Venous Endothelial Damage Produced by Massive Sticking and Emigration of Leukocytes

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A scanning and transmission electron microscope study of canine jugular and femoral veins revealed that large numbers of white cells adhered to the vessel walls, passed through the endothelial intercellular junctions and accumulated in pockets between the endothelium and basement membrane. This led to extensive separation and desquamation of endothelial cells with exposure of subendothelial structures in many areas. The white cell invasion was caused by surgical trauma to adjacent tissues (rather than to the vessel itself) followed by 1 or 7 minutes occlusion by pressure applied externally just below the areas of dissection. The invasion was blocked by lidocaine (Xylocaine®), an agent known to inhibit white cell migration. However, white cell invasion occurred when veins in lidocaine-treated dogs were perfused with normal blood. No white cell invasion occurred in dogs made neutropenic with vinblastine but did occur when these veins were perfused with normal blood (Am J Pathol 74:507–532, 1974).

IN THE NORMAL HEALTHY ANIMAL, leukocytes in the blood flow freely with little attraction to the vessel wall. However, under various pathologic conditions many leukocytes are attracted to the vessel wall. In small vessels, especially venules, this is expressed by the sticking of white cells to the walls and their subsequent migration across the vessel wall and into the surrounding tissues. This has been recognized as part of the response of tissues to sublethal injury since the inflammatory process was described in the nineteenth century.[1] It was later recognized that white cells contribute to the damage of tissues in the affected area by releasing a number of cytotoxic substances.[2] However, there is no evidence that white cell passage per se causes serious damage to these vessels.[3] In many instances of the inflammatory response, the attraction of white cells to the wall of small vessels appears to be the result of injury to adjacent tissue rather than to the vessel itself.[4]

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However, it appears that in large vessels, leukocytes may respond to injury of the cells composing the vessel wall. Thus, the wall itself rather than surrounding tissues is the site of the inflammatory response. In some instances, such as periarteritis nodosa, leukocytic infiltration of the vessel wall occurs as a secondary event with the original injury to the vessel being due to trauma, infection or an allergic reaction.[5] Leukocytic infiltration of the wall of the rabbit inferior vena cava was observed following partial obstruction for 2 hours.[6]

In a recent study we found evidence for another association of white cells with the vessel wall, previously not described. By using a combination of scanning and transmission electron microscopy we found that white cells stick to medium-sized veins, emigrate across the endothelium and accumulate in pockets between it and the basement membrane.[7] This results in extensive separation of endothelial cells from each other and from the basement membrane leading to endothelial desquamation with exposure of the subendothelial surface. In this case, the white cells appeared to be directly responsible for initiating the injury to the vessel wall itself since there was no morphologically detectable injury in the absence of white cell invasion. This is in contrast to the role of white cells in association with small vessels where, as previously mentioned, apparently little damage resulted.

In the present study we were concerned with the means by which white cell invasion of the vessel wall could be induced experimentally in healthy, well-cared for dogs and with proving that white cell invasion was the means for producing the endothelial damage. The use of scanning electron microscopy allowed us to examine the entire surface of each vessel at magnifications ranging from 100 to 10,000 times and with a resolution of 200 Å or better. Transmission electron microscopy of the same material allowed us to identify relevant cells and fibers. The role of white cells in producing the observed pathology was confirmed by using Xylocaine to inhibit white cell migration and vinblastine to produce severe neutropenia.

Materials and Methods

Surgical Exposure and Perfusion of Veins

"Conditioned" dogs weighing between 11 to 20 kg were anesthetized with intravenous sodium pentobarbital (32.5 mg/kg) and tracheostomies were performed. The jugular and femoral veins were carefully dissected free from their surrounding tissues in the neck and hind limb respectively.

In the neck, the terminal 2 to 3 cms of the external and internal maxillary veins which join to form the proximal external jugular vein and the jugular vein itself

down to the level just below the point of entry of the proximal communicating branch of the cephalic vein were dissected out. All of the small side tributaries, including the proximal communicating branch of the cephalic vein, were ligated and divided leaving an 8- to 12-cm length of exposed external jugular vein.

The femoral veins were exposed in the superficial femoral triangle and dissected out from a point just proximal to the junction of the medial saphenous vein to the level of the inguinal ligament where the femoral is joined by the deep femoral vein. The caudal and cranial femoral veins and all other tributaries were ligated and divided. A 2- to 3-cm segment of the medial saphenous and more proximal femoral vein were also exposed leaving a segment of vein 4 to 6 cm in length.

The jugular and femoral veins were prepared for perfusion by cannulation of the external maxillary or medial saphenous vein respectively while allowing blood flow to continue through the larger veins. Perfusion with Tyrode's solution at 20 cm H_2O pressure was started and the incoming blood flow was then interrupted by ligation of the internal maxillary or femoral vein respectively upstream to the infusion; this allowed clearance of all the blood from the exposed veins. When the blood was washed out of the vein segment, the downstream end was cannulated. Controlled distension of the vein segment was accomplished by altering the level of the outflow catheter which was maintained at 10 cm above the level of the vein. The vein was washed out with 250 ml of Tyrode's solution prior to *in vivo* fixation by perfusion with 250 ml of 1% gluteraldehyde in Tyrode's solution. The vein segments were then ligated between the perfusion catheters while distension was maintained and excised. The excised veins were then pinned out on boards, correcting as much as possible any small longitudinal shrinkage that had occurred following excision. They were then placed in 1% gluteraldehyde in Tyrode's solution for further fixation.

Preparation of Veins for Scanning and Transmission Electron Microscopy

After pinning out, the excised veins were submerged in the same gluteraldehyde solution for 1 to 3 days in the cold. They were then opened longitudinally, spread out and tied to glass microscope slides, luminal side up, by wrapping ordinary sewing thread around the slide and vessel. These were placed in 2.5% gluteraldehyde in 0.1 M cacodylate buffer, pH 7.4, for 24 hours in the cold. Then the gluteraldehyde was rinsed off briefly with cacodylate buffer and replaced with 1% osmium tetroxide in 0.2 M cacodylate buffer. This was held in the cold overnight. The excess osmium fixative was removed by rinsing briefly with distilled water. The samples were dehydrated with three changes of pure ethanol, the first two for at least 1 hour each and the last one overnight. The alcohol was replaced by amyl acetate according to the same schedule. The samples were dried from liquid carbon dioxide in the Denton critical point apparatus according to the method of Anderson.[8]

Some samples were taken after the veins were dried but before they were gold coated. Still others were taken after coating and examining in the scanning scope. These samples were re-dehydrated with ethanol and processed through propylene oxide and Epon.

A JSM-2 or JSM-50A scanning and a Philips 300 transmission electron microscope were used for examining specimens.

Experimental Groups

The basic method of surgery and perfusion was modified to produce three groups of experiments. These consisted of dogs receiving no medication other than anesthesia, those receiving lidocaine and those rendered neutropenic by vinblastine treatment.

Group 1 (No Treatment)

These dogs received no medication other than anesthesia. This group was divided into three subgroups consisting of: A, control veins which were exposed and perfused as described above; B, veins in which stasis was produced prior to any surgery by occlusion of the downstream end of the veins by percutaneous pressure for 7 minutes; and C, veins that were dissected out and then occluded for 1 or 7 minutes. All of the veins were then prepared as previously described.

In the remaining two groups the effects of lidocaine pretreatment or vinblastine-induced neutropenia on the response to occlusion of veins after surgical exposure and dissection were studied. These veins were subsequently perfused, fixed and excised as described before.

Group 2 (Lidocaine)

This group was divided into three subgroups and consisted of dogs which were either given lidocaine (Xylocaine®, Astro Co) systemically (A and B) or whose veins were perfused with blood incubated with lidocaine (C). In the first two subgroups the dogs were given 20 mg lidocaine intravenously prior to surgery and an infusion of lidocaine of 2 mg/min was maintained until surgery was completed. No surgery was performed for 30 minutes after the initial injection of lidocaine. In subgroup A, control veins were obtained by using the standard method of preparation and the remaining veins were occluded for 1 or 7 minutes after dissection. In subgroup B half of the veins were occluded while containing lidocaine-exposed blood and the remaining veins were perfused with and occluded while containing normal blood which had been withdrawn from the dog prior to the administration of lidocaine. In the third subgroup of normal nontreated dogs, 50 ml of blood was withdrawn and incubated with 5 ml of sodium citrate and 25 mg of lidocaine for a minimum of 20 minutes. These veins were then perfused with and occluded for 1 or 7 minutes while containing blood that had been incubated with lidocaine.

Group 3 (Vinblastine)

Dogs were rendered neutropenic by injecting vinblastine (Eli Lilly Company) (0.2 mls/kg) intravenously, and the veins were subjected to the experimental procedures only when the polymorphonuclear neutrophil count was less than 1000 cells/cu mm. There were two subgroups. In the first subgroup, A, nonoccluded control veins in the neutropenic dogs were obtained and the remaining veins were subjected to 1 or 7 minute occlusion while containing neutropenic blood. In the second subgroup, B, the veins of neutropenic dogs were perfused with and occluded for 1 or 7 minutes while containing normal blood obtained from healthy dogs. The remaining veins were similarly occluded while containing the dog's own neutropenic blood.

These groups and their subdivisions are summarized in Table 1.

Results

The endothelium and underlying cells and fibers were well preserved in the material that was dried by the critical point method and then reprocessed for thin sectioning. This was indicated by the well preserved cell organelles including the caveolae of the endothelium and the periodicity of the collagen. However, a comparison of these vein sam-

Table 1—Summary of Veins Examined and Degree of Vessel Wall-White Cell Association

	Jugular	Femorals	None to sparse	Patchy	Uniformly heavy	Total
Group 1 (No medication)						
A Surgery, no occlusion	5	6	10	0	1	11
B Occluded prior to surgery	6	0	2	3	1	6
C Occluded after surgery	5	5	0	0	10	10
Group 2 (Lidocaine-treated dogs)						
A Lidocaine-treated animals with own blood						
Surgery, no occlusion	3	4	7	0	0	7
Occluded after surgery	4	3	7	0	0	7
B Lidocaine-treated animals with normal blood						
Occluded with own lidocaine-treated blood after surgery	2	2	4	0	0	4
Occluded with normal blood after surgery	2	2	0	0	4	4
C Normal animal with lidocaine-incubated blood						
Occluded with normal blood after surgery	2	2	0	0	4	4
Occluded with lidocaine-incubated blood after surgery	2	2	4	0	0	4
Group 3 (Vinblastine-induced neutropenic dogs)						
A Vinblastine-treated dogs with own neutropenic blood						
Not occluded	3	2	5	0	0	5
Occluded after surgery	2	2	4	0	0	4
B Vinblastine-treated animals with normal donor blood						
Occluded with own neutropenic blood after surgery	2	2	4	0	0	4
Occluded with normal donor blood after surgery	2	2	0	0	4	4

ples with conventionally processed vein samples shows that the material of intermediate electron density was lost probably during exposure to amyl acetate (Figure 1). Nevertheless it is apparent that the structural components responsible for topographic characteristics had remained largely unaltered during critical point drying. However, as

might be expected by the prolonged exposure of the sample to 23kV, electrons that penetrate some distance into the sample, material that had been examined with the scanning scope showed considerable electron damage (Figure 6B).

During this study white cells were observed sticking to the luminal surface of blood vessels in the process of passing between endothelial cell junctions and accumulated in pockets between the endothelium and basement membrane. The accumulation of these pockets of white cells resulted in the formation of extensive, confluent elevations of the endothelial sheet, separation of endothelial cells along their boundaries and desquamation of patches of endothelium. As the samples were examined it became apparent that it was difficult to accurately quantitate the extent of these associations of white cells with the vessel wall. However, it was possible to obtain a realistic estimate of the condition of the entire luminal surface. This was done by estimating the extent of both sticking and accumulation of white cells and using this as a basis for grouping the veins into three general categories (Table 1).

In some samples white cell sticking and endothelial whealing were extensive, while in others there were whealing, separation and desquamation of endothelium with white cells visible through the gaps but with very few white cells sticking to the surface. Apparently, these represented different stages of the movement of white cells across the endothelial sheet. Cell separation leading to the formation of gaps and the desquamation of patches of endothelium was inevitably associated with the massive accumulation of white cells between the endothelium and basement membrane.

White cell sticking and the separation and desquamation of endothelium were absent in veins from dogs treated with lidocaine or made severely neutropenic by vinblastine.

A general survey of the results of each group is presented in the following few paragraphs while more detailed information is provided in the figure descriptions. The results are summarized in Table 1.

Group 1 (No Medication)

These dogs received no medication other than sodium pentobarbital. The group was divided into controls and two experimental subgroups, one with occlusion of the vein prior to surgery and one with occlusion after surgery. In the controls there was no occlusion while blood was in the vessel.

Scanning electron microscopy revealed that in ten of eleven controls the luminal surface was a smooth sheet covered with endothelial cells

with slightly protruding nuclei and just discernible cell boundaries. The entire luminal surface of four of these veins was free of any defect, while that of six veins showed occasional single white cell invasion (Figure 2). One vein showed extensive white cell invasion similar to that described later for occluded vessels and shown in Figure 4.

In one set of experiments, six jugular veins were occluded for 7 minutes prior to surgery. The luminal surface of one vein was entirely free of defects. Another vein had pseudopods and occasional single white cell invasion as seen in Figure 2. Three veins showed scattered patches of single white cell invasion and another vein showed moderately heavy white cell invasion over approximately half the surface (Figure 3). The other half of this vein was sparsely invaded.

In the other set of experiments five jugulars and five femorals were occluded after surgical exposure. In all ten cases there was heavy invasion of the vessel wall by white cells. This was shown by extensive sticking of white cells to the endothelium and massive accumulation of white cells between the endothelium and basement membrane that frequently resulted in endothelial desquamation with exposure of the basement membrane (Figure 4). This was confirmed by transmission electron microscopy (Figures 5 and 6). In all cases the valves were completely spared.

These results show that the response of white cells to trauma or a combination of trauma and stasis exhibited considerable individual variability. Certainly Group 1 was radically different from Group 3. Group 2 was characterized especially by "patches of single cell invasion" which were easily recognized.

Group 2 (Lidocaine)

There were three sets of experiments in this group. In the first group the dog was given an intravenous drip of lidocaine. Veins were prepared without occlusion and with occlusion following surgery. The three jugulars and four femorals prepared without occlusion were morphologically indistinguishable from their counterparts from animals receiving no Xylocaine and showed minimal white cell sticking and invasion (Figure 7A and B). When the vein was occluded for 1 or 7 minutes there were a few white cells between the endothelium and basement membrane (Figure 7C and D). This was in contrast to the massive accumulation occuring in dogs without lidocaine (Compare with Figure 4).

In another set of experiments the dogs were given an intravenous

drip of lidocaine and both jugulars and both femorals were exposed. One jugular and one femoral were occluded for 1 or 7 minutes before perfusion (*ie*, both the veins and the blood were exposed to lidocaine prior to and during occlusion). The contralateral vein was perfused first with Tyrode's solution, then for 5 minutes with normal blood (removed before starting lidocaine), occluded for 1 or 7 minutes and then processed as usual. As before, the veins which contained the dog's own Xylocaine-exposed blood during occlusion showed very sparse white cell invasion. However, despite the fact that the blood had been kept outside the body for an hour or more, there was extensive white cell invasion in the vessels that were perfused with normal blood (Figure 8A and B). The ultrastructure of the vein appeared to be normal (Figure 8C).

In the third set of experiments with lidocaine, blood was removed from a normal animal to use for perfusion of exposed veins. Lidocaine was added to this blood and incubated for 20 minutes. One jugular and one femoral were exposed as usual while containing the dog's own blood. The contralateral vein was perfused with blood incubated with lidocaine. Each vein was occluded for 1 or 7 minutes. In the veins containing normal blood during occlusion, white cell invasion was as extensive as that shown in Figure 4. However, in the veins with blood incubated with lidocaine there was minimal invasion similar to that shown in Figure 7.

Group 3 (Vinblastine)

There were two sets of experiments involving vinblastine. In one set one jugular and one femoral were processed without occlusion while the contralateral veins were occluded for 1 or 7 minutes after surgery. There was no difference between the occluded and nonoccluded veins. White cell invasion was absent in both. The veins also appeared normal in thin section (Figure 9).

In the other set of experiments one vein was occluded while filled with the animal's own neutropenic blood. The contralateral vein was washed out with Tyrode's solution, perfused with fresh blood from a normal dog for 5 minutes, occluded for 1 minute while filled with normal blood, followed by restoration of blood flow for 1 minute. As before (Figure 9) the vein that was occluded while filled with neutropenic blood showed no white cell invasion while the one with normal blood showed extensive white cell invasion (Figure 10).

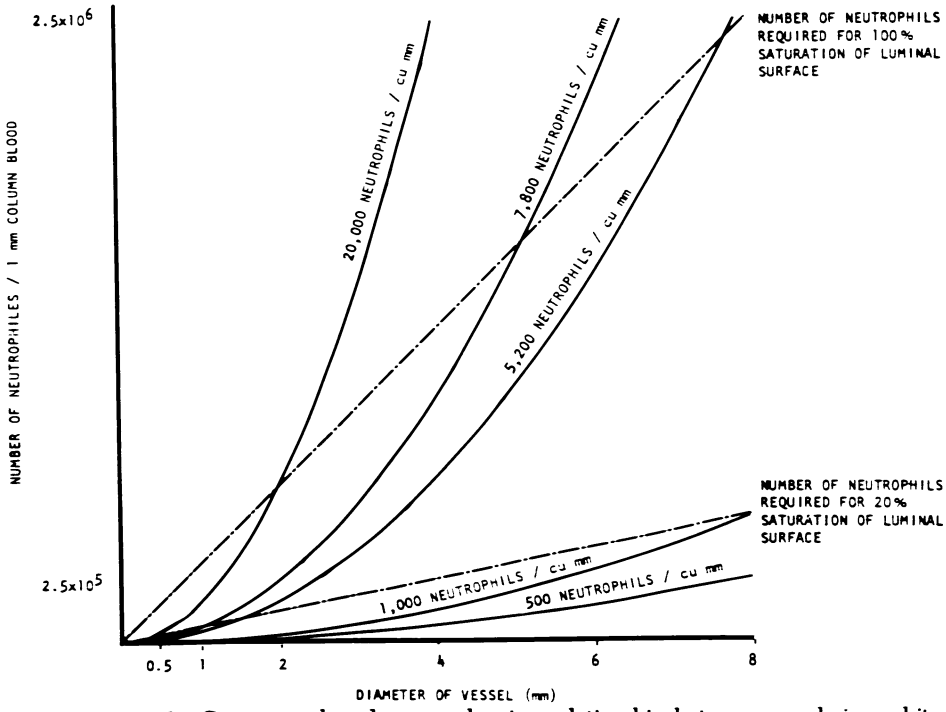
A summary of the results is shown in Table 1.

Discussion

An interpretation of our results must take into account information on the behavior of white cells in small blood vessels and in *in vitro* systems. Of particular relevance is the sticking to and emigration of white cells across the walls of small blood vessels and their emigration through the pores of Millipore filters and along solid surfaces of various kinds. However, two of the numerous differences between venules (100 m) and medium-sized veins (4 to 8 mm) must be considered in interpreting their very different responses to the passage of white cells across the endothelium. The first is the difference in vessel wall thickness.

The wall of small blood vessels is extremely thin in comparison to that of medium-sized veins such as the jugular and femoral veins of dogs. In the case of venules, white cells that pass through the endothelium are held up only briefly before they cross the basement membrane and few perivascular cells and move out into the tissue.[1] However, with jugular and femoral veins the relatively thick collagen-containing wall represents a formidable barrier that is not easily traversed by white cells. The second significant difference between small and medium-sized vessels is the number of leukocytes per unit of luminal surface area. The ratio of volume (number of cells) to surface area increases rapidly as the diameter of the vessel increases (Text-figure 1). This means that in small vessels only a few leukocytes per square millimeter of surface are available for sticking and emigration. However, with 6-millimeter vessels in the dog, there are approximately 10,000 leukocytes/sq mm of surface area. An examination of vessels with the greatest association of white cells indicates that not more than 20% of the vessel wall was covered by "stuck" or "accumulated" leukocytes. Calculations show that in a 6-mm vessel about 30% of the white cells are contained in a 0.5 mm (500 M) shell around the surface of the vessel. Thus, more than enough leukocytes for maximal saturation of the vessel wall are close at hand. Therefore, extent and thus the consequence of white cell sticking and emigration may be related to the availability of white cells for these events should injury occur.

In this study endothelial damage and desquamation in the first group appeared to be a direct consequence of the massive white cell invasion that resulted in multiple forcings of intracellular junctions and accumulation of pockets of white cells between the endothelial sheet and basement membrane (Figures 2, 5 and 6). It appeared that patches of these loosened cells were swept away by the flowing blood. If they had been swept away by the perfusion fluid, there would have been no



TEXT-FIG 1—Computer-plotted curves showing relationship between vessel size, white cell count and covering of luminal surface by white cells.

association of blood calls or fibrin strands with the damaged areas.

The role of white cells in producing endothelial damage was further indicated by the observation that lidocaine, which is known to inhibit white cell sticking *in vivo*,[9] prevented both white cell invasion and endothelial damage. That the protection of the vessel was due to action on the white cells rather than the vessel wall was shown by: a) the invasion of a vein from a lidocaine-treated animal by white cells that had not been exposed to lidocaine (Figure 8A and B) and b) the failure of white cells exposed to lidocaine to invade veins that had not been exposed to lidocaine (Figure 8C).

Further evidence for the role of white cells in producing vascular damage was provided by the experiments with dogs made neutropenic with vinblastine. When the white cell count was reduced to 500 to 1000 and the veins were occluded after surgery, there was minimal white cell sticking and emigration (Figure 9). This was in contrast to the consistent massive white cell invasion produced in untreated dogs (Figures 2, 5 and 6). The protection of the vein was associated with the absence of white cells rather than a change in the vessel or surrounding tissues. This was shown by the fact that when the vein from a vin-

blastine-treated animal was perfused with normal blood, white cell invasion was as extensive as it was in the untreated dogs (Figure 10).

Taken together, these three groups of experiments provide strong support for the concept that massive white cell invasion is the means of producing extensive endothelial separation and desquamation in response to trauma to adjacent tissue followed by even brief stasis. The normal ultrastructural detail of endothelium and white cells in thin sections (Figures 5 and 6)suggests that the damage was partly, perhaps largely, due to physical force. If the white cells had released their enzymes, there would have been ultrastructural evidence of degranulation,[10] and this was not present (Figures 5 and 6). Furthermore, the basement membrane and limiting membrane of endothelial cells showed no evidence of lysis as would be expected if they had been acted upon by hydrolytic enzymes. In the chronic situation endothelial cells in various stages of disintegration are often observed in association with adherent or trapped white cells.[11]

The white cell emigration observed in this study was doubtless similar to the emigration of white cells through Millipore filters and the walls of small blood vessels. It is now widely accepted that an increasing gradient of a chemostatic substance is the means of inducing the migration of white cells *in vitro*.[4,12–14] On the other hand, the measurement of chemotaxis *in vivo* has been difficult and mostly unsuccessful.[1] However, Buckley[15] showed the directional movement of leukocytes toward small, discrete heat injuries produced under aseptic conditions while Hurley[3] used the method of injecting test substances followed by subsequent histological examination of the area of injection. These authors and Sorkin *et al*[4] conclude that chemotaxis results from a gradient of chemotactic substance in increasing concentration from the site of injury to the vessel wall. In our study a gradient of highly active chemotactic substance would be expected from the interaction of minced tissue with serum. The surgical procedure required for exposure of the veins must of necessity have resulted in some bleeding from small vessels (source of serum) and the limited mincing of tissue. Thus, this source of chemotactic factor outside the vessel wall will inevitably be present. It is possible that the same process also occurs on the vessel wall to some extent. Thus, by diffusion across the vessel wall, a concentration gradient of the substance is established which in turn will attract white cells.

Despite the likely presence of a chemotactic substance at the interface of the vessel wall and flowing blood, white cells did not stick or emigrate when the blood flow was not interrupted (Figure 2). This

could be due to continued flushing of the material from the vessel surface so that a critical concentration was never reached. However, when the flow was interrupted for even 1 minute there was massive sticking and emigration of white cells (Figure 4). This could result from the rapid accumulation of leukotactic substances when the material was no longer flushed from the luminal surface of the vessel. The complete absence of white cell sticking on valve surface could be due to the fact that most of the luminal valve surface is separated from the wall by a layer of blood as well as the structural components of the valve itself.

The means by which the leukocytes move from the static blood in all directions, up as well as down, is not clear. It is not known if white cells can "swim" as well as "walk".[16] However, it might not be necessary for them to "swim" at all if they could use red cells as "stepping stones". Calculations show that red cells are some 2 to 3 μ apart in "normal" blood. White cells are 8 to 10 μ in diameter; thus there could be no spatial problem in their movement.

In addition to its role as a nonthrombogenic surface the vascular endothelium has been assigned a major role in the regulation of vascular permeability, a function that is partly dependent on the intercellular junctions. In vascular endothelium the cell-to-cell attachments are of the "tight seal" type in which the membranes of adjacent cells fuse so that the outer leaflet of their membrane is shared.[17] This would seem to imply a stable structural arrangement, a concept that is difficult to reconcile with the ease with which permeability of intercellular junctions to electron dense tracers can be altered by serotonin, histamine and other substances.[18–20] The behavior of the endothelium during and after the passage of white cells through intercellular junctions further challenges the concept of the true fusion of membrane leaflets and the sharing of a segment. In many instances in which leukocytes were caught in transit between endothelial cells the upper edge of one cell was retracted to reveal the membrane of another cell beneath. Yet, in areas of extensive whealing there was no evidence of gaps between cells. Therefore, it must be assumed that the endothelial cells have "resealed" after the leukocytes have passed. The reversible alteration in permeability and the closing of intercellular gaps caused by white cell passage could be accounted for by a concept of surfaces that could be forced apart and readily reassociated when they came into contact again. Such intercellular channels could account for the permeability characteristics of vascular endothelium just as readily as permanent, stable structures.

The sticking of white cells to the vessel wall has been assumed to result from some alteration of the endothelium itself. However, proof of this assumption is still lacking. An alternative possibility and one that has strong support from *in vitro* studies is that the endothelium functions as a porous membrane that poses a barrier between white cells and an attractive substance that is more concentrated on the other side of the barrier (endothelium or entire vessel wall). A relevant *in vitro* model is found in the migration of white cells across a Millipore filter in response to an increasing gradient of chemotactic agent.[21] This was further supported by the recent observation of massive accumulation of white cells on the walls of woven Dacron® tubing 10 and 40 minutes after insertion in the canine thoracic aorta.[22]

White cell-induced damage to the endothelium may be a contributing factor to deep venous thrombosis in man. Two basic problems require solution before this is firmly established. The first problem concerns the distance between the site of trauma and thrombosis and the mechanism of establishing a suitable gradient of chemotactic substance. Since the surgical or accidental tissue destruction usually occurs at a distance from the site of thrombosis, it may be postulated that some substance is formed and transported to the deep leg veins via the blood. However, this would not be adequate to cause the white cells to migrate across the endothelium, since the highest concentration of chemotactic substance would be found in the blood within the lumen. Therefore a further step is required for establishing a gradient in which the concentration of chemotactic substance is higher external to the endothelium than it is in the lumen. If the permeability of small vessels is altered throughout the body in response to trauma, it could be that an inactive substance escaped from the plasma and became activated by contact with cells or fibers external to the endothelium. Then as the active substance diffused across the vessel wall toward the lumen, a suitable gradient would be established. Activation of the complement system may be involved since some of the products are highly chemotactic[23] and apparently represent a necessary means of activating a proesterase that is necessary for chemotactic activity of polymorphonuclear leukocytes.[24]

The second problem that requires solution pertains to the degree of stasis required to produce white cell invasion and the degree of stasis occurring in patients at risk of thrombosis. Either complete stasis for a short time or partial stasis for a long time might suffice to precipitate white cell-induced endothelial desquamation thereby establishing foci for thrombus formation.

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[*Illustrations follow*]

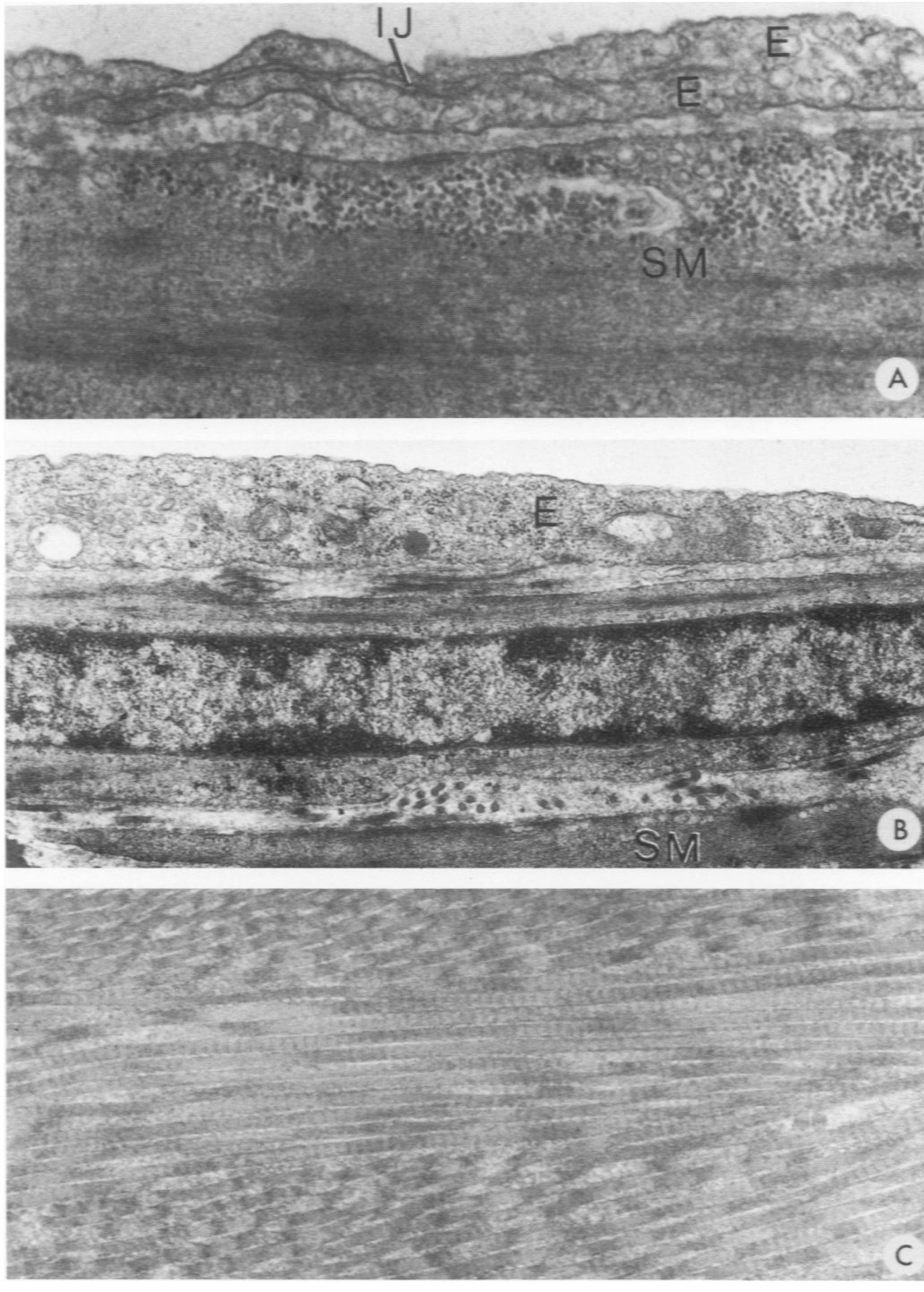

Fig 1—Transmission electron micrographs of normal canine jugular vein. **A**—Shows a vein processed by conventional procedure except that fixation and dehydration were prolonged. The endothelial cells (*E*) have typical caveolae and a long intercellular junction (*IJ*). An underlying smooth muscle cell (*SM*) with its myofilaments is also well preserved (× 43,000). **B**—Shows a section of vein that was dried by the critical point method, redehydrated and embedded in Epon for sectioning. The contrast in the endothelium (*E*) and underlying smooth muscle cells (*SM*) and collagen (*C*), is better than that in the conventionally processed material, is most likely due to loss of diffuse material soluble in amyl acetate (× 20,000). **C**—From the central thickness of the vessel shows collagen fibrils with well-preserved periodicity (× 40,000).

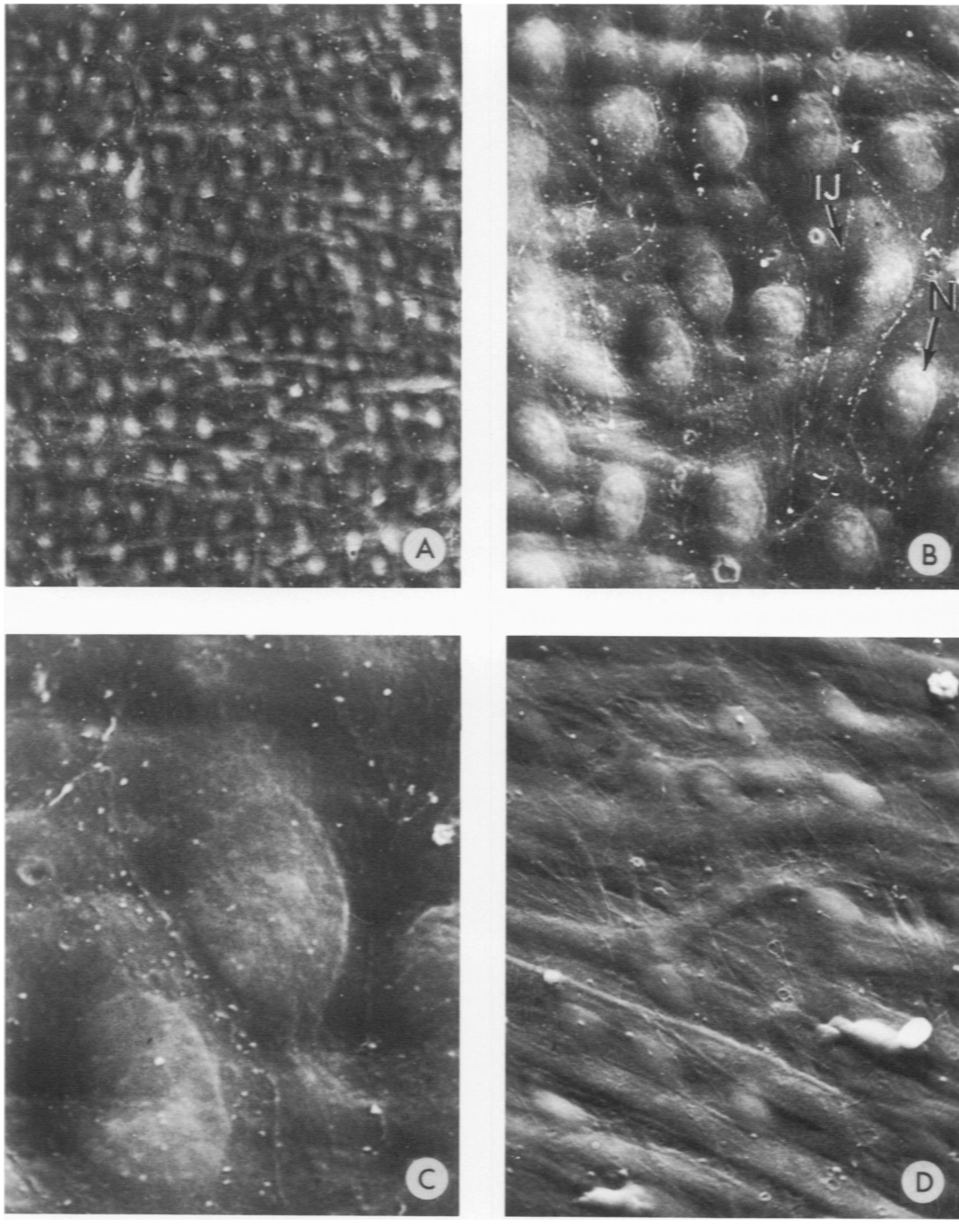

Fig 2A–C—Scanning electron micrographs of typical luminal surface of vessels that were not occluded while containing blood. The smooth intact sheet of elongated endothelial cells with their slightly protruding nuclei and just discernible intercellular junctions was considered normal and used as a basis of comparison for all subsequent treatments. **A**—Low magnification showing many cells and underlying cords of fibers and cells (× 300). **B**—Intermediate magnification shows the nuclei (*N*) intercellular junctions (*IJ*) and mosaic arrangement of the cells (× 1000). **C**—Higher magnification shows the detail of the nuclei and intercellular junctions. The small white dots on the surface are pseudopods (× 3000). **D**—Scanning electron micrograph of luminal surface of blood-filled vessel occluded for 7 minutes *prior* to surgery. The surface has an occasional adherent white cell and a few pseudopods and pits. From transmission electron micrographs it appears that the pits or craters might result from the collapse of vacuoles (× 1000).

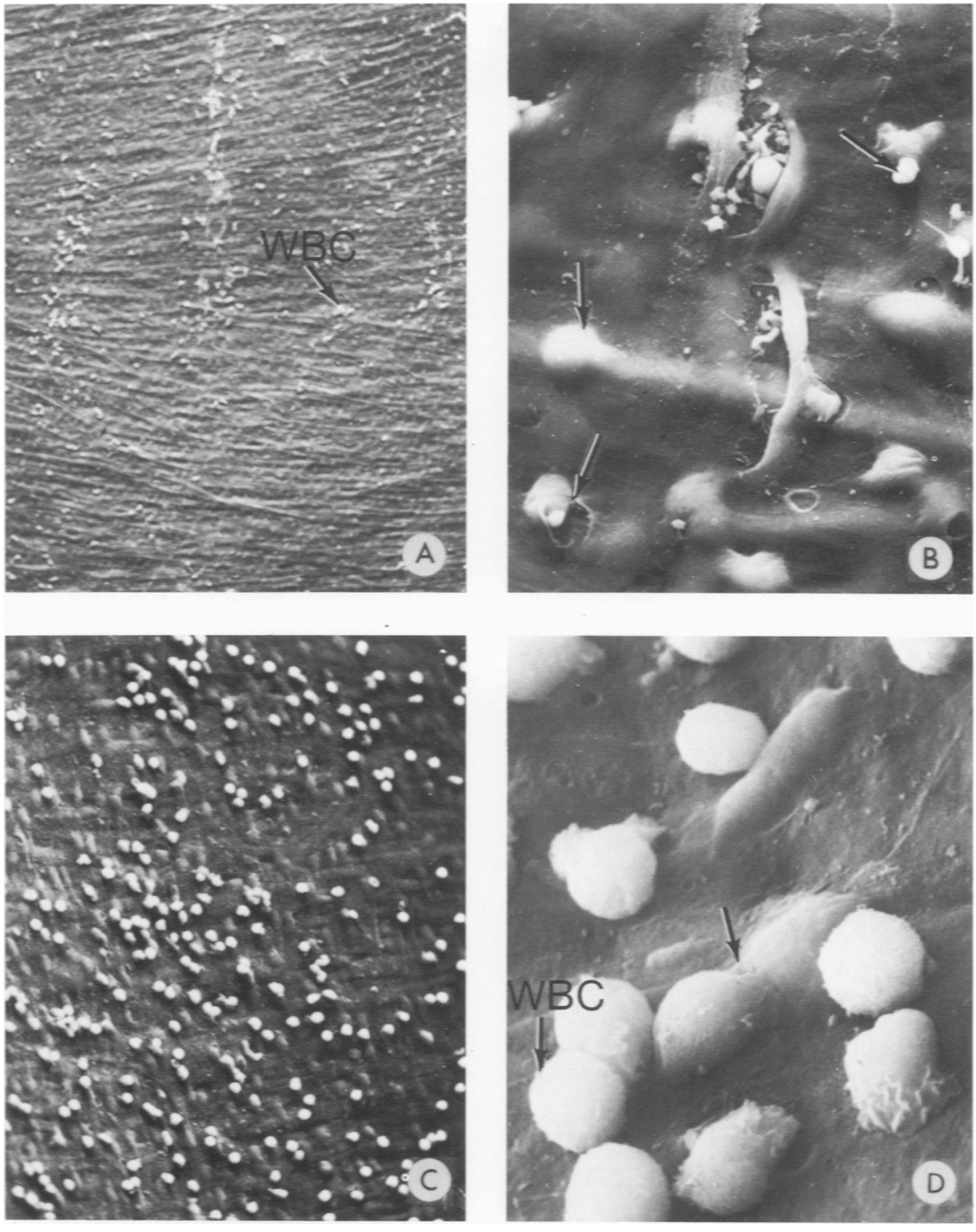

Fig 3—Scanning electron micrographs of jugular veins that were occluded for 7 minutes prior to surgery. **A**—Low magnification survey shows scattered patches of adhering white cells (*WBC*) (× 100). **B**—Higher magnification shows white cells (*arrow*) in the process of passing through the intercellular junction (see transmission Figures 5 and 6 for confirmation). A single white cell between the endothelium and basement membrane (*double arrow*) causes a hump slightly larger than the adherent white cells (× 1000). **C**—A survey of an area with moderately heavy sticking and emigration of white cells (*white spheres*) (× 300). **D**—Details of part of the same area. The surfaces of the white cells (*WBC*) are rough. A double hump apparently representing two white cells is seen (*arrow*) (× 3000).

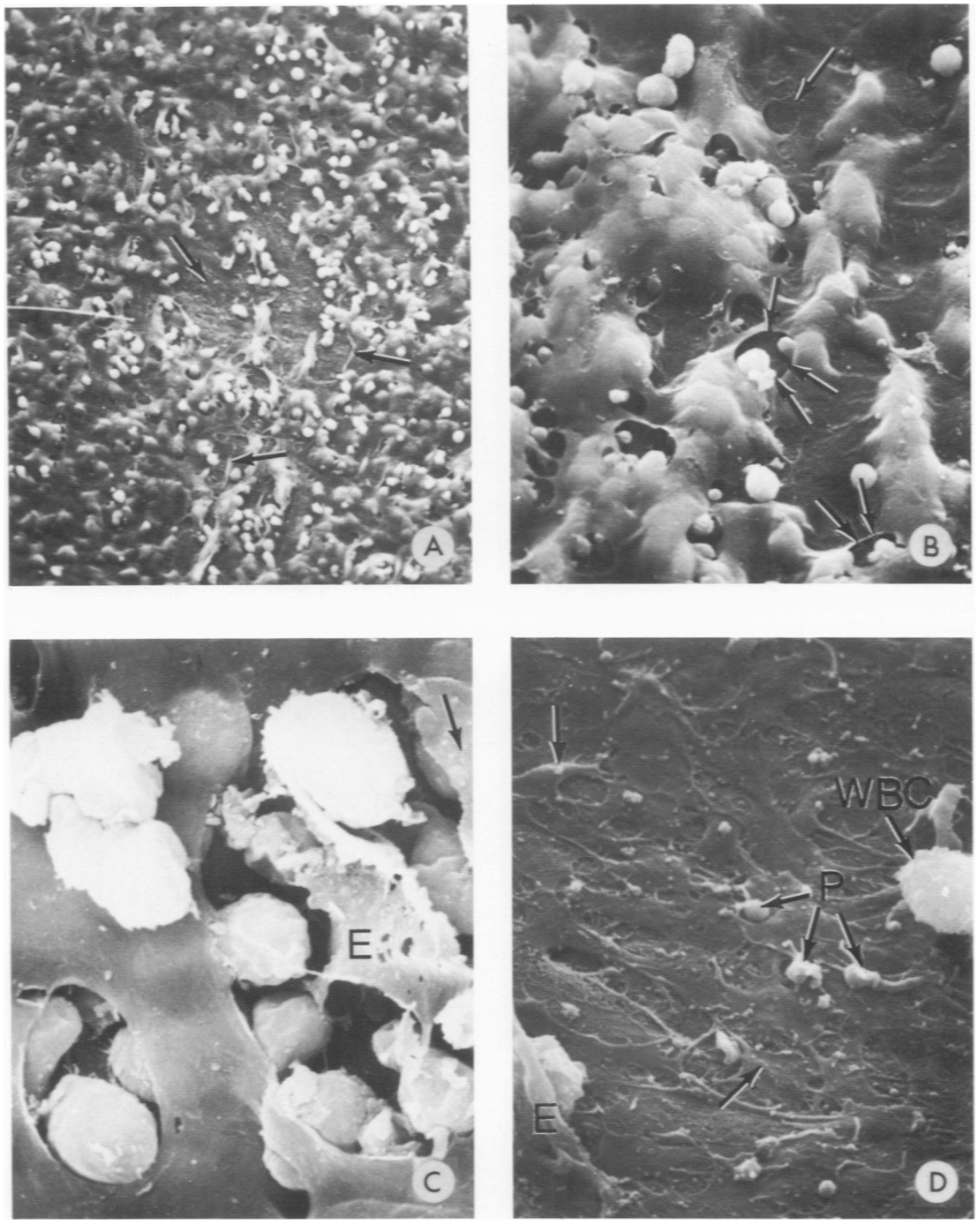

Fig 4—Scanning electron micrographs of the luminal surface of veins that were occluded after surgery. White cell sticking and emigration were always massive in these veins. **A**—Survey of an area about 0.25 sq mm in which extensive white cell (*white spheres*) emigration led to almost continuous whealing (*raised areas*) and areas of endothelial desquamation (*arrows*) (× 300). **B**—Intermediate magnification shows the extent of coalescing of pockets of white cells and the frequency of gaps (*arrows*) between endothelial cells. White cells were caught partially in and partially out in several places (*double arrows*) (× 1000). **C**—A large and a small gap through which white cells are visible. A loose, partially degenerated endothelial cell (*E*) is stretched across part of the larger gap. A loose endothelial cell with pseudopods still attached to the sheet at the upper light of this gap (*arrow*) (× 3000). **D**—An area of exposed basement membrane with a loose wrinkled endothelial cell (*E*) and a white cell at the lower right. A white cell (*WBC*) and a few platelets (*P*) are stuck to the exposed membrane. Thin sections show that the flat, spread cells (*arrows*) are probably smooth muscle and the fibers are collagen (Figure 5) (× 3000).

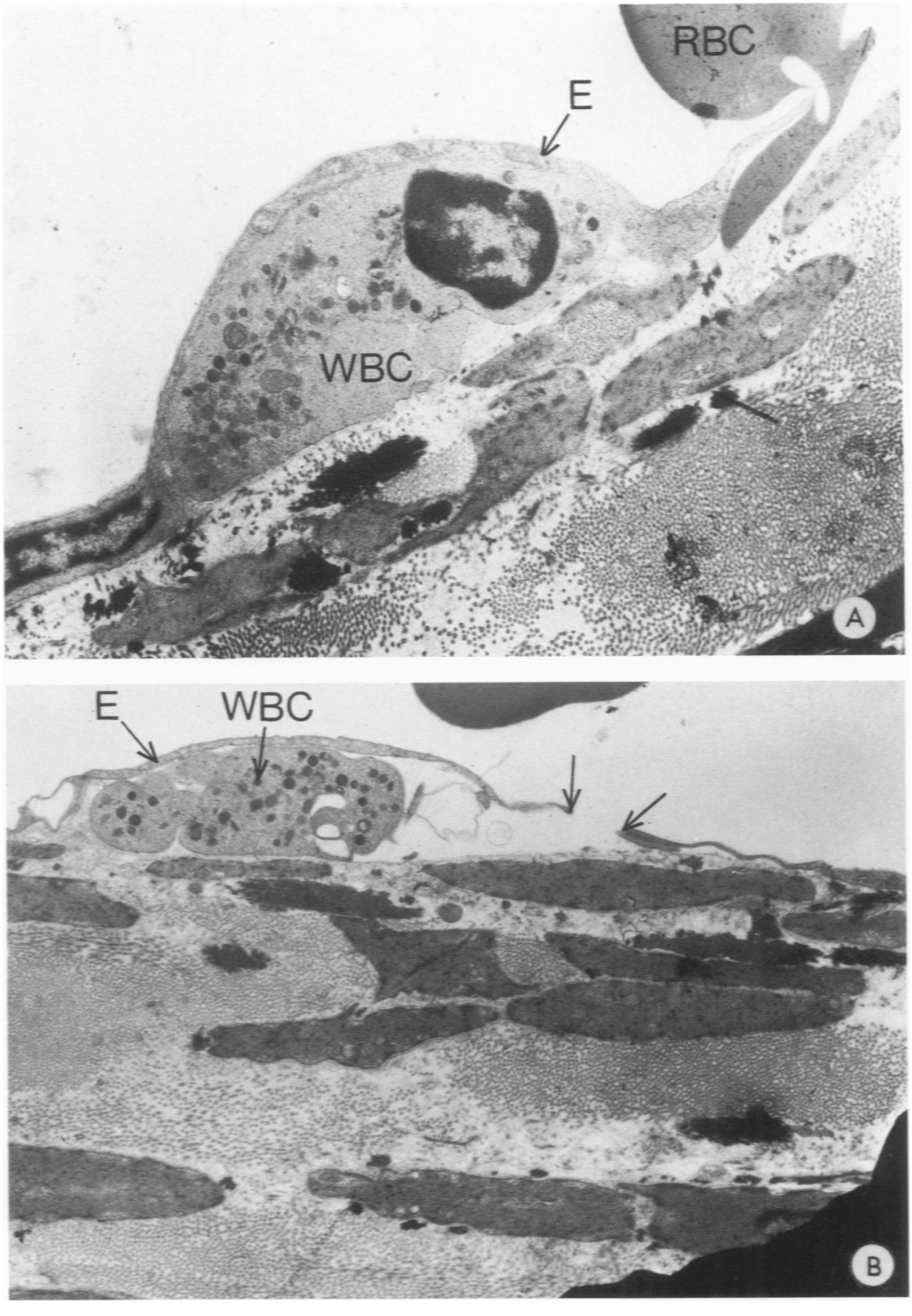

Fig 5—Transmission electron micrographs showing white cells (*WBC*) between the endothelium (*E*) and underlying vessel wall. **A**—A red cell (*RBC*) partially in and partially out (× 8600). **B**—A gap (*arrows*) between two endothelial cells close to an invading white cell (*WBC*) (× 7800).

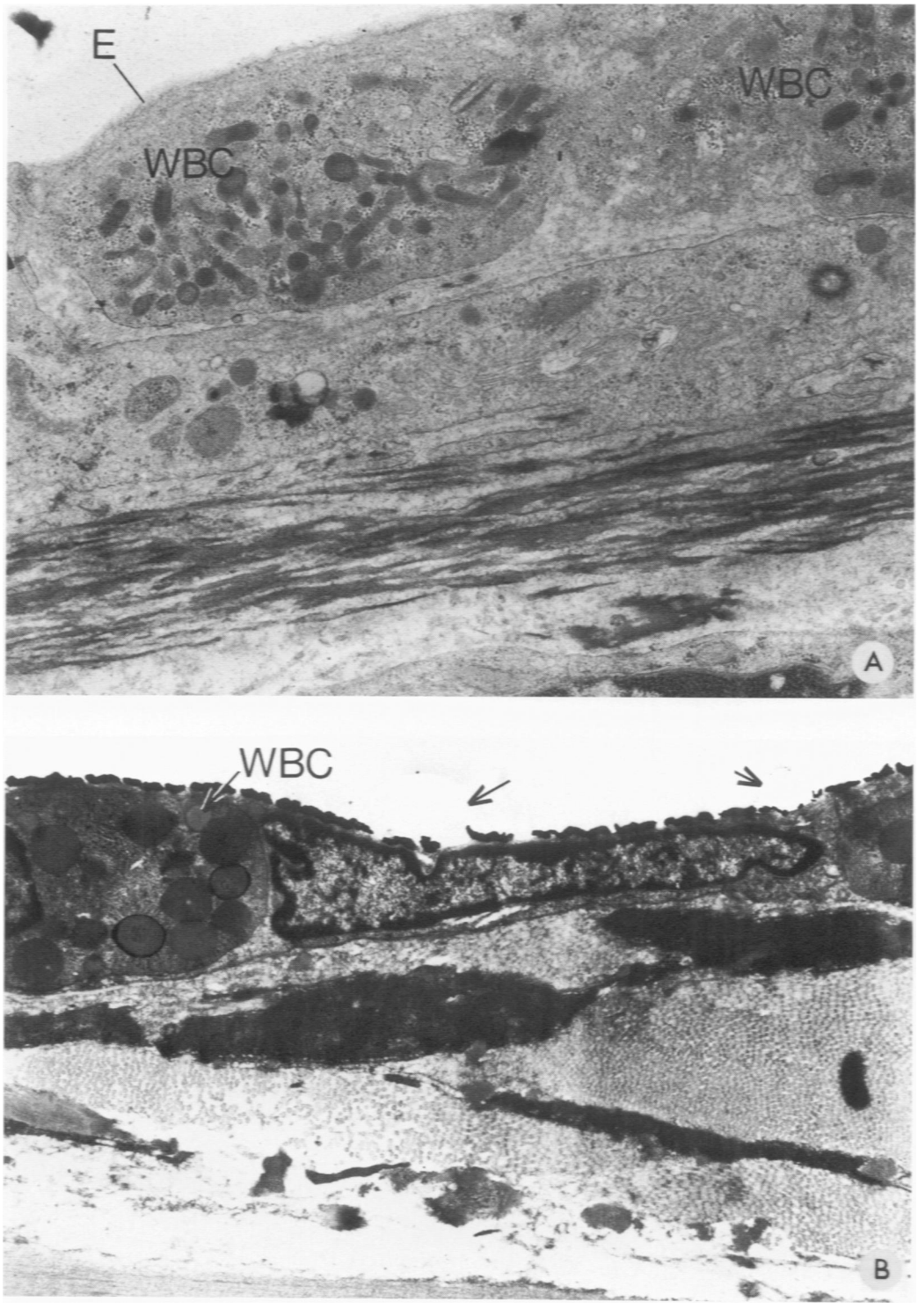

Fig 6—Transmission electron micrograph of vein showing white cells (*WBC*) immediately beneath the endothelium (*E*). **A**—A vein that had been dried by the critical point method but not gold coated or examined in the SEM. The granules are not noticeably different from those shown in Figure 5 (× 26,000). **B**—A gold-coated sample that had been examined with the SEM before reprocessing. The gold coating (*black line along the surface*) was discontinuous. The white cell (*WBC*) granules showed considerable electron damage and the Epon tended to separate from the sample along the gold coating (*arrows*) (× 9400).

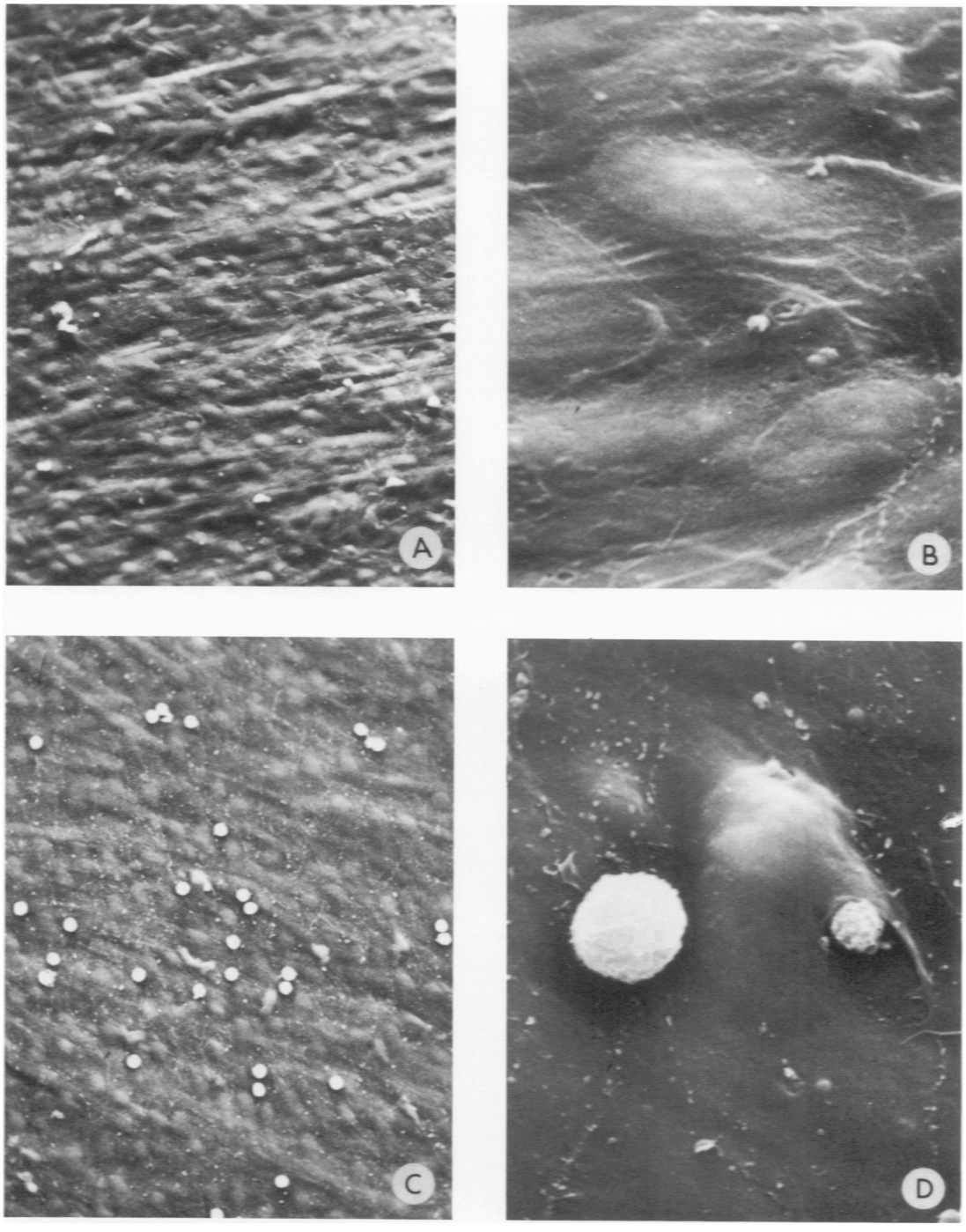

Fig 7—Scanning electron micrographs of veins from dogs given lidocaine before surgery. **A and B**—Veins that were not occluded. The edothelium is intact and indistinguishable from the controls shown in Figure 2 (**A**, × 300; **B**, × 3000). **C and D**—Veins that were occluded after surgery. There is scattered white cell sticking (**C**) and invasion (**D**) (**C**, × 300; **D**, × 3000).

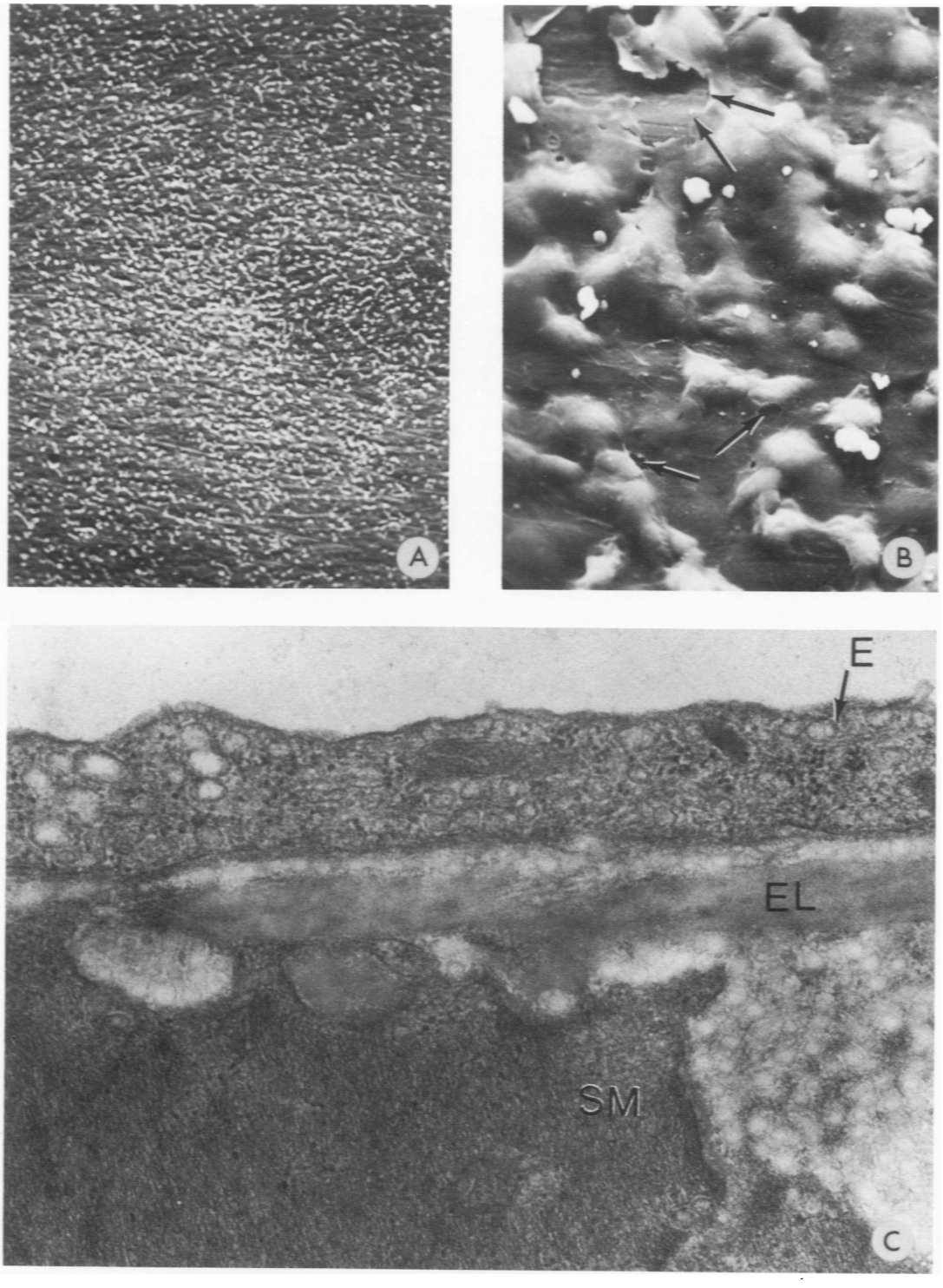

Fig 8—Veins from lidocaine-treated dogs. **A and B**—Scanning electron micrographs of veins that were perfused with and occluded while containing blood that was drawn from the dog before the start of lidocaine administration (normal blood not exposed to lidocaine). Despite the fact that the blood had been *ex vivo* for 30 to 60 minutes before use, the white cell adhesion and invasion was heavy. There were also gaps (*arrow*) and areas denuded of endothelium (*double arrows*) (**A,** × 100; **B,** × 1000). **C**—Thin section of a normal endothelial cell (*E*) and underlying elastin (*EL*) and smooth muscle (*SM*) (× 46,400).

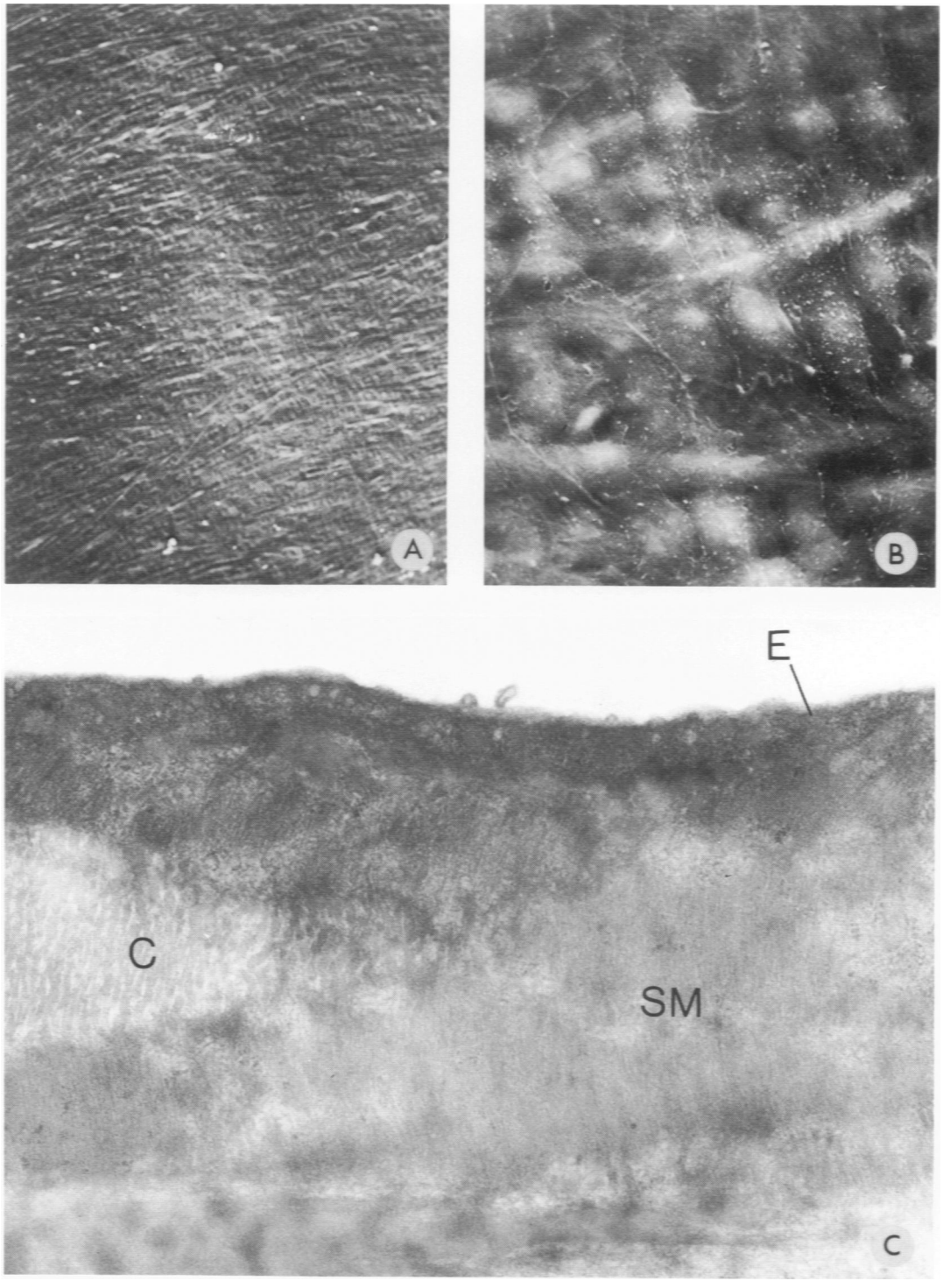

Fig 9—Veins from dogs made neutropenic by treatment with vinblastine. **A and B**—Typical appearance of veins with or without occlusion after surgery. They are indistinguishable from normal veins (Figure 2) (**A**, × 300); **B**, × 1000). **C**—Normal endothelium (*E*), smooth muscle with fibrils (*SM*) and collagen (*C*) (× 30,000).

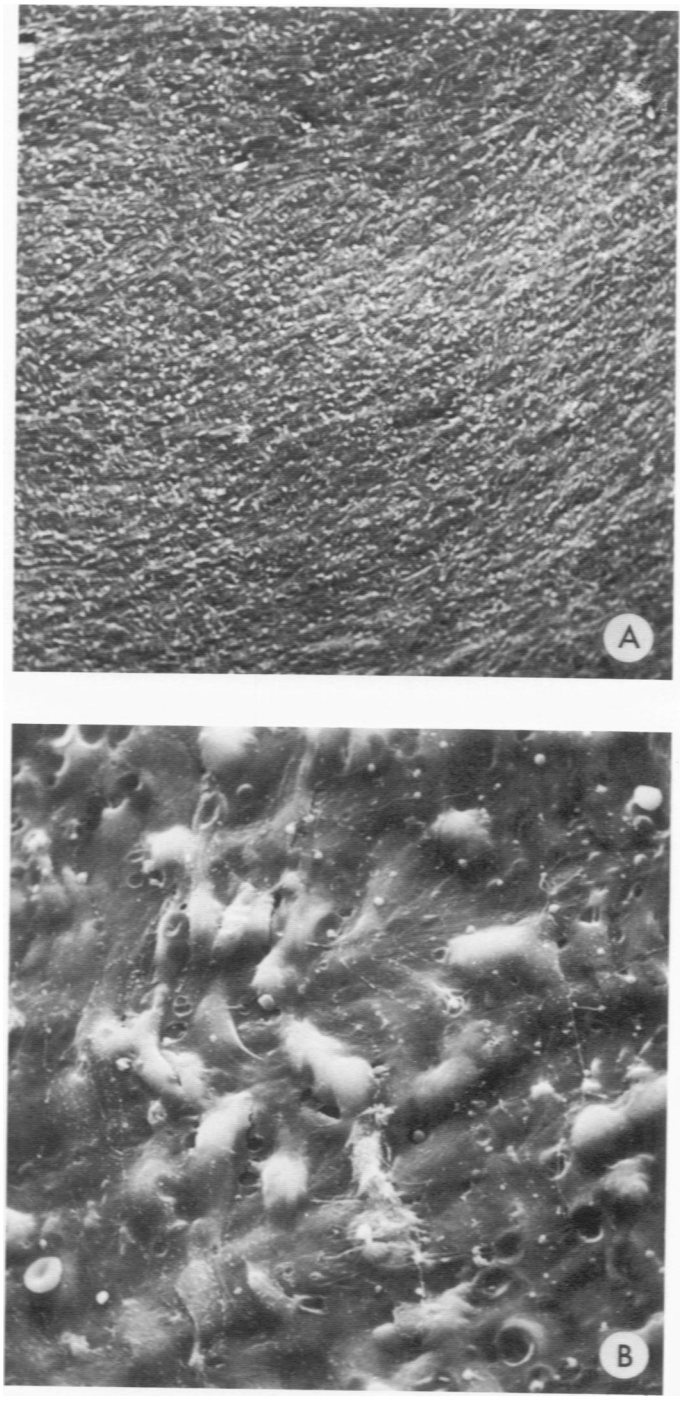

Fig 10—Veins from vinblastine-treated dogs perfused with and occluded while containing normal blood after surgery. **A**—Fairly extensive white cell sticking (× 100). **B**—Pockets of white cells beneath the endothelium (× 1000).