RESOLUTION AND ORGANIZATION OF PLATELET-RICH MURAL THROMBI IN CAROTID ARTERIES OF SWINE

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Thrombosis is recognized as an important factor in the development of arterial disease and its complications. It has been shown experimentally that platelet-rich emboli in the pulmonary circulation of rabbits give rise to foam cell-rich intimal thickenings.[1] However, fibrin emboli in the pulmonary circulation of rabbits produce fibrous thickenings.[2,3] These observations suggest that a platelet-rich arterial thrombus incorporated into the arterial wall should give rise to a foam cell-rich intimal thickening, whereas a fibrin-rich thrombus should give a fibrous thickening.

Arterial mural thrombi in man have not been reported to produce foam cell-rich intimal lesions.[4,5] Most of these thrombi appear to have been fibrin-rich at the time they were studied. In view of the evidence that arterial thrombi begin as aggregates of platelets,[6–11] this may mean that they are subsequently transformed to fibrin. Welch[7] and Aschoff[8] observed many years ago that this was actually the case. Because of these observations and the possible role of platelet-rich thrombi in arterial disease, we studied, by both light and electron microscopy, the fate of platelet-rich mural thrombi in the carotid arteries of swine.

MATERIALS AND METHODS

Animals

The animals used in these experiments were normal swine of either the Yorkshire or crossed Yorkshire-Landrace breeds, between 10 and 30 kg. in body weight. The 46 animals studied were given a standard low-fat hog ration. The composition of this has been previously described.[12]

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Experimental Thrombi

The animals were anesthetized by the intravenous administration of pentobarbital sodium in a dose of 30 mg./kg. body weight. An incision was made so as to expose both common carotid arteries and a Size 0 to 000 sterile silk suture or a fiber of collagen was inserted into the lumen of the artery by means of a fine needle. The collagen was prepared from pig tendon as described by Hovig,[13] and then drawn into fine threads. The incisions were sutured in animals which were allowed to survive for more than 2 hr.

Tissue Preparation

The carotid arteries were removed from the living animal at intervals ranging from 1 hr. to 4 months after the injury (9 at 1 hr. 4 at 2–3 hr., 11 at 7–24 hr., 4 at 2 days, 3 at 3–4 days, 2 at 7–8 days, 5 at 11–12 days, 2 at 14–15 days, 2 at 19–21 days, and 4 at 2–4 months). For light microscopy a portion of the segment containing the suture or collagen fiber was placed in buffered formalin or Helly's solution for 24 hr. In the latter case the tissue was rinsed in running tap water for another 24 hr. The specimen was dehydrated in increasing concentrations of ethanol, embedded in paraffin, and cut transversely at several depths. The following stains were used:

1. Hematoxylin-phloxine-saffron (HPS)
2. Lendrum's martius-scarlet-blue method (MSB)[14]
3. Mallory's phosphotungstic acid-hemotoxylin method (PTAH)
4. Weigert's stain for elastic fibers

Frozen sections of thrombi older than 7 days were stained with Fettrot 7B.

For electron microscopy the remaining portion of the injured carotid artery was cut transversely into sections 1- to 2-mm. thick in the hollow of a wax block containing chilled 1% osmium tetroxide in Tyrode's solution (pH 7.4). The sections were then placed in chilled osmic acid solution for 1½–2 hr. The specimens were dehydrated in increasing concentrations of ethanol, after which they were treated in propylene oxide for ½–1 hr. They were then placed in 50% Epon 812 and 50% propylene oxide for 8–12 hr., and thereafter embedded in Epon 812. Sections ½-μ-thick were cut with a Porter Blum microtome, using either diamond or glass knives, and stained with azure II. A suitable area was then selected for preparation of thin sections. These were stained with lead hydroxide and/or uranyl acetate, and examined in an RCA EMU-3F, JEM, or Philips 200 electron microscope.

Results

One Hour

Light microscopic examination of the injured carotid arteries at 1 hr. showed platelet-rich thrombi adhering to the suture or the collagen fiber, as well as to the adjacent vessel wall (Fig. 1). These thrombi did not occlude the lumen. The masses of platelets were densely packed and were surrounded by thin layers of fibrin. Fibrin was usually present in the region where the platelets came in contact with the silk suture or collagen fiber (Fig. 2). In areas near the fibrin many of the platelets appeared empty and swollen (Fig. 3). A small number of red and white blood cells were incorporated in the thrombus, chiefly in association with the peripheral fibrin layers.

Electron microscopic examination of 1-hr. old thrombi showed that the platelets in the central region of the mass were tightly adherent to

each other (Fig. 4A). They showed pseudopod formation, but most of them contained their granules, mitochondria, and glycogen. In some of the platelets fine filamentous material could be seen extending into the projections (Fig. 5), and tubular-like structures were observed (Fig. 4B). In addition, in some areas there were dark zones at points of platelet adherence, resembling attachment plates or desmosomes (Fig. 4 and 5). Around the periphery of the platelet aggregates, the platelets had undergone marked structural changes (Fig. 6). They had lost their granules, they were swollen, and, in some instances, the platelet membranes appeared to have gaps. In addition, fibrin was interspersed among the platelets. In some regions the fibrin appeared to be in close contact with the platelets (Fig. 7). Some white and red blood cells were present on and near the thrombus surface, and fibrin was also found in close association with them. In some areas a new mass of platelets had formed on top of the layer of fibrin and the degranulated platelets. These platelets were similar in appearance to those observed in the more central region of the thrombus. At the base of the thrombus the platelets showed swelling and loss of internal structure where they were in contact with the suture or collagen fiber (Fig. 8A). The platelets in contact with the collagen frequently showed gaps in their membrane (Fig. 8B). Strands of fibrin were interspersed among the platelets and the collagen fibrils.

Where the thrombi came in contact with the endothelium, the platelets appeared to be adherent to the endothelial cells at some points: the gap between the electron-dense membranes of the cells and the platelets was of the same order of magnitude as that between adherent cells in general (Fig. 9). The endothelial cells in contact with platelets often showed increased vacuolization and swelling.

Two to Three Hours

Light microscopic examination of thrombi 2- to 3-hr. old showed the platelets to be less densely packed in many areas and, invariably, the fibrin layers were thicker (Fig. 10). The number of leukocytes, mainly neutrophils, had increased both on the surface and within the thrombus.

Electron microscopic examination of these thrombi confirmed that the platelets were separated from each other (Fig. 11A and B). The platelets were rounded and the pseudopods were less prominent. The platelets showed increased vacuolization and a number had undergone degranulation. In some platelets the outer membrane appeared to have gaps and, in these areas, fibrin could often be identified between the platelets (Fig. 11). Toward the periphery of the thrombus there were increased amounts of fibrin. In the cytoplasm of some platelets from most regions there were strands of fibrillar material which sometimes

appeared to have the characteristic cross striations of fibrin (Fig. 12A and B). The endothelial cells underneath the thrombus showed signs of severe damage with increased electron density, loss of structural details, and shrinkage. Often the endothelial cells had disappeared completely or were replaced by an unidentified granular material.

Seven to Twenty-four Hours

Examination of 7- to 24-hr.-old thrombi with the light microscope gave the impression that the volume of the thrombus was reduced (Fig. 13). There was a marked increase of fibrin throughout most of the thrombus, particularly in areas near the lumen. Between the fibrin strands there were granular platelets or poorly defined cellular material (Fig. 14). Polymorphonuclear neutrophils and monocytes were lying on the surface and had invaded the thrombus (Fig. 14). Recent platelet aggregates had formed in the area of the fibrin-rich thrombi in several instances (Fig. 13).

Electron microscopic examination of the thrombi at 7–24 hr. revealed that the majority of the platelets in areas near the lumen had disintegrated and had been replaced by thick strands of fibrin (Fig. 15 and 16). Residual platelet debris was trapped between the strands. Polymorphonuclear neutrophils, as well as mononuclear cells, adhered to the thrombus surface. Some of the mononuclear cells were adherent to each other, giving the impression of a "pseudoendothelium" (Fig. 16). Polymorphonuclear leukocytes and macrophages within the thrombus had already phagocytosed considerable amounts of cellular debris (Fig. 15). Fibrin, however, was rarely encountered in the macrophages.

Two to Four Days

It was evident from examination of thrombi 2- to 4-days old that the further alterations were not as extensive as during the first 24 hr. The main changes during this period were, as seen by light microscopy: (1) reduction of fibrin; (2) degeneration of polymorphonuclear neutrophils; (3) growth of endothelium from the sides; and (4) proliferation of elongated cells into the thrombus from the vessel wall.

In certain areas, particularly near the lumen, fibrin had disappeared, leaving behind a granular mass—probably cellular debris. Here and there patches of dense fibrin remained with no obvious relationship to the presence of leukocytes or macrophages. Many red blood cells appeared to have penetrated the thrombus. A large number of polymorphonuclear neutrophils were present, but their granules were mostly lost and many of their nuclei had assumed a rod-like form. Around some of the leukocytes there was a clear zone. An increased number

of macrophages were observed within the thrombus. The intact endothelium adjacent to the thrombus showed proliferation and appeared to be growing over the thrombus from the sides. Furthermore, at the sites where the silk suture or collagen fiber penetrated the arterial wall, elongated fibroblast-like cells appeared to be proliferating, and cords of these cells seemed to be growing into the thrombus where it came in contact with the damaged wall. However, even in areas remote from the proliferating endothelial and fibroblast-like cells, there were scattered elongated cells on and near the surface of the thrombi 3 and 4 days old.

Electron microscopy of the thrombi 2–4 days after the injury also showed a decrease in the amount of fibrin in some areas. The disintegration of platelets was more widespread, although occasional intact platelets were still encountered. Most of the polymorphonuclear neutrophils showed signs of degeneration with loss of granules and condensation of nuclear chromatin. Both polymorphonuclear neutrophils and macrophages had cytoplasmic inclusions of debris, probably including platelet material (Fig. 17 and 18). The clear zone around some of the macrophages was seen in the electron micrographs (Fig. 18). In some of the macrophages lipid inclusions were present (Fig. 17).

Six to Eight Days

Examination of the thrombi by light microscopy 6–8 days after the injury showed them to have a layered appearance. The surface facing the lumen was now covered by flat endothelial-like cells, and underneath there was young granulation tissue with fibroblasts, macrophages, eosinophis, and red blood cells (Fig. 19). There was still residual fibrin and cellular debris, particularly in the deeper layers of the thrombus.

Electron microscopy of the lesions at this stage showed, near the lumen, elongated cells with well-developed rough type endoplasmic reticulum (Fig. 20 and 21). Many of them contained phagocytosed material and lipid. The surface was covered with somewhat similar cells which also had a prominent endoplasmic reticulum (Fig. 20). Other elongated cells contained some bundles of myofibrils in the peripheral part of their cytoplasm and were partly or completely surrounded by a basement membrane (Fig. 22). Collagen was forming between the cells (Fig. 20 and 22), and deep in the mass, fibrin, residual cellular debris, and areas of slightly altered platelets were still present. There were no foam cells.

Eleven to Fourteen Days

At 11–14 days the layered appearance of the lesions was still apparent as seen by light microscopy (Fig. 23). Near the lumen there was now a layer of elongated cells with rod-like nuclei and a cytoplasm which

stained dark blue with PTAH. Deeper than this layer was a layer of young connective tissue, and at the base there was still some unresorbed fibrin and cellular debris. There was little evidence of lipid.

Electron microscopic examination of the lesions at this stage showed that the cells in the layer near the lumen were immature smooth-muscle cells with no apparent lipid inclusions (Fig. 24 and 25). The surface of the thrombus was now covered by endothelial cells.

Nineteen Days to Four Months

The final result of the organization of the thrombi was, as seen by light microscopy, an intimal thickening covered by endothelium and composed of smooth-muscle cells, collagen, and some elastic fibers, particularly just underneath the endothelium (Fig. 26). There were few or no lipid deposits. In one instance calcification—probably of unresorbed fibrin and cellular debris—had occurred (Fig. 27). Vessels were rarely encountered within the organized thrombi, and those which were found seemed to originate from both the main lumen and from the vasa vasorum.

Electron micrographs of the older lesions showed the same characteristics as the 11- to 14-day-old thrombi, although the smooth-muscle cells were now more mature. In addition, strands of homogenous material, probably elastic fibers, were observed between the smooth-muscle cells.

Discussion

The present experiments were designed to obtain mural platelet-rich thrombi in a large artery by using a silk suture or a collagen fiber as a surface stimulus for platelet adherence and aggregation. Platelets readily adhere to collagen[15,16] and undergo release of constituents, including adenosine diphosphate (ADP),[17,18] which causes platelets to adhere to each other. Experiments in vitro have shown that silk sutures produce structural and biochemical changes in platelets similar to those caused by collagen.[19] Although the presence of the silk suture or collagen fiber and its penetration at two sites through the vessel wall probably influences the organization of the thrombus, it is not likely to produce a marked effect on the alterations occurring within the thrombus during the first 3–4 days, the period with which we have been particularly concerned in this study.

The appearance of the arterial thrombi in the early stages is in keeping with the classic descriptions[7,8] and more recent observations.[9–11] Fibrin was frequently found among these platelets at this stage, but it must not be inferred that coagulation was involved in the initial interaction of platelets with the introduced material, since observations in the earliest

stages of thrombus formation do not show fibrin formation in the region of platelet contact with surfaces.[9,10,20] Furthermore, collagen-induced platelet adherence and aggregation is independent of blood coagulation.[16,18,21] It is suggested that collagen can activate Factor XII,[22] and ADP-induced platelet aggregation makes available the platelet lipid and accelerates clotting.[23,24] It seems likely, therefore, that the fibrin formation was secondary to the interaction of the platelets with the introduced material and to the effect of these materials on coagulation. The loss of platelet internal structure in these regions was likely caused by both the surface stimulus and thrombin.

The majority of the platelets in the central region of the thrombus at 1 hr. were tightly adherent to each other and had formed pseudopods. This appearance is compatible with that of platelet aggregates formed by ADP in vitro.[13] It seems likely that the large platelet aggregates at this stage were formed not only by the effect of ADP released by the interaction of the platelets with the silk suture or collagen fiber, but ADP may also have been released through the action of thrombin on the platelets[25,26] and possibly from the damaged wall.[27] In the platelets, particularly in their pseudopods, fine fibrillar structures were often present. These may represent the actomyosin-like proteins known to be present in platelets.[28,29] The diameter of these structures appears to be too small for them to be the microtubles which have been described in platelets.[30,31] Structures similar to the microtubules were observed in a number of platelets, even though osmic acid was the fixative used in the present study. We are uncertain, however, whether these are microtubules or rearrangement of fibrillar material. The desmosome-like structures seen in the central region of the platelet aggregates may represent an additional form of platelet binding or the early stages of fibrin formation between platelets.

Around the periphery of the thrombus the platelets were swollen; some were degranulated with fibrin strands between them. These changes were probably caused by thrombin formed in the area of contact between the platelet mass and the flowing blood.

The platelet masses were sometimes seen in contact with the endothelium. As judged by the gap between the electron-dense parts of the platelets and endothelial cell membranes, they appeared to be adhrent to each other. The endothelium in regions in which platelets were adherent was, at 1 hr., often swollen and vacuolated, and at 2 hr. or later, shrunken and electron dense. Finally, the endothelial cells disappeared. These changes are unspecific signs of endothelial damage.[32-34] They may be due to (1) the initial injury by the introduction of the silk suture or collagen fiber, (2) anoxia because of the overlying thrombus, and (3) re-

lease of damaging substances from the thrombus constituents, particularly the platelets.[35] One cannot infer from the lack of endothelium underneath the thrombus that a denuded intima is a prerequisite for thrombus formation.

After 2–3 hr. the platelets in the central area of the thrombus became less densely packed and assumed a more rounded shape. ADP is rapidly broken down in plasma[36] and platelet aggregation induced by ADP is reversible.[37,38] The separation of the platelets in the thrombus may indicate loss of ADP effect. The increased fibrin at the periphery of the thrombus may be an important component in keeping the mass together.

The fibrin laid down during the following 24 hr. was found between the platelets. An important source is probably the fibrinogen in plasma seeping in between the platelets as they separate. Indirect evidence is provided for this by the fact that the early increase in fibrin was most evident in the region near the lumen. The platelet disintegration which accompanies the laying down of fibrin is probably in part related to the action of thrombin.[13,39] Because some of the platelet granules are lysosomes,[40] it seems reasonable to suggest that lysosomal enzymes may also be factors in the disintegration of platelets. Further, the lysosomal enzymes from leukocytes are probably involved.

Although it is possible that the dense fibrillar material formed within degranulated platelets is phagocytosed fibrin, it seems more reasonable to assume that it is fibrin formed from intraplatelet fibrinogen.[41]

The initial platelet-rich thrombus is transformed into a fibrin thrombus [7,8] after several hours. This has also been observed in both early and more recent [10,20] studies. Most authors who have discussed the role of thrombosis in the pathogenesis of atherosclerosis have stressed the importance of fibrin thrombi.[4,5] This has led to speculation about primary fibrin precipitation on the endothelial lining of arteries.[42] However, any theory which stresses the role of thrombosis in atherosclerosis must take into account the function of the platelets.[1,11]

In agreement with other observations,[7,8,43] there was an increasing number of polymorphonuclear neutrophils and other white blood cells on the surface and within the thrombus during the first 24 hr. The accumulation of these cells on the luminal surface suggests that many of them originate from the blood in the vessel lumen.[43] Since blood monocytes can develop into tissue macrophages,[44] it is reasonable to assume that many of the macrophages within the thrombus are derived from invading blood monocytes.

At 1–2 days many of the macrophages contained cellular debris, some of which was platelet material. Poole [45] has shown by electron microscopy that macrophages phagocytose platelets in thrombi, confirming the

earlier light microscopic observations by Hand and Chandler.[1,46] In another electron microscopic study of organized thrombi, Wiener and Spiro[47] did not observe phagocytosis of fibrin. We too found little evidence of phagocytosis of fibrin by the macrophages, although it did occur. In agreement with earlier observations,[7] a clear zone was found around some of the leukocytes in the fibrin mass which may indicate extracellular lysis of fibrin by leukocytic enzymes.[48]

The decrease in the fibrin content of the thrombi during the remainder of the first week was likely due to the action of fibrinolytic enzymes carried or stimulated by the blood flow, and not so much by the action of leukocytes and macrophages, because the reduction of fibrin was most marked in areas near the lumen and unrelated to cellular elements. This is in agreement with previous observations.[10] At this stage elongated cells appeared with a well-developed endoplasmic reticulum similar to that seen in some macrophages. Likely, these cells were fibroblasts, possibly derived from the mononuclear cells in the thrombus. Some probably originated from the arterial wall. Evidence from studies of the organization of thrombi on synthetic material exposed to the flowing blood also indicates that some fibroblast-like cells are derived from blood cells.[49,50] However, cell-culture studies on the transformation of blood cells into fibroblasts have given conflicting results.[51–53]

The mononuclear cells which were initially present on the luminal surface of the thrombus were either replaced by, or transformed into, elongated covering cells which, in the beginning, had an appearance similar to the young fibroblasts. Later, the covering cells had the characteristics of endothelial cells. There was evidence that endothelial cells proliferated from adjacent intact endothelium at the margins of the thrombus. However, even before this proliferation was far advanced, scattered elongated cells were found on the surface in areas remote from the margins. Studies of the lining which forms on synthetic material exposed to the flowing blood[49,50] suggest that endothelial cells can also arise from blood cells.

By one week, cells with characteristics of young smooth-muscle cells were seen in areas near the lumen, and the number had increased by 14 days. At 3 weeks or later they were found throughout the lesion. The smooth-muscle cells may derive from the fibroblast-like cells. Again, the fact that smooth-muscle cells can be found in organized thrombi on synthetic material in contact with flowing blood[49,50] suggests that they can be derived from blood cells. If so, it is likely from our observations that the blood-borne cell with a potential to develop into a smooth-muscle cell must pass through a fibroblast-like stage.

The final lesion contained only small amounts of lipid compared with the lesions produced in the experiments of Hand and Chandler.[1] This could be due to differences in species, diet, vessel, and mode of introducing the thrombus. In contrast to the lesions produced in Hand and Chandler's experiment,[1] the thrombi in the present study did not at any time occlude the lumen. This could explain the more marked platelet disintegration and fibrin formation in the early stages in the present investigation. If it is true that phagocytosed platelets provide the source of the lipid in the lipophages of the late lesions,[1,46] the paucity of lipid in the plaques of our study could be related to the early platelet disintegration with little phagocytosis of intact platelets. This may mean that the mechanisms affecting the early changes in the thrombi have an important bearing on the subsequent characteristics of the resulting vascular lesion.

Summary

Mural thrombi were produced in carotid arteries of swine by intraluminal introduction of a silk suture or a collagen fiber prepared from pig tendon. The animals were killed at various intervals ranging from 1 hr. to 4 months, and the lesions were studied by light and electron microscopy.

At 1 hr. the thrombus was composed of densely packed platelets surrounded by strands of fibrin. At 2–3 hr. the platelets were separated from each other, and at 7–24 hr. they had undergone disintegration and there was increased fibrin between them. At the same time, many leukocytes—including monocytes or macrophages—were observed on the luminal surface and within the thrombus. The endothelium underneath the thrombus showed early degenerative changes and disappeared.

At 2–4 days the amount of fibrin was reduced. Cellular debris was removed by phagocytosis, mainly by macrophages. Endothelium started to grow in on the luminal surfaces from the sides, and cords of fibroblast-like cells proliferated into the thrombus from the wall. However, there was also evidence that endothelial and fibroblast-like cells were probably derived from mononuclear cells.

At 6–8 days cells with the appearance of immature smooth-muscle cells were present. Some of them were possibly derived from fibroblast-like cells.

The end stage of the organization was an intimal thickening consisting of smooth-muscle cells, collagen, and elastic fibers, and covered by endothelium. There was little or no lipid and few vessels in the lesions.

It appears that a thrombus examined several hours after it has formed may have only a few characteristics of its initial structure. Thus, what appears to be a fibrin thrombus in an artery may well have started as a

platelet thrombus. The mechanisms affecting the early disintegration of the platelets and the accompanying fibrin formation may have an important bearing on the characteristics of the vascular lesion resulting from the organization of the thrombus.

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The technical assistance of Mrs. M. Smith, Miss L. Skibo, and Mrs. D. Bachman is gratefully acknowledged.

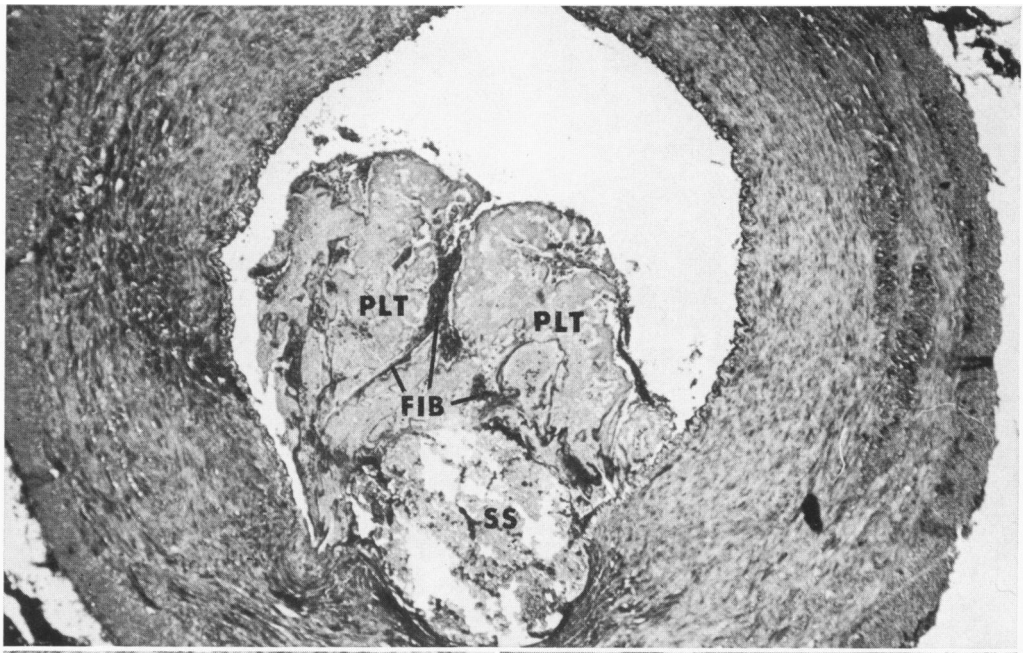
[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Cross section of pig's carotid artery 1 hr. after insertion of silk suture (SS). Nonoccluding thrombus is adherent to suture and adjacent vessel walls. Platelets (PLT) are light gray mass with dark material (fibrin, FIB) around platelet aggregates. Martius-scarlet-blue stain. × 50.

FIG. 2. Thrombus seen in Fig. 1, showing region adjacent to silk suture. Individual platelets (PLT) are evident. Martius-scarlet-blue stain. × 200.

FIG. 3. Zone (seen in Fig. 2) between platelets and fibrin (FIB). Around fibrin are clear zones with some faint dark material between them that may represent swollen and degranulated platelets. Structures at right, which are not readily identified, could represent lysed red blood cells. Martius-scarlet-blue stain. × 1970.



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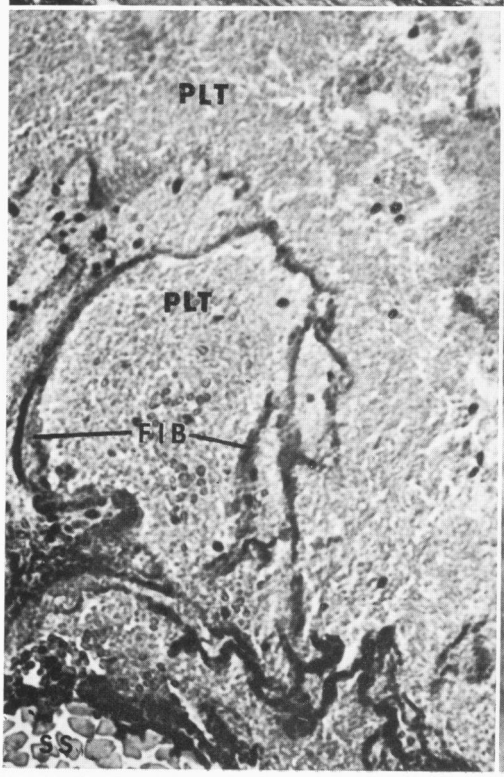

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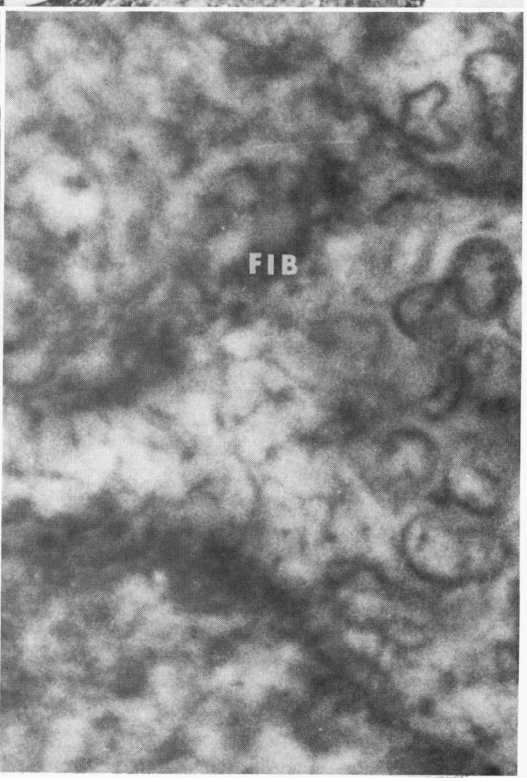

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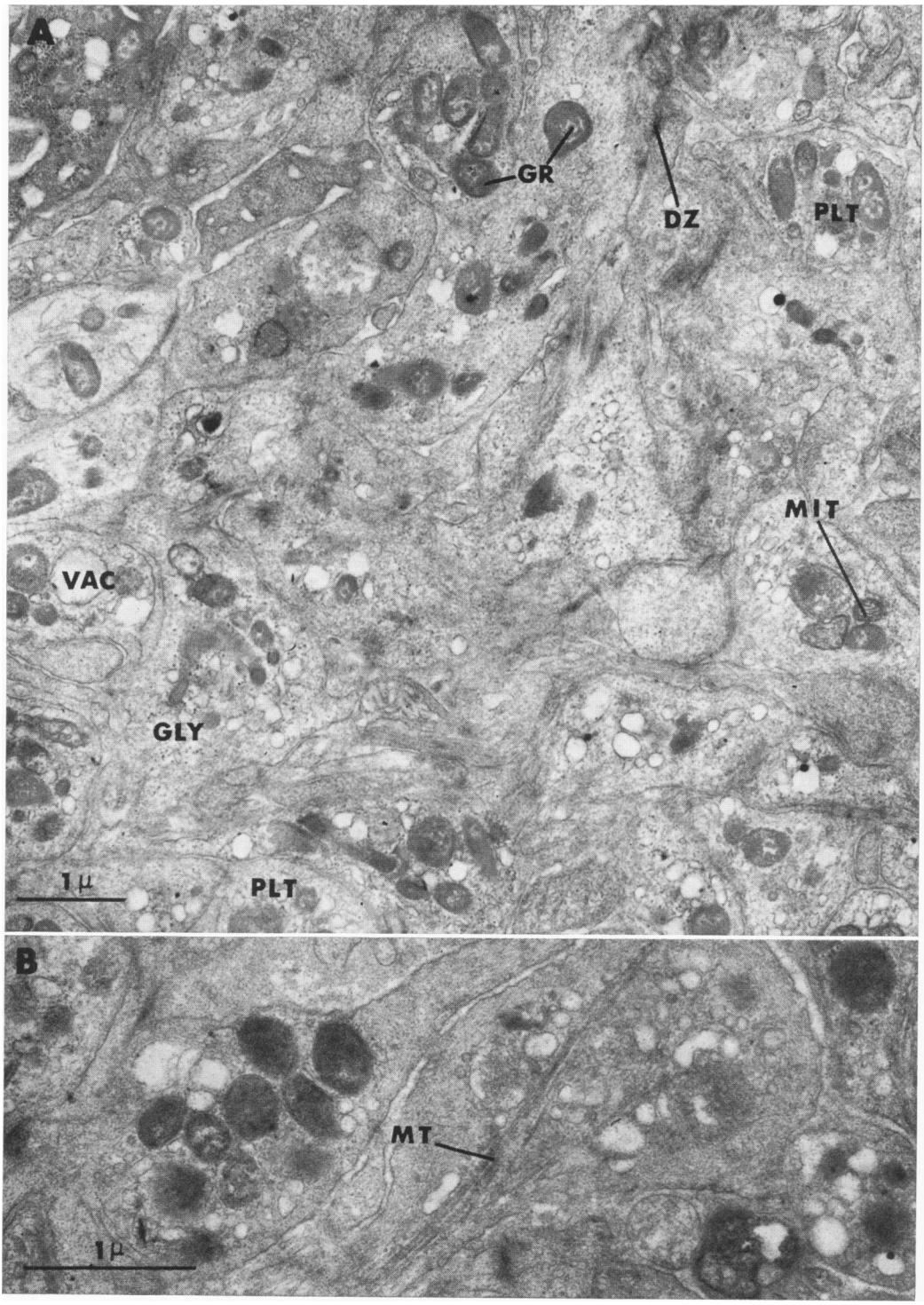
FIG. 4. A. Electron micrograph of section through central region of 1-hr.-old platelet-rich thrombus. Most platelets (PLT) contain their internal organelles—granules (GR), glycogen (GLY), and mitochondria (MIT). Some platelet vacuoles (VAC) are evident. Dark zones (DZ) are apparent between platelets. × 15,600. B. Electron micrograph showing structures with characteristics of microtubules (MT). × 25,200.



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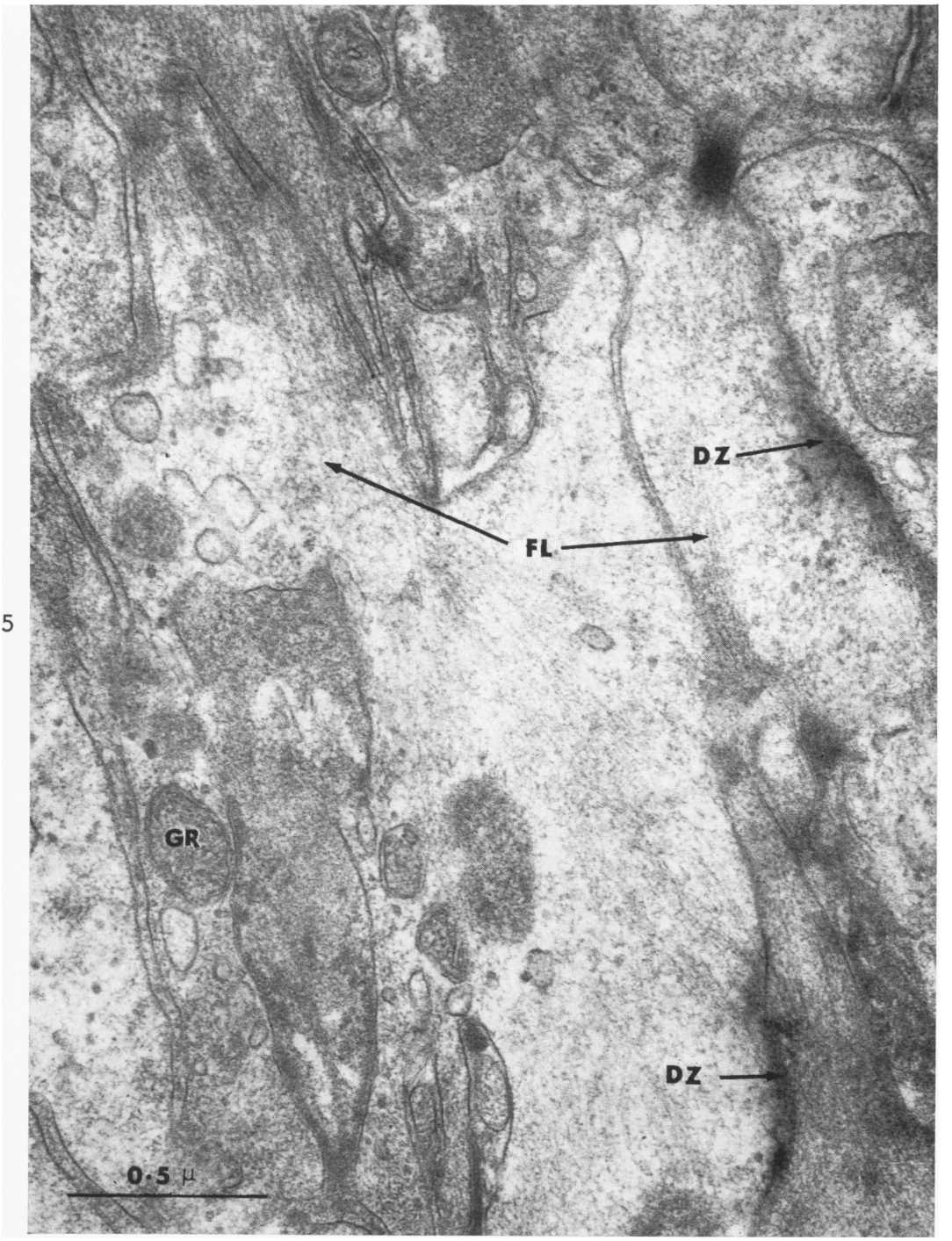


FIG. 5. Central region of 1-hr.-old thrombus. Dark zones (DZ) and fine filamentous-like structures (FL) within platelets are more evident. Dark zones sometimes appear to be in continuity with filamentous-like structures. Granule, GR. × 59,800.

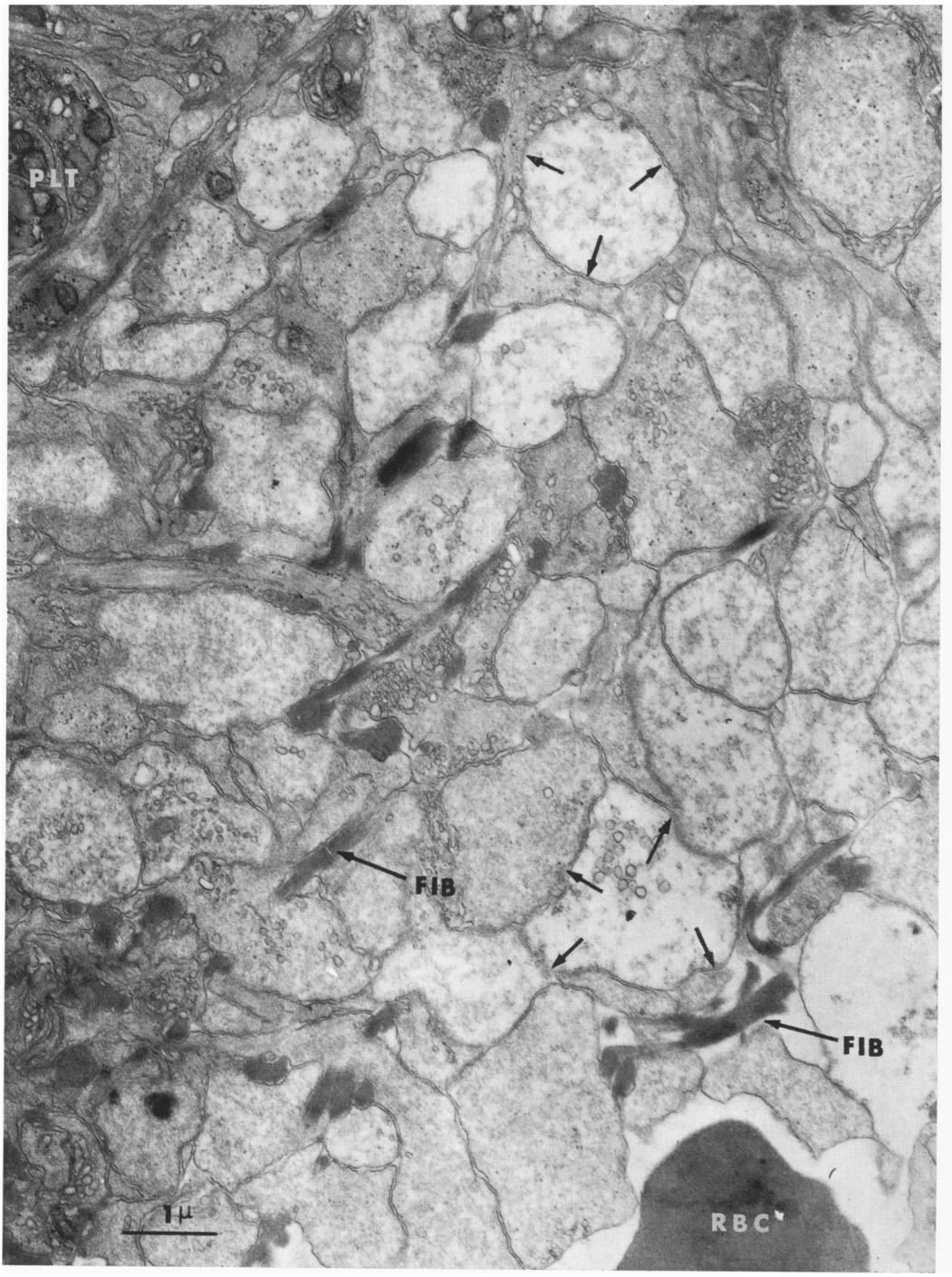


FIG. 6. Section of 1-hr-old thrombus at periphery where fibrin (FIB) is scattered among tightly packed platelets. Red blood cell (RBC) is at luminal side of thrombus. Platelets at upper left contain their organelles and are more electron dense than swollen platelets (outlined by arrows) at periphery. × 13,300.

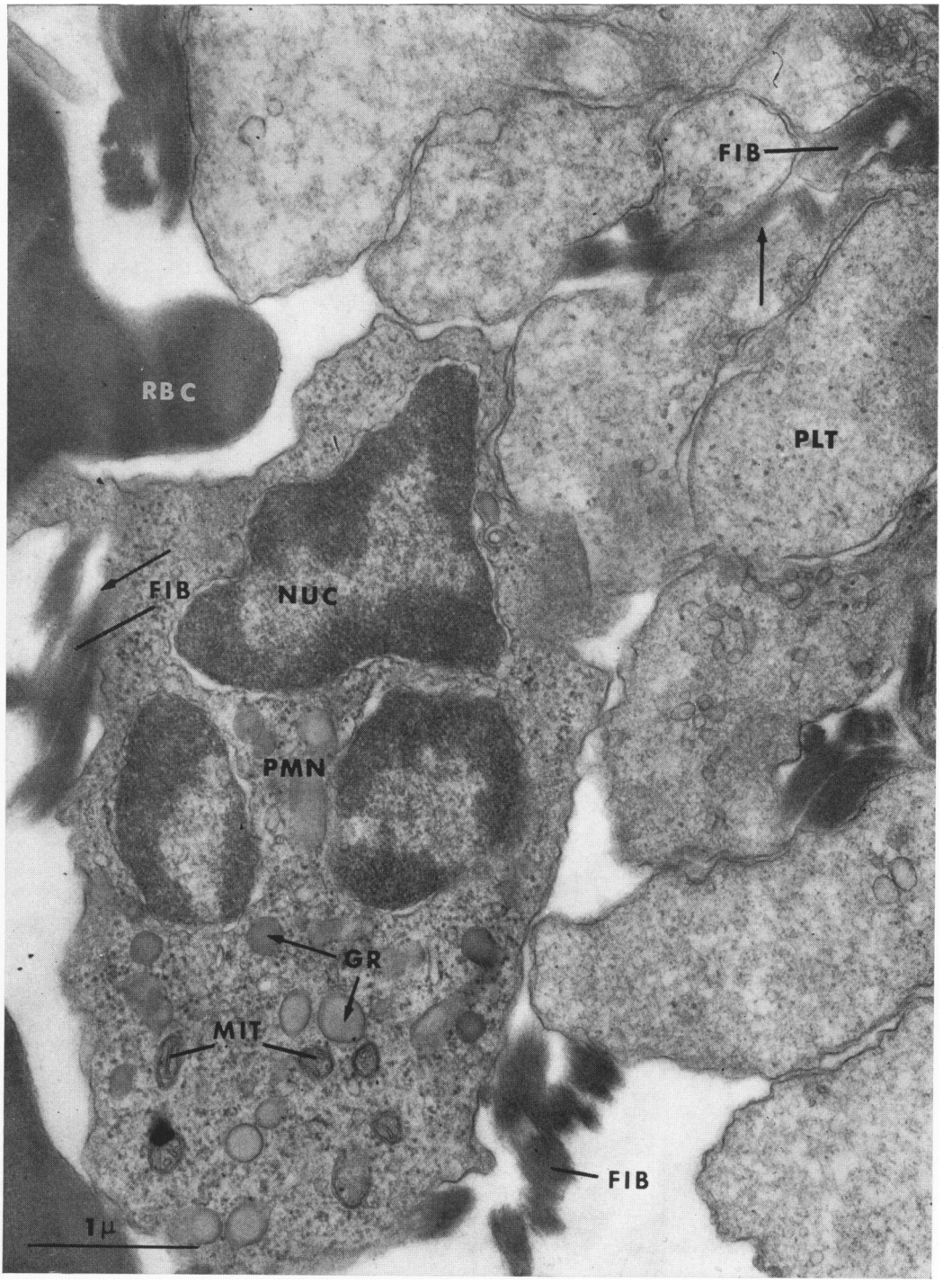


FIG. 7. Peripheral zone of thrombus shown in Fig. 6. Platelets (PLT) are swollen and degranulated. In some zones (arrows), fibrin (FIB) appears to be in close contact with platelet membrane and gives the impression of being in contact with platelet cytoplasm. This is probably an artifact because fibrin shows similar relationship with polymorphonuclear leukocyte (PMN) at left. Granules (GR) and nucleus (NUC) of leukocyte are shown. × 23,400.

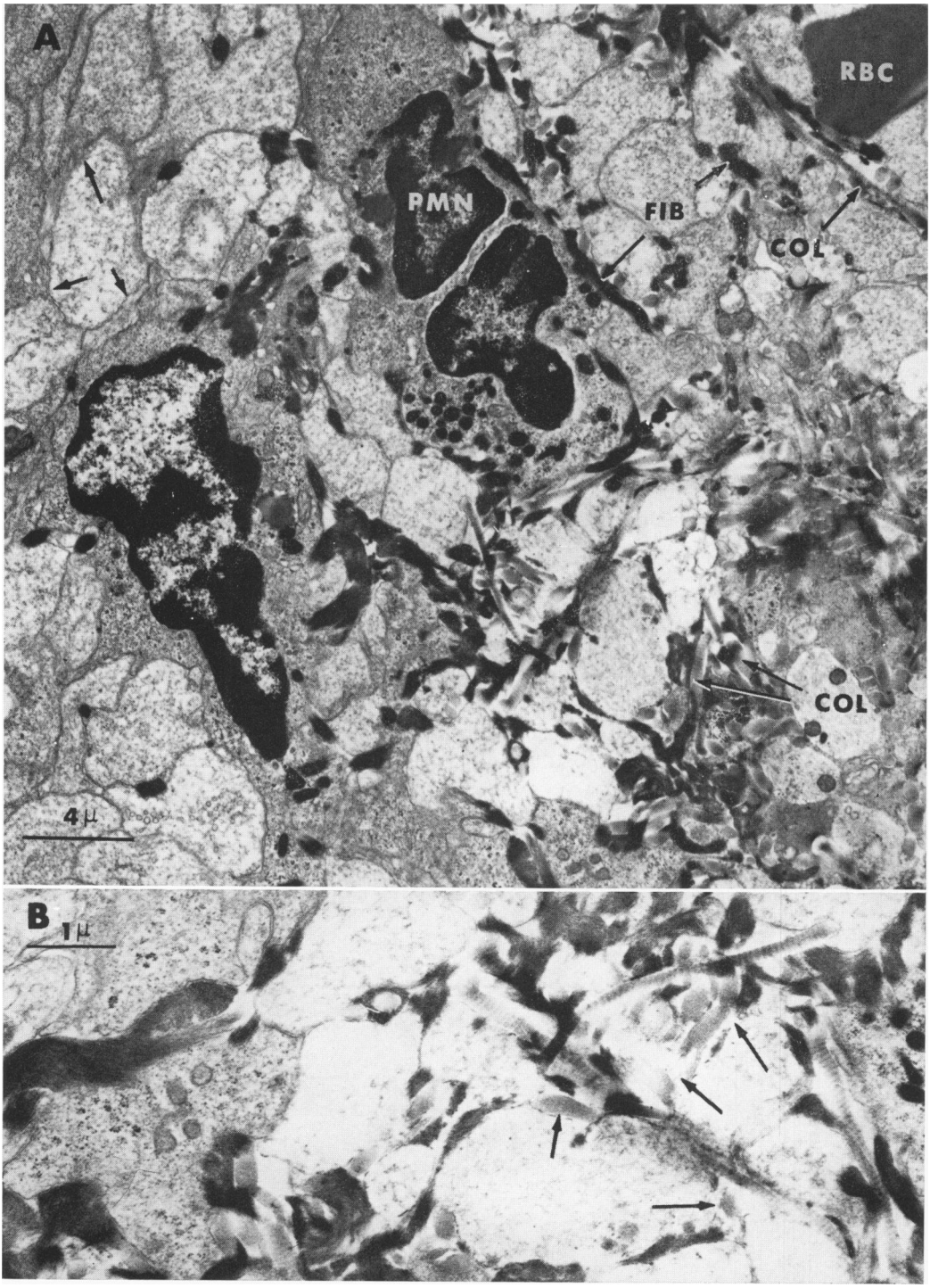
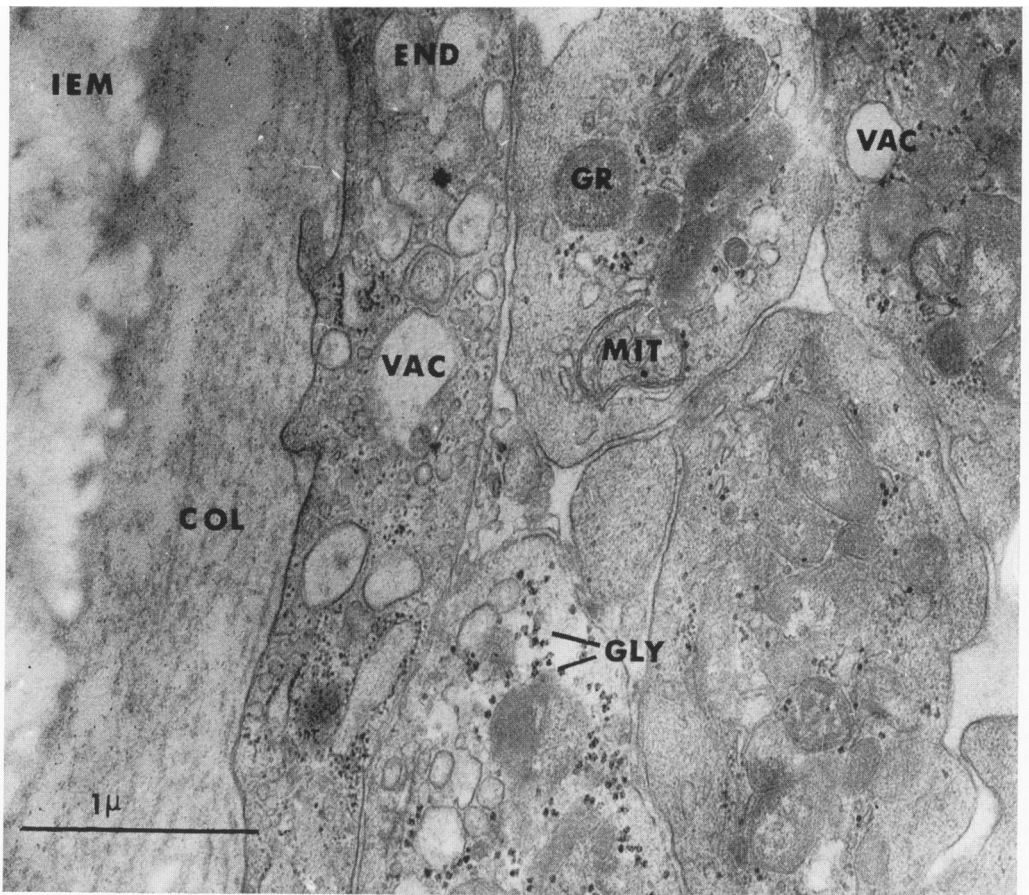


FIG. 8. A. Platelets in contact with collagen (COL) in 1-hr.-old thrombus are swollen and less electron dense than platelets toward center of mass at left. Swollen platelet is outlined by arrows. Fibrin (FIB) is interspersed among collagen fibers and platelets. Polymorphonuclear leukocyte (PMN) and red blood cell (RBC) are present. × 3200. B. At higher magnification, breaks (arrows) in platelet membranes are evident. × 13,500.

FIG. 9. Aggregated platelets in contact with endothelial cell (END) in 1-hr.-old thrombus. Granules (GR), mitochondria (MIT), vacuoles (VAC), and glycogen (GLY) are evident in platelets which have undergone little structural change. Some granules show layered structure within center part, giving appearance of tubules cut in cross section. Endothelial cell has vacuoles (VAC). Gap between electron-dense parts of platelet and endothelial cell membrane is about 150–300 Å. Internal elastic membrane (IEM) and collagen (COL) are indicated. × 30,800.

FIG. 10. Collagen (COL), increased amounts of fibrin (FIB), loosely packed platelets (PLT), and numerous white blood cells (WBC) are seen in this 3-hr.-old thrombus. Martius-scarlet-blue stain. × 376.



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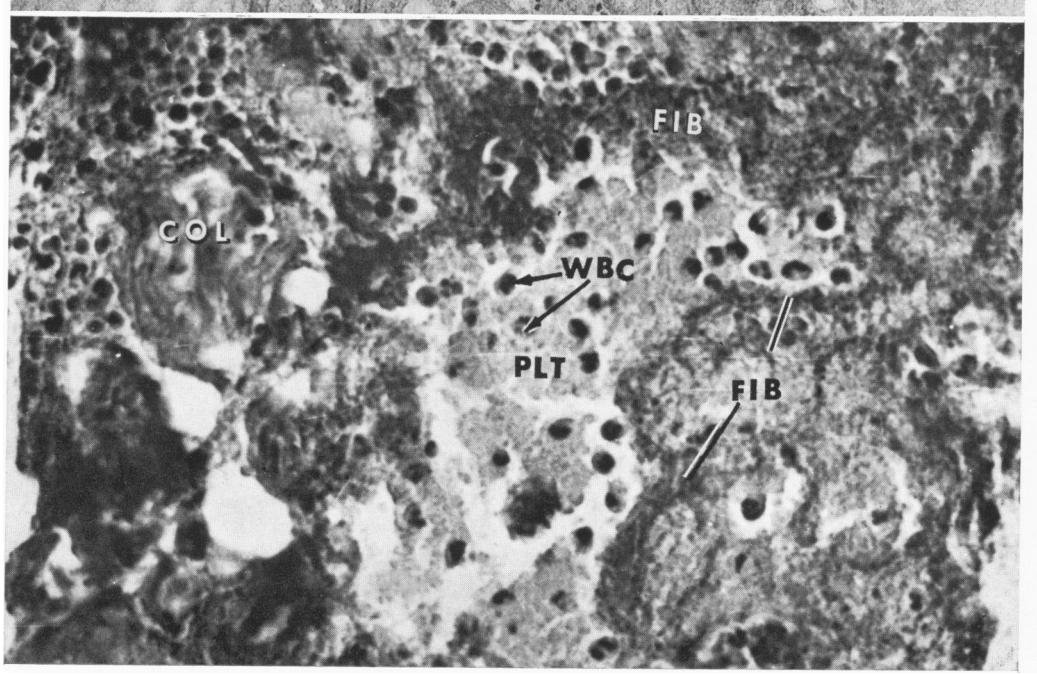

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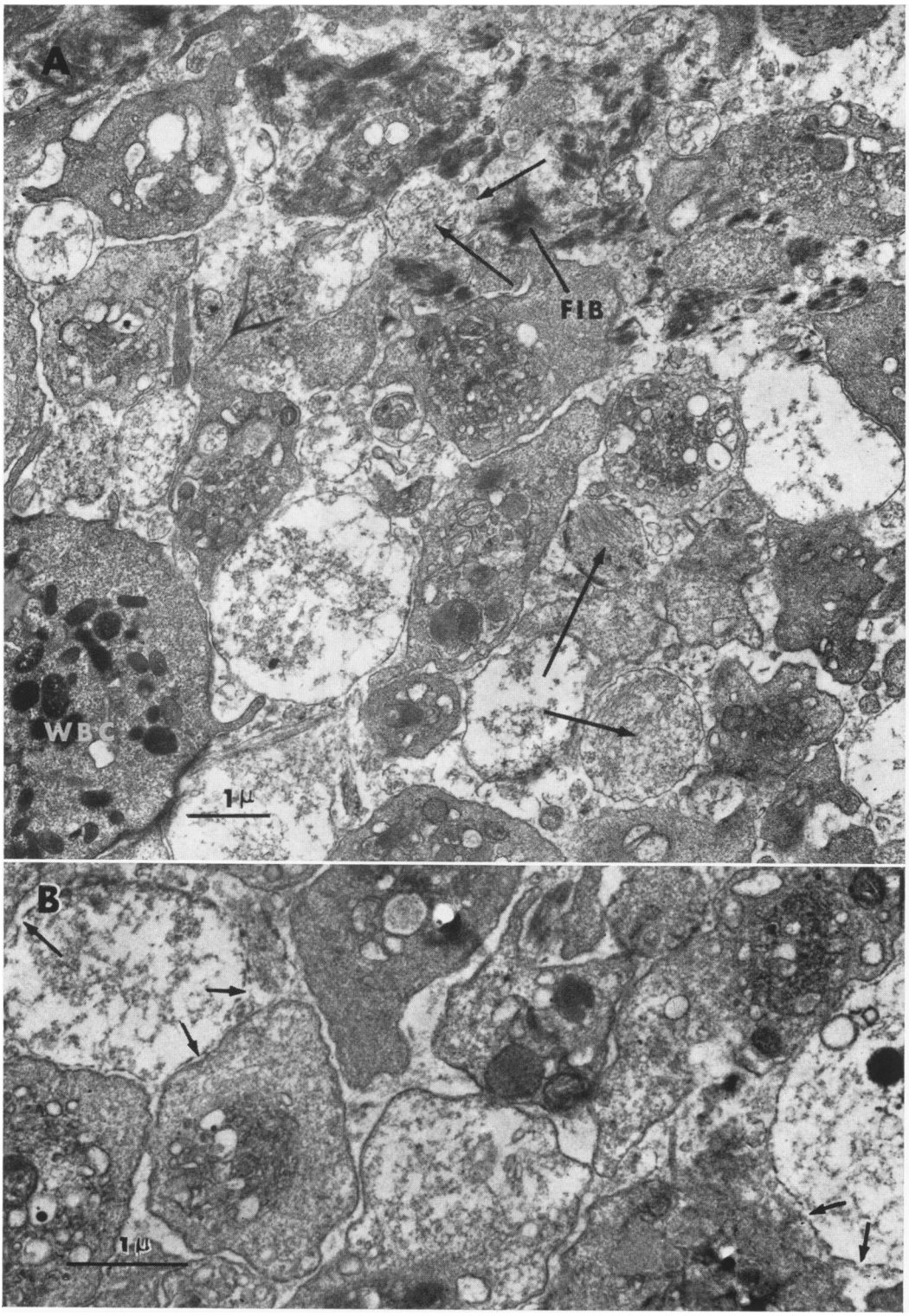
FIG. 11. A. Platelets in center of thrombus 2½ hr. after injury as not as tightly packed and appear more rounded than in earlier sections. Filamentous-like material (arrows) is present in some platelets. Around some platelets is increased fibrin (FIB). White blood cell (WBC) is present. × 13,000. B. Gaps (arrows) in membranes of swollen platelets can be seen. × 18,000.


A
FIB
WBC
1μ
B
1μ

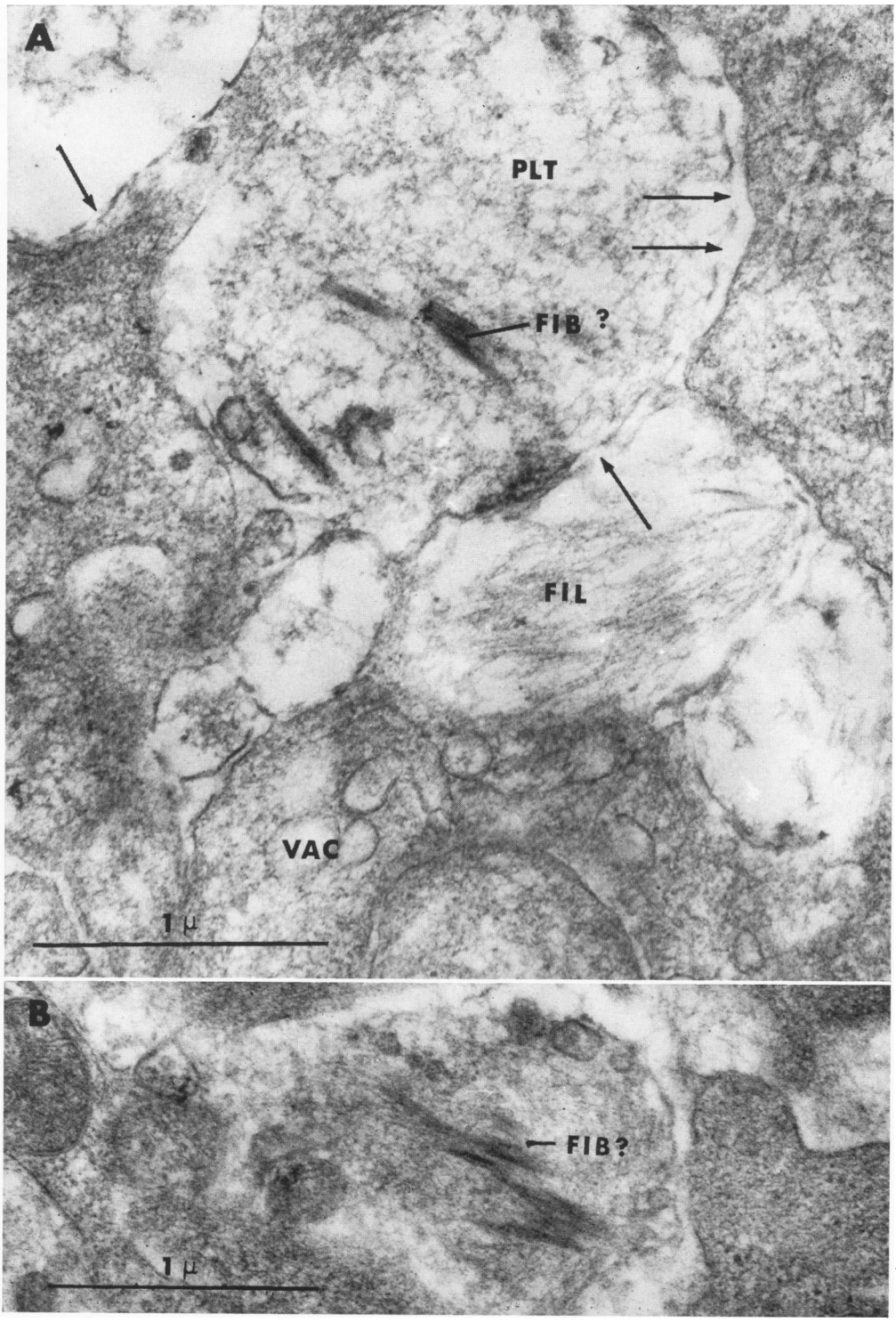
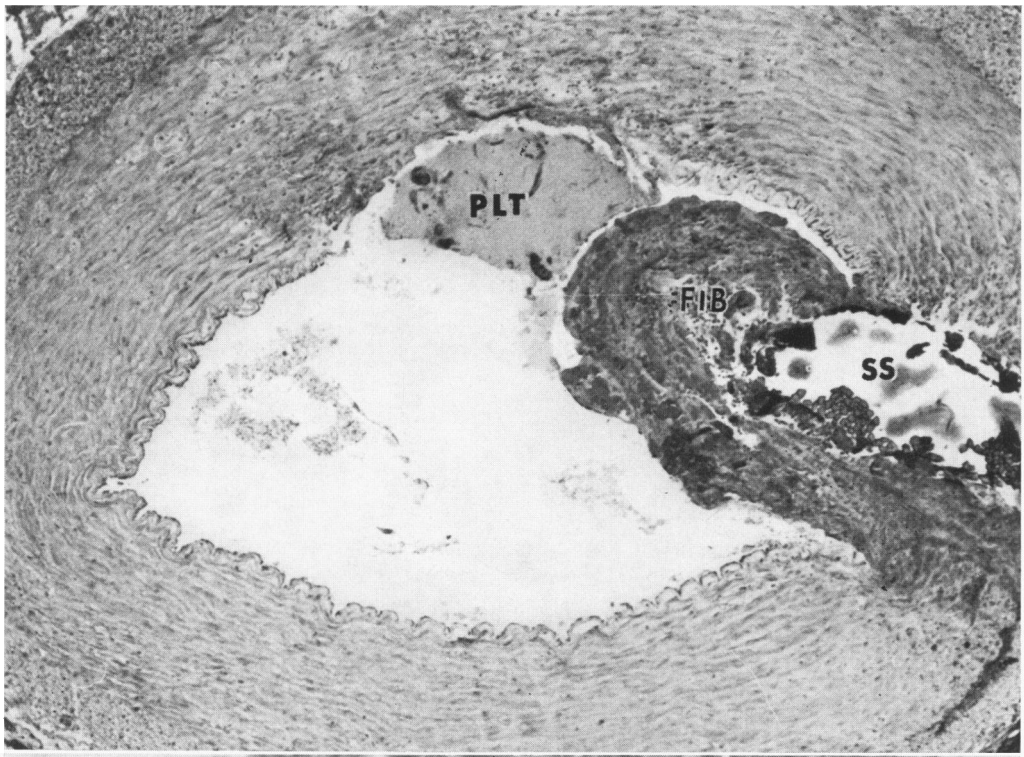


FIG. 12. A. Membrane around some platelets (PLT) in 3-hr.-old thrombus appears to have breaks (arrows). Filamentous-like material (FIL), and dark material which has appearance of fibrin (FIB?) is present in some platelets. Vacuoles are present (VAC). × 43,000. B. Fibrin-like material (FIB) within a platelet. × 40,000.



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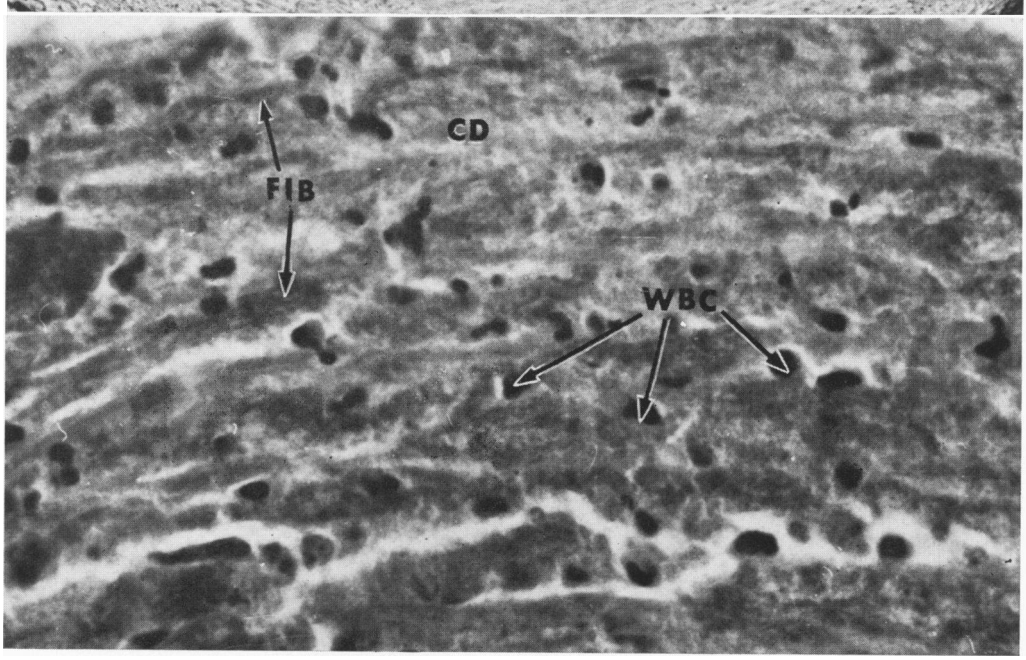

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FIG. 13. Twenty-four hours after insertion of silk suture (SS), thrombus is reduced in size and fibrin (FIB) is increased. There is a recent platelet aggregate (PLT) on the fibrin. Martius-scarlet-blue stain. × 52.

FIG. 14. Fibrin (FIB) in 24-hr. thrombus is interspersed with lighter areas. Cellular debris, CD; white blood cells, WBC. Martius-scarlet-blue stain. × 810.

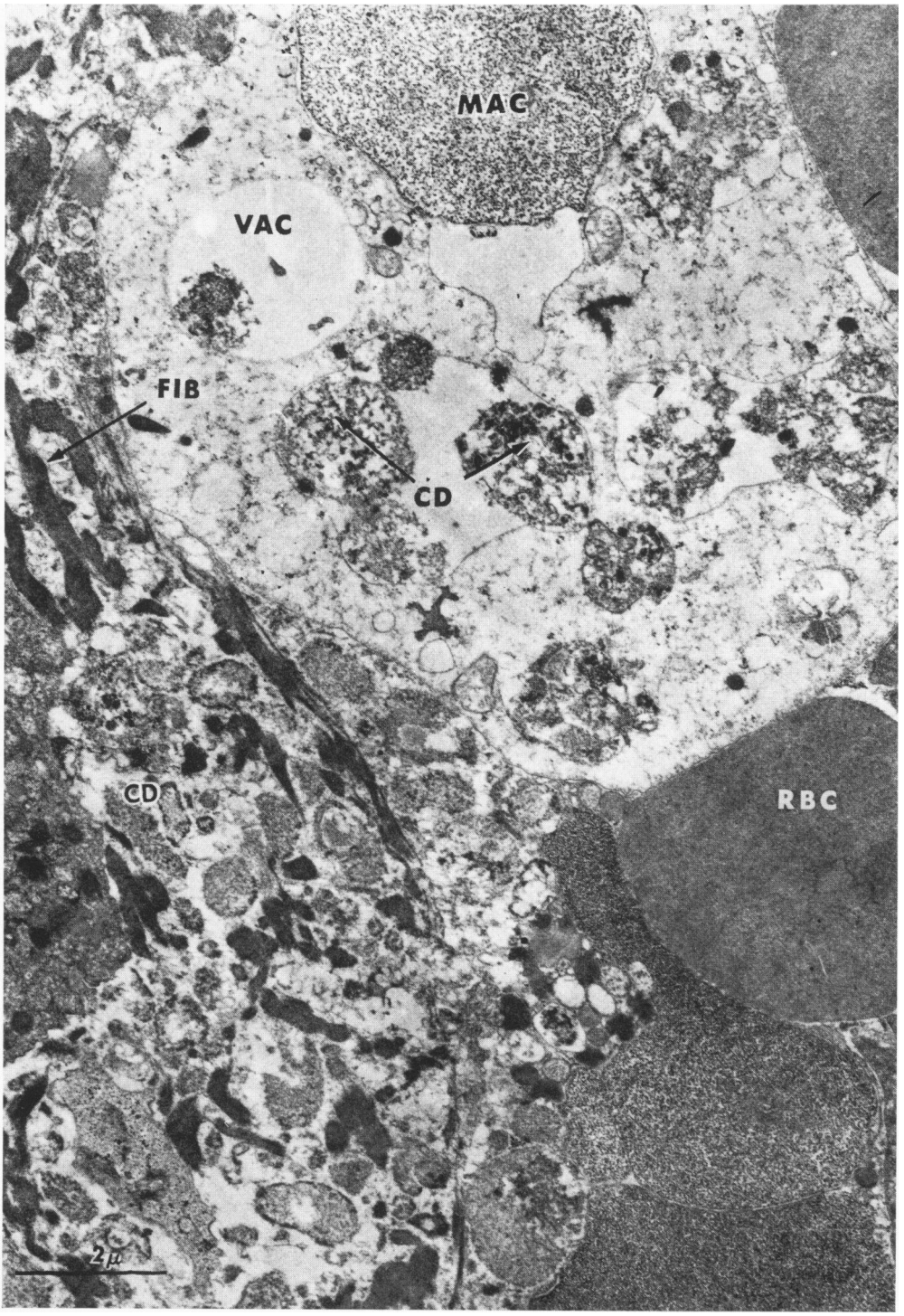

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FIG. 15. Center of 24-hr.-old thrombus shows no clearly identifiable platelets. Cellular debris (CD) is interspersed with fibrin (FIB). Macrophage (MAC) with several vacuoles (VAC) containing cellular debris is present. Red blood cell, RBC. × 12,000.

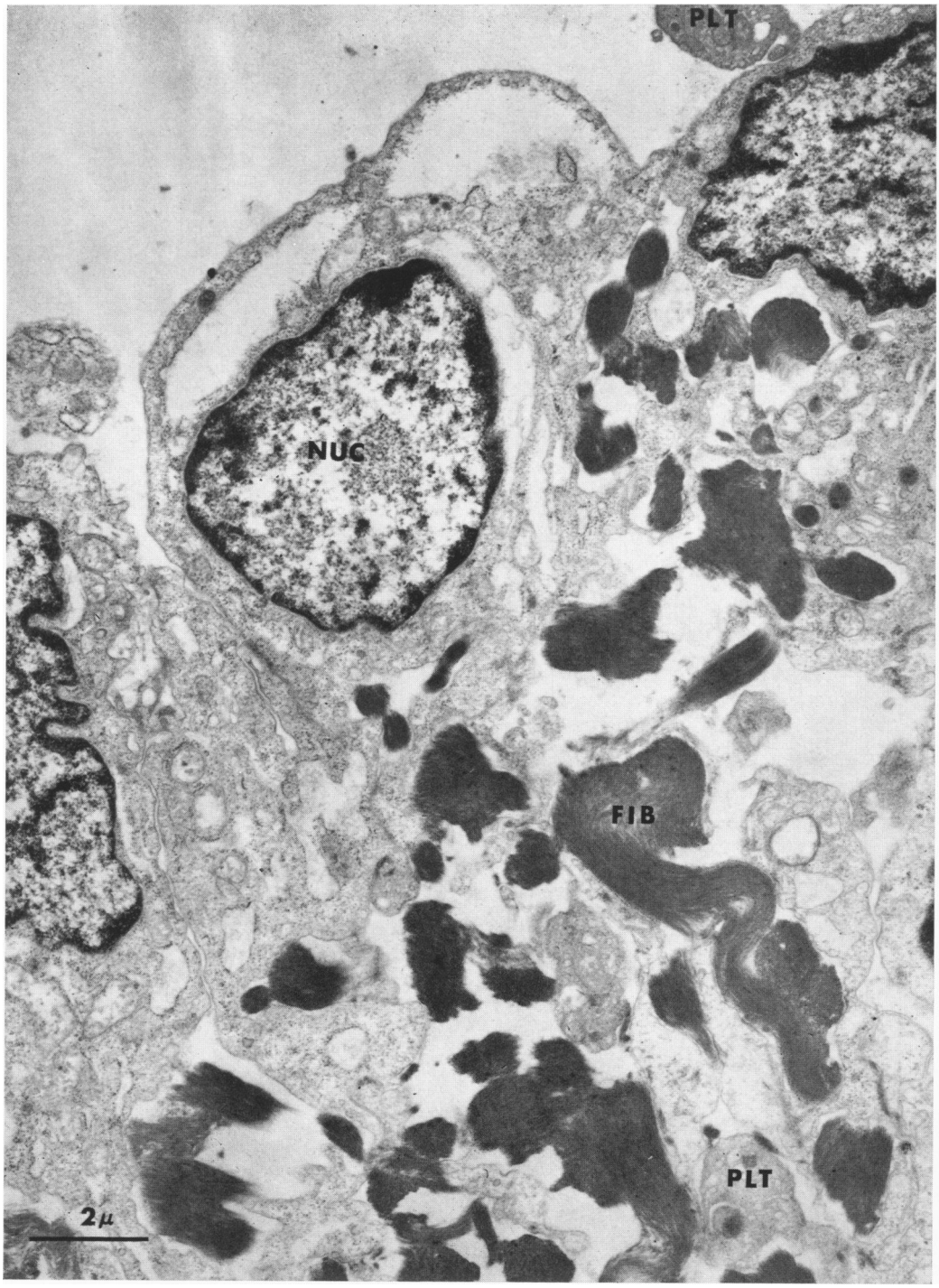

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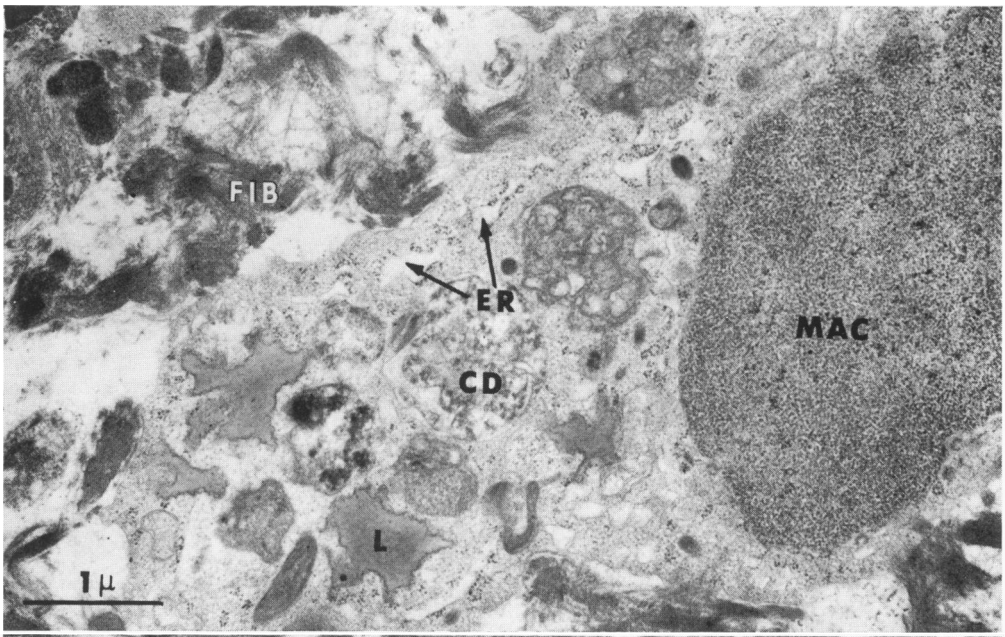
FIG. 16. Surface of 1-day-old thrombus is lined with mononuclear cells which appear to be adherent to each other (nucleus, NUC). Fibrin (FIB) is thick and interspersed among mononuclear cells and residual platelet material (PLT). × 11,800.

FIG. 17. Macrophage (MAC) with lipid inclusions (L) and phagocytosed cellular debris (CD) in 2-day-old thrombus. Endoplasmic reticulum, ER; fibrin, FIB. × 18,000.

FIG. 18. Macrophage (MAC) which has phagocytosed cellular debris (CD) has clear zone between it and dense fibrin (FIB). × 16,000.



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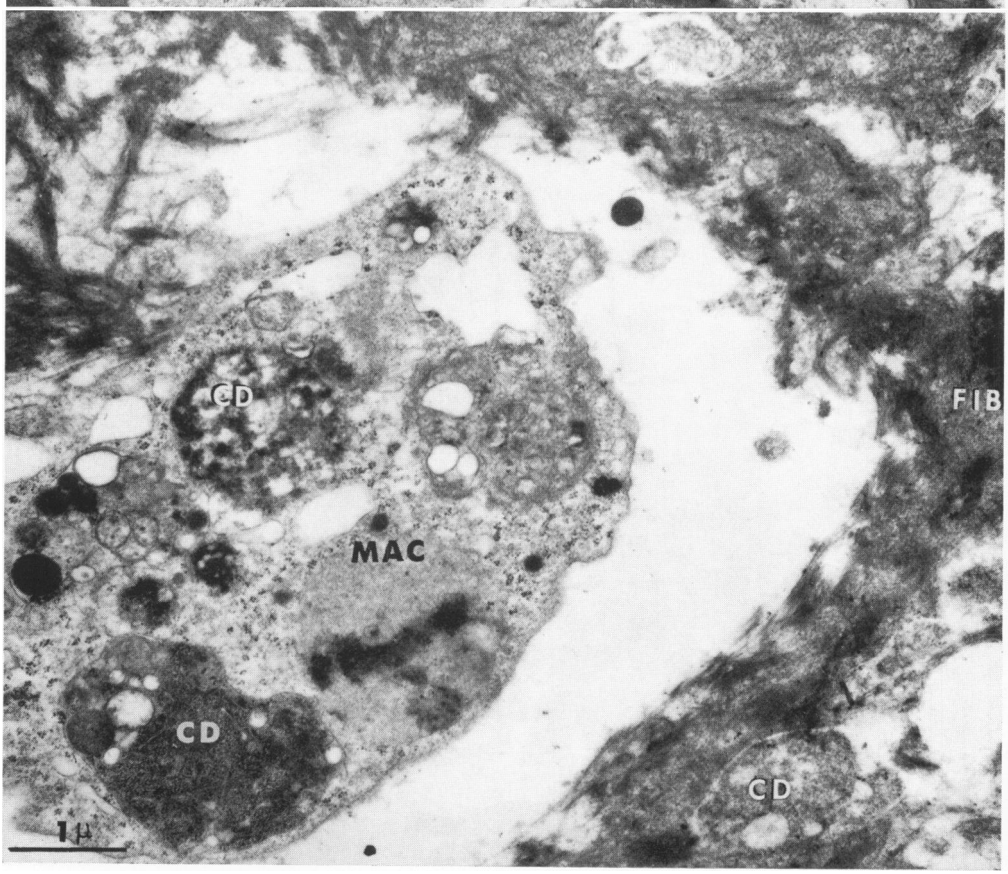

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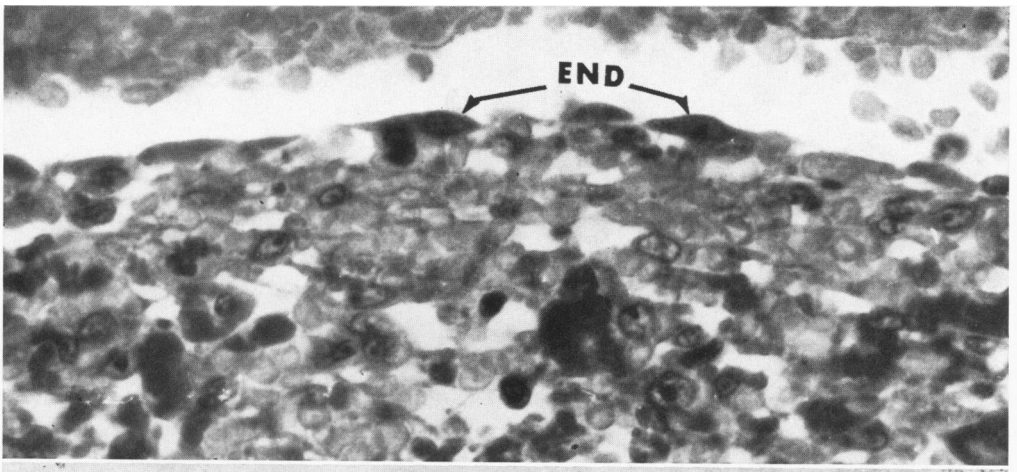
FIG. 19. Luminal surface of 7-day-old thrombus is covered by flat, endothelial-like cells (END). Granulation tissue contains fibroblast-like cells, macrophages, and eosinophils. Martius-scarlet-blue stain. × 810.

FIG. 20. Surface of 7-day-old thombus lined with young endothelium (END). Nucleus, NUC; endoplasmic reticulum, ER. Red blood cells (RBC) vary in electron density. There is some collagen (COL). × 5500.



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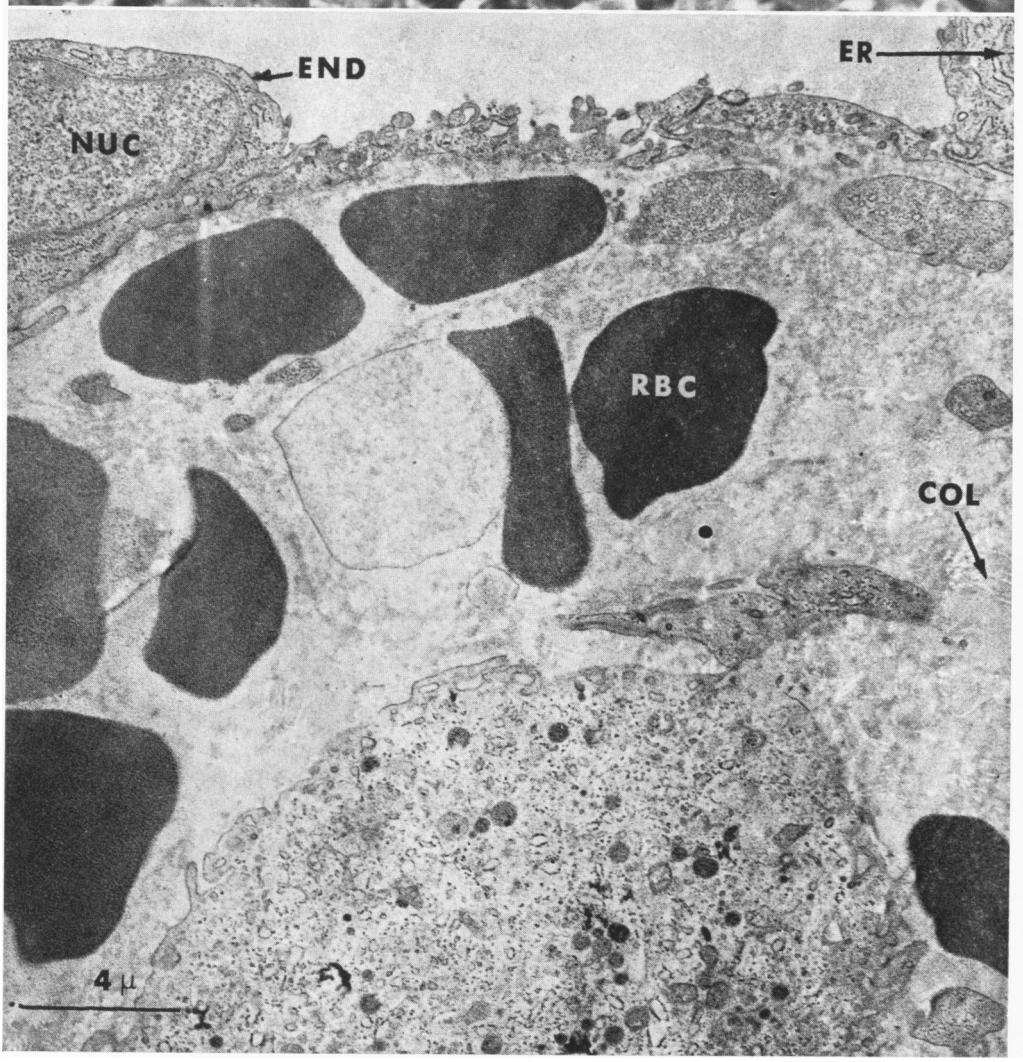

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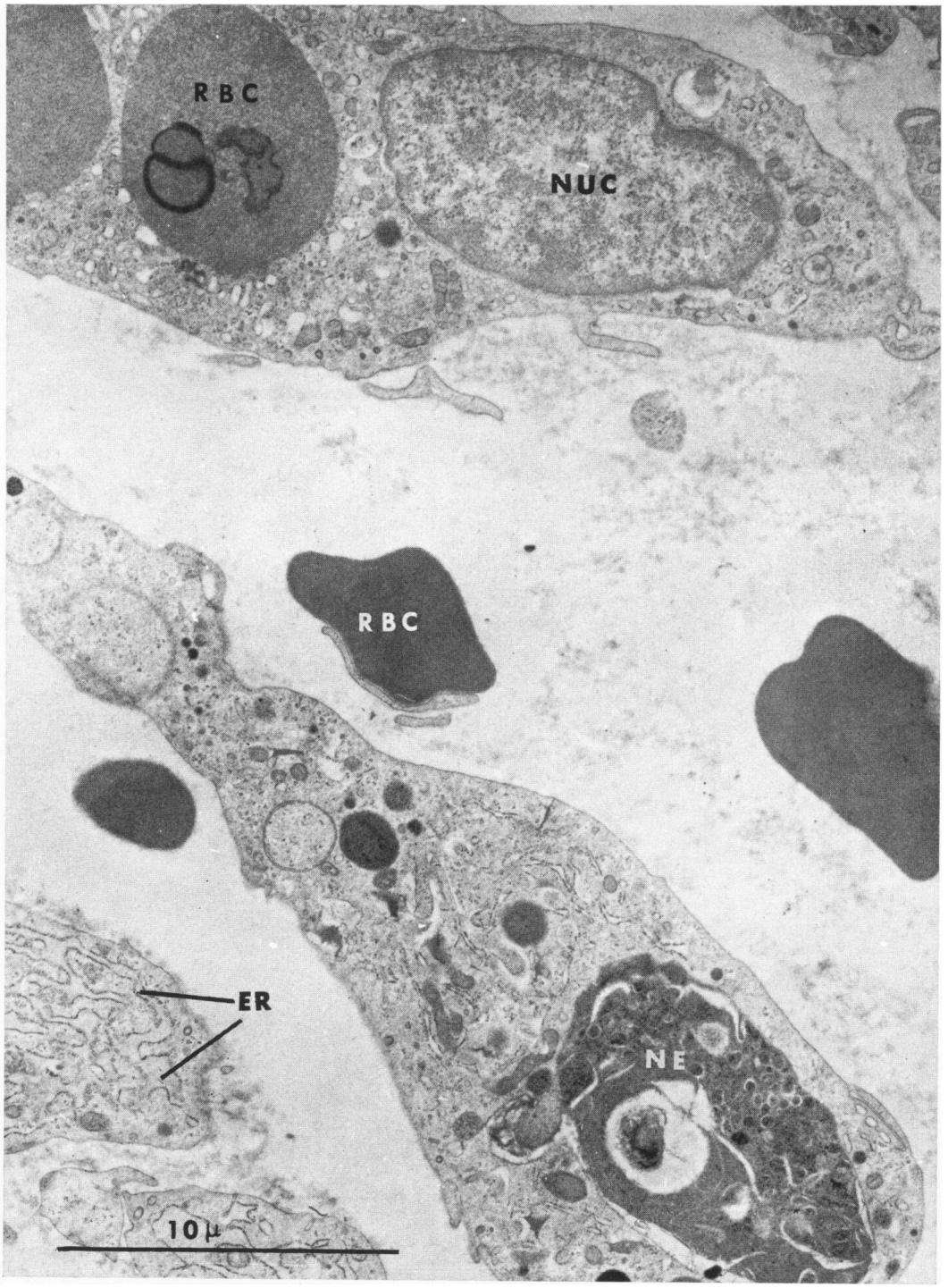

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FIG. 21. Elongated cells (nucleus, NUC) containing rough endoplasmic reticulum (ER) and considerable cellular debris, some of which seems necrotic (NE) in 7-day-old thrombus. Cell at top contains what appears to be phagocytosed red blood cells (RBC). × 5000.

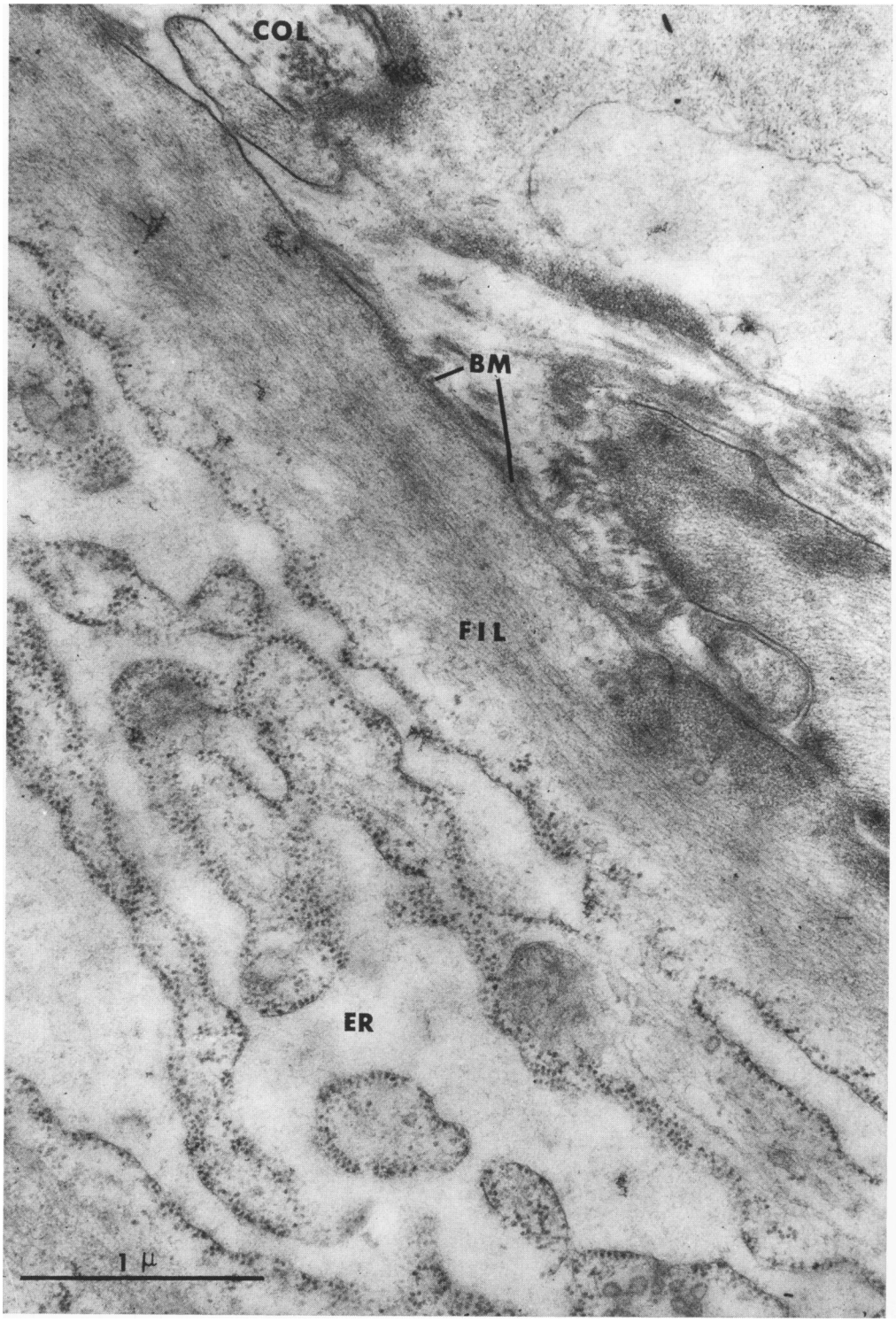

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FIG. 22. Elongated cell with fibrils (FIL) and endoplasmic reticulum (ER) at 8 days. Dark material along cell margin is probably beginning of a basement membrane (BM). Collagen, COL. × 35,700.

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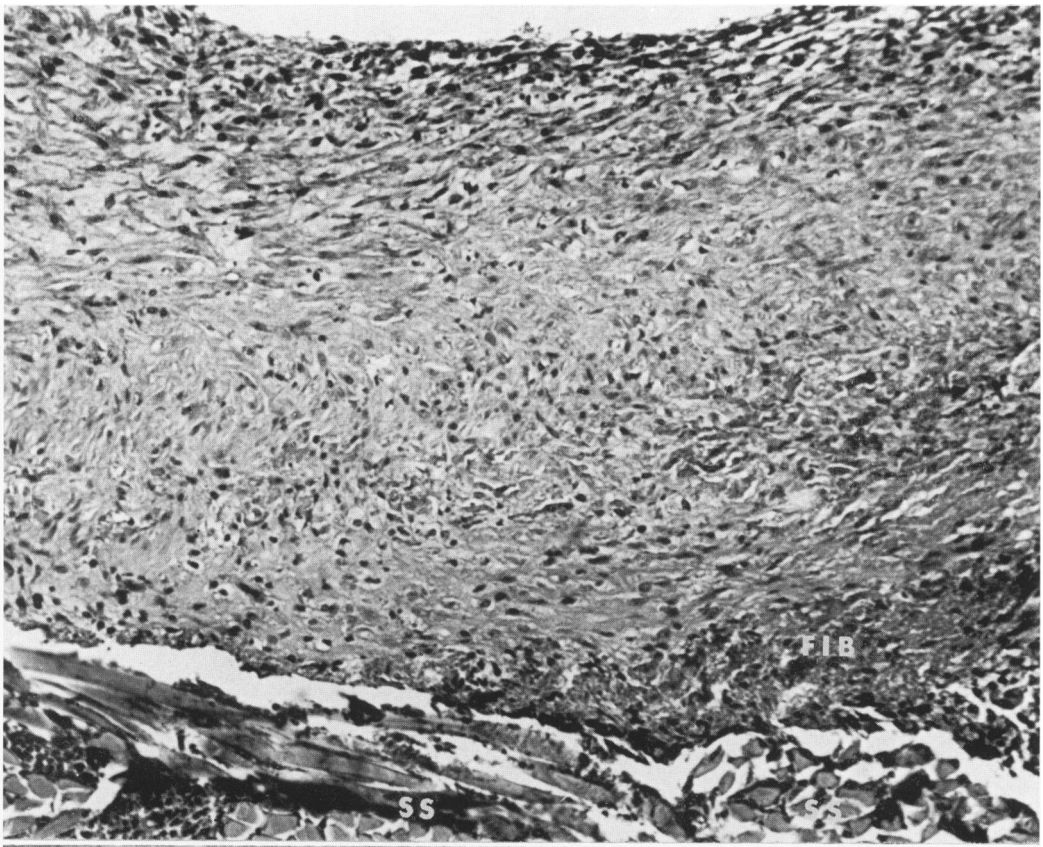

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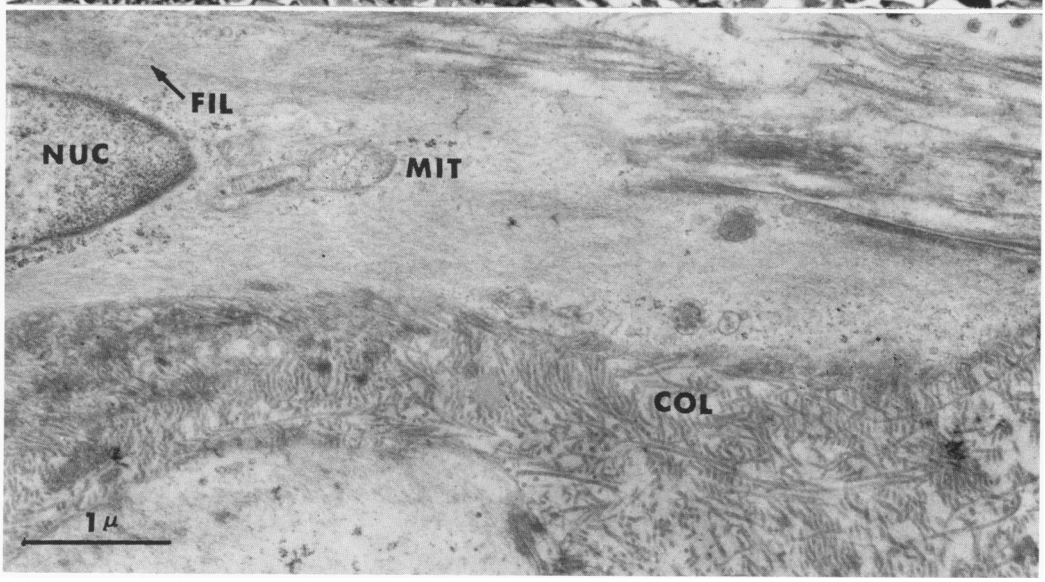

FIG. 23. Silk suture (SS) seen at base of thrombus at 11 days. Surface is lined by elongated cells; beneath it are elongated cells and connective tissue. Dark-staining material at bottom is probably residual fibrin (FIB). Martius-scarlet-blue stain. × 128.

FIG. 24. Collagen (COL) is extensive between cells in 14-day-old thrombus. Mitochondria, MIT; filamentous material, FIL; nucleus, NUC. × 20,000.

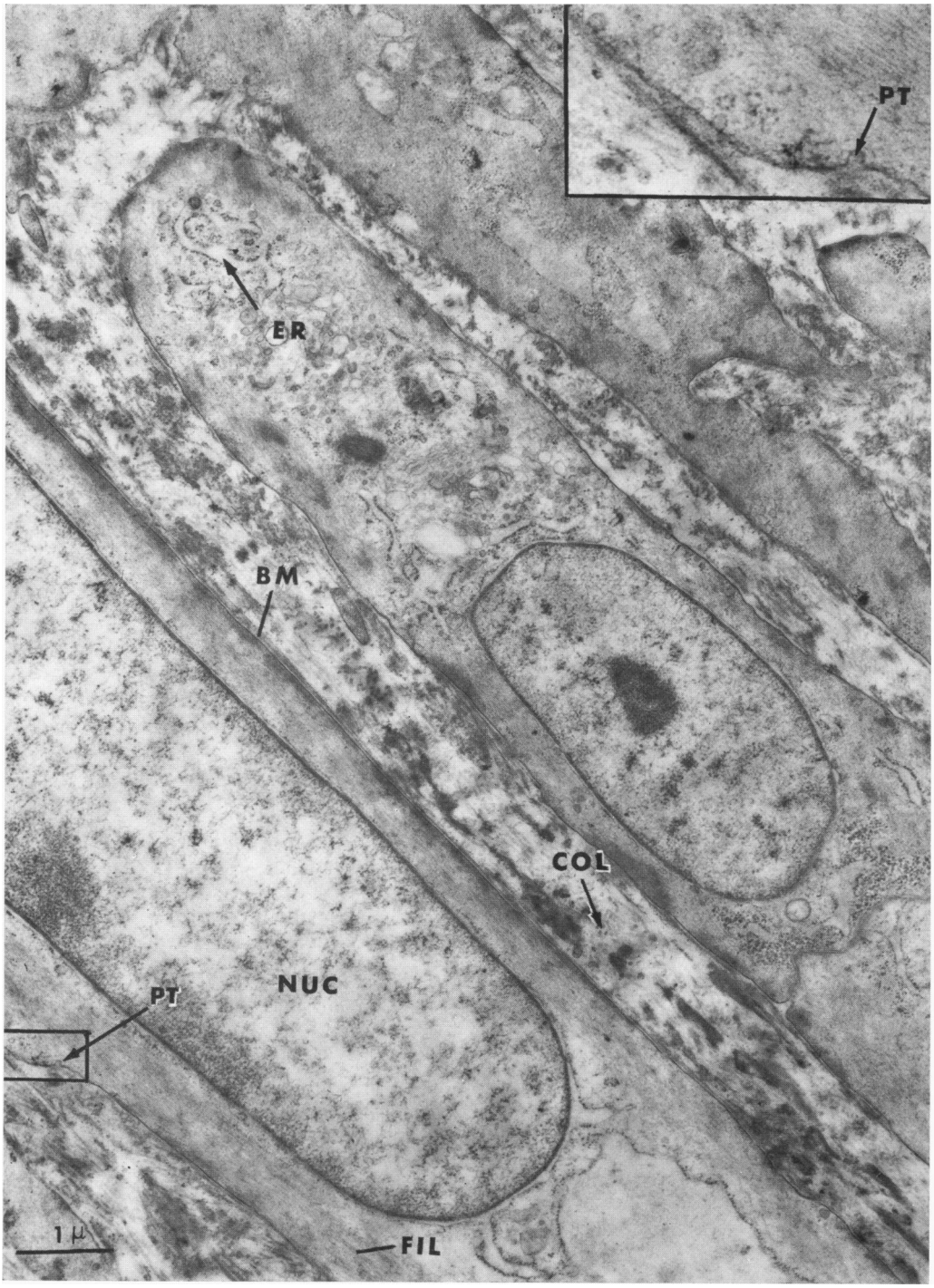

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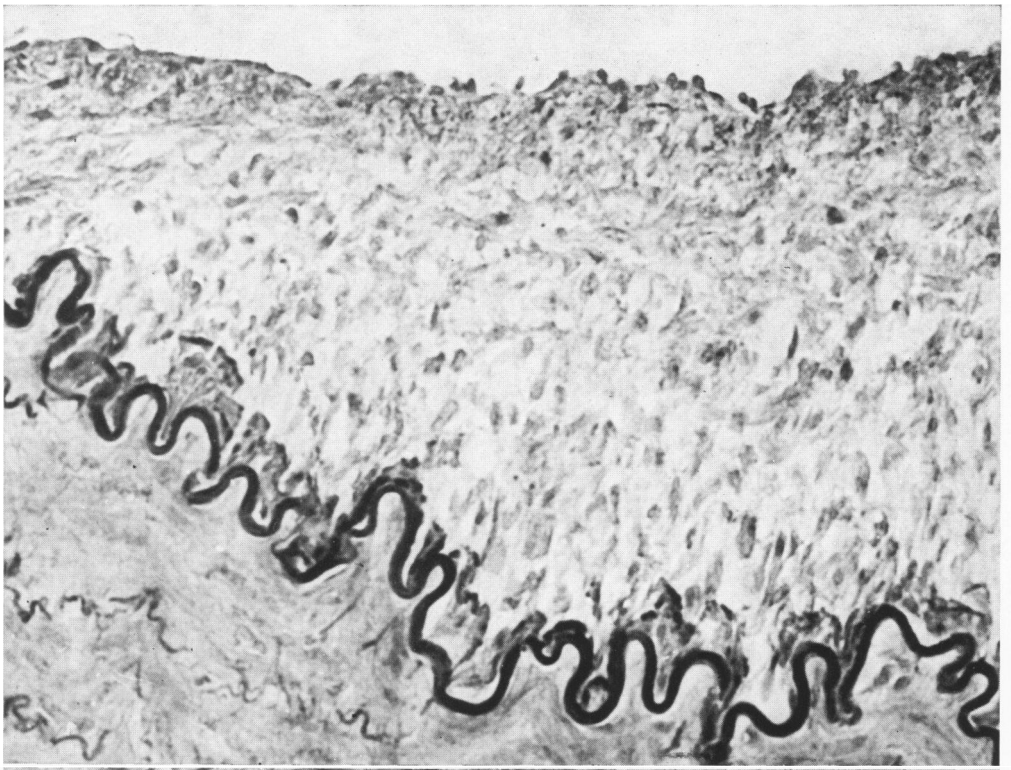
FIG. 25. Elongated cells in 15-day-old thrombus. Nucleus, NUC; endoplasmic reticulum, ER; basement membrane, BM; collagen COL. Some dark material between cells may be elastin, other portions may be residual fibrin. There is filamentous material in cells (FIL). × 14,000. Inset is area at lower left of figure, showing invagination pit (PT). × 40,000.

FIG. 26. Three-week-old thrombus appears to be composed of smooth-muscle cells and collagen. No evidence of lipid accumulation or vascularization in this lesion. Weigert's stain. × 330.

FIG. 27. Base of fibromuscular intimal thickening 4 months after injury. Calcified black masses (C) are evident. There are some small vessels (V). Internal elastic lamina (IEL) of original vessel wall is still evident. Martius-scarlet-blue stain. × 128.



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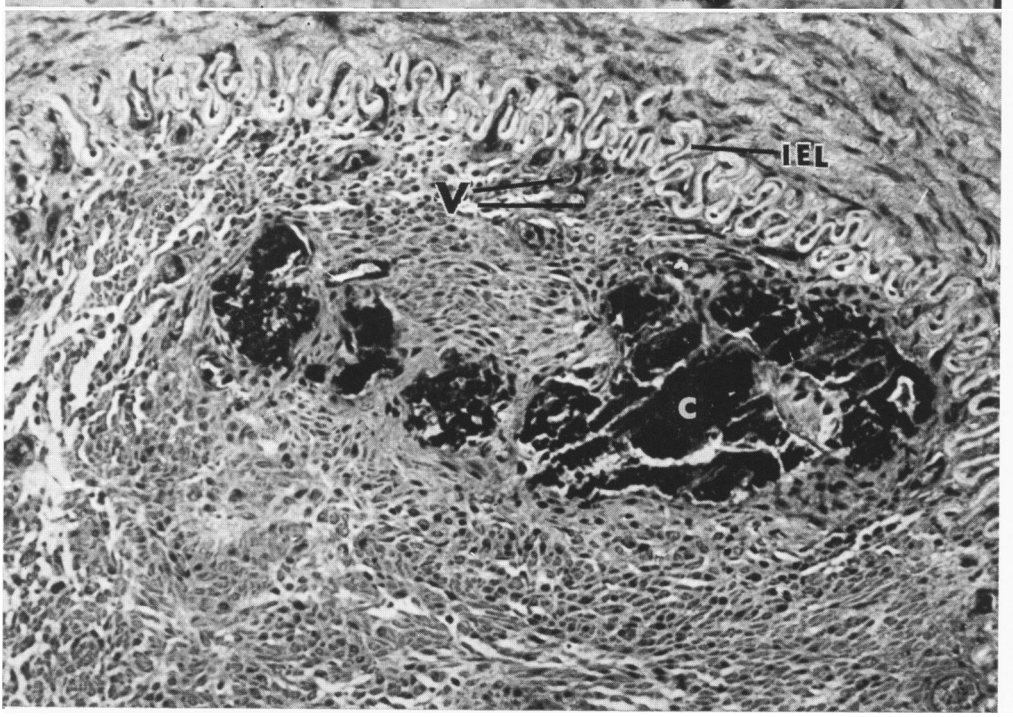

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