Electron Microscopy of the Spleen

I. Anatomy and Microcirculation

Jerome S. Burke, MD,* and Gérard T. Simon, MD

THE OFT-QUOTED PHRASE of Galen, "the spleen is an organ full of mystery," may be a cliché, but it is nevertheless true. While the spleen is now understood to play an important role in erythrocyte destruction, phagocytosis and antigen-antibody interactions, the circulatory pathways through the spleen remain controversial.[1,2] Moreover, although the spleen is known to participate in many physiological processes, the exact mechanisms are usually not well understood. This is particularly true of the immunological functions of the spleen which have become of critical importance since the advent of organ transplantations, and the description of clinical syndromes resulting from the congenital inability to manifest the cellular and/or humoral mechanisms of immunity.[3]

Before initiating studies of normal and abnormal splenic function, it is mandatory to acquire more accurate knowledge of the normal ultrastructure and microcirculation of the spleen. Simple questions as to the types of cell which occur in the spleen, the structure of the red pulp and its sinuses and cords, and the nature of the white pulp cannot be answered with certainty. Whether or not the splenic circulation is open or closed is in dispute even today.[2] The injection of foreign materials,[4,5] transillumination of living spleens,[6,7] silver impregnation of serial sections,[8,9] and electron microscopy[2,10-13] have all been tried, but the question remains open.

Because of these difficulties, it was decided to review again the anatomy and microcirculation of the spleen before undertaking an investigation of its function.

Materials and Methods

Eight normal albino rabbits were anesthetized with intravenous sodium pentobarbital and maintained in surgical anesthesia with ether. Their spleens were

From the Department of Pathology, Banting Institute, University of Toronto, Ontario, Canada.

Supported by Grants MT2189 and MA3703 from the Medical Research Council of Canada.

Accepted for publication October 1, 1969.

Address for reprint requests: Dr. Gérard Simon, Department of Pathology, University of Toronto, 100 College St, Toronto 2, Canada.

* Research Fellow of the Medical Research Council of Canada.

perfused cephalad through a cannula placed in the abdominal aorta above the renal arteries. The thoracic aortas were clamped and the splenic veins cut at the start of the perfusion. The perfusion pressure of 120–140 mm Hg was maintained for 5 min. The perfusate was 50–60 ml of 1% osmium tetroxide buffered to pH 7.2 with 0.1 M Sorensen's phosphate buffer.[14] At the completion of each perfusion the spleens were black. Portions of splenic pulp, hilus, and capsule were then immediately immersed in cold buffered 1% osmium tetroxide solution for fixation. Spleens from 2 additional rabbits and 3 Wistar rats were fixed in formalin for light microscopy after similar perfusion with a commercial India ink and gelatin mixture.*

Following the fixation in cold buffered 1% osmium tetroxide for 1 hr, the tissue fragments were dehydrated in graded alcohols and propylene oxide according to the method of Luft,[15] and were embedded in a mixture of Epon-Araldite as described by Givan, Turnbull, and Jézéquel.[16] Sections were cut with glass or diamond knives on a Porter-Blum MT1, LKB, or Reichert OmU2 ultramicrotome. Thick sections (0.5–1.0 μ) were stained with toluidine blue. Sections for electron microscopy were stained either with lead hydroxide,[17] lead citrate,[18] or with an aqueous solution of uranyl acetate[19] followed by lead hydroxide. These sections were examined and photographed in an RCA EMU-3F or Phillips EM-300 electron microscope.

Results

Examination of the white pulp with the electron microscope reveals this region to be composed predominantly of lymphocytes with a high admixture of reticulum cells. No germinal centers can be recognized. The cells are arranged in clumps or nests. Each clump contains both lymphocytes and ovoid reticulum cells. The nests are separated by a loose irregular meshwork composed of basement membrane-like material, collagen, and the cellular prolongations of fibroblasts and stellate reticulum cells (Fig 1 and 2). At the periphery of the white pulp, adjacent to the marginal zone, there is an irregular collection of plasma cells, macrophages, erythrocytes, and platelets.

The lymphocytes of the white pulp vary in size and are often difficult to differentiate from reticulum cells. In general, they are round to oval and characterized by a high nuclear-cytoplasmic ratio. The cytoplasm is very sparse and inactive in appearance. There is occasionally a little perinuclear rough endoplasmic reticulum, a few free ribosomes, and small mitochondria with few cristae. The smooth endoplasmic reticulum is in the form of small vesicles dispersed randomly in the cytoplasm and the Golgi zone is not prominent.

The reticulum cells are extremely polymorphic. The ovoid cells are similar in appearance to lymphocytes. However, they are usually larger and irregular in shape. They have a smaller nuclear-cytoplasmic ratio and more organelles in the cytoplasm. There is a slightly greater amount

* Higgins Ink Co. Inc., Brooklyn, NY.

of rough endoplasmic reticulum, more free ribosomes, a more prominent Golgi zone, and larger, better developed mitochondria. Vesicles are often arranged in a single or in an "Indian file" order. Other reticulum cells are stellate, with long cytoplasmic prolongations extending between the nests of lymphocytes and ovoid reticulum cells. The prolongations of reticulum cells can be differentiated from those of fibroblasts by their less dense and less fibrillar cytoplasm. Cells, virtually identical to reticulum cells, both ovoid and stellate in shape, but which contain phagocytic debris are found particularly at the periphery of the white pulp (Fig 3). These phagocytes have been termed macrophages, and are identical to the macrophages in the red pulp.

The fibroblasts of the white pulp often appear flat and are stellate with extremely long cytoplasmic prolongations which are sometimes U-shaped. They have a strikingly dilated rough endoplasmic reticulum filled with a very fine flocculent material (Fig 4). The smooth endoplasmic reticulum contains similar material, some of which may appear fibrillar. The smooth endoplasmic reticulum cytoplasmic prolongations of the stellate fibroblasts appear to be continuous with the extracellular basement membrane-like material and collagen fibres. These components together with the stellate projections of reticulum cells make up the loose meshwork which compartmentalizes the while pulp. This meshwork is not dense enough to form solid barriers or prevent the free passage of cells within the pulp.

The plasma cells of the white pulp are more frequent at the periphery. They are recognized by their well-developed system of rough endoplasmic reticulum which extends throughout the cytoplasm. The rough endoplasmic reticulum generally contains a find flocculent material and occasionally Russell bodies. Their mitochondria are round, larger and denser than in lymphocytes or reticulum cells, and have prominent cristae. There are numerous ribosomes both free and attached to the dilated endoplasmic reticulum.

Occasionally an unmyelinated nerve is found in the white pulp. The nerves are always in close association with the arteries of the pulp. They have a variable number of axons and one can usually discern the cytoplasm of the Schwann cell which surrounds them.

In addition to individual cells and nerves, the white pulp also contains blood vessels—arteries, arterioles, and arterial capillaries. The largest vessel of the white pulp is the central artery, which is a branch of one of the arteries of the trabecula. This artery is a typical muscular artery with a smooth muscle layer one or two cells thick and an internal and external elastic lamina. Perhaps characteristic of the arteries of

the spleen are their rather tall endothelial cells, though the cells are otherwise unremarkable. There are no clearly identifiable adventitial cells around the arteries or arterioles. The fibroblasts and large reticulum cells present in the adventitial region are mixed with lymphocytes and not distinct from the similar cells elsewhere in the white pulp. The external elastic lamina blends between these cells with the surrounding collagen and basement membrane-like material of the white pulp.

Arterioles are the first branches of the central artery. They are structurally identical to the central arteries except for their size. Often their lumens are surrounded by one continuous muscle cell only (Fig 5). The arterioles branch into capillaries either in the white pulp, in the marginal zone, or in the red pulp. The transition of an arteriole into a capillary is shown by the replacement of the muscle cell by a pericyte, which is similar in appearance to a fibroblast, and by the conversion of the elastic lamina into an amorphous basement membrane.

The capillary endothelium in the white pulp is continuous with high nonfenestrated endothelial cells. Its basement membrane is often duplicated and surrounds the pericytes. Most of the capillaries are from 12μ to 18μ in diameter, but there are also very small capillaries with luminal diameters of approximately 8μ. These small branches have low endothelium, a continuous single basement membrane, and lack pericytes. Capillaries in the red pulp and marginal zone are similar to the larger capillaries in the white pulp (Fig 6).

Arterial terminations in the spleen were never postively identified. The impressions, however, from both electron microscopic and India ink-gelatin perfusion studies are that the small capillaries of the white pulp terminate in the marginal sinuses surrounding the white pulp follicles, and the larger capillaries either terminate in the same marginal sinuses or extend further into the marginal zone or red pulp to terminate directly into the sinuses of the red pulp. However, no direct connections between capillary and sinus were ever recognized. On the other hand, certainly no capillaries were ever seen to terminate in the Billroth cords and no muscular arteries or arterioles were ever seen in the red pulp. The only muscular vessels found in the red pulp are splenic veins, which are recognized by their long, slender, spindle-shaped endothelium, a continuous basement membrane without elastica, and a smooth muscle layer.

The marginal zone of the spleen is a transitional area between the white and red pulp. It contains many more lymphocytes than the red pulp proper, and these cells are slightly larger than those in the white pulp. There are also numerous reticulum cells and plasma cells and

towards the outer edge, and increasing number of macrophages containing phagocytic debris. The sinuses of the marginal zone vary in size. The largest is called the marginal sinus and is located circumferentially around the white pulp. This marginal sinus is lined by large endothelial cells. Between these endothelial cells and the subjacent white pulp is a basement membrane which is interrupted or fenestrated (Fig 7). The interruptions in the basement membrane provide a passage for cells from the white pulp into the marginal sinus and from marginal sinus into white pulp. It is not uncommon to find cells in these gaps in the basement membrane. Many sinuses varying in size from small channels to large main sinuses branch into the red pulp from the marginal sinuses.

The splenic red pulp is composed of a complex series of anastomosing, tortuous sinuses which vary in size from small channels to large vascular pathways. These sinuses are separated from each other by solid partitions of red pulp called the Billroth or pulp cords. The Billroth cords vary in thickness from one cell to many (Fig 8). Within the cords are mainly reticulum cells, macrophages, and fibroblasts, but plasma cells, leukocytes, platelets, and red blood cells are also present. The Billroth cords are often irregularly partitioned by extracellular reticulum which has the appearance of amorphous basement membrane-like material. This extracellular reticulum sometimes is fibrillar, with a periodicity suggesting collagen (Fig 8).

The sinuses of the red pulp are lined by endothelial cells which vary in size according to the size of the sinus. The larger endothelial cells are in the larger sinuses, do not have as prominent a vesicular system or as well developed an endoplasmic reticulum, and are less stellate than most of the endothelial cells lining the medium and smaller sinuses. As the endothelial cells in medium and small sinuses are more stellate, their nuclei are not always present in every section (Fig 9). The nuclear portion of the cell bulges into the lumen of the sinus while the remainder of the cell is roughly cuboidal. The cytoplasm is characterized by a very large number of vesicles and vacuoles many of which are at the luminal surface. Occasionally, one of the vacuoles contains some dark, dense, coarse material which may perhaps represent a product of endothelial phagocytosis. There is also a well-developed rough and smooth endoplasmic reticulum and the mitochondria are round with a light matrix and well-developed cristae.

The basement membrane separates the endothelial cells from the Billroth cord. This basement membrane is characteristically fenestrated throughout the red pulp as well as in the marginal zone (Fig 8–11). The basement membrane may have a focal filamentous appearance and,

in some locations, the cells on the luminal and cordal sides of the basement membrane have filamentous intracytoplasmic densifications.

The majority of the cells lining the cordal aspect of the basement membrane are reticulum cells. These cells are stellate in shape and similar to those in the white pulp. Their long cytoplasmic prolongations commonly underlie the basement membrane, and often resembles those of a fibroblast. They differ from the endothelial cells of the sinuses, for though the reticulum cells have prominent Golgi zones their cytoplasm is sparse with few organelles (Fig 8 and 10). Their mitochondria are generally close to the nucleus, with a dark matrix, and the cristae are not prominent. Other reticulum cells in the cord are more ovoid in shape and it is relatively common to see cytoplasmic prolongations of these cells extending through the fenestrations of the basement membrane and between the endothelial cells into the sinus. Macrophages which are identical to the reticulum cells except that they have phagocytic vacuoles in addition are also common in the Billroth cords. The phagocytic vacuoles of the macrophages frequently contain products and debris of degradated erythrocytes and/or leukocytes (Fig 8). These cells also occasionally partially protrude into a sinus. The portions of macrophages and reticulum cells which project into a sinus contain fewer organelles than do the portions of the cells in the cords.

The most common cells in the red pulp are erythrocytes. These cells are present in great numbers in the sinuses and are also present in the cords. They display a wide variety of shapes and are frequently observed passing through the interruptions of the basement membrane (Fig 11). The portion of the red cell in the gap of the basement membrane is often squeezed and attentuated and may give the impression of "pitting."[20]

There are many other varieties of blood cells present in smaller quantities in the red pulp, both in the Billroth cords, in the sinuses, and in transit between them. These include reticulocytes, neutrophils, eosinophils, plasma cells, lymphocytes, monocytes, and platelets. Occasionally megakaryocytes, or platelet aggregates without fibrin deposition, are seen.

The trabecula of the spleen vary in thickness and size and are predominantly composed of fibroblasts, collagen, elastica, and basement membrane-like material. The portions of the trabeculae adjacent to the red pulp also contain reticulum cells and macrophages and are bordered by a sinus with normal-appearing sinus endothelial cells and a fenestrated basement membrane (Fig 12). In the white pulp, the trabeculae

lie in immediate relation to the fibroblasts, lymphocytes, and reticulum cells of this zone.

The splenic capsule is composed primarily of cells which have features of both smooth muscle cells and fibroblasts (Fig 13). These cells are stellate, with dense fibrillar cytoplasm, small mitochondria and glycogen-like granules. They are associated with large bundles of collagen and occasionally with some elastic tissue. The inner surface of the capsule is usually lined by a typical large sinus with endothelial cells of the usual type and a fenestrated basement membrane. A few reticulum cells and macrophages are found in the capsule adjacent to the sinus, and frequently a macrophage or reticulum cell can be found in the gaps of the capsular basement membrane. Elsewhere, capsular fibroblasts are found against the basement membrane. The external aspect of the capsule is lined by a single layer of mesothelial cells, characterized by small irregular microvillous projections at their free border.

Discussion

These studies still do not answer clearly the question of whether the circulation in red pulp of the spleen is open or closed. No direct connections between either capillary and sinus or capillary and Billroth cord were identified. In particular, and in contrast to the prevalent opinion,[11,12,21,22] no capillary or arteriole was ever seen to terminate in a Billroth cord. Indeed, muscular arterioles were never seen beyond the white pulp of the spleen.

Though no definite statement is justified, our impression from perfusing the spleen with India ink-gelatin or osmium tetroxide is that the major part of the perfusate flowed directly into the sinuses. Certainly, the osmium tetroxide perfusion tended to dilate the sinuses and not the cords.

However this may be, the issue of whether the splenic circulation is open or closed is probably not of great importance with respect to the functional capabilities of the spleen. As Koyama, Sadako, and Deguchi[21] noted, the circulation can be regarded as both open and closed, in a physiological sense, because even if the main blood flow is in a closed system the presence of many fenestrations in the basement membranes allows easy passage between the sinuses and Billroth cords. The fenestrations could permit the free passage of all blood cells and splenic cells from the sinuses into the cords or from the cords into the sinuses. As a result, cells which are damaged or aged could enter the cords and come in contact with the macrophages in the Billroth cords and marginal zones for sequestration.[4,7,9,12,23] Cells which are essentially

normal probably pass directly through the sinus system or cross the cord from sinus to sinus without sequestration. It is interesting to note that these fenestrations are not a new discovery for they were suggested or described by early investigators.[4,24]

The Billroth cord can then be regarded as a modified type of cellular channel containing plasma and blood cells, even though it is not lined with endothelial cells. It is not a true vascular channel, not only because of the lack of endothelial cells, but also because of its extensive partitioning by basement membrane-like material which has an irregular periodicity suggesting collagen. The presence of fibroblasts in the cords, as well as macrophages and reticulum cells, is also against the idea that the cord is a true vascular channel or even a collapsed sinus, as has been suggested.[10,25,26]

The apertures or fenestrations in the basement membrane of the sinuses are not only present in the red pulp, but are also found in the marginal sinuses between the red and white pulp and in the sinuses lining the capsule and trabeculae. The presence of a fenestrated basement membrane between the red and white pulp, rather than a continuous one, is important because these fenestrations could allow free passage of the cells and antigens between the white and red pulp and provide a possible pathway which may be important in immunological reactions.[27]

The demonstration of high endothelial cells in arteries, arterioles, and arterial capillaries agrees with the description of Weiss.[11,12] However, unlike Weiss, we saw elastica in both central arteries and arterioles. Again in contrast to Weiss, no definite adventitial cells were noted. Cells which were adventitial in location around the central arteries and arterioles of the white pulp could not be differentiated from the fibroblasts or reticulum cells elsewhere in the white pulp, and were therefore referred to as such.

Most importantly, and again in contrast to Weiss,[13] no arteriolar or capillary terminations were recognized in the white pulp. In our opinion, the arterioles and capillaries, small or large, end mainly in the marginal sinuses, although some capillaries do extend further into the red pulp. The great difficulty in recognizing the junctions between capillaries and sinuses is that the main difference between a capillary wall and the wall of a sinus is that the sinus has a fenestrated basement membrane. Both vessels have tall endothelial cells of similar character. Thus, even if the junctional zone were seen in an electron micrograph, it would be difficult or impossible to recognize it. The electron micrographs purport-

ing to show capillaries opening directly into the white pulp or Billroth cords[11,13] seem to be rather a tangential section of the vessels.

The sinuses of the red pulp appear to originate mainly from the marginal sinus. Some of the sinuses are very small and others large. Large sinuses may originate from the capillaries which enter the red pulp, and these large sinuses have many smaller side branches running into them.

The sinus-venous connections were only seen under the light microscope. The veins appeared to originate directly and abruptly, without any transition zones, from the sinuses. Some sinuses joined formed veins as side channels.

The findings as to the cellular components in the white pulp in this study agree essentially with other investigators.[13,28] No germinal centers were recognized in the white pulp. Rather, the lymphocytes and reticulum cells of the white pulp were mixed together in varying proportions to form nests or colonies, separated by a loose, irregular meshwork. This meshwork was composed of the cytoplasmic prolongations of fibroblasts and stellate reticulum cells, together with basement membrane-like material and collagen. The loose meshwork is supportive but is unable to impede the movement of cells in and out of the white pulp.

Nerves are also present in the white pulp particularly near muscular arteries and arterioles and occasionally in the capsule. These nerves are unmyelinated.[28] Ballantyne[29] has demonstrated that the splenic nerve fibers in these periarterial plexuses have butylcholinesterase activity, and no acetylcholinesterase. He suggests that the splenic nerves are therefore of the postganglionic-sympathetic type and probably regulate the flow of blood in the spleen.

The splenic capsule and trabeculae are similar in that both are composed predominantly of fibroblasts together with collagen and basement membrane-like material. Elastic tissue is found in both trabeculae and capsule but is more plentiful in the trabeculae. Smooth muscle cells have been described in both areas.[1] In this study these cells were not positively identified. In the capsule, the "muscle" cells could not be distinguished from cells which could be interpreted as mature or "resting" fibroblasts.[30] It is interesting to note that both the internal capsule surface and the surface trabeculae of the red pulp are lined by the endothelial cells and fenestrated basement membrane of a sinus. Cells such as marcophages, reticulum cells, and platelets are often seen between the basement membrane and the capsule or trabeculum, giving these structures the aspect of fibrotic Billroth cords. The outer layer of the capsule is lined by a single layer of mesothelial cells.[1]

Summary

Ultrastructural examination of osmium tetroxide perfused spleens failed to show any direct capillary-sinus or capillary-Billroth cord connections. However, the presence of fenestrations in the basement membranes throughout the red pulp, marginal zone, and periphery of the white pulp make the question of whether the circulation is open or closed of little functional significance. The fenestrations permit the free movement of plasma and cells between the sinuses and cords and between white pulp and marginal zones, thereby allowing contact between macrophages and damaged or aged blood cells, and between lymphocytes or reticulum cells and antigen-carrying macrophages. The marginal zone consists of elements of both white and red pulp and is delineated by the large marginal sinus from which the majority of red pulp sinuses arise. The Billroth cord is not a collapsed sinus or vascular space but a unique structure containing loose aggregates or macrophages, reticulum cells, and fibroblasts and partitioned by basement membrane-like and collagen material. The ultrastructural aspects of the splenic white pulp, arterial and venular systems, nerves, capsule, and trabeculae are also described.

References

1. MOORE, R. D., MUMAW, V. G., and SCHOENBERG, M. D. The structure of the spleen and its functional implications. *Exp Molec Path* 3:31–50, 1964.
2. BLOOM, W., and FAWCETT, D. W. In *A Textbook of Histology* (ed 9). Saunders, Philadelphia, 1968, p 408.
3. KRETSCHMER, R., SAY, B., BROWN, D., and ROSEN, F. S. Congenital aplasia of the thymus gland (Di George's syndrome). *New Eng J Med* 279:1295–1301, 1968.
4. MACNEAL, W. J. The circulation of blood through the spleen pulp. *Arch Path* 7:215–227, 1929.
5. BJÖRKMAN, S. E. The splenic circulation. With special reference to the function of the spleen sinus wall. *Acta Med Scand 128* (*Suppl.* 191): 1–89, 1947.
6. KNISELEY, M. H. Spleen studies. I. Microscopic observations of the circulatory system of living unstimulated mammalian spleens. *Anat Rec* 65:23–50, 1936.
7. MACKENZIE, D. W., WHIPPLE, A. O., and WINTERSTEINER, M. P. Studies on the microscopic anatomy and physiology of living transilluminated mammalian spleens. *Amer J. Anat* 68:397–456, 1941.
8. SNOOK, T. The guinea pig spleen. Studies on the structure and connection of the venous sinuses. *Anat Rec* 89:413–421, 1944.
9. SNOOK, T. The histology of vascular terminations in the rabbit's spleen. *Anat Rec 130*:711–723, 1958.
10. WEISS, L. A study of the structure of splenic sinuses in man and in the albino rat with the light microscope and the electron microscope. *J Biophys Biochem Cytol* 3:599–610, 1957.

11. Weiss, L. The structure of fine splenic arterial vessels in relation to hemoconcentration and red cell destruction. *Amer J Anat 111*:131–179, 1962.

12. Weiss, L. The structure of intermediate vascular pathways in the spleen of rabbits. *Amer J Anat 113*:51–59, 1963.

13. Weiss, L. The white pulp of the spleen. The relationships of arterial vessels, reticulum and free cells in the periarterial lymphatic sheath. *Bull Hopkins Hosp 115*:99–173, 1964.

14. Gomori, G. "Preparation of Buffers for Use in Enzyme Studies." In *Methods in Enzymology*, Colwick, S. P., and Kaplan, N. O., Eds. Acad Press, New York, 1955, p 138.

15. Luft, J. H. Improvements in epoxy resin embedding method. *J Biophys Biochem Cytol 9*:409–414, 1961.

16. Givan, K. F., Turnbull, C., and Jézéquel, A. M. Pepsin digestion of virus particles in canine hepatitis using epon-embedded material. *J Histochem Cytochem 15*:688–694, 1967.

17. Karnovsky, M. J. Simple methods for staining with lead at high pH in electron microscopy. *J Biophys Biochem Cytol 11*:729–732, 1961.

18. Venable, J. H., and Coggeshall, R. A simplified lead citrate stain for use in electron microscopy. *J Cell Biol 25*:407–408, 1965.

19. Watson, M. L. Staining of tissue sections for electron microscopy with heavy metals. *J Biophys Biochem Cytol 4*:475–478, 1958.

20. Crosby, W. H. Normal functions of the spleen relative to red blood cells: a review. *Blood 14*:399–408, 1959.

21. Koyama, S., Sadako, A., and Deguchi, K. Electron microscopic observations of the splenic red pulp with special reference to the pitting function. *Mie Med J 14*:143–164, 1964.

22. Sakuma, S. Electron-microscopic studies on arterial blood vessels of the spleen, especially on their relationship to the reticuloendothelial system. *Tohoku J Exp Med 94*:23–29, 1968.

23. Rifkind, R. A. Destruction of injured red cells in vivo. *Amer J Med 41*: 711–729, 1966.

24. Sokolloff, N. Über die veröse hyperamie der milz. *Virchow Arch Path Anat 112*:209–236, 1888.

25. Galindo, B., and Freeman, J. A. Fine structure of splenic pulp. *Anat Rec 147*:25–42, 1963.

26. Roberts, D. K., and Latta, J. S. Electron microscopic studies on the red pulp of the rabbit spleen. *Anat Rec 148*:81–102, 1964.

27. Goldschneider, I., and McGregor, D. D. Migration of lymphocytes and thymocytes in the rat. I. The route of migration from blood to spleen and lymph nodes. *J Exp Med 127*:155–167, 1968.

28. Galindo, B., and Imaeda, T. Electron microscope study of the white pulp of the mouse spleen. *Anat Rec 143*:399–416, 1962.

29. Ballantyne, B. The reticuloendothelial localization of splenic esterases. *J Reticuloendothel Soc 5*:399–411, 1968.

30. Movat, H. Z., and Fernando, N. V. P. The fine structure of connective tissue. I. The fibroblast. *Exp Molec Path 1*:509–534, 1962.

The authors would like to thank Professor A. C.. Ritchie for his critical review of the manuscript and Miss Robin Johnston, Mrs. Anne Evans, and Mr. Roger Lamb for their technical assistance. Mrs. Jean Cetkovski typed the manuscript.

Legends for Figures

Fig 1. Topographic aspect of white pulp demonstrates dominance and polymorphism of lymphocytes *(Ly)* and reticulum cells *(Rc)* which makes them often difficult to differentiate. These cells lie in clusters or nests which are loosely separated from surrounding nests by meshworks *(arrows)* composed of basement membrane-like material, collagen, and cellular prolongations of fibroblasts *(Fb)*. Lead hydroxide stain. × 7500.

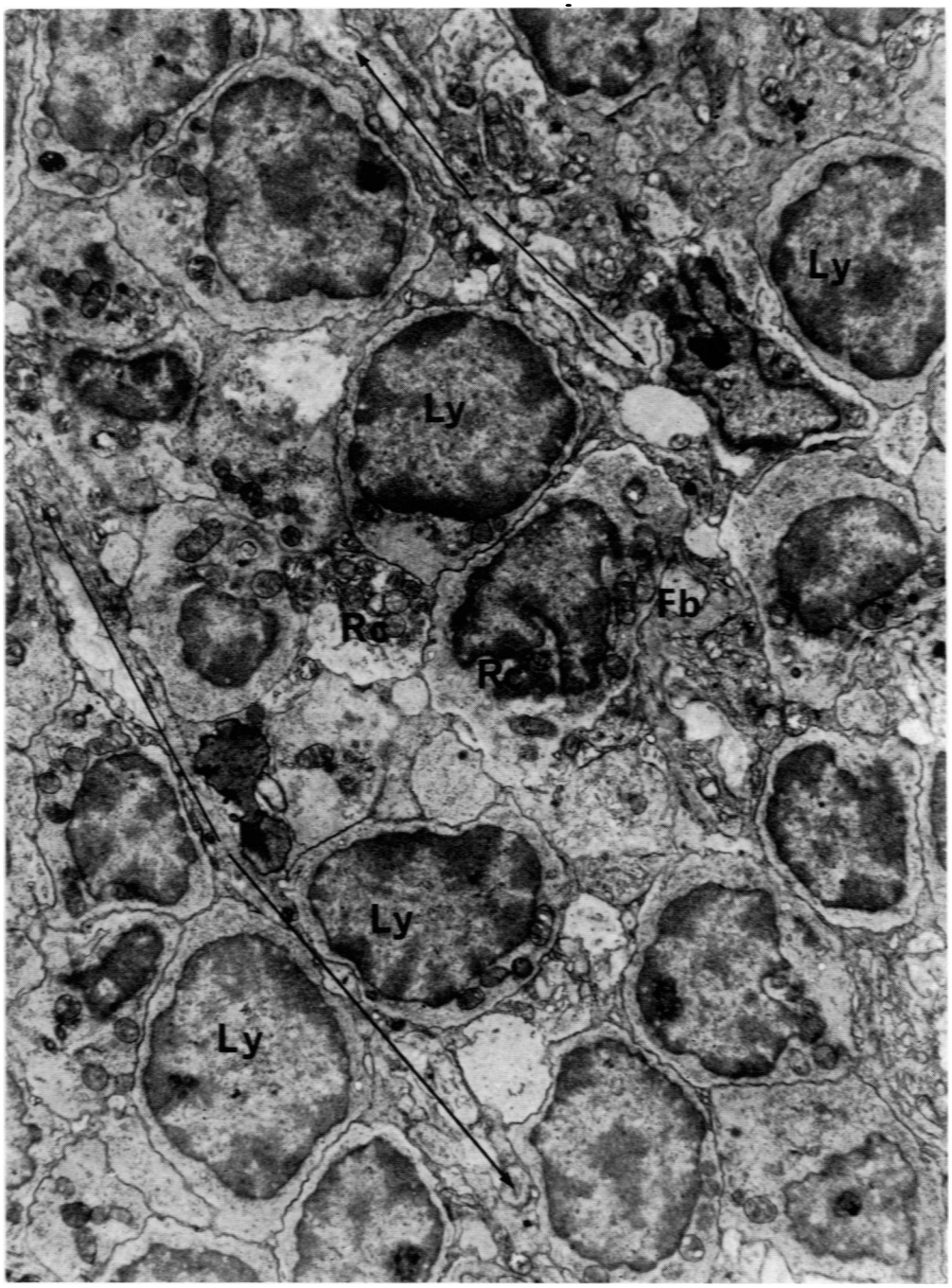

Ly
Ly
Ly
Fb
Rc
Rc
Ly
Ly

Fig 2. *(upper)* Detail of a meshwork of collagen *(c)* and basement membrane-like material *(bml)* with cytoplasmic prolongation of a fibroblast *(arrow)* which separates two lymphoid colonies. These colonies contain lymphocytes *(Ly)* and reticulum cells *(Rc)*. Lead hydroxide stain. × 12,000.

Fig 3. *(lower)* Reticulum cell of white pulp contains phagocytosed material *(arrow)* and is therefore designated a macrophage *(Ma)*. This cell is extending stellate projections *(large arrows)* into the intercellular spaces between surrounding lymphocytes *(Ly)*. Uranyl acetate. Lead hydroxide stain. × 13,000.

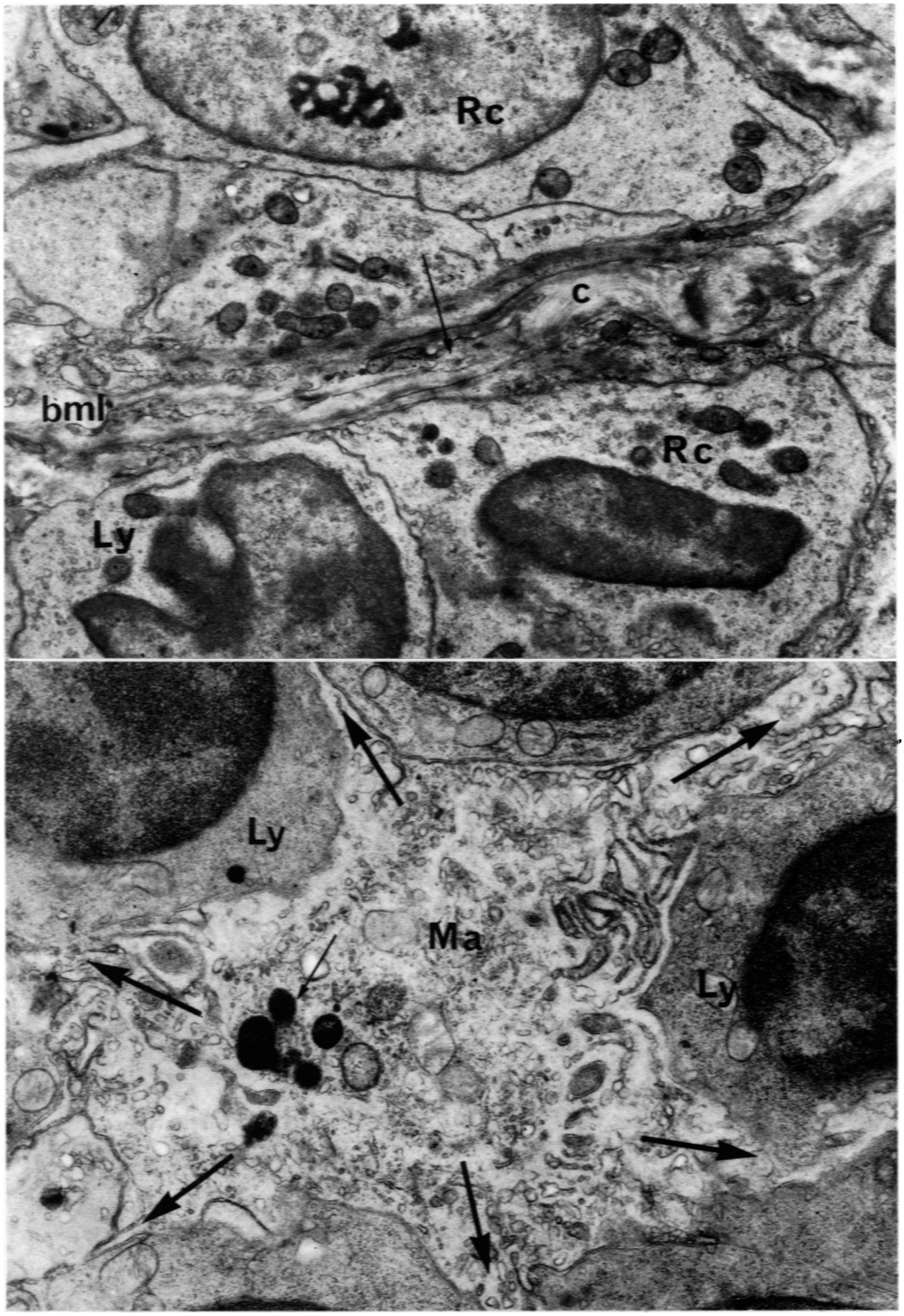

Rc
c
bml
Rc
Ly
Ly
Ma
Ly

Fig 4. Portion of fibroblast lies next to reticulum cell *(Rc)* in white pulp. Different steps in collagen formation are present. There is a prominent dilated rough endoplasmic reticulum complex *(rer)* which contains fine granular flocculent material—without fibril formation. Smooth endoplasmic reticulum *(ser)* is filled with similar material, and in addition contains fine fibrillar material, some of which appears to have a periodicity *(circle)*. At periphery of cell there are tonofibrils *(arrow)*. Nucleus *(N)*; Golgi zone *(G)*. Lead hydroxide stain. × 22,000.

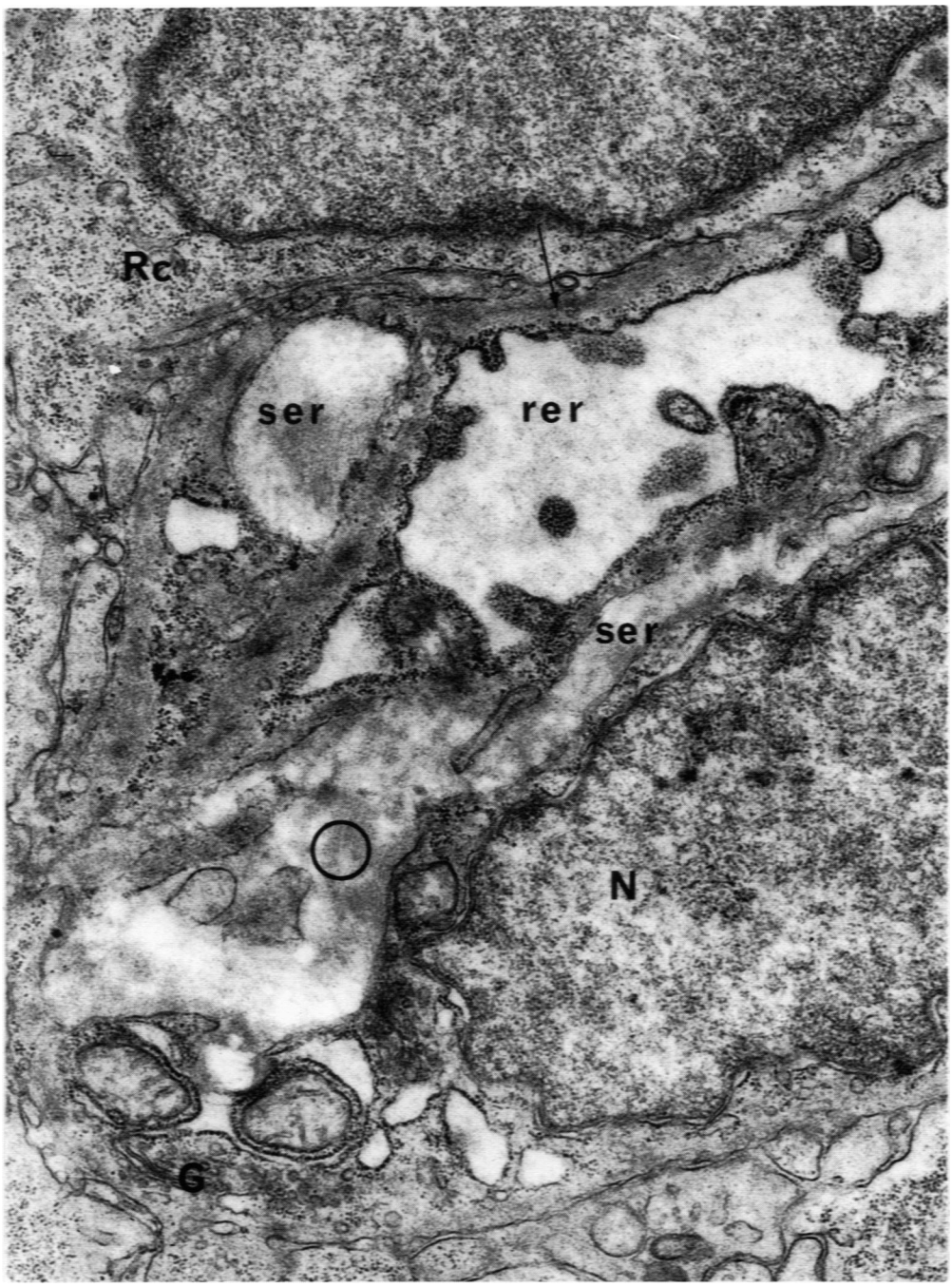
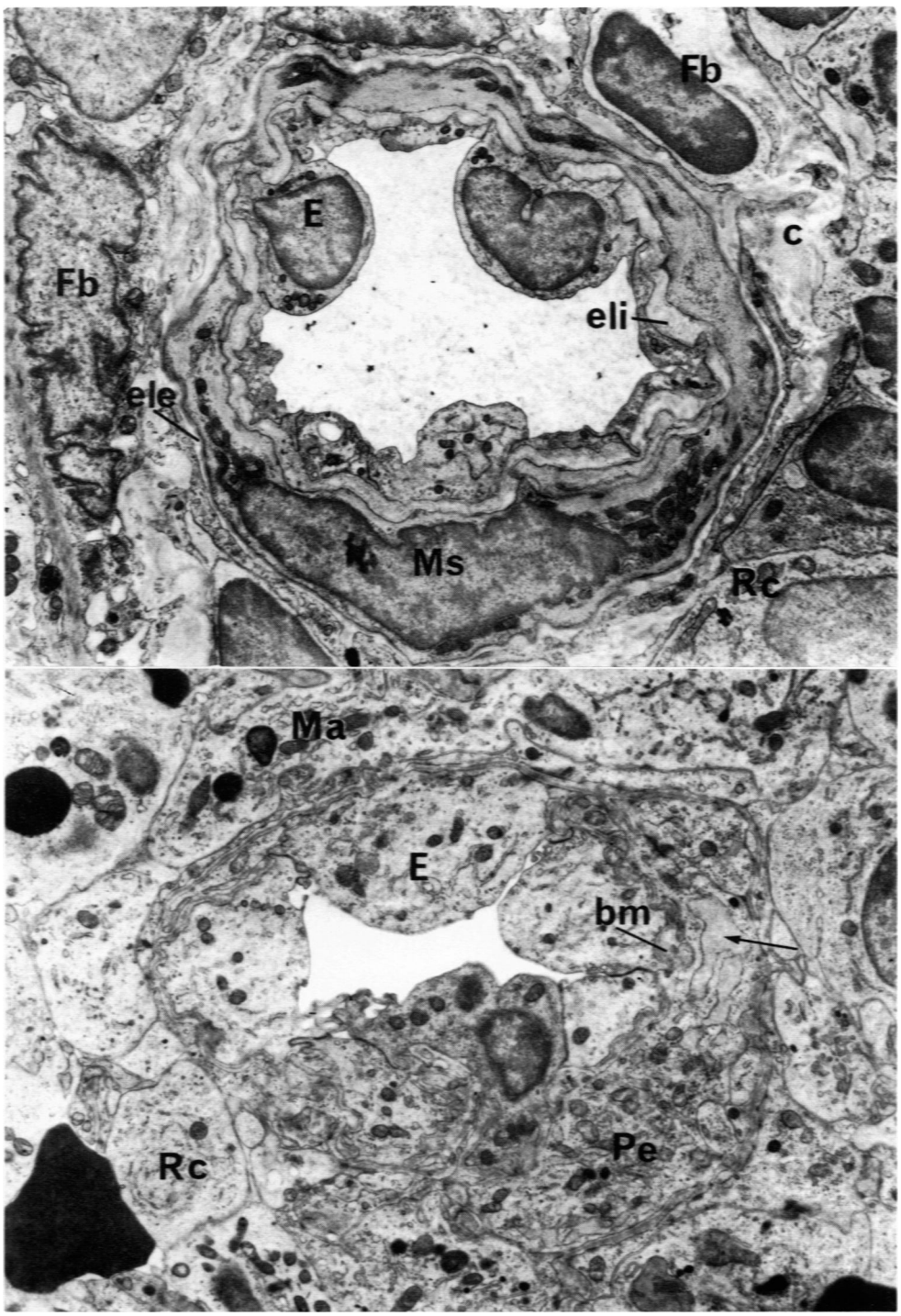

Rc
ser
rer
ser
N
G

Fig 5. *(upper)* Small muscular arteriole in white pulp is characterized by muscle layer formed by one continuous muscle cell *(Ms)*. Endothelium *(E)* is moderate in height and elastica interna *(eli)* is extremely irregular in thickness. Elastica externa *(ele)* is also irregular and merges in random fashion with surrounding collagen *(c)* of white pulp. Cells surrounding this arteriole are adventitial in location and appear to be fibroblasts *(Fb)* and reticulum cells *(Rc)*. Lead hydroxide stain. × 8000.

Fig 6. *(lower)* Small capillary in marginal zone has continuous basement membrane *(bm)* which is duplicated and envelops the pericytes *(Pe)*. Presence of large dilated rough endoplasmic reticulum *(arrow)* containing fine granular material gives this pericyte the appearance of a young fibroblast. Endothelium *(E)* is high in this small capillary. Surrounding the vessel are reticulum cells *(Rc)* and macrophages *(Ma)*. Lead hydroxide stain. × 8000.


Fb
E
c
Fb
eli
ele
Ms
Rc
Ma
E
bm
Rc
Pe

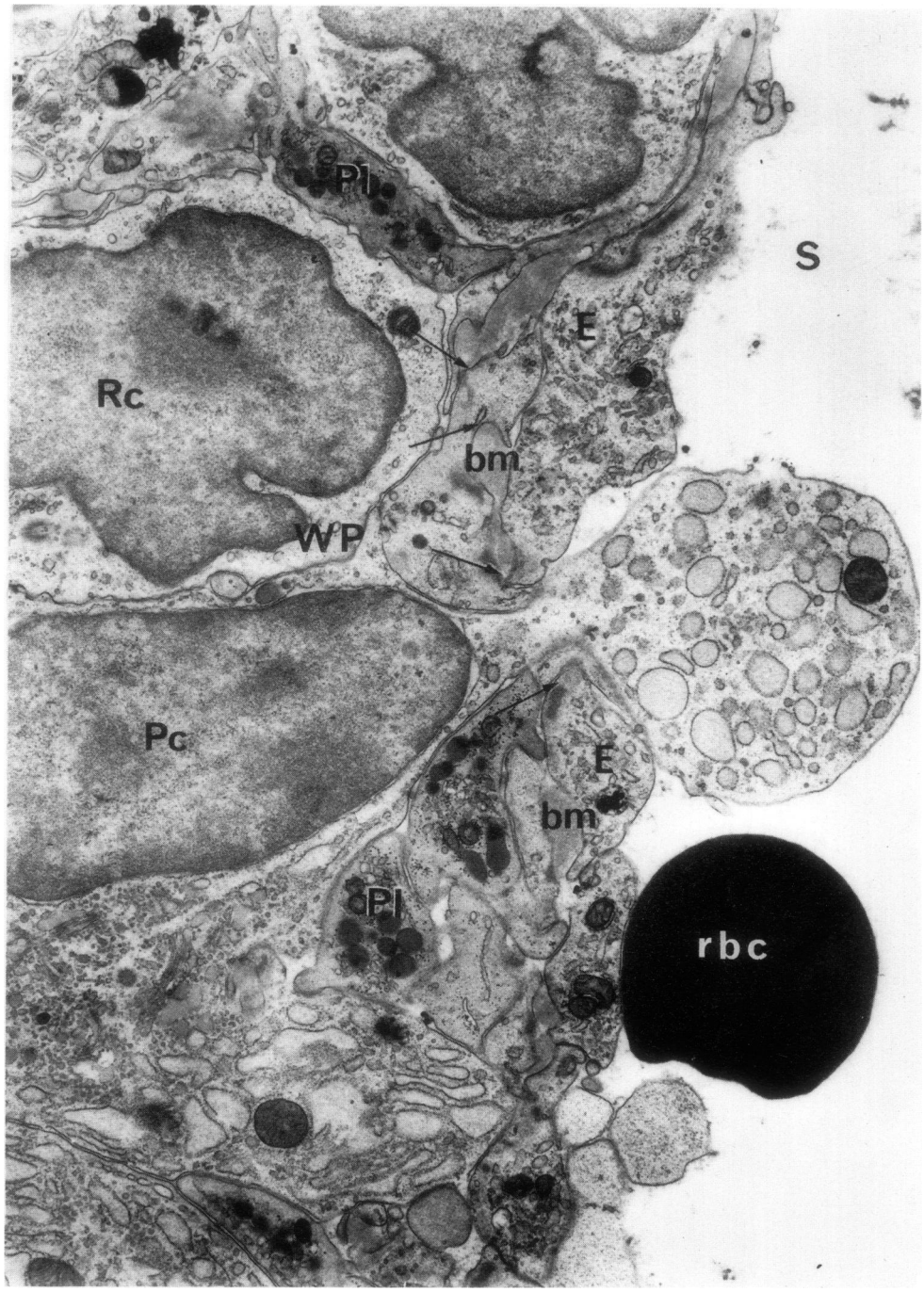


Fig 7. Sinus *(S)* of the marginal zone is lined by endothelial cells *(E)*. Between endothelial cells and adjacent white pulp *(WP)* is an interrupted *(arrows)* basement membrane *(bm)*. Plasma cell *(Pc)* is seen with portion of its cytoplasm passing through one of fenestrations in basement membrane. Other cells seen in periphery of white pulp are platelets *(Pl)* and a reticulum cell *(Rc)*. Red blood cell *(rbc)*. Lead hydroxide stain. × 14,000.

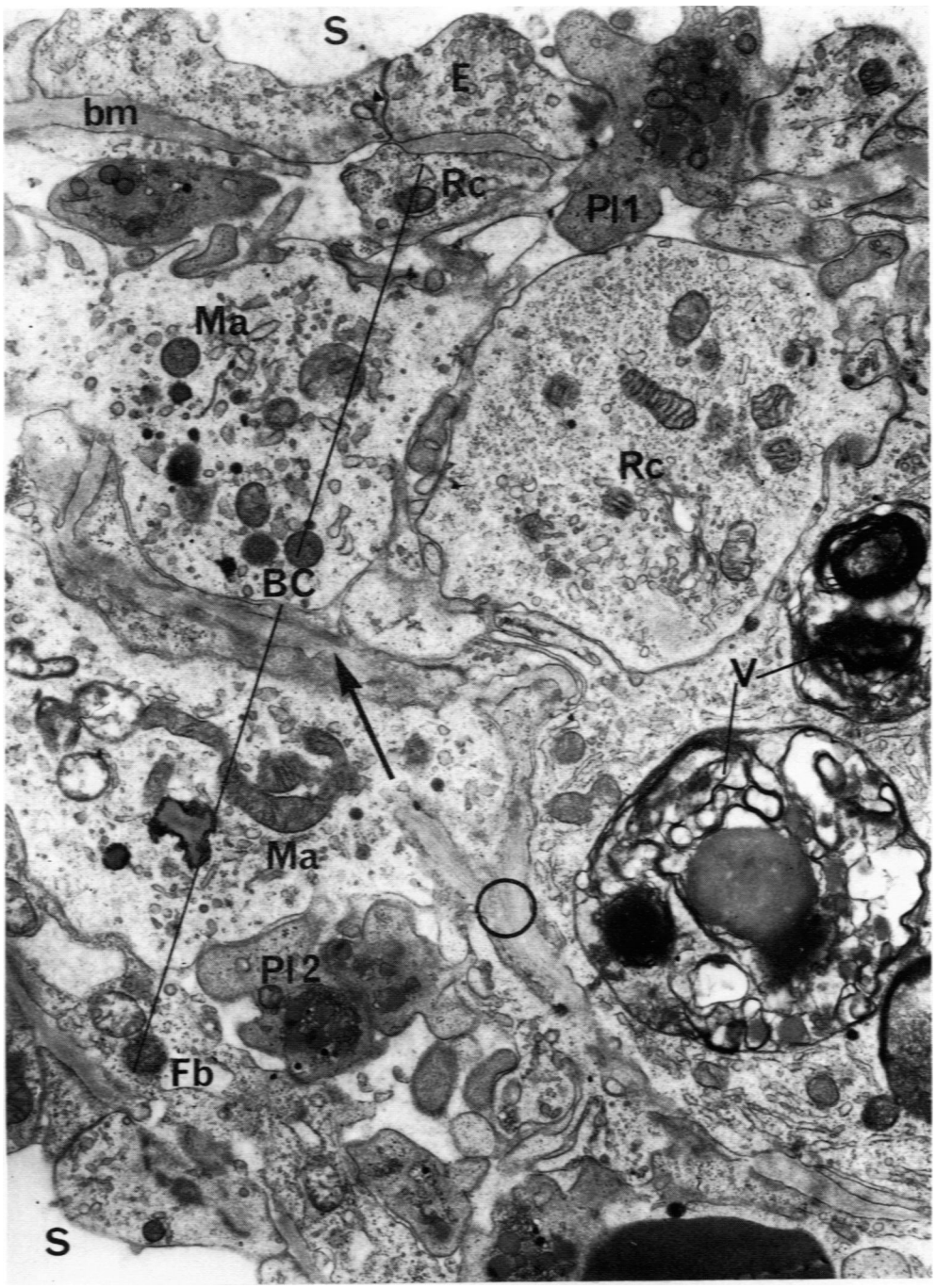


Fig 8. Cross-section of a Billroth cord *(BC)* is seen between two sinuses *(S)* in red pulp. Within cord are mainly reticulum cells *(RC)* and macrophages *(Ma)*, one of which contains large vacuoles *(V)* with debris from degradated leukocytes and/or erythrocytes. Fibroblast *(Fb)* is located in cord at bottom of picture subjacent to basement membrane of one of the sinuses. Crossing Billroth cord in random fashion is extracellular reticulum *(large arrow)* which has appearance both of basement membrane and protocollagen because of focal fibrillar and periodic structure *(circle)*. Also present are platelets *(Pl_1)* lying in gap of sinus wall and within cord *(Pl_2)*. Endothelial cells *(E)*; basement membrane *(bm)*. Lead hydroxide stain. × 15,000.

Fig 9. Sinus *(S)* demonstrates various appearances of its endothelial cells. One endothelial cell *(E_1)* bulges into lumen with large round nucleus *(N)* at its tip. Another cell *(E_2)* appears cuboidal without nucleus. This appearance probably represents section of prolongation of stellate endothelial cell. In both cases cytoplasm is similar and contains small round mitchondria *(M)* with few cristae, and multiple small empty vacuoles. Close to lumen, the vacuoles resemble pinocytotic vacuoles *(pv)*. Some of endothelial cells contain large vacuoles *(V)* both empty and with fine granular material. Basement membrane *(bm)* is erratic in thickness and demonstrates fenestrations *(between arrows)*. Billroth cord *(BC)*. Lead hydroxide stain. × 7500.

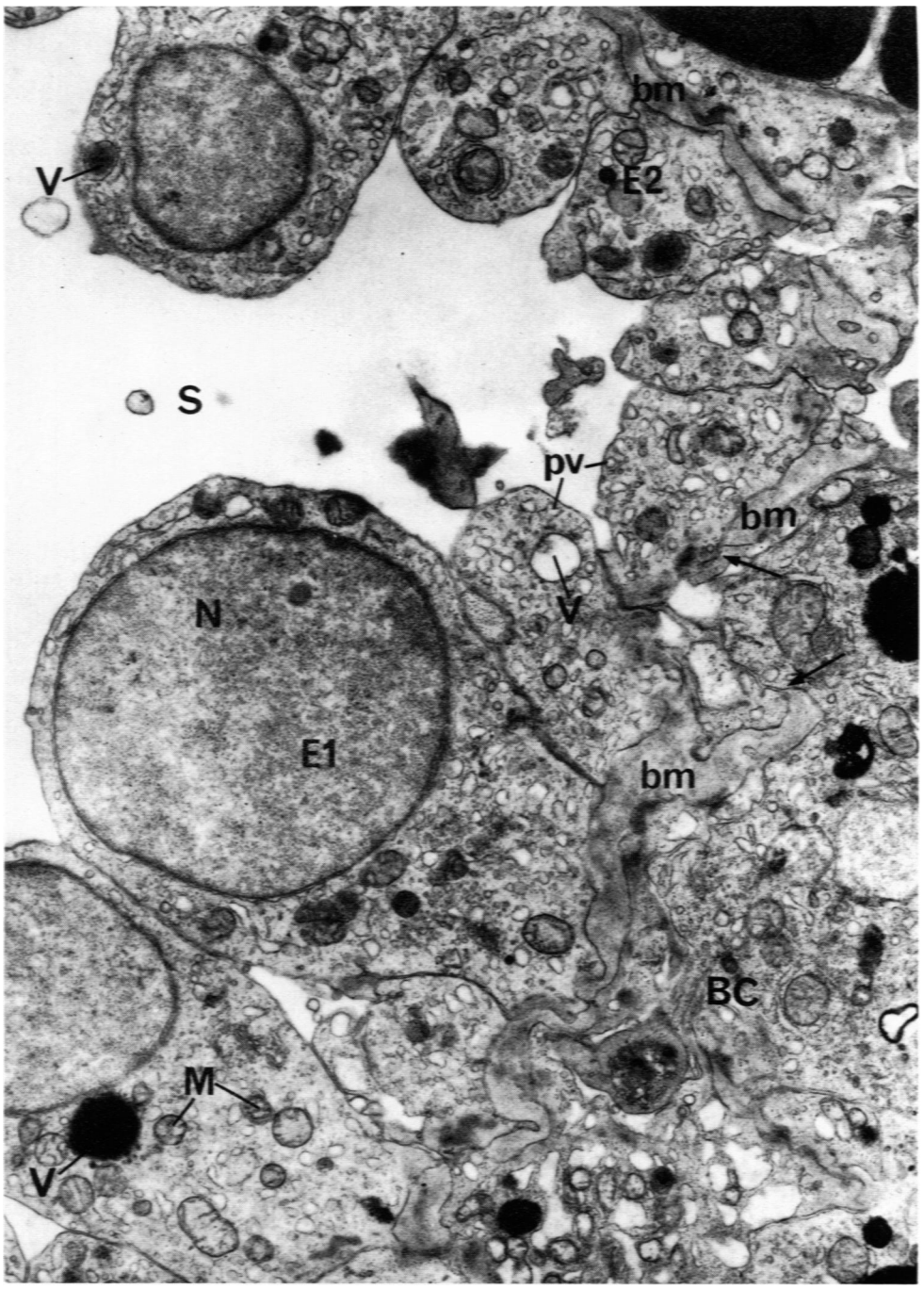

bm
V
E2
S
pv
bm
N
V
E1
bm
BC
M
V

Fig 10. *(upper)* In Billroth cord *(BC)* nucleated portion of stellate reticulum cell *(Rc)* is seen against basement membrane *(bm)* of sinus *(S)*. This reticulum cell has prominent Golgi zone *(G)* with characteristic vesicles, but elsewhere cytoplasm is rather sparse. This is in contrast with prominent vesicles *(arrows)* in endothelial cells *(E)*. Reticulocyte *(ret)*. Lead citrate stain. × 14,000.

Fig 11. *(lower)* Plastic or elastic quality of red blood cell *(rbc)* is shown as it passes through two apertures of basement membrane *(arrows)*. Two globular portions of cell are in a sinus *(S)*, and attenuated connecting portion lies deep to basement membrane *(bm)* in Billroth cord. Endothelial cells *(E)*; reticulum cell *(Rc)*. Lead hydroxide stain. × 17,000.

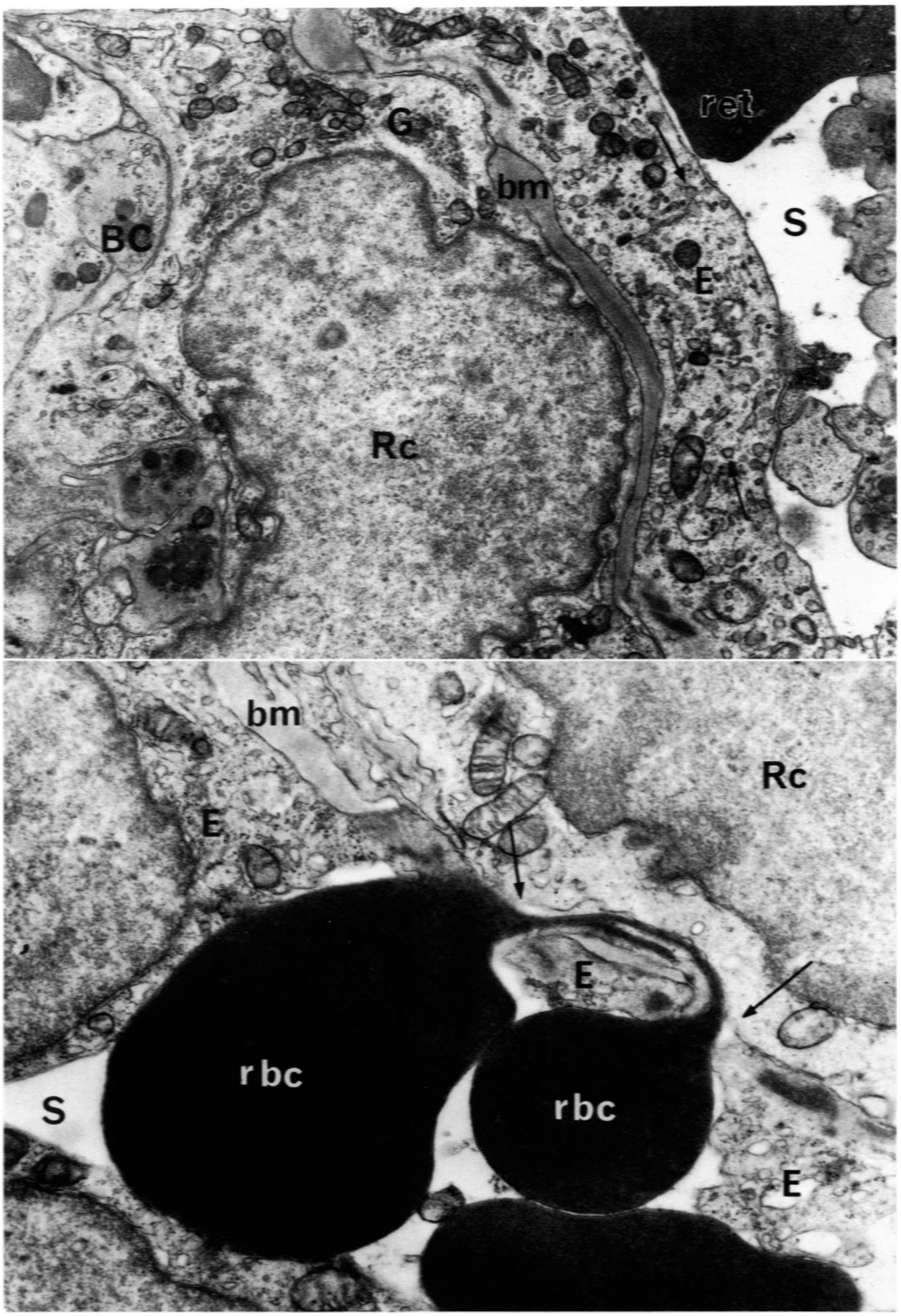

ret
G
bm
BC
S
E
Rc
bm
Rc
E
E
rbc
rbc
S
E

Fig 12. Portion of trabecula *(TR)* is seen between two sinuses *(S)* in red pulp. Trabecula contains mainly fibroblasts *(Fb)* but also contains some macrophages *(Ma)* and reticulum cells *(Rc)*, one of which is seen protruding into lumen of sinus. The intercellular spaces of trabecula are filled with collagen *(c)*, elastica *(el)* and basement membrane-like material *(bml)*. Note that normal appearing endothelial cells *(E)* and interrupted basement membrane *(bm)* line both sinuses. Lead hydroxide stain. × 7500.

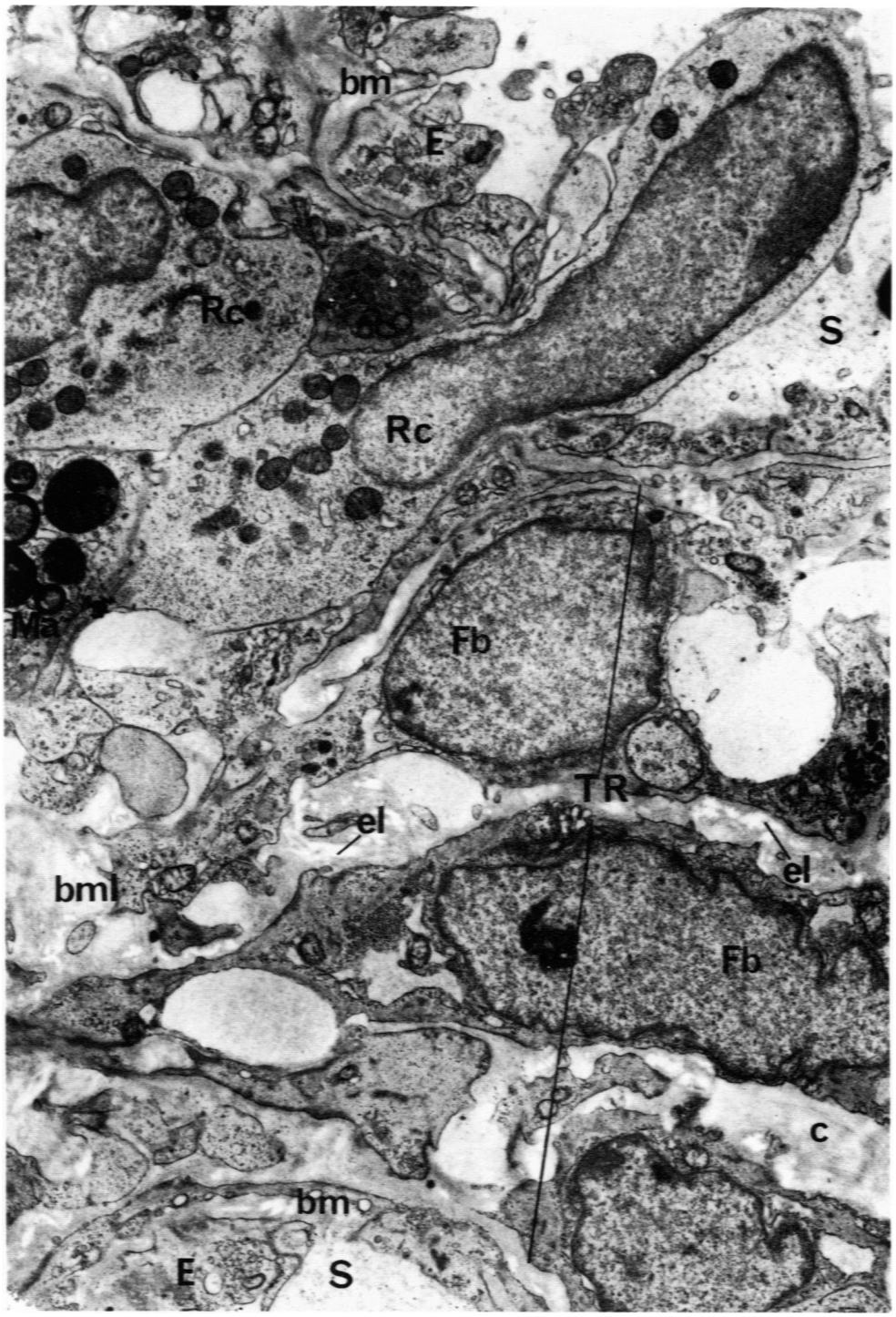
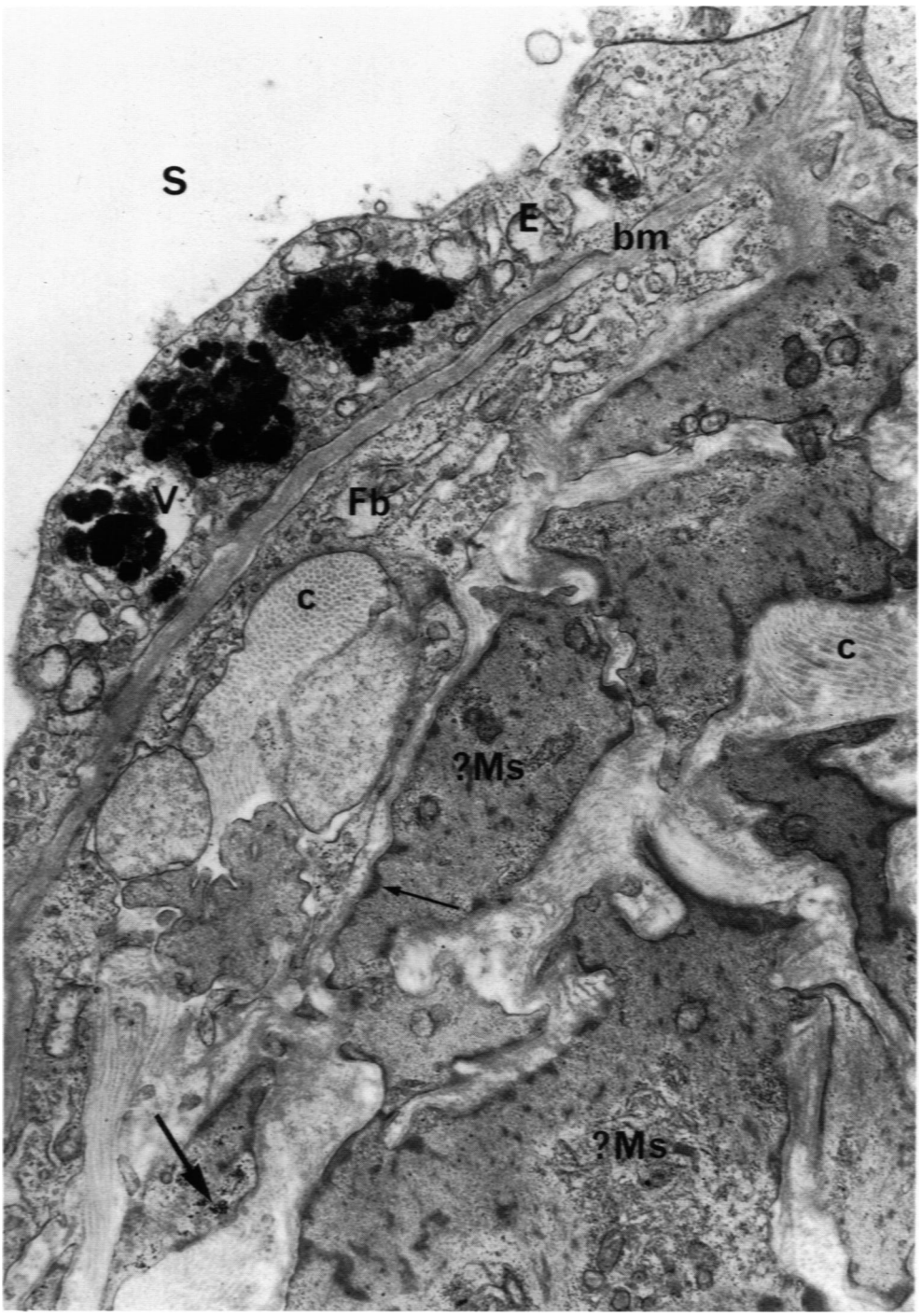

bm
E
Rc
S
Rc
Ma
Fb
TR
el
el
bml
Fb
c
bm
E
S

Fig 13. Typical aspect of capsule is seen with its subcapsular sinus *(S)*. Capsule is formed primarily of stellate irregular cells *(? Ms)* entangled in bundles of collagen *(c)*. These stellate cells have dark fibrillar cytoplasm. Their mitochondria are small and few in number with poorly developed cristae. Dark cytoplasmic granules *(large arrow)* suggest presence of glycogen. Close to cell membrane irregular triangular densities are present *(arrow)*. This cytoplasmic appearance is similar to that of smooth muscle cells. However, extreme irregularity and complex involvement with collagen suggest that these cells may be old fibroblasts. A young fibroblast *(Fb)* is present along basement membrane *(bm)* beneath subcapsular sinus. Endothelial cell *(E)* contains multiple vacuoles *(V)* with very dense granular inclusions. Lead hydroxide stain. × 14,000.


S
E
bm
V
Fb
c
c
?Ms
?Ms