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Experimental Aortic Lesions of Acute Serum Sickness in Rabbits

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The mechanisms by which serum sickness cause lesions in the aortic intima are not known. The early aortic lesions of serum sickness detected by permeability to Evans blue dye were studied morphologically to determine the chronologic sequence of lesion initiation and immunochemically with fluorescein-labeled rabbit IgG and bovine serum albumin to detect the presence of antigen and antibody. The topographic localization of lesions and the morphologic changes observed suggest that endothelial injury occurs first, probably due to immune complex deposition, and that platelets play a secondary or reactive role in the process. (Am J Pathol 88:255–266, 1977)

SERUM SICKNESS COMBINED with hyperlipidemia in experimental animals enhances lipid deposition in the aorta,[1] and when repeated episodes of serum sickness are induced, atherosclerotic lesions result that are similar to human plaque lesions.[2] These findings suggest that increased endothelial permeability associated with the immune complex disease is the cause of or at least is contributory to enhanced arterial lipid deposition and plaque formation. Immune complexes could directly damage the endothelium by deposition in or on the cells with subsequent complement activation or indirectly damage the endothelium by causing platelet aggregation and release. The present experiment was done to investigate the early lesions of serum sickness in the aorta detected by Evans blue permeability for endothelial injury and the presence of platelets.

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Materials and Methods

Twenty-one young New Zealand white rabbits (test) were injected intravenously with 250 mg bovine serum albumin (BSA)/kg body weight. Eleven control rabbits were injected intravenously with a similar volume (2 ml) of physiologic saline. The rabbits were killed in equal groups on the 17th, 18th, and 19th days after injection (5 controls on the 19th day). Two hours prior to killing, each rabbit was injected intravenously with 2 ml of 5% Evans blue. Four rabbits from each test group were anesthesized by intravenous sodium pentobarbital and killed by perfusion with 0.9% saline for 5 minutes followed by 2% glutaraldehyde in 0.15 M phosphate buffer, pH 7.4, for 15 minutes through the heart at mean arterial pressure. Three rabbits from each test group and 3 control rabbits were killed on the 19th day by an overdose of sodium pentobarbital without perfusion fixation to provide tissues for immunofluorescence studies.

Complete necropsies were done. The aorta was opened longitudinally and the intimal surface from the heart to the common iliac arteries examined for foci of Evans blue staining. Blocks of stained and unstained aorta and blocks of renal cortex were further fixed in 2% glutaraldehyde in 0.15 M phosphate buffer for 4 hours and washed in 0.15 M phosphate buffer. For transmission electron microscopy, stained and unstained aortic tissue, and renal cortex were postfixed in 1% osmium tetroxide, dehydrated in graded alcohols, and embedded in Spurr low viscosity epoxy resin. One-micron-thick sections were cut and stained with toluidine blue and screened to select those oriented to show the intimal surface. Ultrathin sections were cut from selected blocks, stained with saturated uranyl acetate and lead citrate, and examined with a Hitachi-12 electron microscope. Tissue for scanning electron microscopy was dehydrated in graded alcohols and processed through a graded series of ethanol/amyl acetate. The tissue was critical point-dried, mounted, coated with carbon and gold, and examined with a Cambridge Stereoscan electron microscope.

For immunofluorescence, blocks of stained and unstained aorta and blocks of renal cortex were removed immediately after killing and rapidly frozen in liquid nitrogen. Immunofluorescence staining was performed for rabbit IgG and BSA as previously described [3] and examined with a Zeiss Universal fluorescent microscope.

Results

Seventeen of the 21 test rabbits showed more numerous foci of staining on the inner surface of the aorta and more intense staining than was seen in 5 of 8 control rabbits. The localization of staining was similar in test and control rabbits, being localized to the arch and proximal descending thoracic aorta (Figure 1). No consistent pattern of staining was evident in test or control rabbits with regard to anterior or posterior surfaces or orifices of emissary arteries. The stained areas were discrete, ovoid in shape, and less than 2 mm in diameter.

Transmission Electron Microscopy

The aortic intima from unstained areas of control rabbits was covered by normal endothelial cells with centrally placed nuclei. The intima in unstained areas was thin and composed of endothelium, basement membrane material, an occasional smooth muscle cell, and fibers of the internal elastic lamina (Figure 2). Sections of stained aorta from controls generally appeared normal. There were scattered foci of intimal edema

and widened subendothelial tissue space in the stained aorta of controls which were associated with small vesicles in the cytoplasm of the endothelium and focal separation of endothelial cells.

The unstained aorta from test rabbits in most cases was similar to that of controls. There were, however, rare areas where the subendothelial space appeared slightly widened; the overlying endothelium in such areas appeared normal.

Two types of intimal lesions were found in stained areas from test rabbits. One was characterized by marked widening of the subendothelial tissue space and intimal edema and was associated with the presence of small vesicles in the endothelial cytoplasm and focal separation of endothelial cells similar to that seen rarely in stained areas from control rabbits (Figure 3). The widened subendothelial space was largely electron lucent and contained scattered flocculent-appearing material resembling basement membrane, collagen fibers, elastic fibers, and occasional electron-dense fibrillar material appearing to be fibrin (Figure 3). Endothelial cell nuclei were folded in appearance, suggesting cell contraction (Figure 4). Cytoplasmic bleb formation was frequently seen in endothelial cells. Endothelial cytoplasm contained increased numbers of vesicles, most of which had no apparent content (Figure 5). Occasional endothelial cells showed increased numbers of profiles of granular endoplasmic reticulum and scattered electron-dense bodies in an electron-dense hyaloplasm (Figure 5). Occasionally on the lumen surface of the endothelium there was electron-dense fibrillar material appearing to be fibrin. Platelets were not seen adherent to the endothelium.

The second lesion type was characterized by focal loss of endothelial cells with a layer of platelets and fibrin on the intimal surface. The intima generally appeared slightly to moderately widened. The surface denuded of endothelium was covered by a pavement of platelets of variable thickness ranging up to six layers. Areas with a thick layer of platelets typically showed the platelets adjacent to the aorta to be degranulated (Figure 6). Occasionally there were small electron-dense fibers between the platelets and fibrin. Cells were found infrequently in the subendothelial space that had numerous mitochondria and profiles of granular endoplasmic reticulum (Figure 7). These cells resembled plasma cells. Plasma cells were not identified in light microscope sections. The identity of these cells is unknown.

Scanning Electron Microscopy

The surface of control unstained aorta was smooth, almost flat, showing slight protrusion into the lumen of endothelial cell nuclei (Figure 8).

Crater-like gaps in the endothelial lining were seen infrequently in stained portions of control aortas. These gaps usually were near the orifice of an emissary vessel.

The aortic surface of stained areas from test rabbits showed two types of alteration. One was a cobblestone appearance due to the widening of the subendothelial space (Figures 9 and 10). The endothelial cells showed numerous small villous-like projections of cytoplasm into the lumen. Crater-like gaps in the endothelial lining were numerous and varied widely in size and shape (Figures 10 and 11). The margins of the gaps had a ragged appearance and in some areas were partially covered by flap-like extensions of the cytoplasm of adjacent cells. Many endothelial cells showed defects in the central portion of the cytoplasm having a fish-mouth appearance (Figure 12). Fibrin and platelets commonly were found in and around the large crater-like defects (Figures 11 and 12).

Immunofluorescence

Stained and unstained areas from control and test rabbit aortas were examined for rabbit IgG and BSA by immunofluorescence. All sections from controls and unstained test rabbit aorta were negative for rabbit IgG and BSA. Stained areas from test rabbit aorta showed focal staining for rabbit IgG and BSA at the surface of the intima, and in occasional areas the staining extended into the subendothelial intimal space.

Renal glomeruli from control rabbits showed no staining for rabbit IgG or BSA. Glomeruli from test rabbits showed immunofluorescent staining for rabbit IgG and BSA in the capillary basement membranes. The pattern of staining was granular. Immunofluorescent staining for rabbit IgG and BSA also was seen in occasional interlobular arteries and peritubular capillaries. Transmission electron microscopy revealed focal electron-dense deposits on the epithelial side of the basement membrane.

Discussion

Immune complex–induced aortic injury, coupled with an induced hyperlipemia, results in arterial lesions that closely resemble atherosclerotic plaques in humans.[2,4,5] This potentially valuable model for human plaque lesions prompted the present study which was designed to observe morphologically the early lesion in the aorta associated with immune complex disease. That the test rabbits had immune complex disease is evidenced by localization of rabbit IgG and BSA in the aortic lesions and the presence of typical antigen–antibody type complexes in the kidney. The absence of rabbit IgG and BSA in control and unstained test rabbit aorta

indicates that the observed lesions in test rabbits were not artifacts related to anesthesia, agonal shock, or tissue handling. Evans blue dye was used to detect the early lesions for morphologic and immunologic study.

By some mechanism, circulating immune complexes enhanced endothelial permeability in the aorta, as evidenced by the increased uptake of Evans blue dye, but the complex disease did not alter the topographic localization of lesions from that observed in controls. The localization of rabbit IgG and BSA in the aortic lesions of test rabbits could be the cause of the observed increase in endothelial permeability or alternatively could be the result of increased permeability. Kniker and Cochrane [6] concluded that abnormal or increased endothelial permeability is necessary for immune complex deposition in the aorta. The localization in the arch and proximal descending thoracic aorta was considered to be a reflection of endothelium altered by the hemodynamic forces operative in this area under normal circumstances. The finding in the present study of similar localization in test and control rabbits would appear to confirm the results and conclusions of Kniker and Cochrane;[6] however, the finding of greater permeability, deeper staining lesions, and more numerous foci of staining in test rabbits suggests that the explanation is incomplete. If there is a spectrum of variable endothelial permeability in the aorta that is governed in part or completely by local hemodynamic stresses, it is possible that the observed increase in staining and more numerous foci of staining in test rabbits reflects endothelial damage due to immune complex deposition added to that of hemodynamic injury. If such a spectrum does exist normally, an additive or potentiating effect of immune complex disease was not evident at any other topographic area; the pattern of staining was the same in test and control rabbits.

An alternative mechanism which might explain in part the observed effect of immune complex disease on the aorta is through the effect of immune complexes on platelets. Immune complexes are known to cause platelet aggregation [7] which could affect endothelial permeability either by aggregates depositing on the endothelium or by the release of vasoactive substances such as serotonin into the blood.[8] Platelet aggregation on the endothelium was not seen in the present experiment. Platelet aggregates on the intimal surface were only evident where the endothelium was missing. If intravascular platelet aggregations and release of vasoactive substances were the cause of the endothelial damage, we would expect to have seen a change in the pattern of staining of the aorta from that observed in controls; the pattern of staining was the same in test and control rabbits. The findings of the present experiment suggest that

platelets do not play a role in the pathogenesis of the aortic lesions until the endothelium has been severely altered by immune complex deposition.

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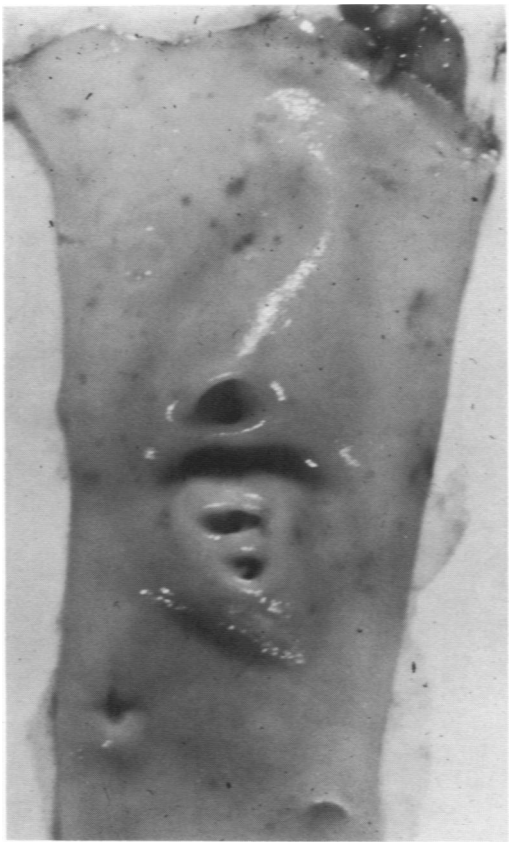


Figure 1—Surface view of test rabbit aorta 18 days after injection of BSA. Notice the presence of Evans blue-stained areas. (× 3)

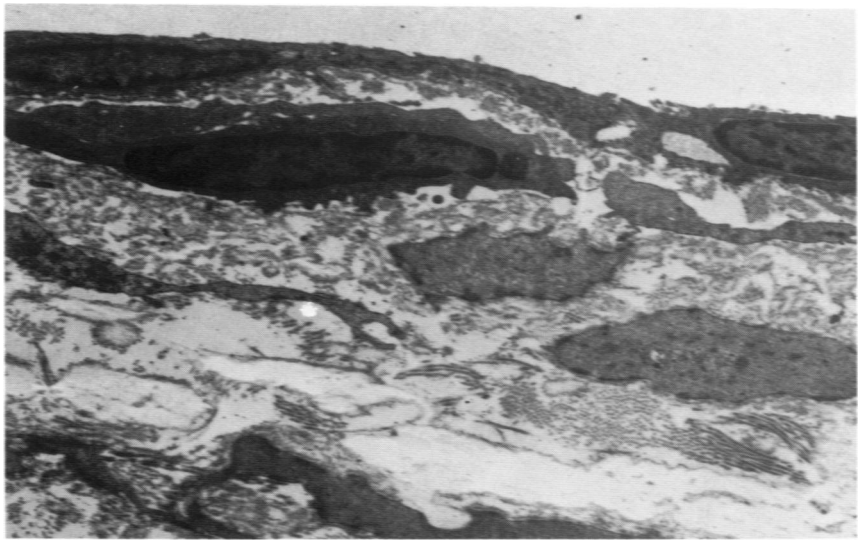


Figure 2—Control rabbit aorta. The intima is lined by a single layer of flat endothelium. (Uranyl acetate and lead citrate, × 3500)

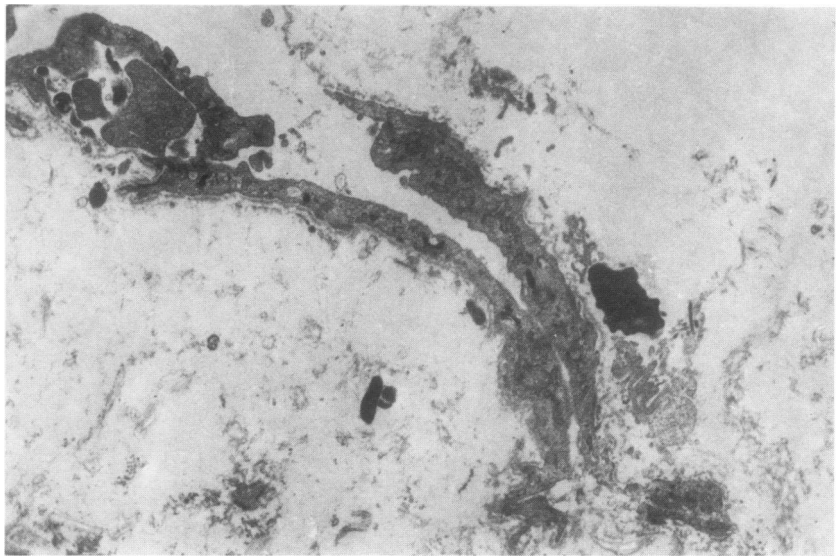


Figure 3—Test rabbit aorta, stained area. Notice widened interendothelial space and subendothelial edema. (Uranyl acetate and lead citrate, × 2500)

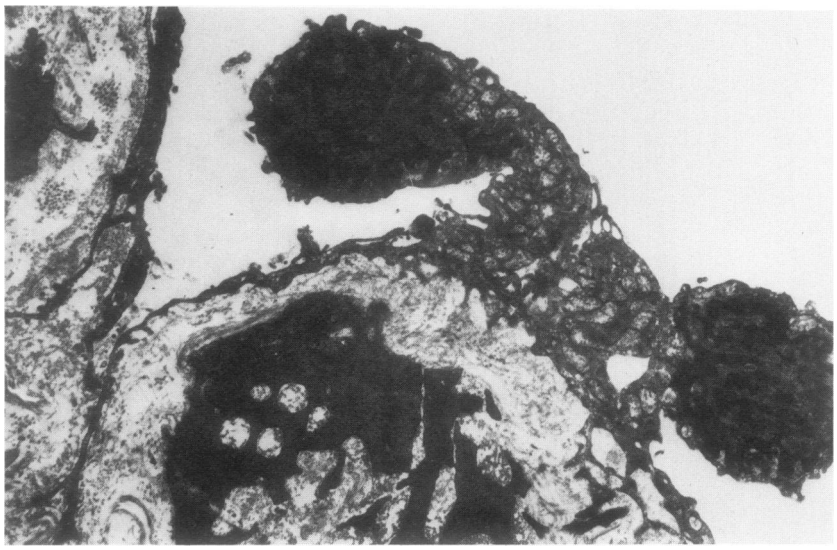


Figure 4—Test rabbit aorta, stained area. The endothelial cells are projecting into the lumen and show indented nuclei, few vesicles, and numerous mitochondria. (Uranyl acetate and lead citrate, × 7200)

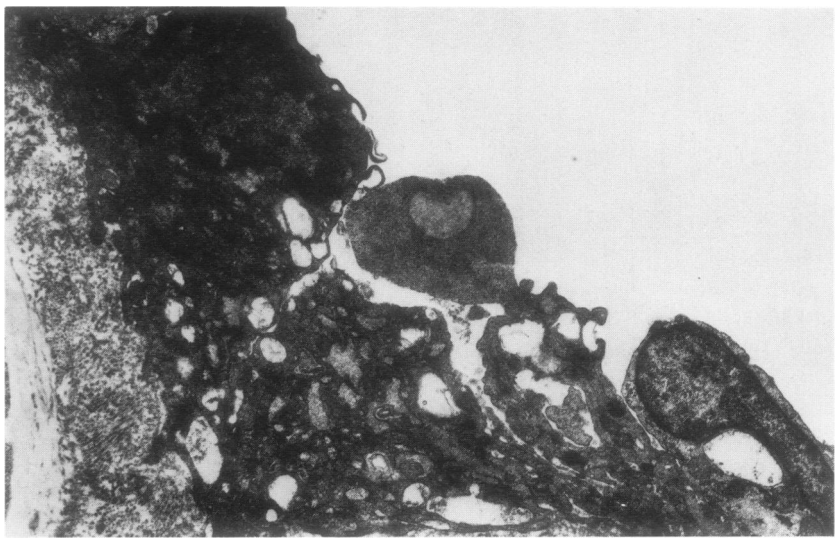
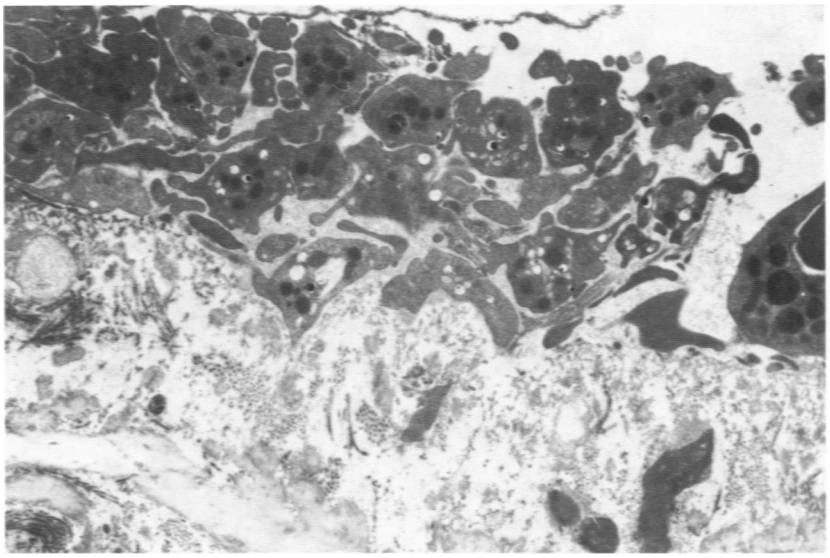


Figure 5—Test rabbit aorta, stained area. Endothelial cell has few vesicles and shows cytoplasmic projection covering nearby endothelial cell. (Uranyl acetate and lead citrate, × 6500)



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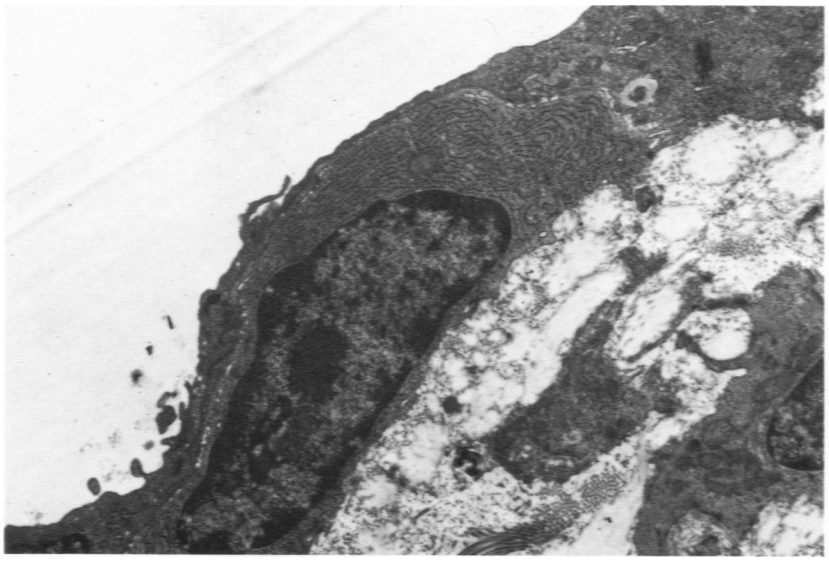

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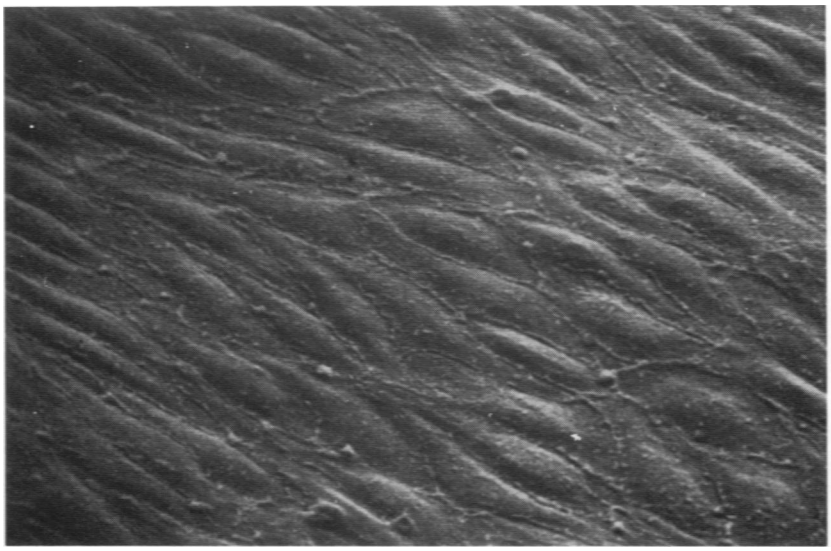
Figure 6—Test rabbit aorta, stained area. Notice platelet aggregates over intima devoid of endothelium. Platelets close to intima are degranulated as compared to platelets facing lumen. A polymorphonuclear leukocyte is also seen lying over ulcerated intimal surface. (Uranyl acetate and lead citrate, × 5000) **Figure 7**—Test rabbit aorta, stained area. A large subendothelial cell with prominent rough endoplasmic reticulum is present. (Uranyl acetate and lead citrate, × 6500)

Figure 8—Control rabbit aorta. The luminal surface shows relatively flat endothelial lining. A few villi are seen projecting from the endothelial surface. (× 900)

Figure 9—Test rabbit aorta, stained area. The luminal surface shows edematous endothelium separated from each other by deep grooves. The endothelial surface is covered with small villi. (× 1800)

Figure 10—Test rabbit aorta, stained area. Luminal surface is covered with edematous endothelium. Notice the numerous "crater-like" (dark) areas in the endothelium. Some of these areas are covered with fibrin and platelet aggregates. (× 180)



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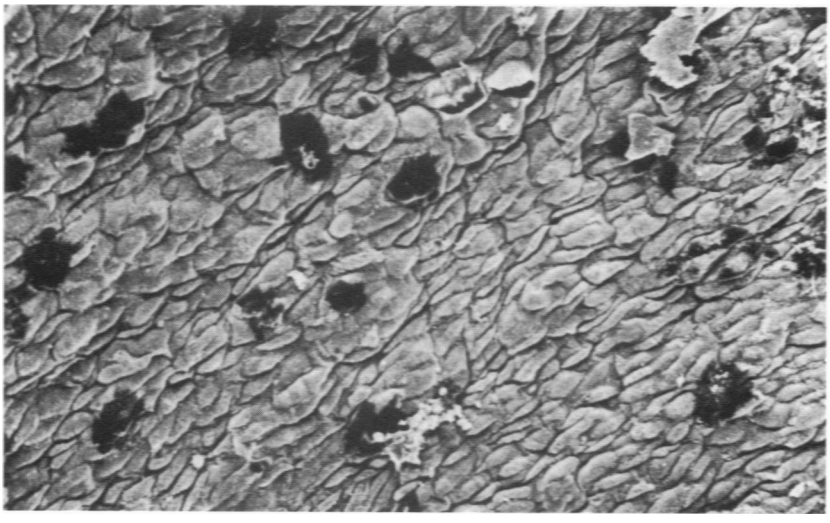

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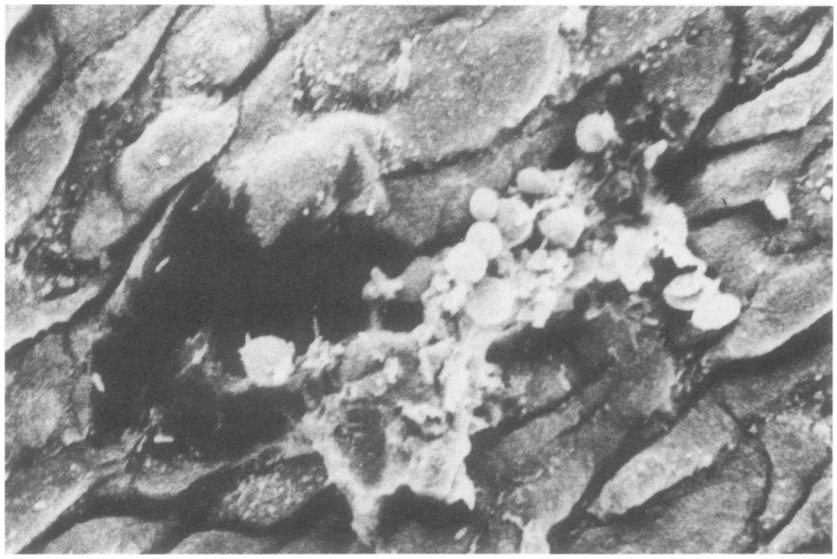

Figure 11—Higher magnification of Figure 10. The crater-like defect has ragged edges and shows endothelial cytoplasmic projections over it. Also the crater-like area is partly covered on one side with platelet and fibrin aggregates. Edema of endothelial cells is present. (× 700)

Figure 12—Test rabbit aorta, stained area. Notice numerous fish-mouth projections with crater-like defects in edematous endothelial surface. (× 850)