zone and the PALS contains numerous reticular cells, which have smooth muscle actin and myosin (Toccanier-Pelte et al., 1987), which are not present in red pulp. These "myoid" cells have long, slender processes, which are intimately associated with reticular fibers. Similar contractile

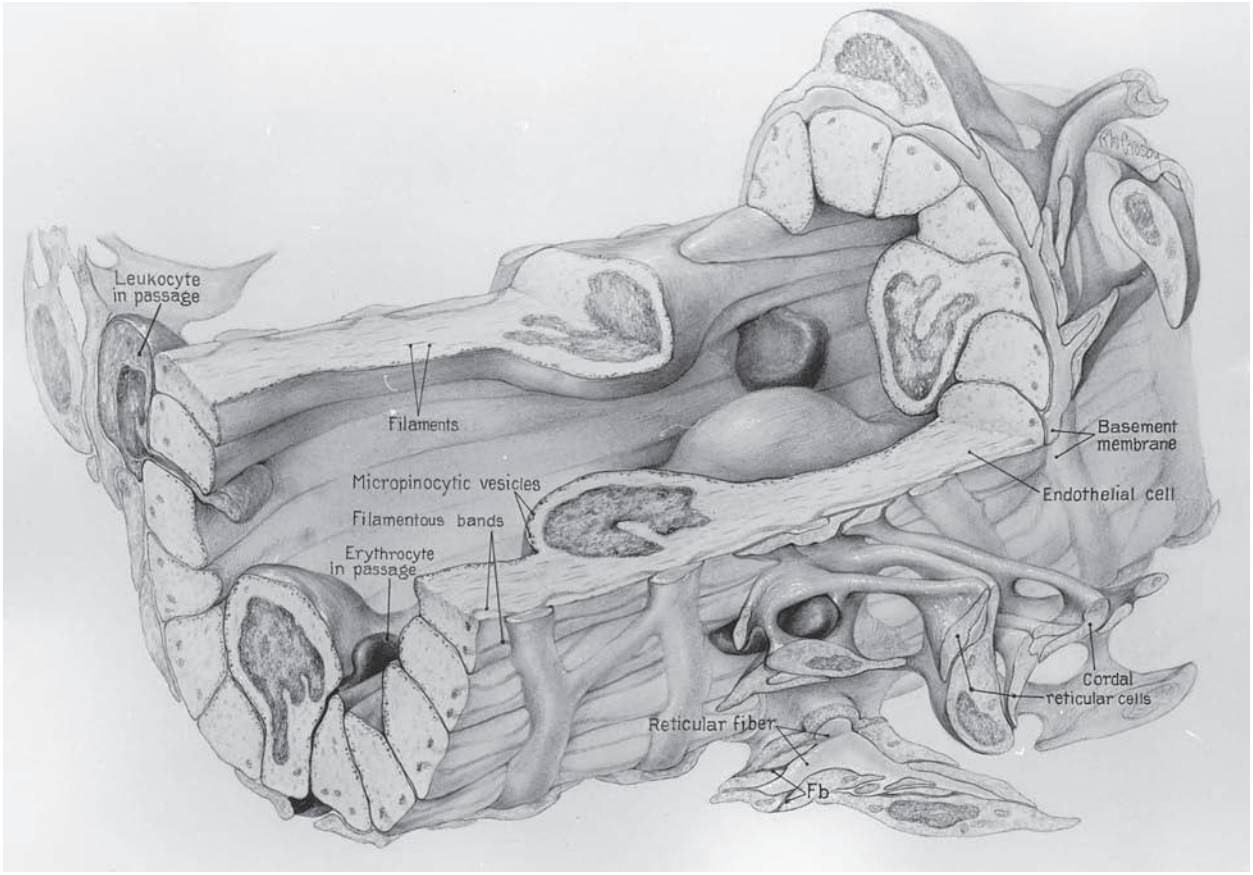

Fig. 2.16. Schematic diagram of a red pulp sinus. The parallel orientation of the sinus endothelial cells is shown, with endothelial nuclei causing protrusion into the sinus lumen. On the abluminal surface of the sinus is the fenestrated basement membrane, to which the cordal macrophages are attached. A blood cell, in this case a leukocyte, is passing between adjacent endothelial cells, to enter the lumen from the pulp cord. (Reproduced with permission from Chen, L. T. and Weiss, L. [1972]).

reticular cells have been shown to be present throughout horse and dog spleen, and are associated with sympathetic nerve terminals (Tablin and Weiss, 1983; Blue and Weiss, 1981). Smooth muscle cells and fibroblasts are closely related, and many fibroblastic cells are contractile. This group includes reticular cells of the spleen, as discussed previously, barrier cells discussed in Subheading 3.3., the myofibroblasts of wound healing, and the myoepithelial cells encircling epithelial structures, notably the ducts and acini of glands. This arrangement, in addition to the smooth muscle cells associated with PAMS, may regulate cellular traffic in areas of high blood flow.

Blood that enters the red pulp is in an open anatomical system, in which significant filtration can occur. This blood continues through the pulp sinuses, pulp veins, and trabecular veins, and subsequently the splenic veins at the hilus. Because the splenic vein enters the portal vein, any increase in portal pressure increases the blood contained in the spleen, and consequently the blood volume of the spleen. When this volume increase is long-standing, it may result in congestive splenomegaly. The myoelastic structure of the splenic vein suggests that the diameter of the vein can be actively varied to modify both intrasplenic pressure and venous flow (Reis and Ferraz de Carvalho, 1988). Secondary splenic distention could in turn modify the relationship of the arterial terminations to the sinus walls, increasing the length and duration of the flow pathway, and improving the filtration efficacy of the red pulp.

2.3.1. FILTRATION PATHWAY The tissues between the terminal arterial vessels and the initial venous vessels comprise a reticulum or reticular meshwork, composed of reticular cells and their associated extracellular matrix "reticular" fibers. In the human spleen, and indeed in spleens in general, the pulp consists of a reticular meshwork in which arterial and venous vessels are supported by adventitial reticular cells, which branch out perivascularly and contribute to the reticular meshwork. If the meshwork is extensive enough, it will contain reticular cells that are entirely confined to it, without an adventitial relationship to blood vessels. In the human spleen, as well as rat, dog, and horse spleens, the reticular meshwork is well-developed. These cells form a system of thin-walled domains reinforced by extracellular matrix fibers. These domains communicate with one another and, as a system, are open, directly or indirectly, to the blood that enters the spleen. Variation in the location and arrangement of the reticular cells, and their matrix components, occurs in a variety of meshworks, termed, by Weiss (1985), "filtration beds."

Two types of filtration beds make up the white pulp, which serve to direct T-cells to the PALS, and hold them there for a period of hours, and B-cells to lymphatic nodules or their mantles, and, for a somewhat longer time than T-cells, hold them there. Reticular cells, dendritic cells, and macrophages probably regulate this traffic. Lymphocytes that do not immunologically react in the white pulp, are cleared by the deep efferent lymphatics. In pathologic

states, filtration beds, through which lymphocytes normally flow rather rapidly, may hold them for long periods, as in the parasinusal red pulp (i.e., splenic cords) in malaria.

In sinusal spleens, the filtration beds comprising the marginal zone are unusually fine-meshed, and are associated with the large number of terminal arterioles present in this region. These extensive arterial terminal vessels deposit large volumes of blood into the marginal zone, making it one of the most highly trafficked parts of the spleen. Blood cells entering the marginal zone may migrate selectively to other filtration beds, e.g., lymphocytes, may go to the white pulp, to be sorted into T- and B-cell zones. In addition, blood cells may be held in the marginal zone and processed there. Damaged erythrocytes are pooled and phagocytosed, monocytes are sequestered and differentiate into macrophages, and platelets may be stored in the marginal zone, in ready reserve for quick release into the circulation.

The reticular meshwork of the red pulp consists of additional terminal arterial vessels, which empty into this specialized domain, and venous vessels, which drain it. Red pulp filtration beds are known as "pulp spaces," and are especially extensive in nonsinusal spleens, because of their role as storage spaces. In sinusal spleens, this meshwork is more limited, because of the vast anastomosing system of venous sinuses. Macrophages present in this domain may quickly increase in number, as a result of trapping and differentiation of circulating monocytes. In addition, the resident macrophage population in the PAMS also participates in cellular surveillance. Red pulp filtration beds contain reticulocytes, which undergo final maturation to erythrocytes before their release into the circulation, and, at the end of their life-span, lead to their phagocytosis. In murine spleens, these filtration beds regularly support extramedullary hematopoiesis, particularly, erythroid stem cell pro-liferation and differentiation (colony-forming unit, burst-forming unit, erythrocytes). This diverse filtration bed also is the site of lymphocyte sequestration and plasma cell proliferation, as well as antibody production in infectious disease, as in malaria, cited previously.

2.3.2. CLEARANCE OF BLOOD BY THE SPLEEN The spleen possesses a remarkable capacity for clearance of blood cells, infectious organisms, particles, and macromolecules. This clearance is dependent on the splenic filtration beds, which provide the appropriate microenvironment for phagocytosis, as well as cell proliferation and differentiation. Under normal conditions, aged erythrocytes and platelets are cleared from the blood by the filtration beds of the marginal zone and red pulp, and, in sinusal spleens, inclusion-containing erythrocytes, such as those containing Heinz bodies, may be pitted of their inclusions, as they squeeze through the interendothelial slits of venous sinuses in their passage out of the spleen, and return to the circulation (Fig. 16; *see also* Chapter 12). Lymphocytes and their accessory cells are sequestered in the filtration beds of the white pulp, and are arranged so as to be able to undergo immune stimulation and responses, or to leave the spleen via deep efferent lymphatics. Monocytes are cleared from the circulation in all beds of the spleen and readily differentiate into macrophages. Bacteria and other infectious particles, such as the plasmodial-parasitized erythrocytes of malaria, are cleared from the circulation by the spleen. Slightly to moderately damaged erythrocytes, such as occur in such congenital hemolytic diseases as hereditary spherocytosis, and erythrocytes damaged by such extrinsic factors as autoantibodies, parasites, and heat, are cleared by the spleen, and are phagocytosed or repaired.

2.3.3. BARRIER-FORMING SYSTEMS Reticular cells constitute a large, stable component of the filtration beds of hematopoietic tissues. Like many fibroblastic cell types, notably the myofibroblast of wound healing, they appear to be contractile, as shown by the significant numbers of actin filaments they contain. Indeed, extracellular matrix formation and contractility are common properties of these cells. Smooth muscle cells are girdled by a sleeve of reticular fibers and the elastic fibers they synthesize. Curiously, reticular fibers (preponderantly collagen type III) lie on the reticular cell cytoplasm, as closely as the elastic fibers on their smooth muscle cells. Myoepithelial cells, whose contractile tentacles squeeze down upon acini and ducts of mammary and other glands, forcing out secretion, and which, by secretion of extracellular matrix of their basal lamina, illustrate the combination of extracellular matrix formation and contractility.

Splenic filtration beds do not normally show a high level of filtration, since more than 90% of the blood circulates through the spleen as rapidly as through tissues with a conventional vasculature. Yet splenic behavior may change rapidly in stress, and the organ can become hypersplenic. We have documented the presence of contractile fibroblasts that are capable of dynamically altering the responsive nature of this filtration domain. These contractile cells, or one or more of their subsets, are capable of fusing with one another in the filtration beds, to form complex, branching, syncytial sheets that form a variety of barriers. These cells have been termed "activated reticular cells" (Weiss et al., 1986), but, on the basis of continued studies, we now define them as "barrier cells" (Weiss, 1991), recognizing their remarkable capacity for diverse structural and functional barrier formation. Barrier cells are present in large numbers in murine and human spleens, under conditions in which splenic clearance appears heightened, pathologically (including sickle cell disease, spectrin deficiency, congenital spherocytic anemia, thalassemia, malaria, and Hodgkin's disease). They may well be evolutionarily conserved, because they are present in the spleen of stressed teleosts. They occur in small numbers in the normal mammalian spleen. Barrier cells proliferate and show morphological signs associated with intense protein synthesis: large nucleoli, dense cytoplasm, and widened perinuclear cisternae continuous with endoplasmic reticulum, so branched and expanded that it imparts a lacy appearance to the cytoplasm.

Splenic barrier cells originate by activation of fibroblasts on the surface of trabeculae and the adventitial aspect of blood vessels; activation is signaled by increased cytoplasmic density, accompanied by increases in rough endoplasmic reticulum, as well as dilated mitochondria. Parallel changes occur in bone marrow, the barrier cells differentiating from the bone-lining layer covering trabeculae and myeloid diaphyseal bone. Barrier cells also originate from circulating precursors; circulating blood contains fibroblast stem cells (colony-forming unit, fibroblastoid), as determined in tissue culture assays. Barrier cells initially accumulate in the spleen, perivascularly, as dense, round cells with relatively short cell processes. Fusing with one another, they migrate from their initial perivascular location, and, in many instances, adhere to existing extracellular matrix, and associate with established reticular cells. They move apart, remaining associated with the resident reticular meshwork, and attached to one another by extended cell processes. Barrier cells thereby augment the functions and structure of the basic reticular cell filtration beds.

Barrier cells enclose blood vessels, providing or enhancing an adventitial layer. They tightly surround single blood cells and

multicellular hematopoietic colonies, isolating and protecting them. Such barrier cell enclosures form a blood–tissue barrier in the spleen, in the precrisis phase of reticulocyte-prone plasmodia (as with *Plasmodium berghei* in murine malaria), preventing bloodborne parasites from parasitizing reticulocytes and their precursors. A remarkable splenic synchrony occurs at crisis. At the same time, the moment of crisis, barrier cell–cell associations are disrupted, relieving the isolation of the hematopoietic, notably erythroid, colonies. The colonies reach the precise point of maturity that permits their erythroid cells, no longer confined by barrier cells, to be released into the circulation, and the parasite (contained in parasitized erythrocytes), excluded precrisis from the splenic filtration beds by the intact barrier-cell barrier, enters these beds, readily crossing the now-disrupted barrier-cell barrier. The postcrisis filtration beds of the spleen are open to the circulation, in contrast to the precrisis spleen, in which they are shut off from the circulation by the intact barrier-cell barriers.

The precrisis spleen accordingly embodies a paradox: It is a large spleen exhibiting splenomegaly, yet it is not hypersplenic, because, with the spleen blood barrier intact, its level of clearance is reduced. It is hyposplenic, or "asplenic," rather than, as would be more characteristic of a large spleen, hypersplenic. These changes are marked and evident in malaria. Yet, on close evaluation of other splenomegalies, such as those of sickle cell disease and thalassemia, they too display hyposplenia in the course of splenomegaly. It may well be, moreover, that the splenic fibrosis in chronic sickle cell disease is not caused, as has been inferred, by cumulative, successive small infarcts, but by the accumulation of barrier cells, which, with chronicity, become fibroblastic, resulting in the fibrotic splenic nubbin that had been the spleen. Examination of the spleen in murine malaria, and the spleen in human sickle cell anemia, moreover, reveals that fibrosis is not figured as a flame-shaped fibroblastic aggregate at the end of a splenic vessel, as would occur in infarction, but rather as a perivascular cuff, where barrier cells lay.

Barrier cells infiltrate existing circumferential matrix reticulum, and adhere to its marginal zone surface, thus transforming the circumferential reticulum into a more effective barrier surrounding and protecting the white pulp. This change may occur after an immune response is initiated in the white pulp, thereby causing that white pulp, already engaged in antibody production, to be refractory to further stimulation by antigen. In the marginal zone and the cords of red pulp of human and other sinusal spleens, where filtration beds are best-developed, barrier cells intercalate into these domains, and may help to regulate their cellular traffic. In contrast, in murine (nonsinusal) spleen, the matrix reticulum composing the filtration domains in red pulp and marginal zone is scanty and less well developed. Yet, in these spleens, as in the sinusal spleens, barrier cells are present as extensive, branched, syncytial arrays, but, with relatively little reticulum to infiltrate, barrier cells appear to be tethered to nonfibrillar matrix components, as well as to the adventitial reticular cells present on the abluminal surface of blood vessels. We believe that barrier cells augment the basal filtration activity of the filtration beds, and serve to regulate their traffic.

Barrier cells provide dynamic, diverse blood–spleen barriers, which, acting in coordination with macrophages and other stromal cells, regulate splenic filtration and its intrasplenic consequences, including blood flow, cell homing and migration, hematopoietic and immune responses, and the clearance of infectious organisms. Barrier cells trap circulating infectious organisms and monocytes on their cell surfaces, clearing them from the blood, providing a selective environment for monocyte differentiation into macrophages and subsequent phagocytosis of the microorganisms. Barrier cells enclosing hematopoietic colonies are positioned to confine factors controlling colony growth and differentiation. They may protect colonies from parasitism, e.g., as erythroblastic colonies in malaria. Activated barrier cells and their associated matrix molecules may effectively close off white pulp. An initial antigen stimulus is thereby met by a complete response, which confines the lymphokines, cytokines, and other regulatory substances, to the white pulp, and prevents secondarily derived antigen from dissipating immunological resources. Closing off the white pulp, in the presence of contagious cellular damage, would reduce autoimmune responses. As barrier cells close off the selected filtration domains, they constitute a shunt, permitting an efficient closed circulation between arterial terminals and veins. The spleen, unlike the marrow, lacks a cellular barrier between hematopoietic tissues and the blood. These specialized cells provide such barriers, thereby conferring on the spleen certain attributes of the marrow.

2.4. CONCLUSION

In a normal spleen, the level of filtration activity may well be regulated by the degree of contraction of the filtration beds, the capsule and trabeculae; the placement of the terminating arterial vessels; and the capacity of the sheet-like processes of the reticular cells to establish tubular connections between arterial and venous vessels. The normal spleen does not appear to depend heavily upon its barrier cells, although they are present in the circumferential reticulum of white pulp, and, in sinusal spleens, they may also be found in the marginal zone and red pulp. In pathological spleens in which the locules of the filtration bed come tightly crowded, as a result of heightened filtration, the spleen becomes firm and enlarged; consequently, the mechanisms regulating blood flow under normal circumstances cannot function, since the spleen is distended and becomes incapable of contraction. The syncytial membrane and meshworks produced by the fusion of barrier cells become the means by which the character of the blood flow is determined. The character of blood flow, in turn, determines whether or not the filtration beds are perfused, whether the circulation is open or closed, and whether or not the blood is cleared.

REFERENCES

Blue, J. and Weiss, L. (1981a) Periarterial macrophage sheaths (ellipsoids) in cat spleen: an electron microscope study. *Am. J. Anat.* **161,** 115–134.

Blue, J. and Weiss, L. (1981b) Electron microscopy of the red pulp of the dog spleen including vascular arrangements, perarterial macrophage sheaths (ellipsoids) and the contractile innervated reticular meshwork. *Am. J. Anat.* **161,** 189–281.

Borrello, M. A. and Phipps, R. P. (1996) Differential Thy-1 expression by splenic fibroblasts defines functionally distinct subsets. *Cell. Immunol*. **173,** 198–206.

Buckley, P. J., Smith, M. R., Braverman, M. F., and Dickson, S. A. (1987) Human spleen contains phenotypic subsets of macrophage and dendritic cells that occupy discrete microanatomic locations. *Am. J. Pathol*. **128,** 505–520.

Drenckhahn, D. and Wagner, J. (1986) Stress fibers in the splenic sinus endothelium in situ: molecular structure, relationship to the extracellular matrix and contractility. *J. Cell Biol.* **102,** 1738–1747.

Faller, A. (1985) Splenic architecture as reflected in the connective tissue structure of the human spleen. *Experientia* **41,** 164–167.

Ford, W. L. (1969) Immunological and migratory properties of lymphocytes recirculating through the rat spleen. *Br. J. Exp. Pathol*. **50,** 257–269.

Ford, W. L. (1968) The mechanism of lymphopenia produced by chronic irradiation of the rat spleen. *Br. J. Exp. Pathol*. **49,** 502–513.

Fujita, T., Kashimura, M., and Adachi, K. (1982) Scanning electron microscopy studies of the spleen: normal and pathological. *Scan. Electron Microsc.* **1,** 435–444.

Fujita, T., Kashimura, M., and Adachi, K. (1985) Scanning electron microscopy and terminal circulation. *Experientia* **41,** 167–179.

Groom, A. C. (1987) Microcirculation of the spleen: new concepts, new challenges. *Microvasc. Res.* **34,** 269–289.

Hammond, B. J. (1975) A compartimental analysis of circulatory lymphocytes in the spleen. *Cell Tissue Kinet,* **8,** 153–169.

Kashimura, M. and Fujita, K. (1987) A scanning electron microscope study of human spleen: relationship between microcirculation and functions. *Scan. Microsc.* **1,** 841–851.

Koike, R., Nishimura, T., Yasumizu, R., Tanaka, H., Hataba, Y., Hataba, Y., et al. (1996) The splenic marginal zone is absent in alymphoplastic *aly* mutant mice. *Eur. J. Immunol*. **26,** 669–675.

Kraal, G., Schornagel, K., Streeter, P. R., Holzmann, B., and Butcher, E. C. (1995) Expression of the mucosal vascular addressin, MadCAM-1, on sinus-lining cells in the spleen. *Am. J. Pathol*. **147,** 763–771.

Levesque, M. J. and Groom, A. C. (1981) Fast transit of red cells and plasma in contracted versus relaxed spleens. *Can. J. Phys. Pharm.* **59,** 53–58.

Liakka, A., Apaja-Sarkkinen, M., Karttunen, T., and Autio-Harmainen, H. (1991) Distribution of laminin and types IV and III collagen in fetal, infant and adult human spleens. *Cell Tissue Res.* **263,** 245–252.

Liakka, A. and Autio-Harmainen, H. (1992) Distribution of extracellular matrix proteins tenascin, fibronectin, and vitronectin in fetal, infant and adult human spleens. *J. Histochem. Cytochem.* **40,** 1203–1210.

Liakka, A., Karjalainen, H., Virtanen, I., and Autio-Harmainen, H. (1995) Immuno-electron-microscopic localization of types III pN- collagen and IV collagen, laminin and tenascin in developing and adult human spleen. *Cell Tissue Res.* **282,** 117–127.

Lyons, A. B. and Parish, C. R. (1995) Are murine marginal-zone macrophages the splenic white pulp analog of high endothelial venules? *Eur. J. Immunol.* **25,** 3165–3172.

Pabst, R. (1988) The spleen in lymphocyte migration. *Immunol. Today* **9,** 43–45.

Persson, S. G., Ekman, L., Lydin, G., and Tufvesson, G. (1973a) Circulatory effects of splenectomy in the horse. I. Effect on red cell distribution and variability of haematocrit in the peripheral blood. *Zentralbl. Veterinarmed.[A]* **20,** 441–455.

Persson, S. G., Ekman, L., Lydin, G., and Tufvesson, G. (1973b) Circulatory effects of splenectomy in the horse. II. Effect of plasma volume and total and circulating red cell volume. *Zentralbl. Veterinarmed.[A]* **20,** 456–468.

Reilly, F. D. (1985) Innervation and vascular pharmacodynamics of the mammalian spleen. *Experientia* **41,** 187–192.

Reis, F. P. and Ferraz de Carvalho, C. A. (1988) Functional architecture of the splenic vein in the adult human. *Acta. Anat*. **132,** 109–113.

Sasou, S., Satodate, R., Masuda, T., and Takayama, K. (1986) Scanning electron microscopic features of the spleen in the rat and human: a comparative study. *Scan Electron Microsc.* **3,** 1063–1069.

Tablin, F. and Weiss, L. (1983) The equine spleen: an electron microscopic analysis. *Am. J. Anat.* **166,** 393–416.

Toccanier-Pelte, M. F., Skalli, O., Kapanci, Y., and Gabbiani, G. (1987) Characterization of stromal cells with myoid features in lymph nodes and spleen in normal and pathologic conditions. *Am. J. Pathol*. **129,** 109–118.

Uehara, K. and Miyoshi, M. (1997) Junctions between the sinus endothelial cells of rat spleen. *Cell Tissue Res.* **287,** 187–192.

Van Ewijk, W. and Nieuwenhuis, P. (1985) Compartments, domains, and migration pathways of lymphoid cells in the splenic pulp. *Experientia* **41,** 199–208.

van Krieken, J. H. J. M. and te Velde, J. (1988) Normal histology of the human spleen. *Am. J. Surg. Pathol*. **12,** 777–785.

Van Vliet, E., Melis, M., Foidart, J. M., and Van Ewijk, W. (1986) Reticular fibroblasts in peripheral lymphoid organs identified by a monoclonal antibody. *J. Histochem. Cytochem.* **34,** 883–890.

Weiss, L. (1983) The red pulp of the spleen: structural basis of blood flow. *Clin. Haematol.* **12,** 375–393.

Weiss, L. (1985) New trends in spleen research: conclusion. *Experientia* **41,** 243–248.

Weiss, L., Powell, R., and Schiffman, F. J. (1985) Terminating arterial vessels in red pulp of the spleen. *Experientia* **40,** 233–242.

Weiss, L., Geduldig, U., and Weidanz, W. (1986) Mechanisms of splenic control of murine malaria: reticular cell activation and the development of a blood-spleen barrier. *Am. J. Anat.* **176,** 251–285.

Weiss, L. (1991) Barrier cells in the spleen. *Immunol. Today* **12**, 24–29**.**