lienic pulpa, on the one hand, and the lienic capsule, on the other. It is somewhat surprising that the subcapsular zone should contain no white pulpa at all.

Summary

An analysis of the connective-tissue structure of the human spleen can give us information about the basic architecture of the organ. The most important part of the spleen is the lienic center around which the subcapsular zone forms an envelope, like a mantle. This zone has but little depth and develops superficially. The tangential radial beam net ('Tangentialbalkennetz') is formed partly by the radial trabeculae of the capsule and partly by the outer branches of the *arbor trabecularis*. This *arbor* divides into 5–6 branching orders. The branches of orders 1 to 3 surround the parenchyma of the spleen center's inner layer. The lienic lobuli which are found between these branches are relatively large and are connected very extensively with their parenchyma. The branches of orders 4, 5, and 6 enclose the lienic lobuli of the outer layer of the spleen center. The splenic lobuli are defined by the vascular course. Mostly they are provided with one or two arterial influxes and, as a rule, with only one venous drain. Their mutual delimitation is more of a funcitional than of a morphological nature. This led von Herrath[12,13] to coin the term 'functional spleen lobuli'. The lienic envelope lies between the inside of the capsule and the outermost branchings of the *arbor trabecularis*. This *arbor* is subdivided, by the radial trabeculae, which never have any vessels, into elongated lobuli and serves first and foremost to regulate pressure. The lattice fibers are of high tensile strength and are extensions of the collagenous fibers seen at the microscopic level.

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Scanning electron microscopy and terminal circulation

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Key words. Spleen; terminal circulation; scanning electron microscopy; white pulp; marginal zone; red pulp; sinus endothelium; rod cells; cords of Billroth; macrophages; platelets.

Introduction

The spleen is one of the organs whose real, three-dimensional microfabric has been most enigmatic. Recent application of scanning electron microscopy (SEM) has contributed a great deal to the elucidation of its structure but many riddles still remain to be solved. During the last 13 years we have been engaged in fine-structural investigation of mammalian spleen mainly by means of SEM[13,16,17,18,20,32,33,61]. This paper reviews the results of this work, concentrating on the human spleen, and compares them with the reports issued by other research groups.

Materials and methods

It seems to be appropriate to introduce here the main materials and methods used in our study.

Materials. 13 spleens obtained in surgery (gastro-pancreatico-splenectomy) from patients with gastric cancer (both sexes, 39–75 in age) could be used as organs with normal structures. Organs with pathological changes were carefully excluded.
Pathological spleens, including idiopathic portal hypertension or so-called Banti's syndrome (3 cases), hepatic cirrhosis (10 cases), hereditary spherocytosis (HS) (4 cases) and idiopathic thrombocytopenic purpura (ITP) (7 cases) have been also investigated in our research group. The results from the pathological organs will be included in this review as far as 1) a part of them represent a normal structure and supplement the findings from the normal organs and 2) any cell or structure after pathological changes seems to reveal its nature which is unclear or concealed in the normal state.
The spleens of animals examined include those of the dog, pig, sheep, rabbit, rat and mouse, but this review is essentially based on the observation of human spleens.

Scanning electron microscopy (SEM). The human spleens were perfused via the branches of the splenic artery with warmed Ringer solution and then with 2 or 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. In animals the perfusion was made in situ, through either the aorta or the splenic artery after laparotomy. The outflow of the perfusate was prevented by ligating the blood vessels at the splenic hilus and the organ (excised at this stage in the case of animals) was kept for several hours in the glutaraldehyde-phosphate fixative. Tissue blocks, measuring roughly $6 \times 3 \times 3$ mm were cut from the fixed organ and, according to a slight simplification[20] of the 'conductive staining' method of Murakami[36], immersed for 10–12 h in an aqueous solution composed of 2% glycine, 2% sodium glutamate and 2% sucrose (pH 6.2), for 12–24 h in a 2% aqueous solution of tannic acid (pH 4.0), and for 4–8 h in a 1% solution of osmium tetroxide. The tissue acquires electric conductivity as it is heavily impregnated with osmium tetroxide through these procedures. This is of essential importance for such spongy structures like the spleen as they will otherwise easily show the phenomenon of charging up in the scanning electron microscope (SEM) which seriously disturbs the quality of the images.
The conductive-stained blocks are dehydrated in ethanol and transferred to isoamyl acetate. The specimens, either in absolute ethanol or in isoamyl acetate, are quench-frozen in liquid nitrogen and, crossing the elongate shape of the blocks, fractured by a mechanical impact[61]. The specimens immersed in isoamyl acetate are dried by the critical point drying method using liquid CO_2, attached on a metal stub and evaporation-coated with gold-palladium[13,20]. We do not use sputter coating as it sometimes causes damages on the cell surface.
Most of the specimens used for this study were observed and photographed in a field emission type SEM (HFS-2, Hitachi Manufacturing Co.) under the accelerating voltage of 10 kV.
Specimens for TEM and light microscopy were also made from the spleens perfused for the sake of SEM study.

SEM of vascular casts. Observation by SEM of resin of blood routes in the spleen was performed according to the method by Murakami[20,38]. Following the arterial perfusion with warmed Ringer solution, half-polymerized methyl methacrylate (commercially available Mercox resin, Oken Shoji Co. Ltd., Tokyo) is injected until the splenic vein is filled with resin[20,35]. The organ, or the animal is warmed to 60–70 °C overnight and the resin cast is polymerized. The soft tissue is macerated with 20% NaOH, and the resin cast is washed thoroughly. The resin casts of blood vessels thus produced are trimmed into appropriate pieces, either evaporation-coated with gold-palladium or impregnated with vaporized osmium tetroxide[38] and viewed under the SEM.

White pulp and marginal zone

The white pulp of the spleen consists of lymphocytes densely packed in the meshes of reticular cells. As SEM images of these elements and follicular arterioles and capillaries have been demonstrated elsewhere[17,18], and as few findings of essential importance in the white pulp have hitherto been obtained by SEM, this part of the spleen will be omitted from this review.
The white pulp is surrounded by a marginal zone which belongs to the red pulp but is devoid of sinuses (fig. 1). A recent review by Nanba et al.[41] depicts the SEM, TEM and light microscopic structures of the marginal zone in different animals. TEM observation by Saito and Kamiyama[47] are available for human marginal zone. We also demonstrated the SEM images of human marginal zone[17,18]. This zone comprises a network of reticular cells and fibers whose meshes are finer than those in intersinal parts of the red pulp. Numerous medium-sized lymphocytes may fill the marginal zone, especially its inner layer[47]. Typically, the marginal zone is limited against the white pulp by a lamellar structure called the circumferential reticulum which comprises attenuated reticular cells and reticular fibers. Perforations in the circumferential reticulum allow the passage of lymphocytes from the white pulp to the marginal zone[17,18].
The marginal zone is richly supplied with arterial blood. It is derived from follicular arteries and partly from penicilli recurring from the intersinal area[53]. The mode of arterial termination in the marginal zone will be dealt with later.

Red pulp

The general part of the red pulp consists of sinuses and cords of Billroth, the latter containing penicillar arteries. This construction is plastically visualized if an appropriate fracture face of a perfused spleen is observed by SEM (fig. 2). The first SEM studies of the splenic red pulp were published by us on the rabbit[33], dog and rat[32] followed by the report by Suzuki[55] on the normal and

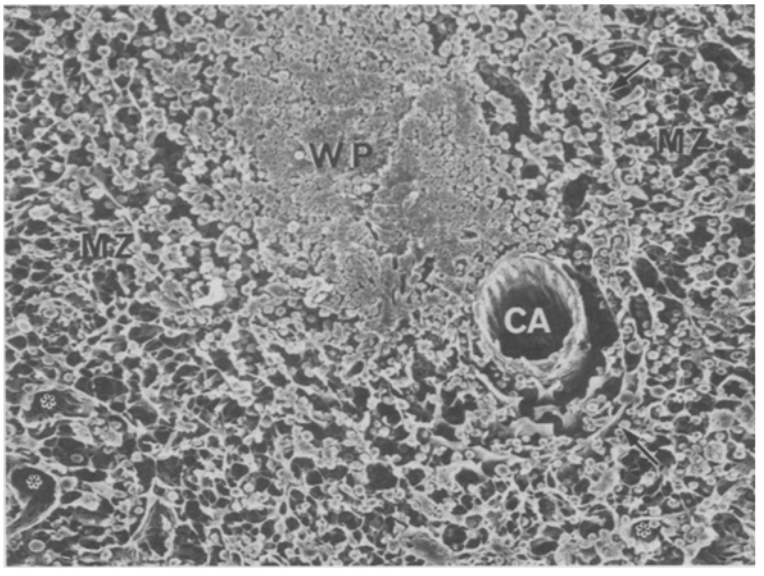

Figure 1. Overview of the central artery (CA), white pulp (WP) and marginal zone (MZ) in freeze-cracked human spleen. Arrows indicate circumferential reticulum. The asterisks indicate sinuses of the red pulp. × 190.

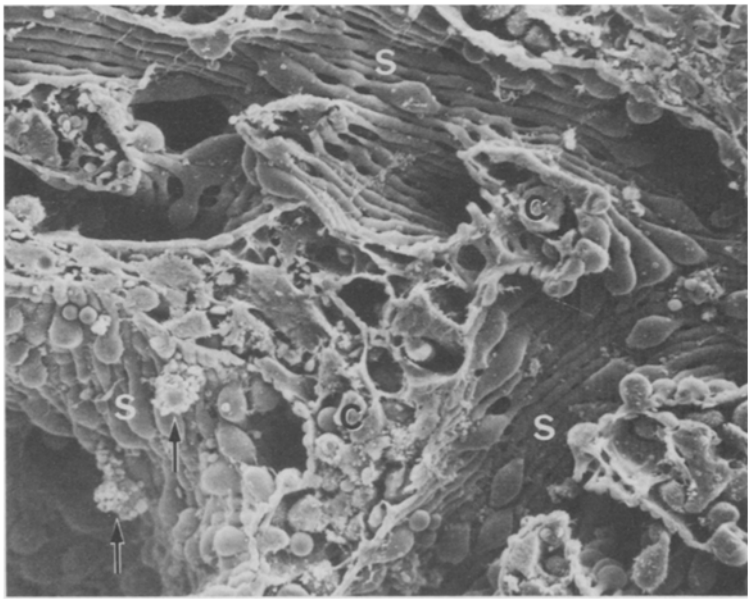

Figure 2. Low power view of human red pulp. The luminal aspect of the sinuses (S) shows a palisade of rod cells with nuclear swellings. Perforations of the sinus wall are clear in this magnification. Arrows indicate macrophages protruding into the sinus lumen. × 580.

pathological (Banti's syndrome and liver cirrhosis) human spleens and by the reports by Leblond[30] and by Weiss[64] on the rat spleen. Application of more advanced specimen preparation techniques has produced more accurate and convincing three-dimensional visualization of the red pulp of human[13,16,18,20] and animal (dog[56], rat[30,48]) spleens.

The structures of the splenic sinus, especially of the human, and their functional implications, will now be reviewed.

Sinus endothelium and its perforations

The sinus endothelium consists of a palisade of rod cells. The rod cells are long and flattened cells with tapered ends. They run parallel to each other and, generally, to the course of the sinus. Their nuclear portion forms a conspicuous ovoid swelling (fig. 2).

The rod cells in animals, e.g. dog, rabbit, rat and mouse, are connected with each other by slender side processes, thus forming a clear lattice structure of the endothelium. This view was established by Mollier[34] in 1911 in his light microscope observation of perfused spleens and confirmed by recent SEM studies[2,32,33,56]. SEM observation revealed that the lattice pattern was much less clear in human sinuses, as the rod cell side processes, although present periodically, were short and broad[13]. Until this understanding was attained using SEM, the view of Mollier[34] had been generally accepted that human and simian rod cells were lacking in the side processes in contrast to other mammals. This view was based on the classical treatise by Weidenreich[63] who described, in his light microscope observation of human spleen in 1901, that rod cells were simple 'Stäbchen' without any side processes.

The TEM studies, meanwhile, were powerless to eluci-

date the relation of rod cells. Early TEM researchers on the spleen mostly conceived that rod cells lie against each other's lateral surfaces and that potential gaps between them may allow the passage of blood cells. Galindo and Freeman[21] denied the occurrence of intercellular gaps and wrote that the plasma membranes of the sinus lining cells 'interdigitate in much the same manner as the plasma membranes of intestinal and renal tubular epithelial cells'. Rappaport[45] declared that the openings in the sinus wall were solely artifacts.

In this chaotic state of TEM studies, mainly caused by ignorance of the studies of early light microscopists, the reports by Thomas[59] in the rabbit and dog and Pictet and associates[44,52] in the rat were exceptional. Fully conscious of the light microscope knowledge of the sinus wall, they observed it in carefully perfused spleens and revealed persistently open spaces between rod cells. Regrettably, they could not attain a precise visualization of bridges between the rod cells. Although Chen and Weiss[8] wrote that the human sinus was lined by simple rods 'arranged side by side without junctional complex', Thomas[59] showed a junctional structure between rod cells in the rabbit and dog. Heusermann and Stutte[25] demonstrated that human rods were connected with intercellular junctions, 'close junctions' and intermediate junctions. A desmosome has not been demonstrated here. Under the SEM the junction straightly traverses the side bridge of rod cells usually showing a suture-like surface appearance (fig. 3).

The gaps or perforations in the sinus wall, as described above, are persistent openings. This, however, does not mean that the gaps are rigid windows. It must be stressed that they may change their shape and size from a literal slit to a round perforation according to changes in local blood pressure and according to possible movement of the rod cells and cordal reticular cells.

The sinus wall perforations represent the routes for passage of blood including its cellular elements, i.e. erythrocytes, leukocytes and platelets. As will be dealt with later, the blood flows through the sinus wall from the cordal to the luminal side. Platelets can pass through the gaps, but larger corpuscles must be strongly constricted in order to do so[15]. During the passage, solid bodies contained in erythrocytes are squeezed out. Howell-Jolly bodies in reticulocytes and erythrocytes are believed to be removed by this mechanism (fig. 4)[4]. The bodies thus thrown out are believed to be phagocytosed by macrophages located in the vicinity. This pitting process[10,18,29,46] is generally accepted as an important function of the sinus perforation. Plasmodia included in red cells of malaria patients are pitted in splenic sinus walls[51], and splenectomy in malaria patients is harmful.

The sinus wall perforations are presumed to serve in the elimination of aged erythrocytes as, owing to their decreased flexibility, they cannot easily pass through the gaps and are caught by patrolling macrophages. This 'mechanical hazard' theory of the erythrocyte destruction has been repeatedly proposed[9,65,66] in parallel to the 'chemical hazard' theory which postulate that changes either in the cell membrane or its coating substances, e.g. IgG and IgM adhering to aged erythrocytes[27], might be detected by macrophages. The latter mechanism may be called 'immunological hazard'. The truth may well be a combination of these different mechanisms.

Rod cell surface structure

Rod cells are usually arranged regularly in parallel. The termination of a rod cell, which occurs as a tapered end, does not disturb the regularity of the cell arrangement. Bifurcation, crossing over and other irregularities, however, may be found occasionally. The branching portions and blind ends of sinuses tend to contain more irregular cells than are found elsewhere.

The rod cells are smooth on the surface but are often provided with drop-like and tread-like microprocesses. The latter measure about 100 nm in thickness and up to 8 µm in length (fig. 5). These microprocesses of rod cells were discovered in 1970 in rabbit[32] and later confirmed to occur also in the human[13]. The function of the thread-like microprojections is unknown. They may be conspicuously increased in hepatosplenic disorders, e.g. idiopathic portal hypertension and liver cirrhosis[16] (fig. 6) and in such blood diseases as HS and ITP[18].

Occlusion of sinus perforations by lateral adhesion of rod cells is also usually recognized in the above men-

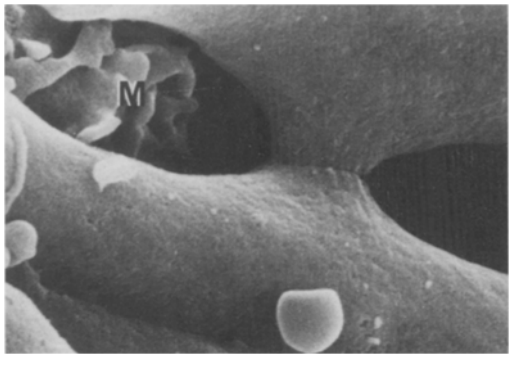

Figure 3. Junction of the transverse processes of human rod cells. M: macrophage. (Reproduced from T. Fujita, Archvm histol. jap. *37* (1974) 187–216.) × 9960.

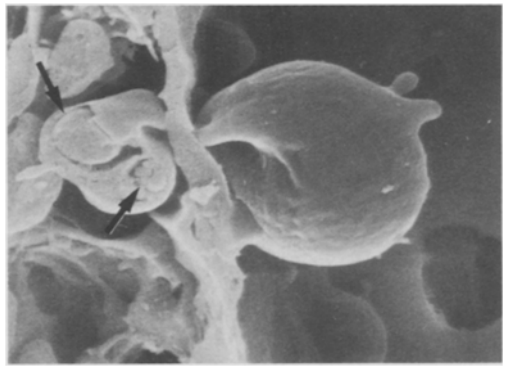

Figure 4. SEM image suggesting the pitting process in human spleen. The erythrocyte is strongly constricted while it passes the sinal slit. The part of the cell remaining in the cord (on the right side) contains inclusion bodies (arrows) which presumably will be squeezed out by the sinal slit. (Reproduced from Fujita et al., Scanning Electron Microscopy/ 1982/I; pp. 435–444. Ed. O. Johari, SEM Inc., AME O'Hare, Chicago.) × 7055.

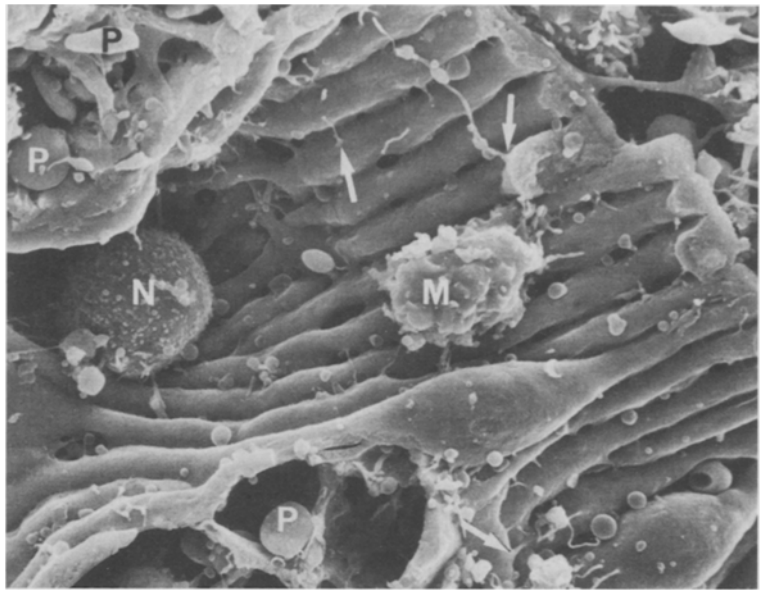

Figure 5. Luminal view of a sinus in a normal human spleen. Longer and shorter filopodia project from the rod cells (arrows). M: macrophage; N: neutrophil; P: blood platelets, × 2240.

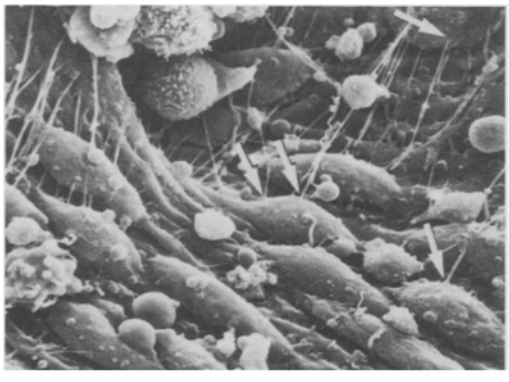

Figure 6. Luminal view of a sinus from the spleen of a 45-year-old patient with Banti's syndrome. Note markedly increased filopodia of the rod cell. Arrows indicate that the filopodia grow from the rod cell. × 1250.

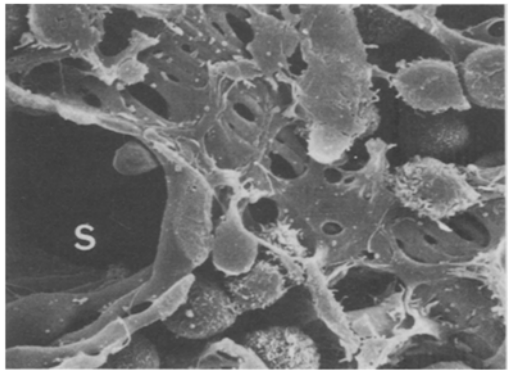

Figure 7. The back side or cordal aspect of a human sinus. S: cross section of a sinus. × 1250.

tioned diseases[16,18]. Furthermore, rough-surfaced swelling of the nuclear portion of rod cells is observed in the cases of ITP and HS[18]. As these changes in the surface structure of rod cells can be easily detected and convincingly recorded by SEM, there seems to be a fruitful field of 'rod cell pathology' to be explored by SEM.

Backside of sinus endothelium

The outside or back side of the sinus as observed by SEM shows ring bands or hoops crossing the rod cells and covering their bridges. They are processes of reticular cells located in the vicinity (figs 7, 8). Usually, a reticular cell extends more than two processes or end feet to the sinus wall, exactly as Weidenreich[63] depicted in his light microscope observation. The reticular fibers or ring fibers are hidden from view as they are sandwiched between the rod cells (including their bridges) and the reticular cell end feet. SEM revealed that each reticular cell end foot is fixed in a corresponding groove on the rod cells, so that the outer surface of the sinus wall remains flat (fig. 8)[13,17]. This elaborate structure had been suggested 50 years ago by Hartmann[22] who noticed that

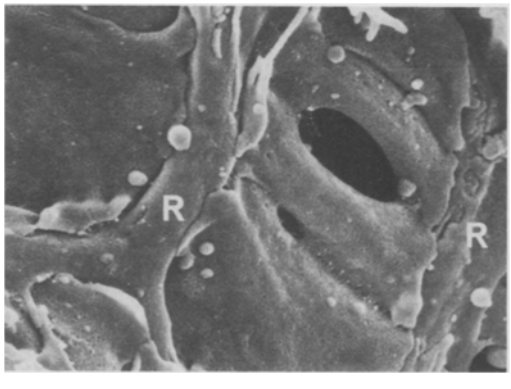

Figure 8. Closer view of the part of the sinal wall shown in figure 7. Reticular cell processes (R) hoop the lattice of the rod cells. × 6000.

ring fibers were pressed into rod cells and, when the former were removed by microdissection, the latter revealed indentations under the light microscope.

Cords of Billroth

The splenic cord is supported by reticular cells and reticular fibers. The former are stellate cells extending

slender processes which connect with those of adjacent cells. As they sandwich and embrace every piece of the reticular fibers, the latter cannot be seen under the SEM unless the former is broken (fig. 8). Adjacent to the sinus, the reticular cells and fibers extend to support it and to embrace it as the cellular and fibrous hoops of the sinus.

The surface of the reticular cells is smooth, except for occasional microprojections. In their meshes are found numerous 'round' or 'free' cells (fig. 9). Neutrophils and lymphocytes are numerous, whereas a few eosinophils and basophils are found. Based on combined light microscope and SEM studies[23], we can presume, if not determine, most of the leucocyte types by their surface structure as seen by SEM[13].

Most leucocytes are rounded in shape. Some are observed under locomotion, showing a pseudopod characterized by ruffled surface and a slender uropod with villous microprojections[18]. Increased instances of leucocyte migration may be regarded as an indication of increased chemotactic factors in the spleen, as suggested in the observation of HS spleens[18].

Macrophages and 'reticuloendothelial system'

Macrophages are characterized by granular, drumstick-like and spinous microprojections densely covering the cell surface[13,14,16]. Long filopodia may also be extended. Macrophages are dispersed in the spaces of the cord as well as on the luminal surface of the sinus endothelium. They may be rounded or may extend pseudopodia, or, otherwise, lie flat on the surface of any structure. It is frequently seen that a macrophage on the outside of the sinus wall issues bunches of microprocesses or some filopodia into the sinus lumen. Macrophages may also occasionally cover extended areas of the endothelial or reticular cell surfaces with their membranous processes. All these attitudes of macrophages can be clearly recognized by SEM, but they may be difficult to visualize from the two-dimensional images of TEM.

SEM observation has convincingly demonstrated that only macrophages are involved in phagocytosis of large bodies, measuring more than a few micra in diameter[14,16]. Foreign bodies may be caught by the processes of a macrophages covering a reticular cell or penetrating an endothelium, and in such a case SEM images clearly indicate that the macrophage and not the cell attached by it is engaged in phagocytosis.

Careful examination of numerous samples by SEM has indicated that there are no intermediate forms between macrophages and reticular cells and between macrophagaes and endothelial cells[13,14,16]. The macrophages are characterized by their rough surface and their migratory and phagocytotic abilities, and the retiular and endothelial cells by their smooth surface and their fixed position. As an additional feature of the macrophage, one may detect its adhesion to a lymphocyte[13,15,17,18] (fig. 10). This image corresponds to the immunologically important process of information transfer from the macrophage to the lymphocyte.

The view that the macrophage is a cell specialized for phagocytotic and other immunological roles and independent of reticular and endothelial cells which are specialized to form the framework of the tissue is compatible with the corresponding understandings reached for other reticular tissues, especially lymph nodes[19] and liver[40]. The concept and term, 'reticulo-endothelial system' or 'RES' (Aschoff, 1924) should be revised, as neither reticular nor endothelial cells will transform into phagocytic cells. We have therefore proposed the term 'macrophage system'[14,15,16], as the protection mechanism of the 'RES' resides neither in reticular nor in endothelial cells, but in macrophages. The term 'mononuclear phagocytic system' or 'MPS' originated by Van Furth[62] also implies the nature of macrophages independent from reticular and endothelial cells. However, the term has been connected with the idea that every macrophage should be derived from a blood monocyte, an idea still disputed among scientists.

Blood platelets

Numerous blood platelets are contained in the red pulp (figs 9, 11). This fact has been known since Aschoff[1] recorded them as 'Milzplättchen' (see also Fujita[13]). Simon and Pictet[52] demonstrated numerous platelets in sinuses and cordal spaces in their nonperfused rat spleen under the TEM (see also Tischendorf[60]). The fine

Figure 9. Numerous blood platelets in a normal human splenic cord. Some of them project one or two pseudopods. In a fractured portion a reticular fiber (arrow head) is exposed. N: neutrophil. × 3570.

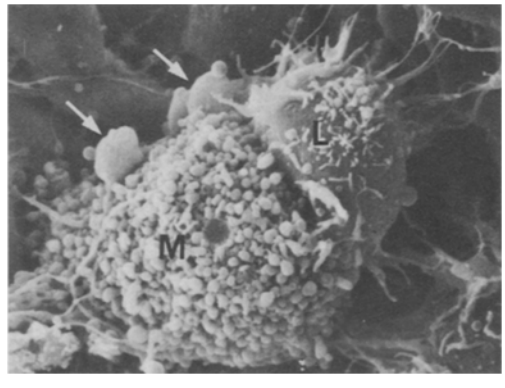

Figure 10. Conjugation of a macrophage (M) and lymphocyte (L) in a human spleen. A few blood platelets (arrows) are also associated with the macrophage. (Reproduced from T. Fujita, in: Three Dimensional Microanatomy of Cells and Tissue Surfaces. Eds L.J.A. Didio et al. Elsevier North Holland, Amsterdam 1981.) × 2900.

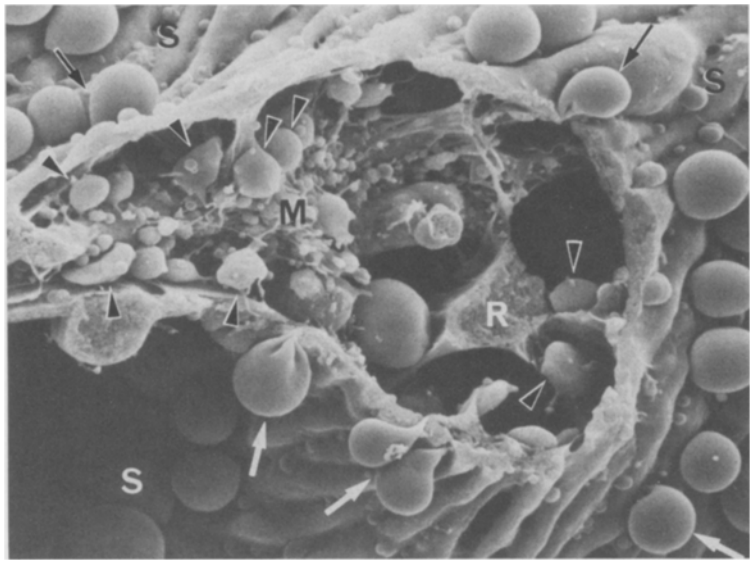

Figure 11. Cord of Billroth surrounded by a sinus (S) in human spleen. A macrophage (M) is attached by numerous platelets (arrow heads). Note also that many erythrocytes hang in a dumbbell shape on the sinal wall (arrows). R: reticular cell. × 2075.

structure and distribution of platelets in the rabbit spleen before and after perfusion was studied by Elgjo[12]. Weiss[64] first reported the SEM images of platelets. He noticed that many platelets, after perfusion, were adherent to the reticulum and rod cells in the rat spleen and remarked: 'Such adherence appeared to be the manner in which the platelets were sequestrated in the spleen.' Besides the possible role of the spleen in destroying platelets, this organ is known to store intact platelets and to release them in response to appropriate stimuli[67]. It is also postulated that platelets which have trapped fine foreign particles and viruses may be gathered in the spleen, thus serving the protection mechanism of the body[5,11,39].

Rosette formation by platelets around a macrophage was reported in our SEM study of normal human spleen[13]. The platelets extend a pseudopod to touch or, possibly, to fuse with macrophage (fig. 11). This peculiar phenomenon seems to correspond to the hypothesis[5] that platelets may trap particulate foreign bodies for presentation to the macrophage (see also Carr[6]).

It seems worthy to add here that in the autoimmune diesease, ITP, platelets are rapidly phagocytosed by macrophages. Macrophages are increased in number in the red pulp and, both in TEM[57] and SEM[18], appear filled with formy spherules corresponding to the platelets being digested.

Arterial distribution

As pointed out by Snook[53], human spleens seem to differ markedly from those in most mammals in the pattern of arterial supply to the white pulp. The central artery in man only rarely issues follicular arteries and, instead, a few penicillar arteries recur to the follicle. Before dividing into end twigs, their branches may run together, forming a bundle of arterioles and capillaries, 'arteriolar-capillary bundle' (Snook) enveloped in a sheath of reticular cells and fibers[53] (figs 12, 13).

Whatever their origin might be, the follicular arteries may extend their terminal twigs to the marginal zone.

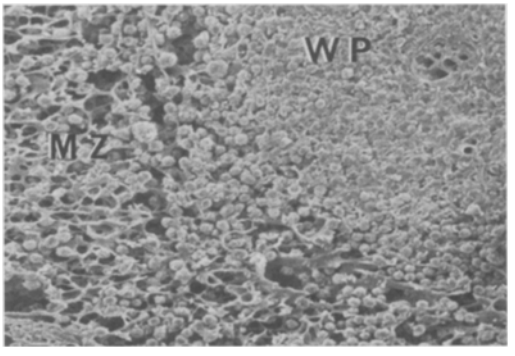

Figure 12. White pulp (WP) and marginal zone (MZ) in the spleen of a patient with ITP. (The disease does not seem to have changed the essential construction of the tissue.) Note the arteriolar-capillary bundle at the upper right. × 190.

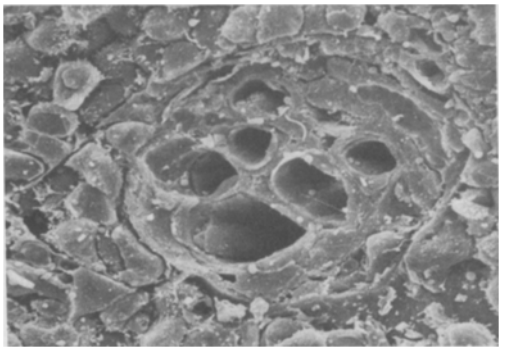

Figure 13. Closer view of the arteriolar-capillary bundle shown in figure 12. Several blood vessels are packed in a reticular sheath. × 996.

Also some penicillar twigs may terminate here[53]. The marginal zone (vide supra) is thus richly supplied with arterial blood.

The proximal portions of penicilli are sheathed arteries characterized by a concentric structure surrounding the endothelium. The endothelium is provided with occasional pores through which erythrocytes may pass. The

sheath consists of alternately piled lamellae of flattened reticular cells, macrophages and reticular fibers[13]. The reticular cells are identified by their smooth surface, whereas the macrophages are densely covered by microprojections (fig. 14). Conspicuously tortured erythrocytes, apparently migrating through the layers of the sheath, may be identified under the SEM. Early light microscopists[58, 60] have recorded this view, suggesting erythrocyte passage through the sheath in the dog and cat. It seems reasonable to postulate that blood plasma and formed bodies, including erythrocytes, are filtrated through the loose structure of the sheath to be checked and, if judged harmful, phagocytosed by the macrophages distributed in it (see also Tischendorf[60]).
The distal portions of the penicilli lack the sheath structure and run usually in intermediate parts between adjacent sinuses.

Termination of arteries

The mode of arterial termination in the spleen has been disputed over eight decades. According to the view originated by Weidenreich[63] arteries terminate in splenic cords and do not directly pour into sinuses. This 'open' theory was opposed by the 'closed' theory of Helly[24] who advocated that the ends of arteries are connected with the sinuses. The dispute between the two theories has been reviewed by Tischendorf[60], who supported the closed theory, and other authors[50, 64]. Studies of living transilluminated spleens of the mouse and some other mammals led two research groups to support the closed theory[28, 43] and two other groups to support then open theory[31, 42]. Experimental investigation by injecting plastic microspheres of specific sizes via the splenic artery suggested that both open and closed circulation occur in the spleen[7].
SEM observation on the end portion of arteries revealed that the open circulation suggested by Weidenreich holds true in mammalian, at least in human spleen. We identified by SEM three types in the terminal structures of arteries in human spleen, although there are gradations between these types.
In the first type the endothelium of the arterial end portion becomes perforated or split and fans out in a funnel shape to the cordal spaces[13] (figs 15, 16).
The second type is represented by sacculated end portions of arteries which may be called 'teloarterial ampulla' (fig. 17). The ampulla is perforated with small round pores, less than 1 µm in diameter, and many erythrocytes are usually seen passing through them by strongly constricting their bodies (fig. 17).
The third type seems to be formed by connection of two or more penicillar ends into a larger ampulla or elongated and twisted cave. In most complicated cases, the cave form a ring or a network into which several arterial ends pour (figs 18, 19). The wall of the cave is provided with pores, as well.
The arterial terminals can thus be classified into a simple funnel type, a simple ampullar or sacculated type and a complex type. In the latter two types, the saccule or cave is traversed by a few trabecules which measure about 0.5–1 µm in thickness and 20–30 µm in length (figs 18, 19). They are covered by the continuation of the endothelial sheet and probably contain reticular

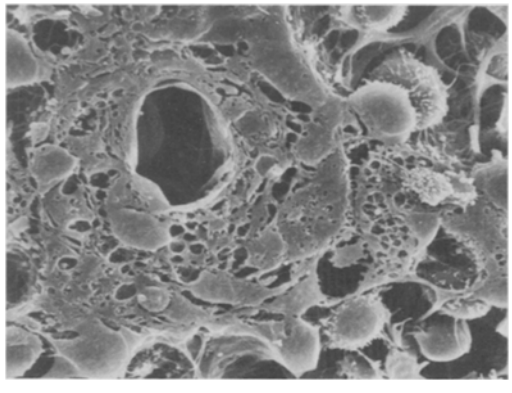

Figure 14. A sheathed artery enveloped by macrophages and reticular cells. This specimen derives from a patient with ITP and therefore the macrophages show honeycomb-like cytoplasm because of the phagocytosed platelets, and this serves, in this micrograph, to identify the cells. × 1330.

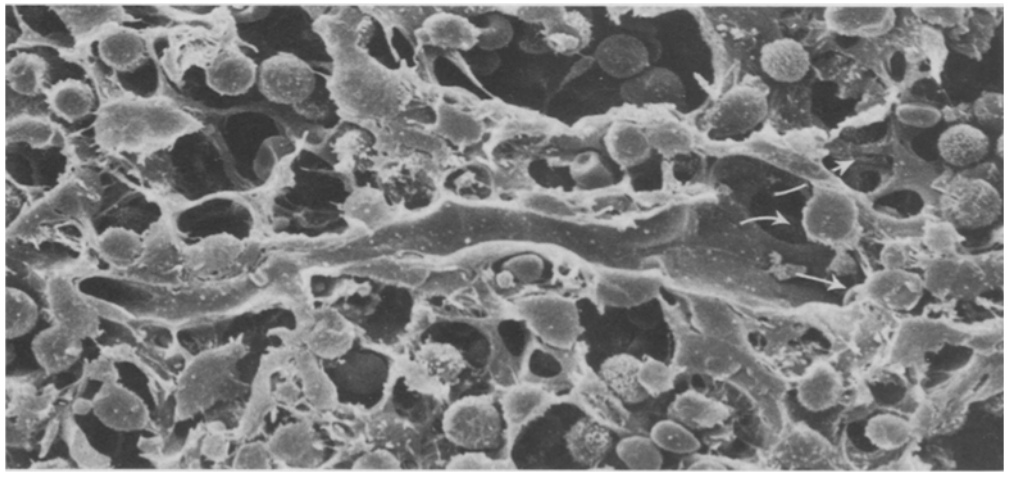

Figure 15. A longitudinally fractured penicillus in the cord of normal human spleen. This arterial capillary fans out on the right hand side, opening into the cordal spaces with fenestrations (arrows). × 996.

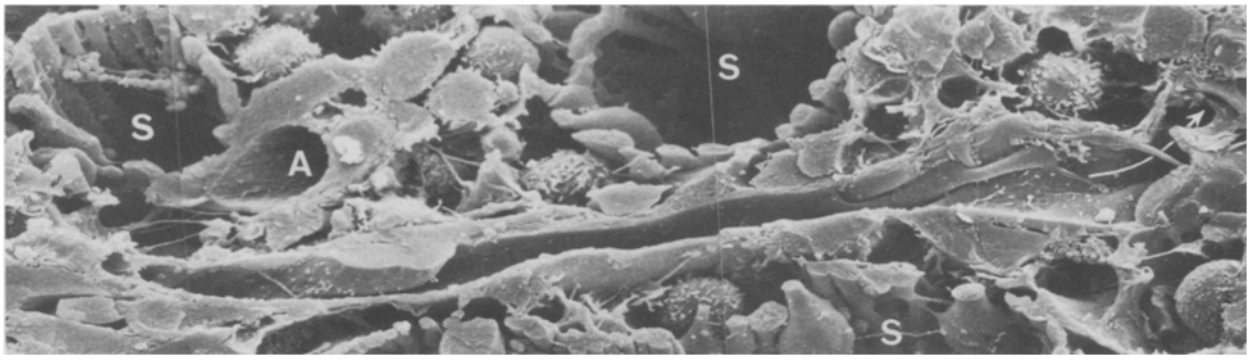

Figure 16. Another penicillus longitudinally hit. The terminal portion opens into the cordal space (arrow). A: an obliquely fractured penicillus which is probably derived from the same stem. S: sinuses. × 1250.

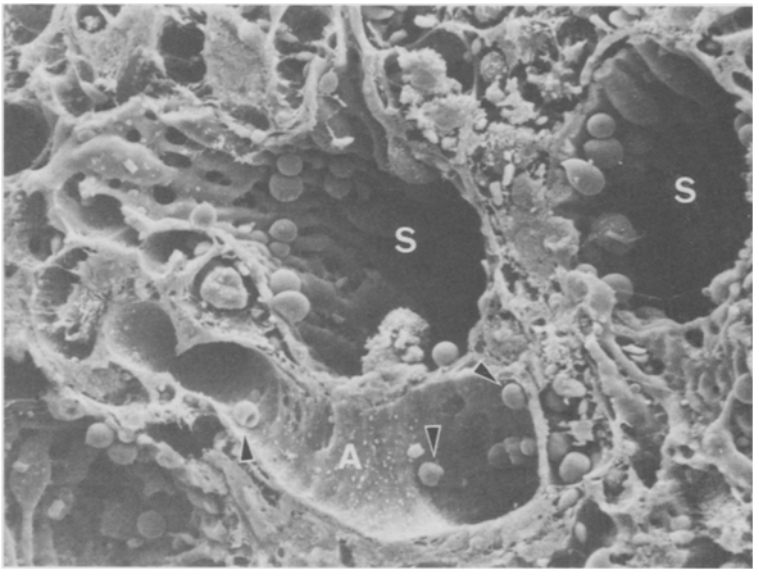

Figure 17. Ampullary terminal (A) of penicillus. The endothelium has pores and some erythrocytes are passing through them (arrow heads). S: sinuses. × 913.

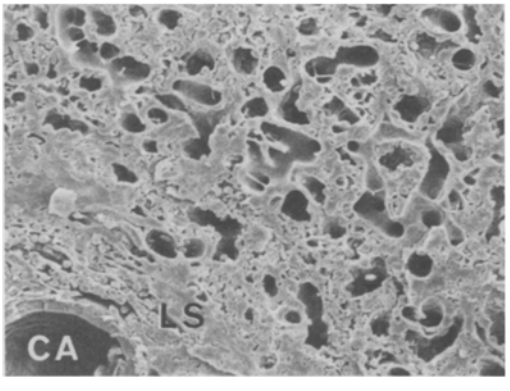

Figure 18. Complex channels of teloarterial ampulae found in the vicinity of a central artery (CA) and periarterial lymphatic sheath (LS). Normal human spleen. × 190.

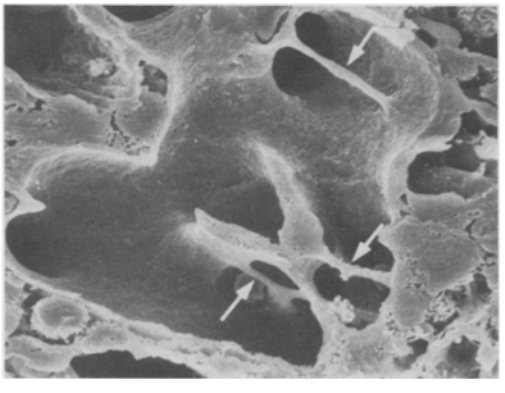

Figure 19. Closer view of one of the teloarterial channels shown in figure 18. They are spanned by thin trabecules (arrows). × 996.

(collagen) fibers in their core. These trabecules apparently support the saccules, preventing their excessive swelling.

The saccular termination was first demonstrated in the SEM studies[17,20,26] of human spleen. A possibly identical structure has been suggested as 'ampulla' by Snook[54] in his observation of silver-impregnated rabbit spleen. The complex caves at the arterial ends have hitherto been unknown as far as the authors are aware. Occurrence of perforations permitting erythrocyte passage as well as that of the specific trabecular structure support the view that the saccules and caves are not artifacts caused by perforation pressure but genuine and regular structures. The funnel-shaped and saccular structures are found

frequently in the terminations of penicilli. The saccular forms often occur at the terminals of follicular arteries at the marginal zone. The third type, the complex caves of arterial ends, tend to occur in groups, often close to pulp veins and in or near the marginal zone (figs 20, 21). Spacious channels around the white pulp which receive follicular arteries on one hand and, on the other, open to the marginal zone have been known as 'marginal sinuses' in the rat and mouse. These structures were recently studied by TEM[47,49] and resin replication-SEM[41]. The complex caves of arterial ends demonstrated in human spleen may possibly be remnants of the murine marginal sinuses.

SEM of vascular casts

The SEM studies of the vascular casts of the spleen still suffer from conflicting results[50], partly because of possible species differences. Barnhart and her associates[2,3] showed casts suggesting the occurrence of both open and closed circulation in the dog and man. Murakami et al.[37] in the rat, and Schmidt et al.[50] in the dog supported the predominant occurrence of closed circulation; criticism of these findings will be given below.

SEM observation of the methacrylate vascular casts of the human spleen in our research group supports the validity of the open theory, although it is difficult to exclude the possible occurrence of closed routes, which must be very rare if present at all. It is constantly being demonstrated that the casts of penicillar arteries are connected not with those of sinuses, but with finer, granular masses of resin, which corresponded to the cordal spaces[26].

Recently micrographs are accumulating in our hands which indicate the occurrence of the three types in the arterial termination (vide supra) in human spleens. As they will be published elsewhere (Kashimura et al., in preparation), only an instance of the funnel-shaped terminal (first type) is shown in this article (figs 22, 23).

Further evidence of open circulation

Careful light microscope observation of paraffin sections confirm that both funnel-like and sacculated (ampullar) terminations of penicillar arteries occur in the human spleen.

Erythrocytes are seen, by light microscopy, TEM[44] and SEM[13,30], to hang in a dumbbell shape on the sinus wall; they are especially numerous in the vicinity of arterial

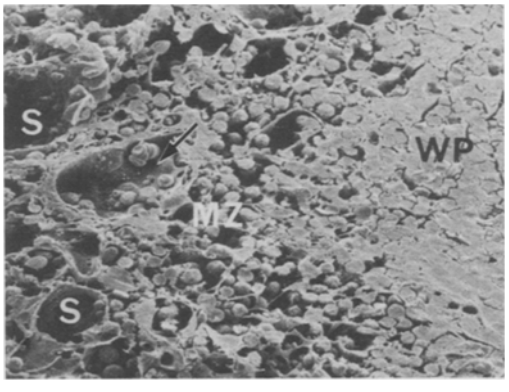

Figure 20. Marginal zone (MZ) of normal human spleen. A teloarterial ampulla is seen (arrow) which is probably the end of a follicular artery. × 307.

Figure 21. Closer view of the terminal cave of an artery shown in figure 20. The endothelium shows nuclear swellings and is provided with larger and smaller gaps (arrows). Red and white blood cells are attached to the endothelial surface. × 913.

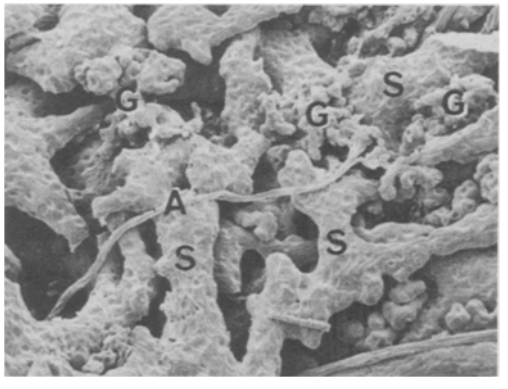

Figure 22. Resin cast of blood routes in human red pulp viewed by SEM. A penicillar artery (A) extends to end in a funnel shape. It continues to granular resin masses (G) which correspond to cordal spaces and not to the casts of sinuses (S). Note also the impressions of rod cells and their nuclei of the sinal casts. × 193.

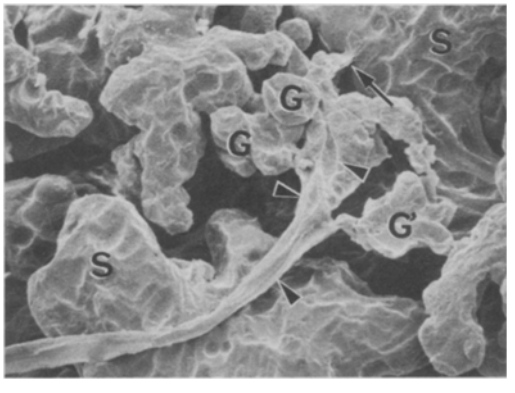

Figure 23. A closer view of the cast of the terminal portion of the penicillus shown in figure 22. The arterial capillary fans out and continues to granular masses (G) which are the casts of the spaces in the cord of Billroth and can be visualized to be connected with the cast of sinuses (arrow). Note also that the arterial cast shows longitudinally extended, spindle-shaped impressions (arrow heads) which are due to the protrusions of long endothelial cells as seen in figure 16. × 730.

terminations. They hang on the lattice of the sinus wall always pushing their heads into the sinus lumen and never in a reverse direction (figs 11, 24). Already Thomas[59] pointed out, in his TEM study of rabbit and dog, that this phenomenon might indicate 'that at least in local areas perfusion fluid flowed from the cord spaces into sinuses'. The SEM observations show that numerous red cells hang in the same direction on sinus walls all over the organ. This regular pattern of red cell hanging is additional evidence supporting the theory that blood moves from the cord to the sinus. If the splenic circulation were 'closed', the blood would flow in the reverse direction and the erythrocytes would hang the other way.

Criticism of the findings supporting a closed circulation

In their early SEM study of vascular casts in rat spleen, Murakami et al.[37] demonstrated that the ends of arteries, especially follicular arteries, were connected with sinus-like swellings and supported the closed theory. More recently, Murakami and associates[68] reexamined the casts with SEM of higher resolution and came to revise their view. As in the case of human spleen, the resin casts of arterial terminals are connected with irregular resin masses corresponding to the spaces in the splenic cords. It seems likely that in their earlier study, leaking resin opened the cordal spaces, especially the loose architecture of the marginal zone, into a funnel shape, which continued to the sinuses, and such images were erroneously taken as the direct connection of arteries and sinuses. Murakami and associates[68] now support the open theory also in this species, although they admit a possibility that a direct connection between the artery and sinus might occasionally occur.

Examining human spleens under the SEM, we notice that the following three structures occasionally occur and suspect that they may be taken, erroneously, for direct routes from the artery to the sinal lumen. First, sinuses are occasionally loose in their wall structure at their tapered ends, showing larger fenestrations than elsewhere (fig. 4 in Fujita and Kashimura[17]). Some of them seem large enough to allow a rather smooth passage of erythrocytes. Second, a slit at an arterial termination may rarely overlap a sinal perforation without the intervention of a recognizable amount of cordal element (fig. 9 in Fujita et al.[18]). Both of these structures may convey arterial blood into the sinus more smoothly than otherwise, but they cannot be regarded as portions of a closed circulation. Third, the splitting preterminal portion of an penicillar artery may occasionally be difficult to differentiate from a thin sinus. The endothelial cells of the artery in this portion are conspicuously elongated longitudinally with nuclear swellings, also elongated, and they may closely resemble the sinus endothelium. Figure 25 shows an enigmatic example of the third possibility mentioned above. In this micrograph, we presume that the longitudinally hit vessel which resembles a thin sinus might be a terminal portion of an artery with a split wall. However, when we look at many images of similar kinds, we must say that we cannot exclude a genuine connection of an artery with a sinus. What can safely be said is that in mammalian, at least in human spleen, such a direct connection (closed route) occurs very rarely if any at all.

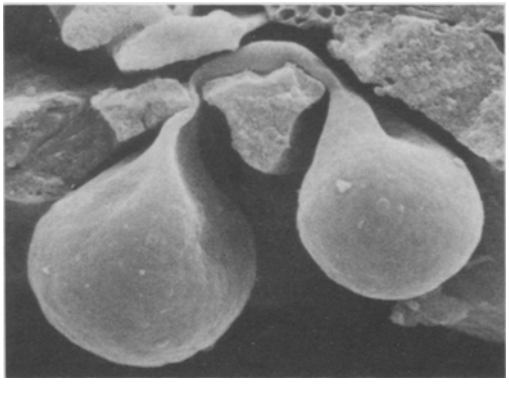

Figure 24. An erythrocyte hanging on the rod cell palisade of a human splenic sinus. The round head always projects into the lumen of the sinus, while the constricted neck remains in the cord. × 7300.

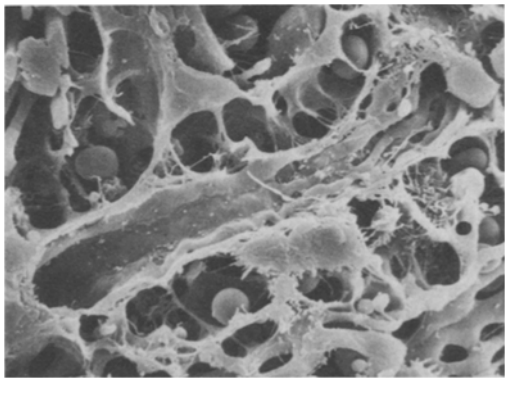

Figure 25. A terminal portion of a penicillus which continues into a perforated vessel. The latter resembles a small sinus but may possibly represent a gradually widened arterial end portion lined with longitudinally extended endothelial cells. × 1250.

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In vivo and electron microscopic studies of the splenic microvasculature in mice

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The spleen of many adult mice is an erythropoietic organ. Since this organ is amenable to in vivo microscopic study, we have used the mouse spleen as a site to study the interrelationships between erythropoiesis and the microvascular compartment of the hemopoietic microenvironment[30–36,43–45].

However, neither the 'open' nor the 'closed' theory of splenic circulation by themselves adequately explained our observations of the microcirculation during conditions of erythropoietic stimulation and suppression in mice with normal and elevated hematocrits (polycythemia). As a result, it was necessary to elucidate the pathway of blood flow through the erythropoietic red pulp. This pathway has been the subject of recurring controversy[48,49] since most in vivo microscopic studies of the mammalian spleen and a few electron microscopic studies suggest that in the red pulp most of the blood flows within channels lined by endothelium[4,27,40–42]. However, other in vivo microscopic studies[18,29,39] and most transmission and scanning electron microscopic investigations[1–3,5–10,12,13,15,19,21–25,28,38,47,50–52] suggest that most of the circulation of blood within the red pulp is not contained by endothelium, but flows through a meshwork of reticular cell processes to reenter the vasculature by penetrating the endothelial wall of the venous sinuses.

In an attempt to resolve this controversy and provide the morphologic data necessary for improving the interpretation of the results of other experiments, the microvascular system of the mouse spleen has been studied using improved, high resolution in vivo microscopic methods[30–38,43,44] and the light microscopic images secured in vivo were correlated with the ultrastructure of the red pulp obtained by transmission electron microscopy.

The spleens of more than 1500 CF_1 and CD mice have been studied by in vivo microscopy[36,37]; 40 spleens were examined electron microscopically. To study the spleen in vivo, the animal first is anesthetized with either Urethane (2.5 mg/g i.p.) or sodium pentobarbital (0.03 mg/g i.p.). Then the tip of the organ is gently exteriorized through a 1-cm, left subcostal incision and positioned over a mica window in a specially designed microscope stage. The window overlies a long working distance condenser of a modified Leitz Panphot microscope. The spleen is covered by a piece of Saran (Dow Chemical) cemented to a movable 'U'-shaped frame. The Saran holds the organ in position and limits movements induced by respiration and the heart, yet is flexible enough to avoid compression of the underlying splenic microvasculature. Homeostasis is maintained by

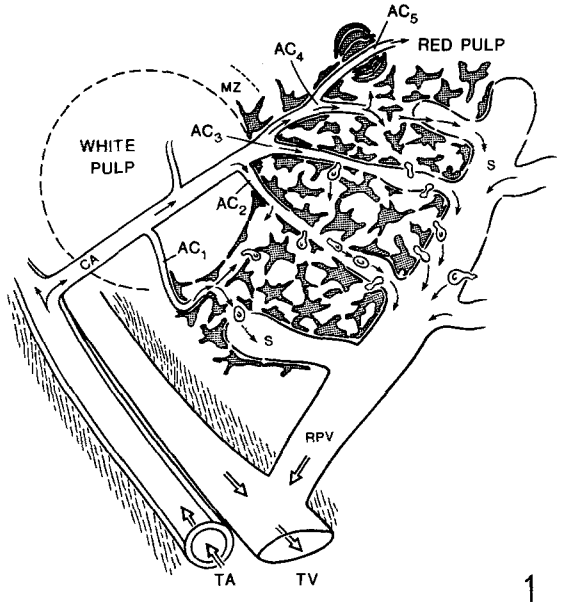

Figure 1. Diagrammatic illustration of the splenic microvasculature[38]. TA: trabecular artery; TV: trabecular vein; CA: central arteriole; AC_1 and AC_2: 'arterial' capillaries terminating in the marginal zone (MZ); AC_3 and AC_4: 'arterial' capillaries terminating in the red pulp, AC_5: sheathed 'arterial' capillary terminating in the red pulp (not developed in the mouse); S: venous sinus; RPV: red pulp venule. Arrows indicate direction of blood flow. Solid black lines indicate the endothelial lining of vessels.