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Terminating arterial vessels in red pulp of human spleen: a transmission electron microscopic study

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Key words. Spleen; red pulp; transmission electron microscopy; terminating arterial vessels; connections.

The subject of the terminating arterial vasculature and its connections has been reviewed a number of times from our own laboratory and, in this multi-authored review, by Fujita and his colleagues and by the McCuskeys. We and they have concluded that the circulation in the red pulp is served by a vasculature in which there is no stable structural endothelial continuity, no mural continuity of a conventional sort, from the arterial terminal to the vein. But it has been amply documented by in vivo studies (see that of the McCuskeys and by the washout experiments of Groom, Song and their colleagues) that blood flow through the spleen may be as rapid and efficient as through other organs and tissues, yet offers several delayed circulations as well.

We shall return to rationalize the structural basis of blood flow through red pulp, but at this point, set out a number of transmission electron micrographs of red pulp of human spleen to demonstrate the nature of some arterial terminals. The material was obtained, with the aid of the Pathology Department at the Hospital of the University of Pennsylvania and with the cooperation of Professor John Glick of the Oncology Division, from patients splenectomized for staging of Hodgkin's disease. Only normal spleens or normal portions of minimally involved (stage I) spleens were used. The spleens were fixed by immersion according to routines already published within 20–30 min of clamping of the vascular pedicle. Well over 100 terminating arterial vessels were found in red pulp. Details are presented in the figures selected, provided with tracings and legends. Our general observations are as follows:

Metarterioles and arterial capillaries before and at termination characteristically contain dark and light endothelial cells, often alternating. Intermediate and microfilaments are present in abundance and stand out clearly in the light cells. Terminating vessels are capillary in character and possess a nonperforate basement membrane except where the endothelial cells separate, providing egress for luminal blood cells. These vessels also possess adventitial reticular cells which by branching

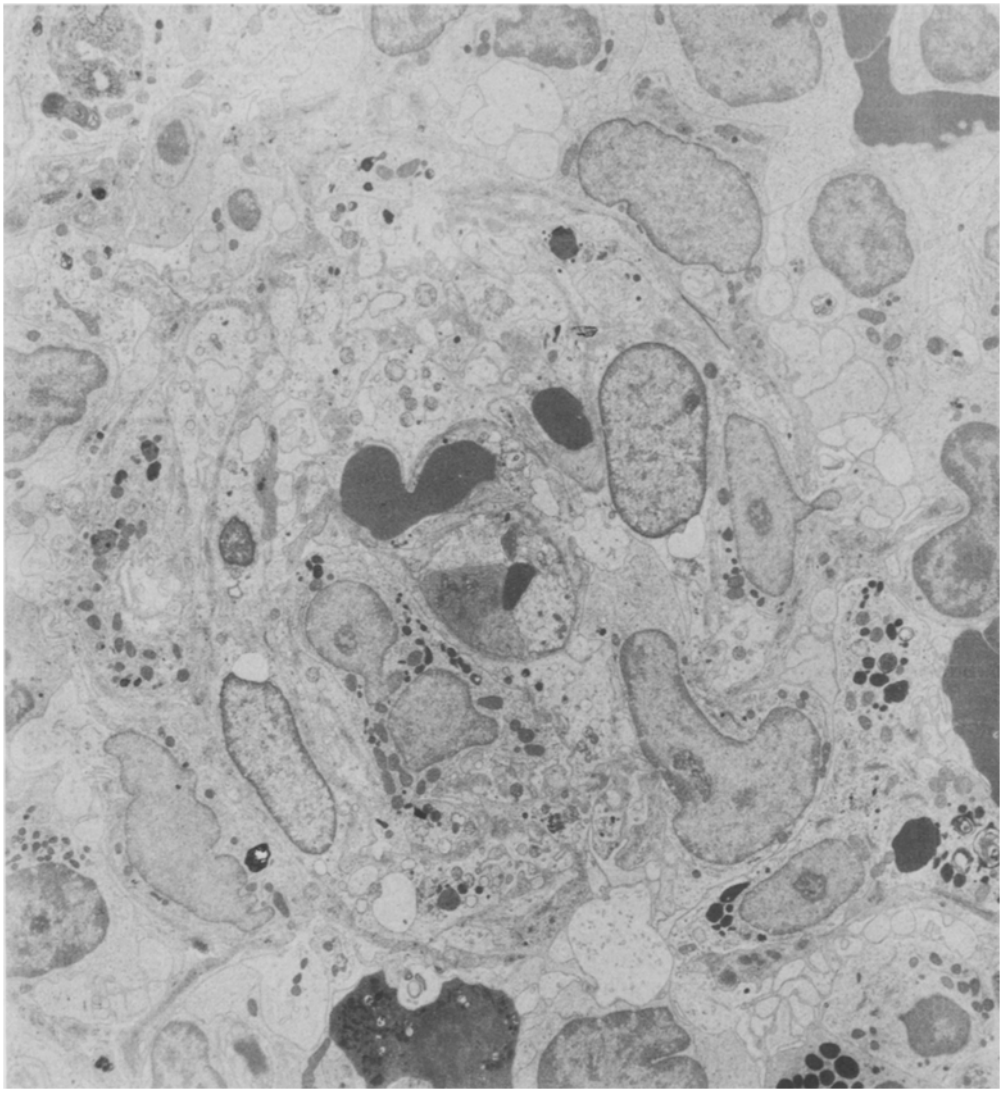


Figure 1. A small sheathed capillary, within a small macrophage sheath in a cord. The capillary is terminal and appears to have bifurcated. The left branch has the endothelium labeled (End A) in the tracing. The right branch may be opening allowing the deeply indented erythrocyte escape into the sheath. The sheath is three or four cells thick and consists of strands of reticulum and reticular cells (R) holding mononuclear cells, most of which are macrophages ar monocytes (MØ). While well-developed sheaths such as this are not present in all terminating arterioles and capillaries, virtually every terminating arterial vessel is encompassed or enveloped at least in part by one or more macrophages as shown in figure 2. ×2975.

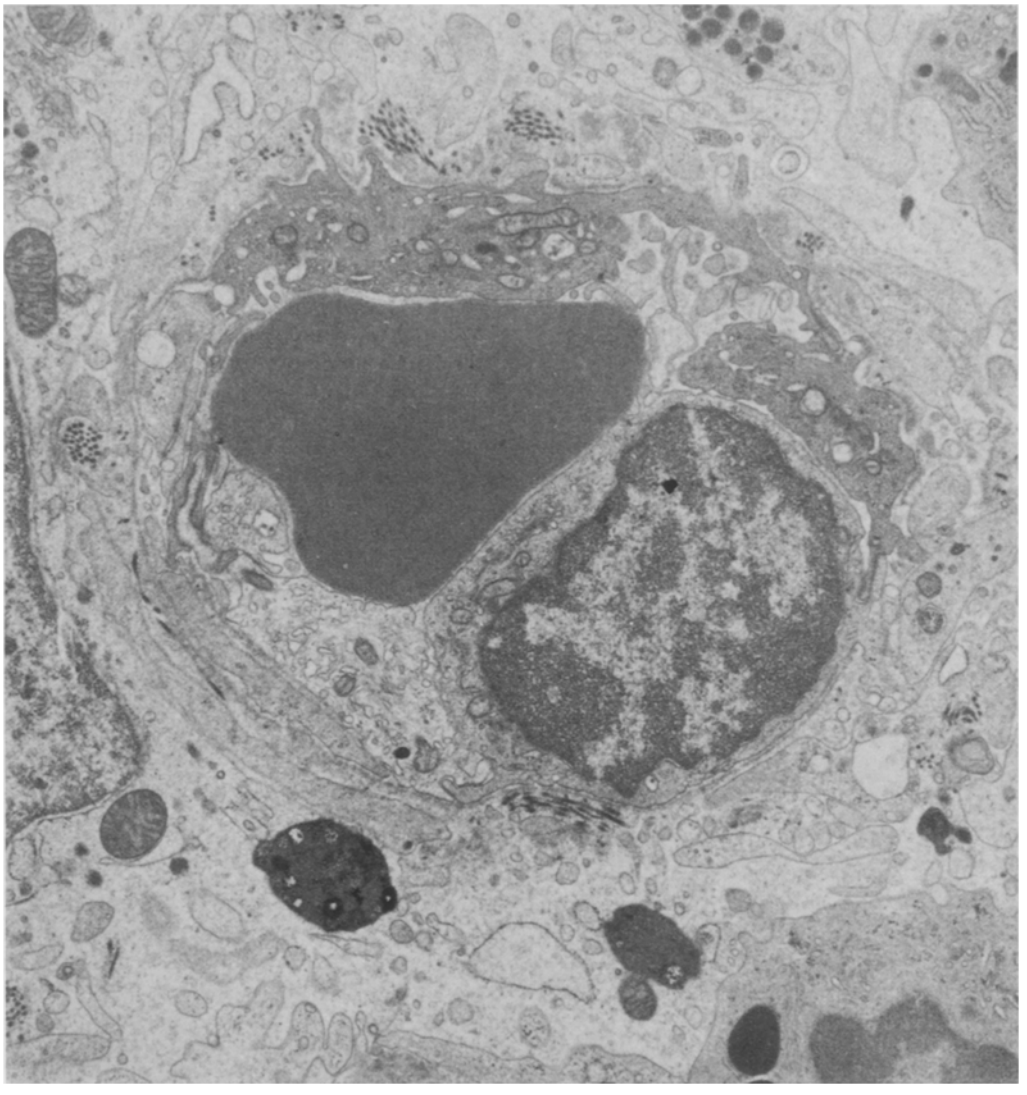


Figure 2. A small arteriole near termination. Endothelial cells are joined by junctional complexes. The alternation of light and dark endothelial cells (End A) is typical. The basement membrane (bm) is slender and may contain some collagen fibers. Portions of macrophages (MØ) surround the vessel. ×5950.

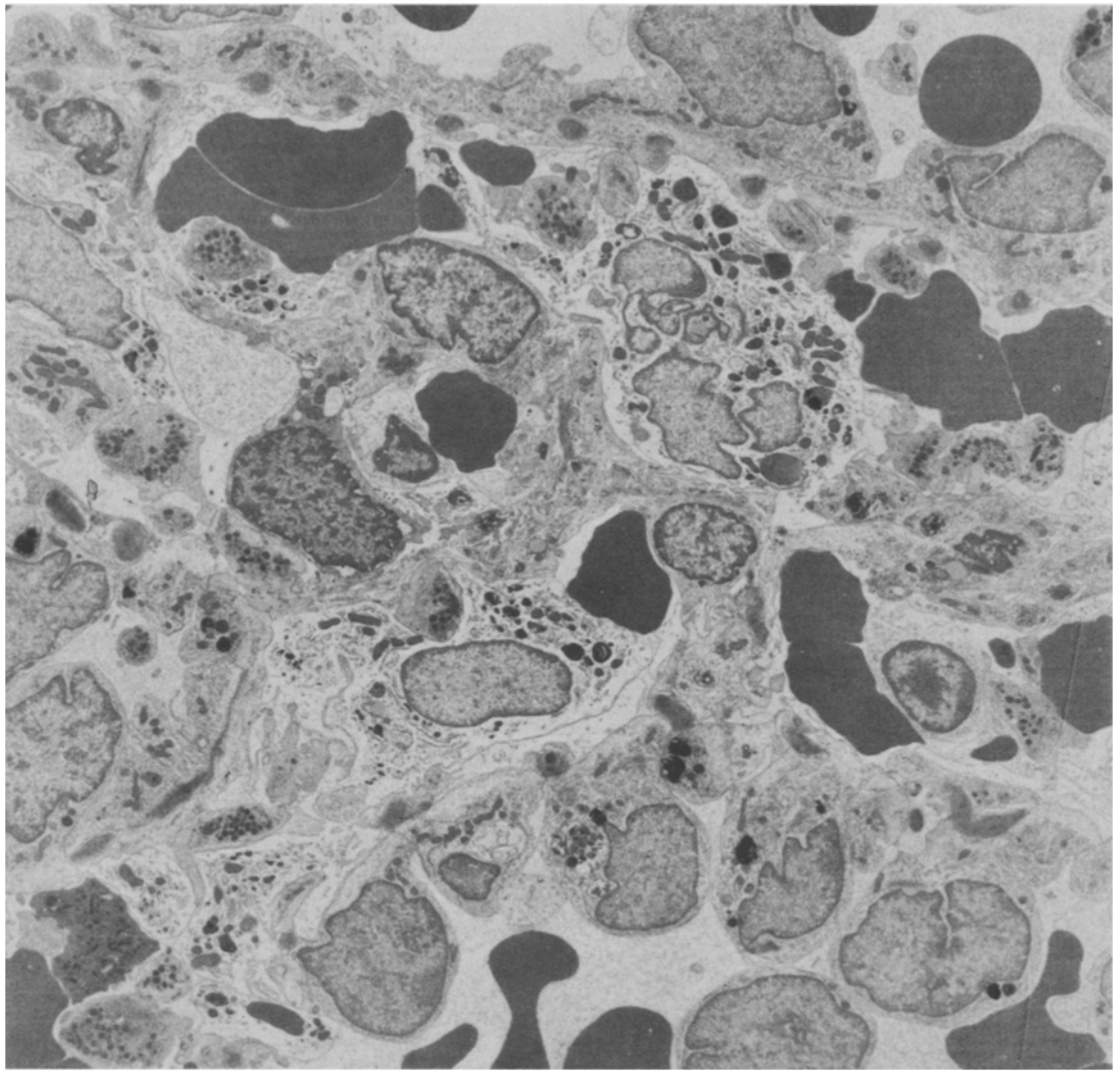
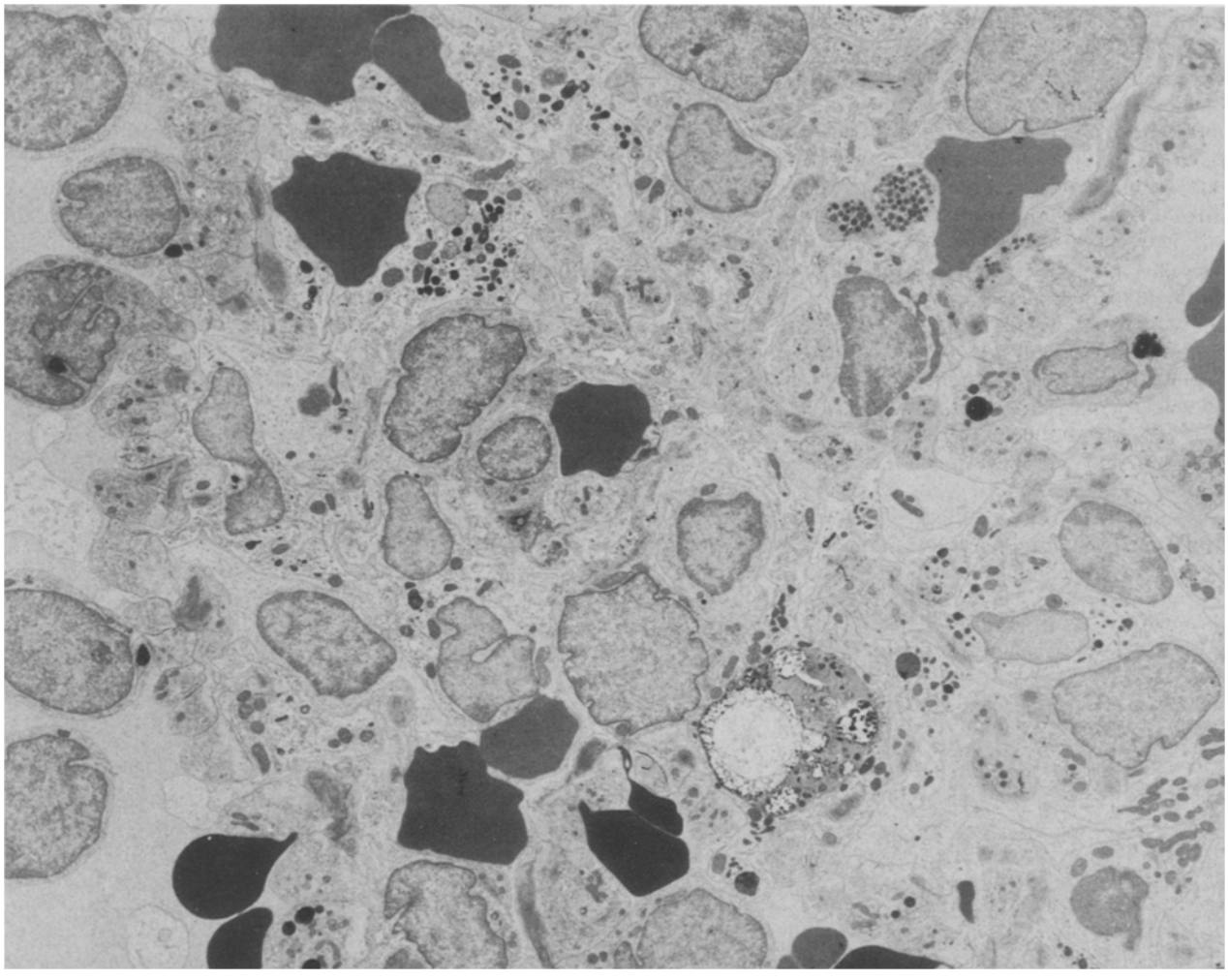


Figure 3. Red pulp. Three sinuses in the left right and upper borders of the field (see tracing Lu_s = lumen sinus) lie in this field. The endothelial cells (End S) contain nuclei, typically indented in their basal aspect. Note the heterolysosomes present in many of the endothelial cells. The basement membrane (bm) is interrupted. A process of a macrophage (arrow) protrudes into the lumen, between endothelial cells of the sinus on the left. The center of this field is occupied by a cord. It contains a terminating arteriole (endothelium = End A) containing an erythrocyte held in the reticular meshwork (the reticular cells = R). Macrophages (MØ) fill much of the space of the cord, surrounding the arteriole and extending processes between sinus endothelial cells. Except where parted by cells the endothelial cells lie with their lateral borders apposed. ×4250.



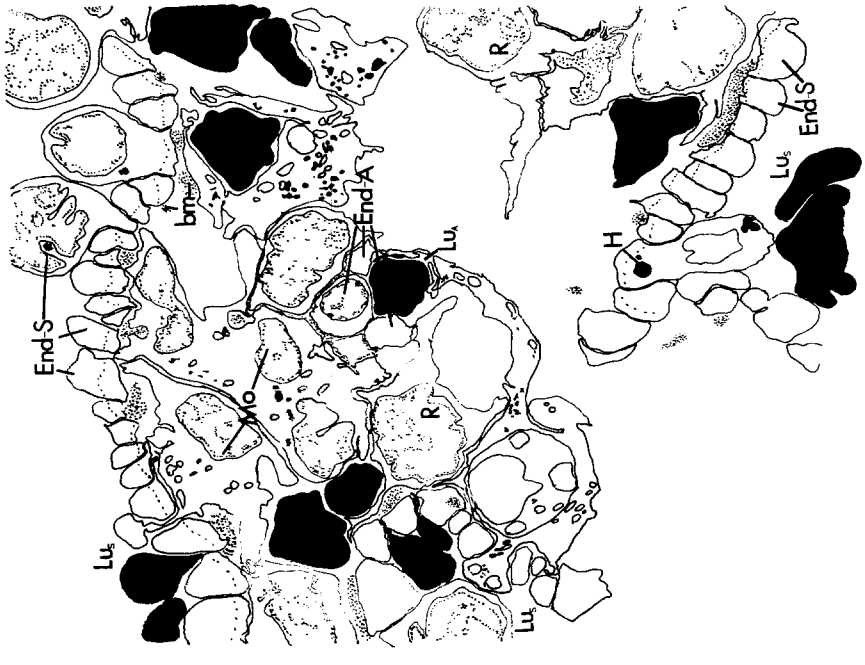


Figure 4. Red pulp. Three profiles of sinuses lie in this field of red pulp. (Lu$_s$ = lumen of sinus). In the sinuses along the upper and left border red cells squeeze between endothelial cells in passage across the endothelium. The endothelial cells (End S) are largely cut in cross section and lie with their lateral borders apposed. They contain heterolysosomes (H). Note their clear basal portions (rich in microfilaments). The basement membrane (bm) is present as an interrupted granular structure. The center of the field contains a terminating arterial vessel, (endothelium = End A, lumen = Lu$_A$). Reticular cells (R) form a reticulum and macrophages (MØ) surround the arterial vessel. Many platelets are present. ×2730.

into the surrounding cord, hold the arterial terminals in the reticulum.

Terminating arterial vessels are typically associated with macrophages. Arterial capillaries just before termination may be surrounded a compact cluster of macrophages and mononuclear cells (including monocytes and cells of the Langerhans antigen-presenting system) held in a reticular meshwork. This is, of course, an ellipsoid as discussed by a number of authors in this volume, notably Fänge and Nilsson in their studies of fish spleen. Blue and I suggested that the term *periarterial macrophage sheath* be used instead of ellipsoid because it is more descriptive, parallel to the name of the more proximal periarterial lymphatic sheath, and more accurate since these formations are seldom ellipsoidal. Further, the term periarterial macrophage sheath anticipates that as the arterial vessel goes on and terminates, passing beyond the periarterial macrophage sheath, it remains associated with macrophages. One, two or more macrophages partially surround the vessel, virtually an attenuated periarterial macrophage sheath. These macrophages, supported by the cordal reticulum, are characteristically interposed between arterial openings and nearby venous sinuses. A macrophage may extend a process between the widened interendothelial slits of a terminating arterial vessel and the very macrophage may reach to the nearby sinus and extend a process between its endothelial cells. Such cordal macrophages are typically rather large and contain a good deal of phagocytized material.

Reticular cells and reticular fibers make up the reticular meshwork of the cords. This meshwork supports arterial terminals, venous sinuses and, in its interstices, holds the erythrocytes, macrophages, monocytes, platelets and lymphocytes and other cells constituting the migratory or circulating population of free cells of the cords. In these human spleens clusters of platelets are common, suggesting the cords as a site of the large-scale platelet storage provided by the spleen. Reticular fibers are usually rather lightly stained, occasionally they can be quite dark. Often reticular fibers, which are predominantly granular, contain collagen fibers. The cytology of the reticular cells has been reviewed elswehere. Here the continuity of the endoplasmic reticulum and the perinuclear space, the plaques of subplasmalemmal microfilaments alongside reticular fibers, and the abundant intermediate filaments may be noted.

The human spleen is a sinusal spleen. Its vascular sinuses are well-developed and easily found. While there are many large-lumened 'typical' sinuses there are also many collapsed vessels and many sinuses that, in cross section, are scarcely larger than small arterial terminals. The rod-shaped endothelial cells of the vascular sinuses lie side by side with lateral borders contiguous. In our material and in other immersion-fixed material viewed by TEM we seldom see sinus endothelial separation or gaps except where free cells are passing through or intruded into the sinus wall. In the SEM gaps are often but not always (see Weiss '74) present, suggesting that omnipresent interedothelial gaps not containing cells in passage, as discussed in the 'Conclusion' of this review are an artefact of SEM. Quiescent interendothelial slits may be viewed as potential spaces, capable of distention when cells or strong fluid waves pass through. Leukocytes being motile, moreover, can force a wider transmural interendothelial separation than erythrocytes. The base of the sinusal endothelial cells, rich in intermediate filaments arranged longitudinally is rather light in most of our specimens. A striking and distinctive characteristic of the sinusal endothelium in human spleen is the large number of variegated heterolysosomes, some of which may be quite large and complex. This may signify moderate phagocytic capactiy of this endothelium. These endothelial heterolysosomes are more marked in human beings than in dogs.

Certain characteristics of arterial terminals warrant comment. Often we observe terminating vessels in cross section midway between two or more sections of vascular sinuses. The circumference of these arterial vessels may be broken or imperfect due to flaring or endothelial separation. Not uncommonly, two arterial capillaries are side by side or even share a basement membrane or adventitial cells along part of their perimeter, suggesting a bifurcation. Erythrocytes or leukocytes may be observed escaping into the cords from such arterial vessels. Terminating arterial vessels may lie close to a sinus. In unusual instances they may lie so close that no adventitial or accessory cells intervene and basement membranes may be the only interposed material. If in the spleen there is no stable structural endothelial continuity in the intermediate vasculature as there is in other tissues, how does the spleen provide flow as rapid and as efficient as through other tissues? It would appear that several factors may be at work:

An effective channel may be created between arterial ending and the wall of the vein or venous sinus by reticular cells which form the meshwork of the pulp. These presumably fibroblastic cells possess broad cellular processes and may, by the alignment and configuration of these processes, produce cylindrical channels quite as competent as conventional vessels. Arterial vessels, moreover, terminate at varying distances from vascular sinuses (in sinusal spleens) and from pulp veins (in nonsinusal or storage spleens) and it is likely that channels through the reticulum operate most efficiently when the distance between arterial and venous vessels is small. Contraction of the reticular cells of the reticular meshwork which supports arterial terminal and venous vessels would, as discussed below, bring arterial and venous vessels together, facilitating rapid, direct flow. Indeed, Groom and his colleagues showed that rapid flow depends upon splenic contraction. There is, further, the case where a terminal arterial vessel is quite close to sinus as described by the McCuskeys, Fujita and his colleagues and in our figure 7, separated only by basement membranes. If interendothelial slits of these vessels are open and in register, direct and rapid flow is likely effected.

With regard to the flow of blood into venous vessels there would appear to be little problem for blood cells circulating through the red pulp to pass into the fenestrated venules present in such nonsinusal spleens as that of the cat, shown by Blue and Weiss. Transmural passage through the wall of vascular sinuses would appear, on the face of it, more difficult since these vessels are not fenestrated and, as discussed earlier, the rod-

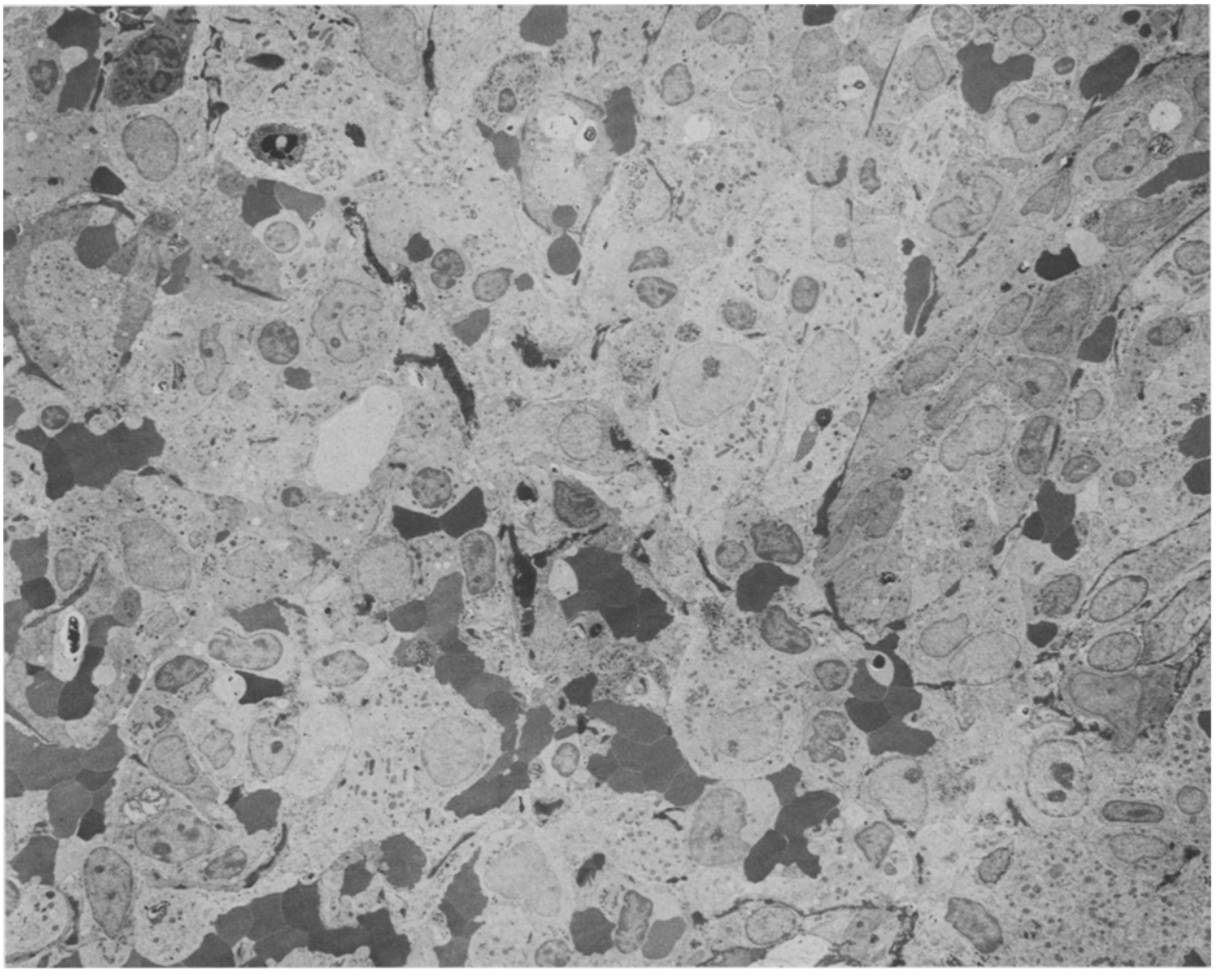


Figure 5. This complex low power field is a cellular portion of the red pulp rich in vascular sinuses many of which are small-lumened or collapsed. Profiles of vascular sinus occur which are approximately the size of arterial terminations. Reticular fibers in this preparation are rather dense. ×1720.

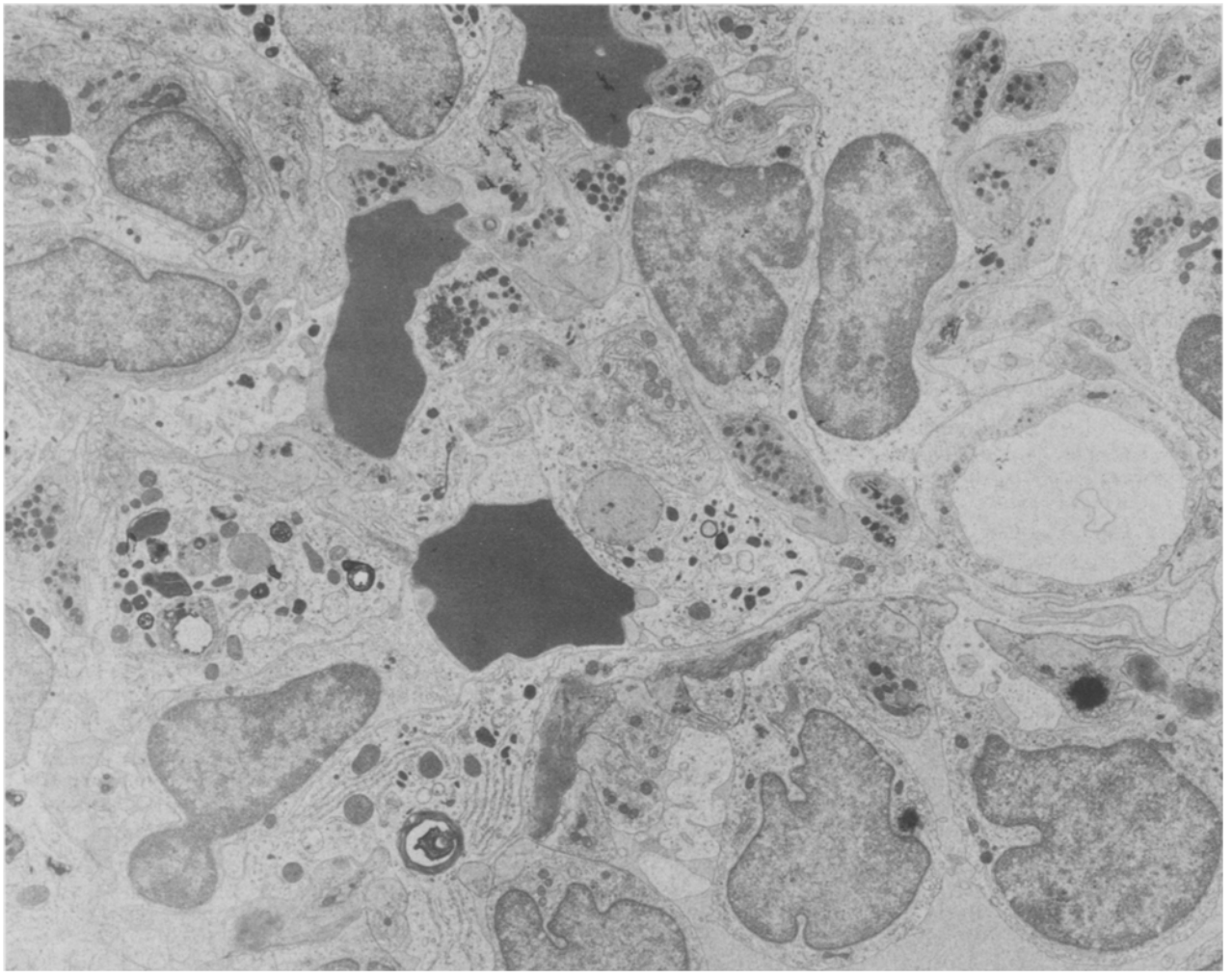
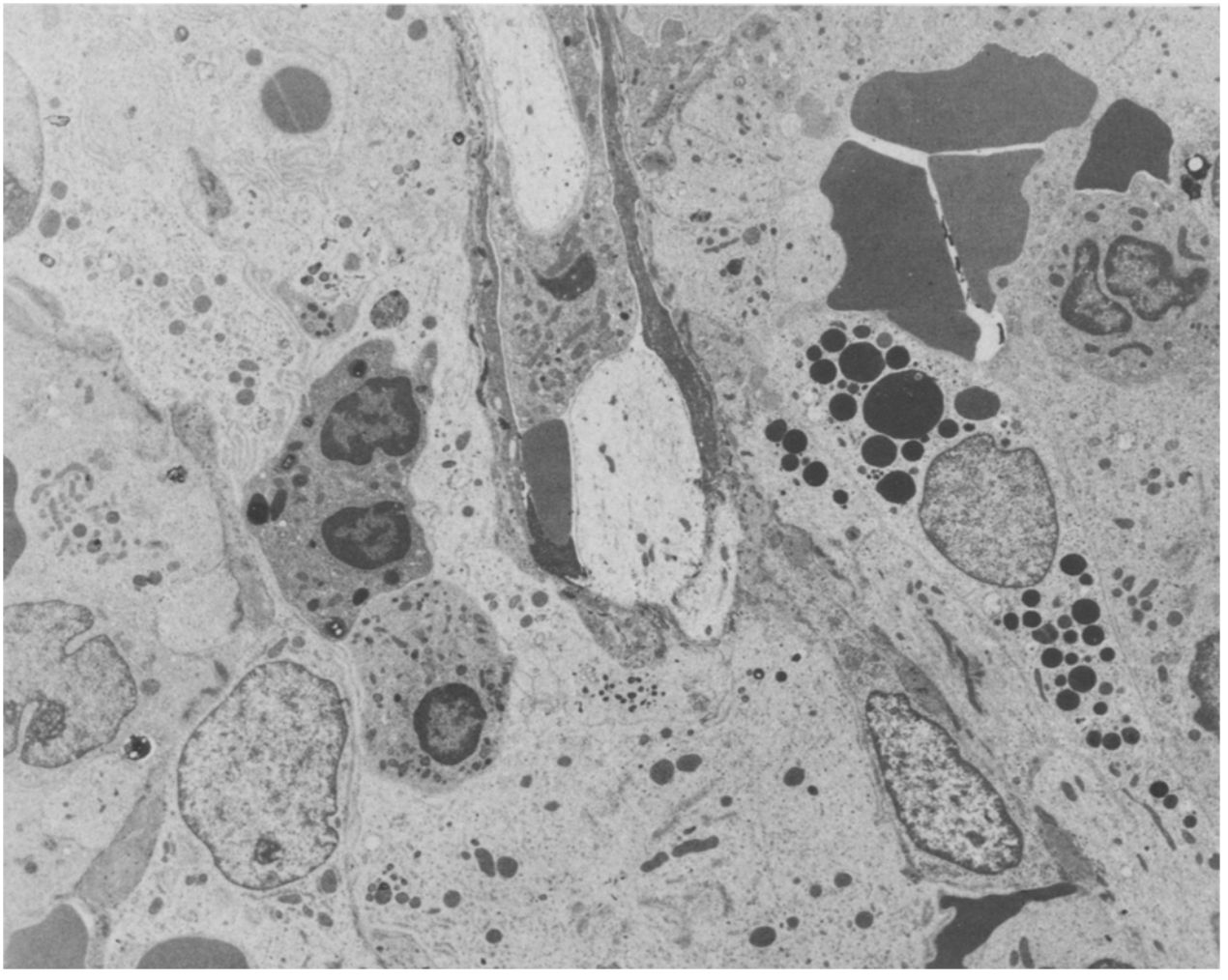


Figure 6. This field includes a slender arterial vessel, a vascular sinus and the cord of red pulp. The artery, in the right upper corner, is near terminal. It is surrounded by a large mononuclear cell (M). The cord in which the artery runs contains macrophages (MØ), erythrocytes and platelets. The sinus is on the left (lumen = Lu_s, endothelium = End S). The endothelial cells are cut in near cross section – there are several cells cut through the nucleus which typically shows a basal indentation. The basal cytoplasm of the endothelial cells sits in the interrupted basement membrane (bm) and may be clear due to the presence of filaments and the exclusion of other cytoplasmic organelles. The endothelium contains heterolysosomes. Endothelial cells lie side by side, occasionally separated by the process (arrow) of a macrophage (MØ). ×5460.



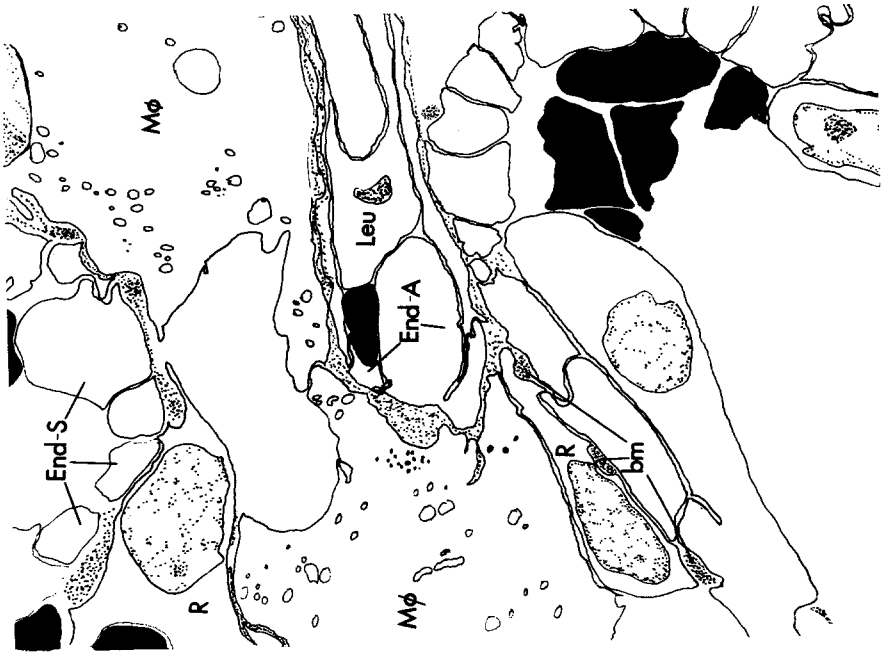


Figure 7. A band of cordal tissue runs across the field separating a sinus, above, from one below. Within the cord, cut longitudinally, lies a terminating arterial vessel, its endothelium (End A) containing as is typical, dark and light cells with filaments evident in the lighter ones. A leukocyte (Leu) lies in its lumen. Note that the arterial capillary appears to share a basement membrane with the sinus. While there appears to be no endothelial continuity between capillary and sinus, if an interendothelial slit of the capillary is in register with one of the sinus, and if both were patent and unimpeded by basement membrane, blood from artery to sinus may flow directly. See discussion and the papers of Fujita and his colleagues and the McCuskeys in this review. ×4680.

shaped endothelial cells lie side by side with only slit-like interendothelial spaces. But the endothelium of small splenic arterial vessels is high and may efface the lumen, reducing it to a slit-like space. (This observation may be made not only in immersion-fixed blocks of tissue but also in living tissue.) Yet when blood flows through such arterial vessels a significant lumen appears and blood flow is quite rapid. The interendothelial slits of the venous sinuses may open in a similar manner. Several factors may account for the opening of these slits. Surely blood pressure and related hemodynamic factors contribute. Cell motility must also be a factor for leukocytes in passage. (See the McCuskeys' in vivo account in this review.) The rod-shaped endothelial cells of the venous sinuses contain, longitudinally arrayed, both intermediate filaments, likely vimentin, and microfilaments, likely actin. If these microfilaments contract they would cause the interendothelial slits to open, facilitating transmural flow. Further, adventitial reticular cells, which may also be rich in microfilaments, adhere to the basal surface of the rod cells and extend out into the reticulum of the pulp. If these cells contract they would have the double effect of causing the interendothelial slit of the sinus endothelium to gape (perhaps, in conjunction with contraction of the rod cell as postulated above), and shortening the distance between arterial vessels and the sinus endothelium. Add to this a synchronized contraction of capsule and trabeculae as may occur due to their smooth muscle (see the classification of spleens by capsule and trabeculae detailed in the paper of the Hartwigs) and direct flow may be further facilitated. What may induce contraction of the contractile elements in the spleen is reviewed in Reilly's paper in this volume. Adrenergic sympathetic innervation may well be a major stimulant since, as discussed in several papers in this volume, this is the primary innervation of the spleen and not only smooth muscle but microfilament-rich reticular cells are innervated.

The important scanning electron microscopy of Schmidt, MacDonald and Groom in which the intermediate circulation of sinusal and nonsinusal spleens is depicted in microcorosion casts indicates direct arteriovenous connections in the sinusal spleen under certain conditions. Such casts are primarily records of flow and only inferentially of structure. That such direct pathways of flow exist is, as stated above, clear. They may be observed on the high-resolution in vivo microscopy of the McCuskeys as well as in the casts of Fujita and of Schmidt and their colleagues. What the structure of these pathways is must remain uncertain through study of casts alone or even through high-resolution in vivo microscopy. In my opinion, we must turn to other means, of which transmission electron microscopy is the most valuable, to ascertain the structure of these pathways. Then follows the attempt, as done here, of rationalizing or understanding one set of data in terms of the other.

A variety of cells resident in the reticular meshworks of the spleen are encountered by the circulating blood as it reaches the spleen. These cells may carry regulatory mechanisms that affect blood flow. It is evident from the work presented and reviewed by van Ewijk and Eikelenboom and their colleagues in this volume that the cells of the blood are offered different vascular pathways through the spleen. Thus lymphocytes may take the selective pathways through white pulp. The assumption of a selective white pulp pathway by circulating T cells, for example, may depend upon the association of the T cells with interdigitating cells in the marginal zone or red pulp where lymphocytes and interdigitating cells are delivered by arterial terminals, as discussed in the Conclusion of this multi-author review. Thus one consequence of the vascular arrangements in the spleen is that it facilitates those cellular interactions among migratory and stromal cells that regulate blood flow and such functions of the spleen as antibody formation.

At the outset of my (L.W.) work on the spleen, I concluded that cords were collapsed sinuses, that the the open vs closed dichotomy was too mechanical to apply to the spleen, and that the entire pulp of the spleen is a vascular tissue, albeit highly specialized. While the notion that cords are collapsed sinuses was quickly found incorrect, the characterization of the entire pulp as a specialized vasculature and the inappropriateness of positing the problem as open vs closed remain valid.

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