A Scanning Electron Microscopic Study of the Spleen
By Leon Weiss

The reticulum and vascular sinuses of the normal rat spleen were studied by scanning electron microscopy. Observations were also made of erythrocytes, macrophages, platelets, and other migratory elements. Reticular cells of the periarterial lymphatic sheath, the marginal zone, and cordal spaces were large, bulky, irregular cells with broad processes that formed a spongework. When marked retraction of these cells was induced in the drying phase of tissue preparation, they showed the slender multiple fingerlike processes characteristic of the argyrophilic reticulum. The reticular cells at the periphery of the periarterial lymphatic sheath were flattened and formed cylinders about the central artery. They were, moreover, associated with unusually heavy extracellular fibers. Vascular sinuses were suspended in the reticulum by attachments of cordal reticular cells and of fibrillar reticulum to the adventitial surface. Adventitial cells of the sinus, moreover, branched into the cords. Endothelial cells typically lay side by side without gaps, except as migratory cells passed through the wall. Erythrocytes were commonly observed in passage across the sinus wall. In sinuses and cords, they were often swollen, irregular, and bore blebs and crenulations. Macrophages displayed rich surface folds and processes. Platelets were abundant and were adherent to the surface of reticular cells and the endothelium of sinuses. Such adherence appeared to be the manner in which the platelets were sequestered in the spleen.

The spleen is an encapsulated spongework or reticulum perfused with blood. Elements of blood are stored in the spleen: approximately one-third of the platelets of the body are normally held in ready reserve. But spleen and blood may interact and each be modified. Thus, the spongework surrounding arterial stems the periarterial lymphatic sheaths becomes selectively loaded with blood-borne T and B lymphocytes; it concentrates antigen and permits the interactions that result in clonal selection, antibody production, plasma cell development, and germinal center formation. Macrophages, derived from the transformation of monocytes carried into the spleen by the blood, are held and concentrated in the reticulum of the marginal zone, of the cords of red pulp, at the perimeter of white pulp, and about lymphatic vessels in central white pulp. Such macrophages, the most consistently present of the migratory elements in the spleen, are operative in red cell destruction, antibody formation, and clearance of colloidal and particulate substances from the blood. Normal erythrocytes pass readily through normal spleen, traversing a red pulp which severely tests them. Thus, the cordal reticulum is charged with macrophages and contains considerable extracellular hydrolytic enzyme activity. With remarkable pliancy, normal erythrocytes squeeze through nar-
row interendothelial slits in the sinus wall. But slightly or moderately damaged erythrocytes or, it would appear, those at the end of their life span, fail to pass through the spleen quickly. They are delayed and modified or destroyed.

The spongework and the vasculature of the spleen, upon which filtration and interactions with blood depend, represent the fixed or substantive elements of the spleen in contrast to the lymphocytes, macrophages, erythrocytes, and other free or migratory elements. The spongework, variously characterized as a three-dimensional web, scaffolding, reticular meshwork, or reticulum, is made of large branched cells, reticular cells, and reticular fibers. Earlier considered as multipotential hematologic or connective tissue stem cells and as fixed macrophages, reticular cells are now recognized as functioning primarily to form a spongework. They are intimately associated with reticular fibers which, as shown by transmission electron microscopy (TEM), they sheath. They would appear to synthesize these fibers and are thereby related to fibroblasts.

The filtration meshwork formed by reticular cells and fibers differs in different parts of the spleen. In the periarterial lymphatic sheath, particularly at its periphery, the reticulum is arranged circumferentially about the central artery. Often several layers of reticulum are laid out in this manner, with the result that the artery is surrounded by two to four coaxial concentric layers of reticulum. The most peripheral layers tend to be more rigorously disposed. In germinal centers, due probably to the rapid accumulation of antibody-producing cells and their precursors, the reticulum appears pushed away; thus at the margin of the germinal center, it is compressed, and in the center, it is absent. In the marginal zone, the reticulum forms a close-meshed network. In the cords, it may form a somewhat looser meshwork, except in the cordal tissue directly surrounding the sinuses, where it is organized in the human as a type of coaxial sleeve or mantle.

The vascular sinuses have been regarded as a modified type of reticulum, and the common presence of distinctively organized microfilaments and other cell organelles establishes similarities between the endothelial cells of sinuses and reticular cells. But there are significant morphological and histochemical differences, so that it is difficult to advocate a fundamental similarity. Sinuses remain perhaps the most distinctive elements, structurally and functionally, in the splenic vasculature.

This paper presents observations on the splenic reticulum and on splenic sinuses of the normal rat with the scanning electron microscope (SEM). The organization of reticular cells and their surface structure is described for white
pulp, marginal zone, and red pulp. The structure of vascular sinuses is described. Observations are presented on the structure of platelets, leukocytes, and erythrocytes, and their relationships to the fixed elements. Implications of these observations on blood flow, cell sequestration, and cell destruction are considered. Methods of preparation of the spleen for SEM are discussed. This work follows the important SEM studies of rabbit red pulp by Miyoshi, Fujita, and Tokunaga, of the dog and rat red pulp by Miyoshi and Fujita, and of the human spleen by Suzuki. Leblond's SEM study of the spleen and bone marrow of the rat is in press (see Acknowledgment section).

While many results of this study confirm those of TEM studies, and it should be possible, in theory, to reconstruct the three-dimensional SEM picture from TEM techniques, SEM micrographs have not only displayed the structure of the spleen vividly but also have provided new information. Among the results reported here by SEM, the shape of reticular cells and the conformation of their processes, the variation and nature of erythrocyte shapes in the cords, the contours of the sinus endothelium, and the shape of the platelets and their relationships to reticular cells and endothelium have been observed for the first time.

MATERIALS AND METHODS

Animals

Twenty-five male rats of the Sprague-Dawley strain, weighing between 175 and 220 g, were used. The animals were anesthetized by ether inhalation for simple splenectomy. For lengthy procedures, such as perfusion, the rats were maintained under surgical anesthesia by intravenous injection of phenobarbital (Nembutal) 1.0 mg/kg body weight in saline.

Preparation of the Spleen

Spleens were fixed by a number of techniques. In the simplest method, the spleen was removed from an anesthetized animal, cut into slices about 2-mm thick, diced into 2-mm cubes, and immersed in fixative. In other instances, it was fixed by interstitial perfusion, which I had earlier used on bone marrow. Here the spleen was exposed in situ by a flank incision through the lateral abdominal wall, and a sharp No. 18 disposable needle dripping fixative was inserted into its medial tip and pushed along the longitudinal axis of the ribbonlike organ to a depth of about 10 mm. The needle was stabilized by clamping or holding by hand and, by gravity flow at 130 cm H₂O, a volume of approximately 50 ml of fixative was run in for about 15 min. The opposite tip and one edge of the spleen, approximately equidistant from the tips, was nicked by scissors cut. Some fixative dripped from these cuts. Some, moreover, appeared to be carried away through the splenic vein. The animal remained seemingly undisturbed, its vital signs maintained through the early phase of perfusion. Later in the process, however, the animal twitched, had some major muscle contractions, and, in some instances, convulsive movements for 20-30 sec. By the end of the perfusion, in most instances, the animal was dead. In successful perfusions, the spleen became dense and rubbery, but showed no change in size and little, if any, in color. The perfused spleen
was removed and cut transversely into slices 2-mm thick, which were immersed in fixative. Most samples for SEM study were taken near the far tip of the spleen, well ahead of the needle point, or alongside the needle track where fixation occurred by back diffusion. Many preparations were checked by TEM for the quality of preservation.

The spleen was usually sliced with a new, wiped stainless steel razor blade. In a number of instances, however, a cut was made to a depth of less than 1 mm. Then, as with a bar of soap, the spleen was bent away from this cut and broken. This procedure, suggested by Dr. T. Kuwabara, of the National Institutes of Health, Bethesda, Md., was useful because the break often followed vascular or other "natural" planes. Thus, both cut surfaces and broken surfaces of the spleen were available for study. While no fixation schedule afforded consistently good results, identified as an absence of shrinkage, detailed rendition of cell surfaces, and good contrast in the tissue, the most useful procedure was as follows:

Glutaraldehyde (3%, in 0.05 M cacodylate buffer, pH 7.2, with 6.8%, sucrose at room temperature) was used both for immersion and perfusion fixations. The tissues remained in this fixative 4-6 hr, or overnight. They could be stored in buffer, but before postfixation, they were washed at least 15 min in distilled water to prevent the appearance of granular deposits over their surfaces and in crevices. They were then placed in osmium tetroxide (1% in 0.05 M cacodylate buffer, pH 7.2, with 1.5%, potassium ferrocyanide) 2 hr at 4°C or at room temperature.

Ten percent formaldehyde, both by immersion and perfusion, and by itself or followed by osmium, caused shrinkage and relatively poor contrast.

Two means were taken to dry the tissues. In each case, the tissue was cut into slices 2-mm thick, and in most instances it was then cubed.

In freeze drying, the tissue was rinsed free of osmium, and from distilled water, was quenched by immersion in liquid isopentane in liquid nitrogen. The isopentane was largely decanted. The tissues lay on the floor of a beaker in a layer of isopentane which just covered them. This preparation was placed in a vacuum evaporator and pumped out to a vacuum of 10 mm Hg: in the process, the isopentane was drawn off. Aside from the bathing in isopentane while being pumped out, the tissues were not kept on a cold plate or maintained cold throughout the vacuum drying.

The second means of drying tissues was a Bomar (Bomar Co., Seattle, Wash.) critical-point apparatus. The tissues were first dehydrated in alcohol. They were brought to freon TF, and after about 1 hr immersion, dried at the critical point of Freon 13.

The dried tissue was mounted on a carbon disk with silver electrical paint and the preparation placed in a dessicator.

Twelve to twenty-four hours later, the tissue-bearing disks were placed in a Jelco Vacuum Evaporator, and with the tissue rotating and rocking, a thin (slight yellowish cast) layer of gold was evaporated over its surface.

Two major technical problems related to tissue preparation were encountered. In one, the tissues appeared well preserved in the SEM, but the cell surfaces were covered by a smooth amorphous coat, probably coagulated plasma, which masked the details of the cell surface. The second was faulty drying with resultant shrinkage and cracks (see Plate VIII).

The best preparations, i.e., those having more detailed surface contours, better retention of free cells, showing least shrinkage, cracking, and retraction, and having a structural consistency with well-fixed TEM material, were rather consistently obtained with critical-point drying. The preparations showing most shrinkage were those dried by freeze drying. Freeze drying was not carried out optimally, however, since cold was not maintained through the vacuum drying. The marked retraction that typically occurred in this procedure was, however, a very useful artifact, since the reticular cells probably dried down and shrank against the reticular fibers on which they lay, and the free cells dropped out. Such preparations thereby revealed the distribution of the

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**PLATE III.** Periarterial lymphatic sheath.

**Fig. 5.** Reticular cells of the periarterial lymphatic sheath. The cells are large and bulky with an irregular surface. A small migratory cell likely lay in the recess marked by the arrow. These reticular cells may retractor with suboptimal drying (see Plate VII). × 2800.

**Fig. 6.** A complex spongework made of reticular cells is evident. The cells have both microvillous and broad velamentous processes. Small dimples mark the cell surface. At a number of places (arrow), fibers are closely associated with the cells. × 6200.
extracellular reticulum. The pattern of retraction of the endothelium of sinuses, moreover, indicated the layout of the ring segments of the basement membrane (see below).

The presence of free cells in the reticulum was variable. There was a tendency for these cells to fall out. To some extent, this could be augmented by a 5-min ultrasonication of the tissue blocks in water after initial fixation. Because the loosening and removal of free cells occurred often enough without ultrasonication, it was seldom used.

**Electron Microscopy**

The microscope used was a Jelco SEM 3. For most of the work, it was used at bias 1, condensor 14, 15 kV, and a 100-μ aperture. A 100-sec scan was used for photography. Kodak 4 x 5-in. professional film was used, developed in Dektol 1:1 and prints made at somewhat less than 2 x enlargement on Kodabromide contrast grades No. 3 and No. 4.

In order to increase the confidence with which cells and tissues were identified in scanning electron micrographs, the following measures were taken:

Samples of tissue obtained from the same spleen were prepared separately, for both SEM and TEM, and the fields compared. The TEMs in a recent paper were drawn from similar material to that used in the SEM study. After examination in the SEM, moreover, blocks of spleen were regularly embedded in plastic, sectioned, stained with toluidine blue, and examined by light microscopy (LM). Sometimes the very structure viewed in the SEM, i.e., a distinctively branched sinus, was found in a LM section of that block.

Certain low-power fields, such as white pulp, can be identified as surely in the SEM as in Giemsa-stained LM sections. It is readily possible, as in the case of the periarterial lymphatic sheath, to single out cells of the arterial wall, reticular cells, lymphocytes, and other unmistakable elements in a low-power SEM field, and then characterize them at high powers.

There have, in addition, been studies of isolated cells by SEM whose results are usefully adapted to the spleens reported upon here. Wetzel has correlated the LM and SEM appearance of leukocytes by a technique whereby a given leukocyte fixed on a glass slide and stained with the Giemsa stain can be photographed by both LM and SEM. Polliack and his colleagues have isolated small lymphocytes, described their distinctive structure, and shown striking differences between T and B cells by SEM. Diverse forms of erythrocytes have been recognized in the SEM and classified by Bessis.

It is through the means given above that such cells and structures as reticular cells, macrophages, and vascular sinuses have been identified in this SEM study of the spleen.

**OBSERVATIONS**

The white pulp stood out clearly. The central artery would often literally stand out above the surrounding periarterial sheath, and the circumferential layout of the periarterial sheath was conspicuous (Figs. 1-4). The marginal zone was a broad dense band containing many connective tissue fibers. It contained sinuses which lay close against the periarterial sheath, cupping or surrounding it (Figs. 1, 2, and 7-9). The red pulp could show branching sinuses and large
veins (Figs. 2 and 20). Beneath the capsule, the cordal pattern was solid rather
than open, and the sinuses tended to be contracted (Fig. 10).

Reticular cells were large, bulky, irregular cells with broad membranous
processes (Figs. 5, 6, and 11). Their surfaces were smooth or had projections,
folds, or irregularities of different sorts. Extracellular fibers were closely asso-
ciated with these cells. By SEM, in contrast to TEM, it was difficult or im-
possible to demarcate fiber from cell. The fiber often seemed to come out of
the cell as a horn or projection (Figs. 3, 6, 9, and 11). Where the preparations
were shrunken, reticular cells had a folded or corrugated perikaryon and were
arborized, with several orders of fingerlike branches (Figs. 12 and 13).

White Pulp

The central artery of the periarterial lymphatic sheath often lay off-center
even in sheaths where no nodules were present. The artery or arteriole was sel-
dom large, 30 μ or less in diameter. Larger arteries were often found in smaller
periarterial sheaths. Arterial vessels showed a complex thick wall that included
connective tissue and muscle layers. Endothelial cells were high, regular, and
oriented longitudinally (Figs. 1, 3).

In the central periarterial lymphatic sheath, the reticulum was often similar
to that of the red pulp and marginal zone (see below). Toward the outer third
of the white pulp, however, the reticular cells formed sheetlike membranes that
took on a circumferential pattern, forming concentric perforate cylinders sur-
rounding the central artery (Figs. 1, 3, and 7, 9). At the rim of the periarterial
lymphatic sheath, representing the outermost two or three layers of circum-
ferential reticulum, the extracellular fibers associated with reticular cells were
unusually heavy, broad, flat branching bands (Figs. 7, 9).

Sinuses

Endothelial and adventitial cells were the only mural structures regularly seen
(Figs. 15, 13, 27). The basement membrane did not stand out. Its presence was
evident only indirectly, primarily in closing the interendothelial gaps in the
sinus wall (Figs. 26, 27).

In well-preserved preparations, the endothelial cells typically lay side by side
without intercellular gaps, save those created and filled by cells in passage (Figs.
14, 15, 20–27). In less well-preserved preparations, the endothelial cells tended
to retract, leaving interendothelial spaces (Figs. 16, 17). Poorly preserved endo-
thelial cells were markedly retracted, held in place only by slender cytoplasmic

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**PLATE V.** Reticular cells of splenic cords.

Fig. 10. This field is typical of the subcapsular region. The reticular cells are polyhedral and
form a rather complete pavement in a tilelike pattern. The oval spaces in the surface represent
sinuses (5). Note the small protuberances that beset the surface of reticular cells. They rep-
resent platelets, as extrapolated from TEM. × 3700.

Fig. 11. Here a more spongelike irregular cordal field than that of Fig. 10 is exposed. Again,
the reticular cells are large structures with platelike surfaces, often wrinkled or dimpled. Two
typical cells are indicated (*); fibers are also evident. A large branching ribbonlike reticular
cell is indicated by the diverging arrows (\(\�\)) in the upper right quadrant. It is probably asso-
ciated with fibers. × 3600.
processes (Fig. 17). These processes could, presumably, give way and the endothelial cells then lift away (Fig. 18).

The endothelial nuclear zone was rounded, protruded into the lumen, and often ran smoothly into the remainder of the cytoplasm (Figs. 20, 21, 23, and 24). The nuclear zones of contiguous cells were often bunched, but seldom assumed a regular pattern. In large veins, in contrast, the nuclei were often regularly spaced. The luminal surface of the sinus endothelium usually was smooth with broad folds. It was sometimes knobby (Fig. 24), however, or clusters of quite long fingerlike projections were present and could lay from one cell to another (Fig. 23).

Often free cells, notably red cells, were in passage across the sinus wall (Figs. 20–23, 26, and 27). Occasionally, the bulk of an erythrocyte lay in an interendothelial slit, but, more commonly, the cell was drawn out and the portion in passage was quite slender. The picture was remarkably like that seen in transmission electron micrographs. Leukocytes lay within the lumen of the sinuses. They were remarkable for surfaces richly beset with microvilli or membranous folds (Figs. 14, 15, 20–23, and 25). Those with microvilli resembled B lymphocytes described by Polliak and his collaborators.

The adventitial or outside surface of a sinus was characteristically set in folds (Figs. 13, 26, and 27). Cordal reticular cells were attached to this surface (Figs. 13, 26, and 27). Where cellular shrinkage was marked, the cordal cells were attached only by slender strands (Fig. 13). In the absence of evident shrinkage, however, cordal reticular cells were attached along their body or by their broad membranous folds (Fig. 27). The reticular cells, as in white pulp and marginal zone, were irregular, large, and bulky (Figs. 14, 15, 26, and 27).

Somewhat dilated sinuses displayed lumen and endothelium clearly. But contracted sinuses were common (Figs. 26, 27), especially in the subcapsular region (Fig. 10). Here they appeared as slender tubules or recesses in a field of broad platelike reticular cells, and their endothelium was folded and obscured.

Within the wall and lumen of the sinuses, erythrocytes were often pleomorphic, being nearly spherical and bearing one or more protrusions or dimples (Figs. 15 and 22). Leukocytes and other elements in such fields did not appear disturbed, and biconcave erythrocytes were often nearby (Figs. 19 and 22).

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**PLATE VI.** Cords and sinus dried by freeze-drying. In contrast to the previous fields, there is maximal cellular retraction or shrinkage. The cells shrink against the reticular fibers around which they lie, leaving a picture which probably corresponds with the disposition of the reticulum, as would be seen by silver stains.

Fig. 12. A reticular meshwork in a splenic cord. The reticular cell perikaryon on the left (*) has a folded or corduroylike surface, the pattern of dried out or folded down tissue, highly characteristic of reticular cells prepared in this way or air dried. A main branch comes off this cell (arrow) to the right and branches at least six times. Some of the branches are very slender and play upon the surface of other cells. Note the reticulum appears to be continuous, its branches merging with other branches. Reticular cells may also appear as flattened structures, again with branches, i.e., the cell at the lower margin of the field (*). Compare with Fig. 11. × 6750.

Fig. 13. A sinus (S), viewed from the outside, runs through red pulp held in a meshwork of reticulum. The adventitial surface of the sinus consists of folded flattened reticular cells. These cells send out processes or have processes continuous with the branches of reticular cells of the cords. Again, as in Fig. 12, a continuous reticular meshwork is found, consisting of cells and fibers. × 6750.
According to Bessis and Weed's nomenclature, most would be classified as echinocytes.  

Cords and Marginal Zone

The SEM picture of the cords and marginal zone which corresponded most nearly to that obtained from classic silver-stained light-microscopic sections occurred where the tissue was severely shrunken and the migratory cells washed free. Here reticular cells were large, branched cells with multiple slender branches of several orders. Their perikaryon was characteristically ovoid, its surface folded. Their terminal branches were smooth and threadlike and rested upon other structures (Figs. 12 and 13). Where little shrinkage occurred, however, a different picture was obtained (Figs. 14, 15, and 27). Instead of cells with long slender branching processes, forming a delicate three-dimensional web, large, bulky, irregular cells were in touch with one another, often by means of sail-like membranous processes, folds, ruffles, or blunt protrusions. They thereby formed a spongework, the hollows or interstices of which were irregular and continuous, but rather limited (Figs. 6 and 11). The reticular cells appeared to have been deformable and yielding, however (Fig. 5), so that the potential interstitial space that could accommodate migratory cells was relatively large. Reticular cells could also lie quite close to one another, set side by side in the manner of tiles, scales, or plates and could thereby form a rather solid block. This cordal arrangement often occurred in the subcapsular region (Fig. 10).

The cords contained white cells and erythrocytes (Figs. 14, 15, 26, and 27). Most of the white cells were likely macrophages. The erythrocytes were often poikilocytopic (Figs. 14, 15, 26, and 27) of echinocytic types.  

DISCUSSION

The Nature of Sinuses

Endothelial cells lie close together and form a delicate ribbed membrane. While some luminal endothelial processes have been observed by TEM, the
degree and variations in luminal endothelial protuberances and projections as observed by SEM were unexpected. Of course, some of these projections may have been extrinsic material attached to the endothelial surface rather than processes actually involving the plasma membrane of the endothelial cells. The extrinsic material may be cytoplasmic, as blood platelets, or extracellular, as plasma or mucopolysaccharide.

The reduced area of basement membrane (due to its large fenestrations) and the absence of any endothelial intercellular junctions in the adult combine to provide only restricted attachments for the endothelium of sinuses and account for the ease with which, in retracted preparations, it is lifted away. Since the basement membrane of the splenic sinus is markedly fenestrated and the adventitial cells cover is incomplete and variable, a significant expanse of the sinus' basal endothelial cytoplasm may be presented to the cords and available for the passage of blood.

The interendothelial slits of splenic sinuses are an important component of the spleen’s vascular pathway, since blood must flow through them passing from cords to sinus lumen.\textsuperscript{56} The slits are simple narrow spaces between elongated endothelial cells lying side by side and close together. These slits are different from the interendothelial spaces of the sinuses of bone marrow where junctional complexes occur in the plasma membranes.

In tissues fixed by immersion or by interstitial perfusion, as in the case in this SEM study and in recent TEM studies from this laboratory,\textsuperscript{58} or by venous perfusion,\textsuperscript{17} the interendothelial slits of splenic sinuses are, as a rule, quite narrow. I believe it likely that these slits are physiologically narrow for the following reasons: in vital microscopical studies,\textsuperscript{23} migratory cells in the sinus wall are squeezed tightly and pop through when crossing the wall; such rigid intraerythrocytic inclusions as Heinz bodies\textsuperscript{6} and malaria plasmodia\textsuperscript{24} delay the erythrocyte’s passage across the sinus wall, and the protoplasm containing the nonpliant inclusion is snapped off, retained on the cordal side of the sinus.
SEM STUDY OF SPLEEN

Intravascular filaments, probably skeletal in character (≈ 100 Å in diameter), are aligned in bands running close to and parallel to the endothelial slits, an arrangement that would keep the slits closed. In material judged well fixed, slits are usually tight in TEM and SEM material fixed by immersion, interstitial perfusion (this study), and venous perfusion; in relatively poorly preserved material, gaps are present between endothelial cells. But because wide interendothelial gaps occur in splenic sinuses due to artifact does not preclude their development physiologically. Occasionally, even in well-fixed material, elliptical gaps do occur. The presence of intravascular filaments that are likely contractile (≈ 70 Å in diameter) and the absence of any junctional complexes that would fix or stabilize the interendothelial slit suggest that endothelial cells may contract or be displaced and the interendothelial slits thereby widened. The occurrence of widened gaps after arterial perfusion as seen in the work of Burke and Simon, Miyoshi, Fujita, and Tokunaga, Suzuki, and Leblond would suggest that under certain conditions of active arterial flow there may be a widening of the slits due perhaps to the force of flow or to a reflex action initiated by distention of the arterial vessels.

I suggest, therefore, that the interendothelial slits of the splenic sinuses are narrow under most circumstances and that they may be widened by drying and other artefacts. It is possible, however, that, under certain conditions of perfusion or flow, they may widen physiologically.

The basement membrane does not stand out clearly in these preparations. It may well be masked since, extrapolating from TEM, the components of the membrane (both transverse and ring segments) are embedded in or folded into the basal surface of the endothelium, or covered by cytoplasmic prolongations of cordal reticular cells. The location of the ring component of the basement membrane is disclosed, however, by the periodic interendothelial closures between the mural gaps. This is shown in Fig. 27 in this paper.

Poikilocytic erythrocytes are common in the sinus lumen, in the sinus wall, and in the cords. Biconcave cells are also present in these places. The echinocytic form of erythrocytes in this material may possibly be an artifact. But the environment of the red pulp is harsh toward erythrocytes. Indeed, crossing the cords, sinus wall, and sinus lumen constitutes a test of erythrocyte viability wherein only sound, pliant cells survive. Erythrocytes subjected to this test may enter the sinus lumen irregularly bullooned, bearing projections, or other-

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**PLATE IX. Red pulp.**

*Fig. 20.* A sinus runs from left to right and bifurcates to the right. Its endothelium is well preserved, the cells lying close together without evident gaps. The endothelial nuclei, often lying in clusters, are smooth swellings on the surface. There are a number of leukocytes lying upon the endothelium, their surface rich in delicate folds and microvilli. Platelets are common, many of them recognizable because they have undergone viscous transformation and are smooth oval structures. A few erythrocytes are present. They are pinched at the point where they appear to be squeezing through the wall. x 1875.

*Fig. 21.* This field is a higher power of the left lower center of Fig. 20. The endothelium has some folds or flat ridges on its surface. The surface richness of the leukocytes may be seen more clearly, and a slipper-shaped erythrocyte at the lower right corner is passing between two endothelial cells, severely constricted in the interendothelial gap (>. Platelets of different size (arrows) are present. Many are associated with strands of fibrin. x 5250.
wise altered. While less vivid, abnormalities in the shape, size, density, and other characteristics of erythrocytes in red pulp are commonly found by transmission microscopy. By light microscopy, for example, there is often evidence of spherocytosis and variation in staining intensity. By electron microscopy, spherocytosis, variation in density of hemoglobin, and vacuolization are observed in red cells. Changes in red cells of red pulp have also been observed by vital microscopy. Thus, the presence of normally biconcave red cells close to the deformed ones, the likelihood that cells in passage through the cords and through the sinus wall and sinus lumen are subject to severe test, and the presence of similar findings by transmission microscopy, indicate that departures from the biconcave shape of red cells in red pulp observed here by SEM may constitute actual, significant findings. These changes may well be reversed with return to a less severe circulation.

The Nature of Cords

The structure of the cordal reticular cells provides significant implications for splenic function and circulation. In the best preserved preparations, cordal reticular cells were bulky, irregular cells with broad processes which formed a spongework of connected narrow, tortuous spaces. By the presence of indentations, the cells appear deformable, so that a limited volume in the empty spongework can open to a much larger one as free cells and fluid enter and expand the space by pressing against the reticular cells that confine the space. Thus, the reticular cells curve, fold, and even enwrap the migratory cells held in the reticulum. There is an enormous surface expanse of potential contact between migratory cells held in the reticulum and the reticular cells, one greatly increased by the surface folds and processes of both reticular cells and migratory cells. If these reticular cells are similar to those reported as capable of binding antigen, their structure would permit an efficient, rich presentation of such antigen to any migratory cells passing through.

The structure of the reticular cells has important implications for the circulation, permitting a more effective rationalization than heretofore of the "physiologically closed, morphologically open" enigma with which students of the splenic circulation have had to contend. It has become clear, for example, that in histologic sections of fixed spleen, the circulation is "open" in that arterial vessels regularly terminate in the cordal spaces. Yet in studies
of the vital circulation, almost without exception, the circulation is "closed" in that blood appears to travel within closed vessels, and arterial vessels appear directly continuous with sinuses. The cordal space is partitioned by the membranous processes of reticular cells, and these membranes may form a rather complete channel closely stimulating conventional vascular channels fabricated of contiguous cytoplasmic sheets. Such a channel could conduct the blood, with the efficiency of an endothelially-lined vessel, from an arterial ending to the interendothelial slits of the sinus wall. The disposition and nature of the reticular cells of the cords may well be responsive to different situations. Thus, the cordal channel postulated above may become reorganized and actually divert blood from the sinus walls forcing a sojourn in the cords. Or the cytoplasmic sheets of the reticular cells may be furled, resulting in fingerlike extended processes similar to those regularly found in shrunken preparations. The cordal spaces would be more fully in communication and capacious under these circumstances, and being less efficient in moving blood through the spleen rapidly, would pool it.

These SEM studies reinforce the position\textsuperscript{79} that, rather than view the cordal spaces as open or extravascular, it is more useful to consider them a type of vascular space, and that the pathway from arterial vessel, through the cords, and through the sinus wall into its lumen is a distinctive, but, as with the cavernous spaces of genitalia and other special vascular segments, nonetheless genuine vascular path.

The delay, destruction or modification of a circulating cell usually depends upon the state of that cell rather than that of the spleen. Even in instances where marked splenic enlargement and pathology can cause a shortened life span of normal or near normal cells, as in methyl cellulose-induced splenomegaly, the anemia is mild. The layout of the cordal reticulum may depend upon interactions of the circulating cells and cordal reticular cells. If the cell is normal, the reticular cells would provide an efficient channel to the sinus. If abnormal, the path would be altered to favor cordal delay.

Even if the blood is efficiently conveyed to the sinus wall, the blood cells must still cross the wall. Normal cells are pliant enough to get through readily, and it is possible that the rapid and continued passaging of cells may induce a slit to become widely enough dilated to simulate, by vital microscopy, conventional intravascular passage (see Discussion above). This possibility is made more plausible by noting that splenic arterial endothelium is high, and that the arterial lumen in fixed preparations may be just as restricted a slit as found between endothelial cells in the sinus wall.\textsuperscript{69} Even having reached the sinus lumen, the splenic test of blood cells does not end. Knisely described contraction of the terminal end of sinuses with the holding of blood within their lumen for some time.\textsuperscript{21} His observations can now be rationalized by the finding of

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PLATE XI. Luminal surfaces of sinuses in red pulp.

Fig. 24. The endothelium is covered with many bodies of uniform size, likely blood platelets. $\times 4500$

Fig. 25. The endothelium presents a corduroy appearance. An erythrocyte (E) is hung saddlebag style upon a long process which extends from a cell which is probably a macrophage (M). The edge of the sinus wall and a wedge of cord are visible in the lower left corner. $\times 4250$. 
filaments within endothelial cytoplasm. Pooling in the sinuses in the presence of macrophages (which have been seen in the sinus lumen both by transmission microscopy and in this SEM study) affords further opportunity for the spleen to destroy or modify circulating cells.

**Platelets in the Spleen**

The capacity of the spleen to sequester platelets and hold them in equilibrium with the blood is remarkable. Observations in this study suggest that large numbers of platelets adhere to the reticular cells of cords, marginal zone, and white pulp, and to the endothelium of sinuses. These observations were confirmed by review of many transmission electron micrographs where, in locations where platelet-sized masses were observed by SEM, platelets, revealed by their distinctive organelles, were found. The mechanism of adherence may relate to the collagen or extracellular connective tissue-secreting capacity of reticular cells and endothelial cells. These cells may secrete from all their surfaces. The extracellular connective tissue of the basement membrane and the reticular fibers are relatively stable because they are protected, respectively, by adventitial cells and other reticular cells. But, as has been postulated, where the secretory cell surface faces on the vascular lumen or the interstice of the reticular meshwork and is thereby unprotected, most of the secretory product is washed away, leaving only a film which can be recognized by the periodic acid Schiff reaction. Platelets aggregate on collagen. There may be enough residual collagen or collagenlike protein on the surface of sinus endothelium or reticular cells, when there is slow flow or relative fluid stasis, to hold platelets and account for the spleen’s sequestering role. Perhaps when blood flow through the sinuses and cords is increased, the double function of washing away
the binding extracellular connective tissue from the exposed surface of fixed cells, and carrying the blood platelets out into the circulation, is served.

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A Scanning Electron Microscopic Study of the Spleen

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