Transmural Cellular Passage in Vascular Sinuses of Rat Bone Marrow

By LEON WEISS

The structure of vascular sinuses in rat bone marrow is modified as cells cross their wall. Endotoxin, by causing withdrawal of granulocytes from the circulation, induces a compensatory discharge of fresh granulocytes through the wall of sinuses. As a result the sinus wall becomes infiltrated by clusters of late-stage hematopoietic cells which appear in transmural passage. Two correlates of such infiltration are elevation of adventitial cells from the wall and the creation of adventitial spurs extending into perisinusal hematopoietic spaces. In untreated rats adventitial cells cover, on the average, about 65 per cent of the sinus wall. After 0.04 μg. endotoxin the cover is reduced, fitting a slope regressing at the rate of 0.22 per cent/min. (t = 3.1038, p = < 0.003), the last determinations made at 120 minutes. Values for the percentage of adventitial cover calculated from observations after endotoxin are 52.5 per cent in 90 minutes and 32 per cent in 120 minutes. Even after endotoxin, a number of sinuses retain full cover while a number of control sinuses, likely reflecting normal cell passages, have little adventitial cover. Endothelial cells form a continuous layer. Apertures occur in the wall normally and increase in number after endotoxin. These apertures may contain cells, apparently in passage, or may be free of cells. Lysosomal disruption occurs in mural cells, particularly in segments of wall associated with cellular passage and with gelatinous change.

This paper deals with the adaptations of vascular sinuses in the bone marrow of rats to the transmural passage of late-stage hematopoietic cells from hematopoietic compartments toward the blood. Drawing upon both normal rats and rats after short-term mild stimulation by endotoxin, new observations and inferences are presented upon the penetrance of the walls of sinuses by hematopoietic cells, variations in adventitial cover, and the formation of adventitial spurs extending into perisinusal hematopoietic spaces.

Terminology

The vessels reported upon here have been termed vascular or venous sinuses or sinusoids. Their trilaminar structure in the rat has been recognized and identified from lumen out as a simple layer of lining cells or littoral cells, a basement membrane, and a simple layer of adventitial cells. Adventitial cells have not been described in the chicken and pigeon or in the guinea pig. The terms lining cells or littoral cells in place of endothelium are of long-
standing use because their layer is different from endothelium as occurs in most other places. Nonetheless, endothelium has gained some currency. I have chosen not to call the cells lying within or crossing the sinus wall, evidently destined for the blood, blood cells both because they are not yet in the blood and because they may not be mature. I refer to them as mature leukocytes or late-stage hematopoietic cells, as appropriate.

Cellular exchange between hematopoietic and circulating compartments of bone marrow occurs through the walls of vascular sinuses. The thickness, density, and other characteristics of each of the three mural layers are subject to abrupt and irregular variation. Lining cells may be attached to one another by tight junctions. On the other hand, adventitial cells, which form an incomplete layer, display junctional complexes only unusually. Cytoplasmic processes, or even whole cells, may extend from the adventitial layer into perisinusal hematopoietic spaces. As a result, the hematopoietic space is, to some degree, compartmentalized. The basement membrane is typically the most defective mural element, being absent over broad stretches. Sinus walls may possess apertures. DeBruyn, Thomas and Michelson, in a study of the guinea pig, stress that these are present where active migration of blood cells through the sinus wall occurs. In the chicken and pigeon, Campbell found that pores were present only in association with blood cells passing through the lining cells: he regards apertures without cells as artifacts.
Fig. 1.—A vascular sinus occupies most of the lower portion and right side of this field. It is closely surrounded by tightly packed hematopoietic cells. A lymphocyte lies in the lumen. The adventitial layer is complete, with the exception of two places (see tracing). The adventitial cell at the lower left corner is voluminous, its cytoplasm undercut (arrow). Both in this cell, and in two others about the circumference, clear irregular membrane-bounded spaces (*) are present. These may be disrupted lysosomes. The endothelium displays a characteristic variation in thickness. The basement membrane has the density and texture of hemoglobin and its substance resembles that of the lumen and of the perisinus extracellular space. The absence of granular membrane, and the marked density of the basement membrane area, of the plasma and of other extracellular spaces, as seen here and in Figs. 2 and 7, may be due to washing out of the basement membrane and lysis of some erythrocytes. Control animal (× 15,200).
Fig. 2.—A vascular sinus runs across the top of the plate. Its endothelium varies considerably in width, being thickest in its nucleated portion. A cell rich in endoplasmic reticulum, likely a plasma cell, appears to press into the under surface of the nucleated endothelial cell. The adventitial layer is incomplete. Note the extensions of adventitial cells into the perivascular hematopoietic tissue. Several of these extensions contain cytoplasmic filaments. The hematopoietic cells are tightly packed. Little extracellular space is present. Four minutes after endotoxin (× 7100).
The sinus wall is responsive. In the massive delivery of reticulocytes and erythroblasts into the circulation during recovery from a phenylhydrazine-induced acute hemolytic anemia, in experimental hepatitis in dogs, and after irradiation deficiencies in basement membranes and adventitial layers become pronounced, apertures develop in the walls, and rearrangements in the contours of the sinuses appear to occur.

Endotoxin damages intravascular granulocytes, perhaps by lysozyme action due to labilization of lysosomes. Such granulocytes are cleared from the blood by the spleen and other reticuloendothelial organs. In compensation, the bone marrow rapidly releases granulocytes to the circulation from its myelocytic reserves. I have used endotoxin in this study to increase the magnitude of the exodus of granulocytes from the marrow.

**MATERIALS AND METHODS**

The bone marrow of rats was examined at intervals after the animals were given endotoxin. The details are as follows.

**Animals**

Eleven male and 16 female Sprague-Dawley albino rats each weighing 150-250 Gm. were used.

**Administration of Endotoxin**

A single injection of 0.04 µg. of S. typhosa lipopolysaccharide B. (Difco Labs., Detroit, Mich.) was made intraperitoneally.

**Fixation and Embedding**

Rats were anesthetized with ether and femoral marrow removed four minutes, 20 minutes, 90 minutes and 120 minutes after the administration of endotoxin. Control marrows were taken without exposure to endotoxin. When marrow was taken for electron microscopy, white blood counts and blood smears for differential counts were made from blood from the retroorbital plexus. Marrow smears and splenic imprints were also made. The smears and imprints were stained with May–Gruenwald Giemsa stain. The marrow was handled in one of several ways.

In six animals the femur was quickly removed and cleanly cracked open.

In four animals the femoral cortex, in an anesthetized animal, was gently scraped away. The marrow so exposed was flooded with fixative for eight to 12 minutes. Then the bone was removed and placed in fixative.

In five animals the procedure recommended by Campbell was used; the cortex was scraped away until only a thin plate of bone remained. Then, after cutting about its perimeter, the plate was lifted off. The whole bone was then removed and placed in fixative.

In 12 animals parenchymal perfusion, a new technic of fixation was employed. It likely bears a relationship to the deep in situ fixation used by DeBruyn et al. The details of parenchymal perfusion are as follows:

A rat is anesthetized with ether and placed in a lateral position so that the thigh is flexed, its lateral aspect uppermost. The skin over the thigh is reflected. The femoral diaphysis is now very close to the surface. By easy blunt dissection the entire lateral femoral diaphysial surface is exposed. Now a hole is drilled through the cortex into the medullary cavity at each end of the exposed diaphysis with an electrically powered dental or hand drill. There may be some bleeding from one or both holes. A No. 19 needle, the bevel ground off, is inserted into one of the holes. The fit is tight. The needle is the open end of a system of syringe and polyethylene tube completely filled with fixative. There are no air bubbles. The syringe is placed in a pump (No. 255-1 Sage Instruments, White Plains, N. Y.) and the fixative pumped into the medullary cavity at the rate of 0.5 ml./min., or slower. Immedi-
Fig. 3.—See opposite page for legend.

ately on starting the pump, blood wells out of the open hole. A mixture of fixative and blood follows, and within 30 seconds the effluent is grossly clear and evidently fixative. In occasional attempts, fixed plasma or, less frequently, some marrow tissue is expressed through the open hole for the first minute of the perfusion. About 15–25 ml. fixative is pumped through. The animal’s vital signs during this period are stable. There are no tremors or convulsions. Only the thigh muscle which is bathed in effluent fixative twitches. The time from skin incision to the outflow of fixative from the medullary cavity may take no more than 30 seconds.

If the line AD represents the femur, B the point of the needle insertion, and C the outflow point, i.e., A—B—C—D, three zones may be recognized. BC is the zone through which the fixative flows. AB and CD are contiguous zones which include the epiphyses. The marrow is fixed from A-B judged by firmness and color and subsequent microscopy. What appears to happen in most instances is that the fixative dissects its way between the marrow and the endosteum in segment BC. In some instances the perfusate reaches the central longitudinal vein or some of the vascular sinuses and flows through them. In other instances the perfusate tears a passage through the parenchyma, rendering a portion of the marrow cylinder too damaged for use. Segments AB and CD are typically well fixed and often minimally disturbed. Under the electron microscope, the cells are usually well fixed and tightly packed, and most of the vascular sinuses and other blood vessels contain blood or hematopoietic cells.

One consequence of perfusing the living animal with the volumes of fixative used here is that certain substances which are liquid or fragile in the living state may be washed out or distorted. Thus, the basement membrane may be washed away and some erythrocytes may be hemolyzed. If the animal is permitted to die and a few minutes let go by—as would ordinarily happen in immersion fixation of blocks—these changes do not occur. The
Fig. 3.—In the sinus which occupies most of this field the adventitial layer is incomplete. On the left a granulocyte is pressed against the abluminal surface of lining cells. On the right at the top, an erythroblast presses upon the vessel. Below it another granulocyte appears to displace adventitial cells. Between the erythroblast and the granulocyte lies a rarefied portion of an adventitial cell which may be undergoing lysis (arrow). An adventitial cell below this granulocyte retains its attachment to the wall but, in its upper portion, curves away from the wall (see tracing). A segment of endothelium on the left (arrow) is thinned but no aperture is evident. Twenty minutes after endotoxin (× 10,400).
Fig. 4.—A sinus, containing a reticulocyte, lies on the left. The layer of lining cells, as does the basement membrane, varies in thickness and density. Note the clear vacuole (arrow) and compare with similar structures in Figs. 1, 2 and 8. The granulocyte on the right lies against the abluminal surface of the lining cell layer. At its upper aspect the granulocyte sends a tongue-like process that fits about an endothelial cell and reaches into the basement membrane (arrow). The basement membrane has a fibrillar character. The cytoplasm in the upper right corner is that of an adventitial cell separated from the wall by two granulocytes. Ninety minutes after endotoxin (× 31,500).
effluent from the beginning is colorless and evidently fixative. The marrow is still excellently fixed but the basement membrane resembles that described by other technics and hemolysis is minimal. Evidently coagulation has occurred post-mortem which stabilizes some elements to the perfusate.

Karnovsky's formaldehyde-glutaraldehyde mixture was used in most fixations. In nine of the parenchymal perfusions this mixture was diluted 1:4 with water, as recommended by Karnovsky. Three perfusions were performed with picric acid-paraformaldehyde-glutaraldehyde mixture.

In five animals, including two from Campbell's procedure, osmium tetroxide was the primary fixative.

On completion of any of the above procedures a length of marrow was removed from the bone, care being taken to avoid deformation, and cut into cross-sections 1–2 mm. thick. That marrow which had no preliminary fixation was placed in gluteraldehyde-formaldehyde. In the other cases the cross-sections were placed in the fixative to which they had been exposed. The tissue remained in gluteraldehyde-containing fixatives 4–12 hours, was then cut into smaller blocks as necessary, the smallest dimension being 1 mm., and placed in one per cent osmium tetroxide buffered to pH 7.4 with cacodylate for two hours. The blocks were rinsed, dehydrated, infiltrated with epon and placed in the tip or cover of a Beem (Bronx, N. Y.) capsule for embedding. Blocks were oriented, in the latter case, to permit sectioning in relationship to any desired marrow axis.

Sectioning

Colorless or faint gray sections were cut with a DuPont (Wilmington, Del.) or Rondkin (Honolulu, Hawaii) diamond knife in a Sorvall-Porter Blum II (Norwalk, Conn.) microtome. Thicker sections (1–4 μ) were cut with glass knives and stained with toluidine blue for light microscopic study.

Electron Microscopy

Sections were stained with lead citrate and uranyl acetate and examined in a Siemens Elmiskop I.

Photography

Electron Image plates (Kodak, Rochester, N. Y.) were developed in HRP developer (Kodak) and enlarged 1–5X in a Durst S 45 enlarger (Italy).

Cell Counts

Leukocyte counts of the blood and differential cell counts on Giemsa stained smears of blood, marrow and spleen were made.

Observations

The results described below were made by study of the marrow perfused with Karnovsky's dilute fixative. By extensive comparisons, similar results were apparent with the other methods of preparation. Only in parenchymal perfusion fixation, however, was there regularly very little extracellular space in the hematopoietic compartments.

Hematopoietic cells were clustered close upon sinuses (Figs. 1–3,8). The cells closest to the vessels were erythroblasts of intermediate to late stages of maturation, late-stage granulocytes, and megakaryocytes.

Adventitial cells lay against the basement membrane and, in the absence of the membrane, against the abluminal endothelial surface (Figs. 1, 3–5,7). The adventitial layer could fail to cover completely the outside of the sinuses. Gaps in the adventitial cell layer were typically filled with late-stage hematopoietic
cells or mature leukocytes (Figs. 1–8). The extent of the adventitial cover was determined by measuring, by means of a map measure, the lengths of both the endothelial layer and the adventitial layer along the perimeter of sinuses in electron micrographs at total magnifications of 1300–3200. The ratio of adventitial and endothelial lengths was expressed as a percentage and tabulated in blocks of 10 or 11 percentiles for each of the following groups: controls and four minutes, 20 minutes, 90 minutes and 120 minutes after endotoxin. A total of 60 sinuses was measured. The results are presented in Fig. 9.

The adventitial layer was subject to the abrupt and irregular variation in volume, density and vesiculation described earlier (Figs. 1,3,7). There was some evidence of phagocytosis. The cells contained rough endoplasmic reticulum and many small round vesicles. In perhaps 20 per cent of the adventitial cells, particularly in the perisinusal processes, filaments or tubules were observed (Figs. 2,8). Large irregular lucent vesicles, limited by unit membrane, were present in adventitial cells, particularly in those whose cytoplasm was both voluminous and rarefied (Figs. 1–4,7,8). Lysis of a mural cell was occasionally observed. The presence of an adventitial layer could be difficult to recognize due to: 1) rarefaction of adventitial cytoplasm so that it appeared to be extracellular where molded by free cells pressed against it; 2) displacement of adventitial cells away from basement membrane and endothelium and 3) extensive defects in the adventitial coat.
Fig. 5—In this field a small cluster of free cells lies between an adventitial cell and the basement membrane. The lumen and the endothelium of the sinus runs along the left margin of the field. The adventitial cell, for most of its extent, is displaced from the wall by two granulocytes and two reticulocytes. At its upper and lower ends, however, the adventitial cell has the normative position on the basement membrane (see tracing). Ninety minutes after endotoxin (× 15,000).
Fig. 6.—The lumen of a sinus lies at the top of this field and is occupied by reticulocytes. Reticulocytes, moreover, have entered the wall and separate the lining cell layer (which has gaps at the arrows) from the adventitial layer (Adv). Three erythroblasts press upon the adventitial cell. One hundred twenty minutes after endotoxin ($\times 22,000$).

Where a gap occurred in the lining cell layer, an underlying adventitial cell would present upon the lumen, assuming endothelial function.

The basement membrane was markedly defective. Like the adventitial layer it was subject to irregular and abrupt variations in thickness and density (Figs. 3,4). It often had the granular structure characteristic of reticular fibers and of the basement membrane in splenic sinuses and in other vessels.$^{23-25}$ In many
Table 1.—Incidence and Content of Mural Apertures in Sinuses after Endotoxin*

<table>
<thead>
<tr>
<th>Time after Endotoxin (in minutes)</th>
<th>0</th>
<th>4</th>
<th>20</th>
<th>90</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Sinuses Measured</td>
<td>10</td>
<td>20</td>
<td>4</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>Total Length of Wall (in mm. real wall)</td>
<td>0.55</td>
<td>0.99</td>
<td>0.25</td>
<td>0.72</td>
<td>0.59</td>
</tr>
<tr>
<td>Total Number of Apertures</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Apertures without Cells</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Apertures per Mm. Wall</td>
<td>1.8</td>
<td>2.0</td>
<td>4.0</td>
<td>4.2</td>
<td>10.1</td>
</tr>
</tbody>
</table>

* Incidence of apertures, expressed as number of apertures/millimeter wall, increases with time after endotoxin. Approximately half the apertures physically contained a cell. Size of apertures free of cell varied between approximately 0.1 μ to 0.3 μ. Total length of sinus wall studied in this work was 3.10 mm.

other places, however, particularly in territories active in cellular transport, the zones of the basement membrane were occupied by material resembling plasma or hemoglobin (Figs. 1,2,7). The granular basement membrane was the most deficient of the mural layers.

The endothelium was the most consistently represented of the mural layers. It was subject to the same variations as the adventitial cells, but the variations were neither as widespread nor as pronounced as in adventitial cells.

**Mural Apertures**

Apertures occurred in the walls of sinuses. They more often occurred in zones where the lining cells remained the only mural element than where the wall was trilaminar. The number of apertures, accordingly, was increased after endotoxin. Apertures would contain a cell, presumably in passage, or be free of any cell. The results obtained by counting apertures along measured lengths of wall are presented in Table 1. The initial observations were made at magnifications of 1300–3200×. Portions containing apparent apertures were also photographed at magnifications of 10,500 and enlarged two to four times to confirm that apertures did in fact exist.

**Adventitial Processes**

(Figs. 2,3,7,9)

The processes were usually well developed where there appeared to be active cellular transit through the sinus wall. These processes often contained filaments and tubules (Figs. 2,8).

**Effects of Endotoxin**

Reference has been made above to the displacement or disappearance of adventitial cells and to the increase in number of apertures after endotoxin. The structure of myeloid granulocytes, as determined in electron micrographs, was not affected by endotoxin. Within the two hours that followed the injection, intraperitoneally, of 0.04 μg. endotoxin a moderate leukopenia occurred. Granulocytes accumulated in the spleen, rising from control values of approximately 18 per cent to approximately 35 per cent in two hours. There was no significant change in the proportions of myeloid cells.

I observed no cytologic changes distinctive to endotoxin-treated animals.
Instead, after endotoxin, larger areas of the sinus wall displayed changes, also present in controls, associated with the passage of cells across the sinus wall.

**Discussion**

Cellular traffic between vascular and hematopoietic compartments of bone marrow is large scale. The intravascular granulocytes of small laboratory mammals number in the order of $35 \times 10^6$ cells and most of these turn over every few hours. Further, at least $1.0 \times 10^9$ erythrocytes are likely supplied to the circulation each day. The exodus of myeloid leukocytes may be increased by endotoxin, that of erythrocytes by phenylhydrazine or other hemolytic agents. Thus, while it may be experimentally useful to increase the rate of cellular passage, it is clear that active and extensive passage of cells through the sinus wall normally occurs.

The extent of adventitial cell cover appears inversely proportional to the volume of transmural cellular passage. In animals where the marrow sinus adventitial cover is more deficient than in the rat, the possibility must be entertained that cellular traffic is greater. Those considerable areas of wall free of adventitial cells in normal rats are taken by late-stage hematopoietic cells and mature leukocytes: the free cells may, in fact, be regarded as occupying a place in the wall. The adventitial layer may be deficient because adventitial cells disappear—perhaps by lysis—round up, retract processes—or, because they are separated from the basement membrane and endothelium by clusters of hematopoietic cells. Such separated adventitial cells may be found deep in the hematopoietic compartments. The likeliest explanation of these findings is
Fig. 7.—In this field, a long segment of the adventitial layer is separated from the wall of a vascular sinus and extends deep into the surrounding hematopoietic space. The lumen of the sinus is on the right and contains two granulocytes. On the left, in relationship to a lymphocyte which lies against the endothelial layer (see tracing), the adventitial layer extends into and beyond the left upper quadrant of the field. The contours of the adventitial layer appear determined by the hematopoietic cells pressed upon it. One hundred twenty minutes after endotoxin (× 10,400).

that hematopoietic cells are wedged beneath the adventitial layer and force the adventitial cells outward. Indeed, in this manner, adventitial processes extending from the sinus wall into the perisinus tissue are created. The data may, of course, be equally well interpreted that adventitial cells primarily move out, creating adventitial processes, and thereby bring hematopoietic cells under their cover. In regions of mural infiltration the substance of basement membrane, as has been described,23,24,25 is usually absent, apparently dissolved or washed out. (See above discussion on fixation.) Infiltrating hematopoietic
Fig. 8.—The lumen of a sinus, its wall incomplete, lies on the left below. Extending upward is an adventitial cell forming an adventitial spur which extends into the perivascular hematopoietic space. Note the large clear vacuoles (arrows) and the cytoplasmic filaments (arrow). Granulocytes, reticulocytes and on the right a small part of an erythroblast complete this field. Twenty minutes after endotoxin (× 27,000).
cells thus become separated from the lumen primarily by the layer of littoral cells. This layer will then thin, break down, and the cells in passage enter the lumen of the sinus. It is evident in this material, as it has been in work previously reported, that cells in the sinus lumen may not be fully mature. They may, nonetheless, be delivered into the blood stream, or they may be delayed in sinuses, released only on maturation.

After exposure to endotoxin, the area of the adventitial layer is reduced. Since endotoxin induces a movement of the marrow's granulocytic reserve into the blood, the reduction in adventitial cover after endotoxin and the presence of hematopoietic cells in the adventitial layer supports the conclusion that adventitial cells are displaced by cells on their way to the blood. It must be noted, however, that variation in adventitial cover was present both in un-
treated animals and after endotoxin. Clearly, even after stimulation by endotoxin a number of sinuses remain inactive in cellular transport while in control animals, a number of sinuses are extensively active.

The first hematopoietic effect of endotoxin is to reduce the circulating granulocytic pool (CGP) by damaging these cells,14,15 perhaps by lysosomal damage and lysozyme action.13 These cells are then sequestered from the circulation by the spleen and other tissues. The marrow compensates by releasing granulocytes to the CGP from its maturation and storage pools of metamyelocytes and mature granulocytes.14,15 The effects of endotoxin as discerned in this study are explicable in this sequence. Specifically the results are explicable as an increase in the movement of granulocytes from hematopoietic compartments across the sinus wall into the sinus lumen. A leukocytosis or leukopenia may occur in the CGP depending on the rate of egress of granulocytes from the CGP, the rate of entrance of granulocytes into the CGP, and the balance of the exchanges between the two vascular pools of granulocytes, namely, the CGP and the marginated granulocytic pool (MGP).14,15

In the animals treated with endotoxin in the present work, leukopenia and splenic sequestration occurred within two hours succeeded by leukocytosis in four hours. While a movement of hematopoietic cells into the wall occurred, no significant differential exit of granulocytes from the marrow took place as determined by differential cell counts.

The adventitial cells in the rat may affect the entrance of hematopoietic cells into the blood. Adventitial cells are capable of becoming fatty.1,2 But short of fatty change they may become rarefied and voluminous and gelatinous. Fatty or gelatinous, they encroach upon the volume of the hematopoietic space and in the rigidly boxed myeloid cavity displace hematopoietic cells. Since the resistance of the vascular compartment is likely relatively low, the cells would be displaced to the blood. This movement would occur even were the swelling cells distant from the mobile cells. The action may be direct where hematopoietic cells lie between littoral and adventitial layers. Here adventitial swelling would press the hematopoietic cells lumenward against the abluminal surface of the lining cells. The process would be enhanced moreover if mural cells are contractile as their filaments suggest.

Lining cells and adventitial cells are cytologically similar and lining cell and adventitial layers (save for adventitial spurs) are similar. In short, in many segments of sinuses there is an absence of luminal-abluminal structural polarity. This is underlined by the presence, along the sinus wall, of adventitial cells presenting upon the sinus lumen, assuming an endothelial role, where gaps occur on the overlying lining-cell layer. The absence of polarity also makes plausible the concept of the reworking of vascular contours and the assumption by the adventitial processes of endothelial functions. Such an assumption is exemplified in the following progression: adventitial cells are displaced from the wall and thereby form at least a partial enclosure about hematopoietic cells pressed into the wall. These hematopoietic cells are then separated from the lumen by but a segment of endothelium. Should that endothelial segment become attenuated and drop out, there would simultaneously occur: the hema-
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topoietic cells though remaining in place be set into the lumen, the displaced adventitial cells become lining cells, and the vessel assume new contours.

The large clear irregular vacuoles in mural cells, particularly in voluminous rarefied adventitial cells, likely represent disrupted lysosomes. This inference is supported by the resemblance of these vacuoles to those in granulocytes where the relationship to lysomes is unmistakable. This suggests that the voluminous rarefied mural cell may result from the action of the lytic lysosomal enzymes released into the cytoplasm. Carried farther, actual cell lysis may occur by this mechanism; apparent examples of this have been seen. While lysosomal damage followed the administration of endotoxin it was also present in control animals, particularly where cellular transport occurred.

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REFERENCES


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