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EARLY LESIONS OF EXPERIMENTAL ENDOCARDITIS LENTA *

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The transmission of infection with *Streptococcus viridans* by intravenous injection of pure cultures into animals with the production of an experimental disease closely resembling the human endocarditis due to infection with this organism has been previously reported.[1] By the simple procedure of intravenous inoculation one may cause endocardial vegetations in various animals such as rabbits, rats and mice. Rabbits, because of their conveniently large ear veins, have been used by us for the most part. It has seemed best to designate this experimental infectious disease, which may be regarded as specific in the bacteriologic sense, as experimental endocarditis lenta. Our collection of material from these experimental animals has been studied in some detail in order to follow the sequence of events in the experimental disease. Photographs of the fully developed valvular vegetation were shown in the previous paper. In this present communication we purpose to deal with the changes observed in the heart in the earlier stages of the experimental disease, where the lesions are, for the most part, so minute as to be readily overlooked in gross inspection of the heart.

Rabbit 36 was inoculated by intravenous injection of 2 to 4 cc. of a culture suspension of *Streptococcus viridans*, strain P, that had been centrifugalized, washed in saline solution, recentrifugalized and suspended in saline solution, daily on May 10, 11, 12 and 13, 1939. The rabbit died early on May 15th. At autopsy there were visible many small renal abscesses and small rough spots on the tricuspid valve, thought to be early vegetations. Sections through this region actually showed definite early vegetations near the margin of one of the tri-

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cuspid flaps. The earliest changes, however, were seen in the endocardial endothelium of the ventricle and in the myocardial capillaries. Figure 1 represents a small portion of the right ventricle and Figure 3 is a drawing of a small area of this section. In almost every microscopic field one can find streptococci contained within endocardial endothelial cells. Bacteria are few and the endothelial cells seem in many instances to be free from other alteration. Some of them, however, are thickened and partly loosened. Sometimes the nucleus appears more rounded than normally or may even exhibit irregular fragmentation of the chromatin. At one place about midway in the figure there is some material adherent to the endocardium and in this a few erythrocytes can be distinguished. The subjacent myocardium appears unaltered, but in it near the center of the drawing there is a capillary almost filled with a thrombus, containing many streptococci, most of them within polynuclear leukocytes. There is a slight excess of wandering cells external to the capillary wall and, at a short distance to the left in the drawing, there are extravasated erythrocytes. At the bottom of the drawing there are represented four serial sections of this thrombosed capillary, the third in the series belonging to the section represented in the main drawing. Several normal capillaries may be seen, two fairly large ones at the upper right.

Rabbit 34 was inoculated by intravenous injection of 2 to 4 cc. of *Streptococcus viridans,* strain P, that had been washed and then suspended in saline solution, daily on May 10, 11 and 12, 1939. Blood culture taken on May 15th was positive. The rabbit died on the morning of May 16th. At necropsy there was seen a small irregular thickening near the margin of a mitral flap. Sections through this leaflet actually revealed an irregular accumulation of fibrin near the free edge and there were many well stained cocci contained in this fibrin. On examining the section of the leaflet nearer to its base there was seen on the auricular surface an irregular displacement of the endothelial cells in which a few streptococci could be distinguished even in the mihroscopic section stained with hematoxylin and eosin (Fig. 4). Erythrocytes also were recognized along with amorphous material, apparently fibrin and platelets, adherent to this auricular endothelial surface. Another section of this same mitral flap, at some distance from the first section, stained by the method of Brown and Brenn,[2] revealed the streptococci more distinctly (Fig. 7). Here also there was distortion of the endothelial cells and of their nuclei, and a small thrombus, containing several erythrocytes, was adherent over an area almost 0.1 mm. in extent. In both drawings the subendothelial edema is distinctly shown and it is worthy of note that the endothelium

on the ventricular surface seems to have escaped alteration to a very large extent. Careful study of several sections failed to disclose any cocci included by phagocytosis in the endothelium on the ventricular surface of the mitral leaflet.

Rabbit 33, weighing 2,000 gm., was inoculated by intravenous injection of 2 cc. of culture no. 353 daily on May 10, 11, 12 and 13, 1939. There was no further inoculation. Blood culture taken on May 15th gave positive growth, as also did a second one taken on May 18th. The animal died at 2:30 p.m., May 19th, or 9 days after the initial inoculation. At autopsy there were gross infarcts in both kidneys and minute vegetations were recognized on the mural endocardium of the right ventricle and of the left ventricle as well as on the mitral and aortic valves. The liver showed considerable coccidiosis.

Figure 2 shows a photomicrograph at low magnification representing a section passing through the left ventricle, an aortic cusp and a portion of the aortic wall. The finer details are illustrated by the colored drawings. In Figure 5 there is pictured a small bit of the ventricular endocardium. The endothelial cells contain engulfed streptococci but seem not to be greatly altered. A capillary just beneath the endocardium is cut longitudinally and appears to be normal. The myocardial cells, however, are separated by edema fluid and are also somewhat fragmented. The endothelial lining of the ascending aorta, shown in Figure 8, also contains many cocci and, for the most part, is without marked alteration of the endothelium or the subjacent stroma. However, over an area of about 0.5 mm. in length the endothelial cells have been destroyed by the massive proliferation of the bacteria and the underlying stroma has been invaded by streptococci with the production of edema and, in places, coagulation necrosis. Over these latter, more severe lesions, the bacteria are seen as compact masses of cocci directly in contact with the fluid within the aortic lumen.

A photomicrograph of the section of the aortic cusp is shown in Figure 10 and more detail is shown in the colored drawings. Figure 6 represents the entire thickness of the valve. On the aortic side one recognizes the scattered cocci adherent to, and included by phagocytosis in the endothelial cells, without much evident alteration of these latter elements. On the ventricular face of the valve, on the other hand, the endothelial cells contain many more bacteria. The endothelial cells themselves are swollen and are elevated irregularly by the accumulation of edema fluid in the subjacent stroma. In places there are small amounts of fibrin adherent to the free surface and these deposits sometimes contain recognizable erythrocytes. Such a small deposit is shown near the upper left corner of the drawing. Figure

13 represents a portion of the same aortic leaflet and shows the ventricular face at the site of the curve or kink, readily identifiable in the photomicrographs. Here phagocytosis of the cocci is also evident and at one place, at the right, a polynuclear leukocyte containing cocci is adhering to the endothelium. Of particular interest, however, is the lesion at the left of the figure, where the streptococci have already multiplied to form a dense bacterial colony with more or less advanced destruction of the endothelial cells. An irregular deposit of fibrin with incarcerated erythrocytes is attached to this altered surface and constitutes a minute, irregular projection which was grossly recognizable as an early vegetation. This is large enough to be seen in many serial sections.

In the sections of this heart there are also lesions in the myocardial vessels. Figure 9 represents a coronary arterial branch of a diameter of about 0.4 mm. and the interesting portion of the arterial wall is shown in Figure 11. At one place the endothelial cells of the intima have engulfed some of the streptococci and here there is a small adherent thrombus, evidently composed of fibrin, platelets and incorporated erythrocytes, and including easily recognizable streptococci. This lesion is evidently similar to those seen in the ascending aorta of this same animal. Figure 12 shows a thrombosed capillary in the ventricular myocardium. The thrombus extends along the capillary and contains polynuclear leukocytes and masses of bacteria toward the upper end in the picture. Toward the bottom of the figure the clot consists chiefly of fibrin and altered erythrocytes without visible cocci, suggesting an extension of the clot by the process of relatively aseptic thrombosis. This would appear to be a rather recent obstruction because of the slight changes in the adjacent myocardium. However, for decision in regard to mode of origin, even this vascular lesion is already too far advanced. Whether it started by endothelial phagocytosis of circulating streptococci or by the attachment of a polynuclear leukocyte already loaded with cocci remains uncertain but the latter mode of origin seems probable.

Discussion

The significance of these observations in relation to the sequence of events in the development of the lesions of endocarditis requires little discussion because the evidence is in itself so clear. Obviously, an endocardial lesion might develop by extension of the inflammatory process from a thrombosed capillary near the endocardial surface, such as that shown in Figure 3 (rabbit 36). This might easily be accepted as the mode of origin of some mural vegetations. In our experimental

rabbits, however, such thrombosed capillaries are found after considerable search, whereas the endothelial cells of the mural endocardium may everywhere serve as phagocytes for cocci. It seems, therefore, that the great majority of the valvular and mural lesions as well as those of the aortic and arterial walls take origin from the intimal implantations of the circulating bacteria. Subsequently the endothelial cells may destroy the included cocci so that the lesion heals without residual damage, and this evidently takes place over considerable areas of the endocardium, as will be evident in the study of later stages of this disease. Failing this, the lesion may progress to cause more serious structural alteration and functional incapacity. This failure to heal evidently depends upon several factors: first, the virulence of the infecting bacteria; second, the general resistance of the host; third, other miscellaneous circumstances. Without entering too fully into these questions at the moment, we may here point out the contrast between the two surfaces of a heart valve. As may be seen in the drawings, it is the auricular surface of the mitral valve and the ventricular surface of the aortic valve which seem more favorable to the proliferation of the bacteria. This, we believe, is due to the greater physical trauma to which these surfaces are exposed. Each mitral leaflet comes into contact with its fellow at each systole of the ventricle so that the endothelial cells on the auricular surfaces over the area of this contact are closely applied to each other and they are peeled apart at each ventricular diastole. Similar contact under pressure affects the ventricular surfaces of the aortic leaflets. Physical relationships of pressure, speed of blood flow, stagnation of the blood and contact with other solid elements doubtless also play a part in the relative frequency of lesions in the ascending aorta, sinus of Valsalva, arteries, capillaries and veins, but the present experimental material does not warrant any extended discussion of these relationships.

Summary

1. Following the intravenous injection of large amounts of washed bacteria as well as untreated cultures of *Streptococcus viridans* into rabbits, the bacteria are taken up extensively by phagocytosis by the endothelial cells of the endocardium and of the intima of the aorta and coronary arteries.

2. The bacteria also lodge in the myocardial capillaries either by direct endothelial phagocytosis of streptococci or by arrest of sluggish leukocytes containing the bacteria.

3. After phagocytosis many bacteria are evidently destroyed without production of recognizable persistent structural changes.

4. In some places, particularly on the heart valves, the included bacteria tend to survive, multiply, and initiate the precipitation of elements from the blood so as to give rise to the vegetations of endocarditis.

5. Local physical factors play some part in the progress of these local lesions.

This paper is a report of the results of joint effort. The experimental work on the animals was performed for the most part by Miss Spence. The microscopic sections were prepared by Miss Slavkin. The illustrations in color were drawn by Dr. MacNeal.

REFERENCES

1. MacNeal, W. J., Spence, M. J., and Wasseen, M. Experimental production of endocarditis lenta. *Am. J. Path.*, 1939, 15, 695–705.
2. Brown, J. H., and Brenn, L. A method for the differential staining of Gram-positive and Gram-negative bacteria in tissue sections. *Bull. Johns Hopkins Hosp.*, 1931, 48, 69–73.

DESCRIPTION OF PLATES

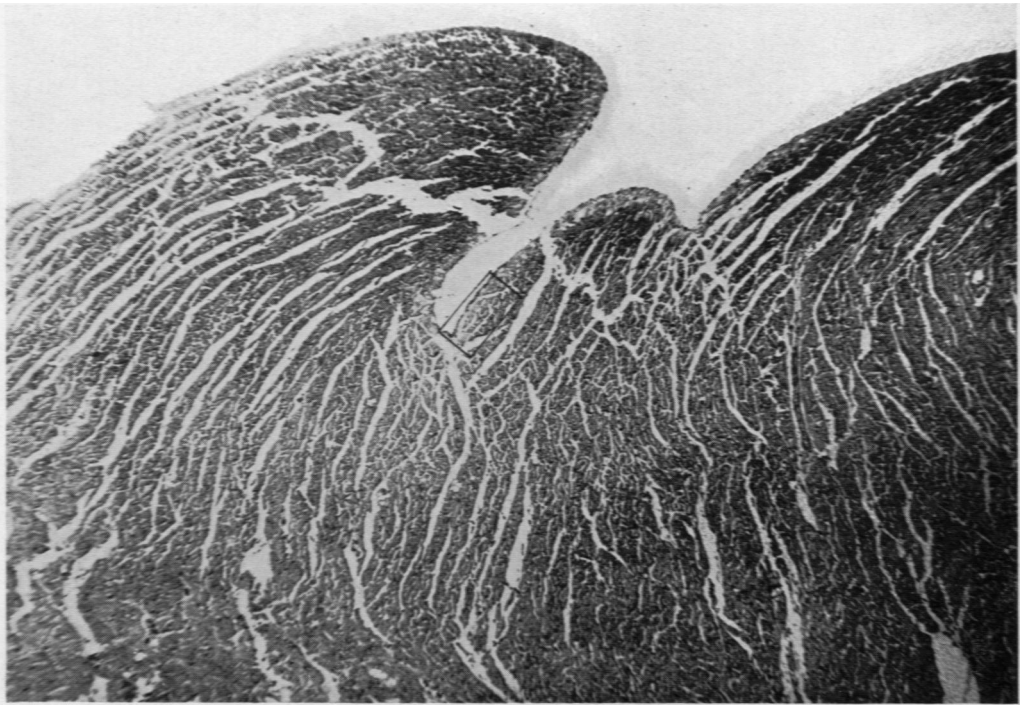
The order and orientation of the illustrations have been arranged to meet the requirements of economy and artistic reproduction. By attention to the legends the reader may avoid confusion.

PLATE 87

FIG. 1. Rabbit 36. Section through the wall of the right ventricle. Photomicrograph at low magnification. Abnormalities might easily be overlooked in this picture. The portion outlined by the rectangle is shown at higher magnification in Figure 3.

FIG. 2. Rabbit 33. Section through the aortic valve including a valve leaflet, part of the ventricular wall and part of the wall of the ascending aorta, stained by the method of Brown and Brenn.[2] Photomicrograph at low magnification. One may recognize small areas of infection on the auricular face of the valve and on the intima of the aorta. The locations of the fields represented in Figures 5 and 8 are indicated by short heavy lines near the endothelium.

1



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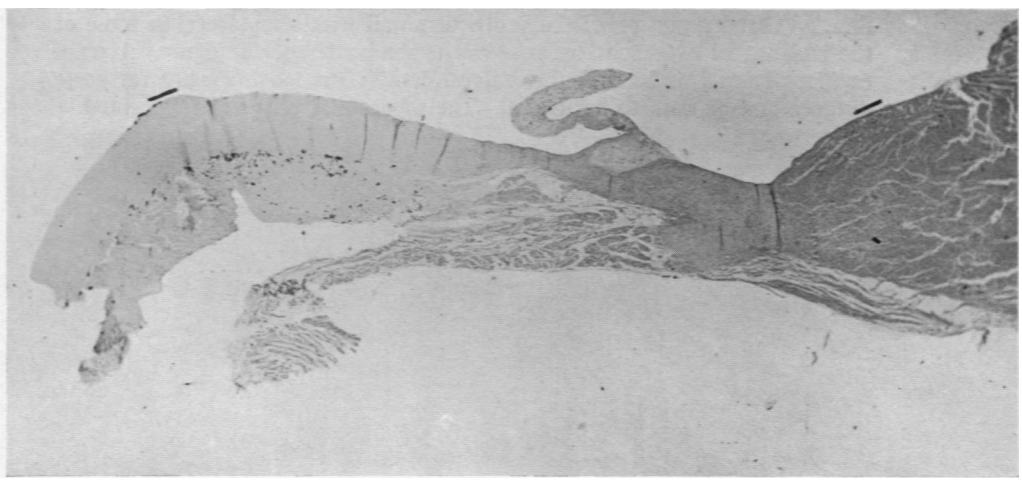

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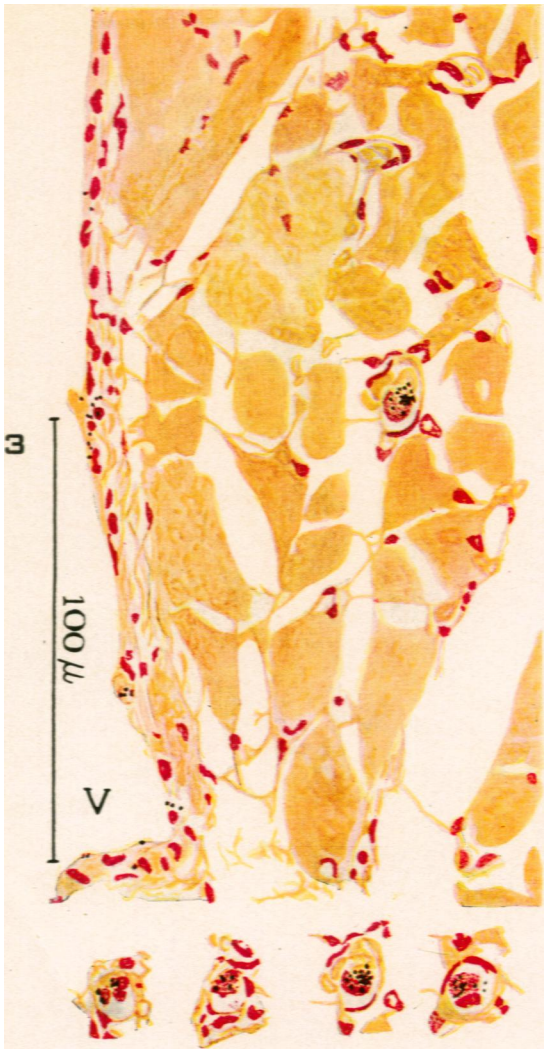
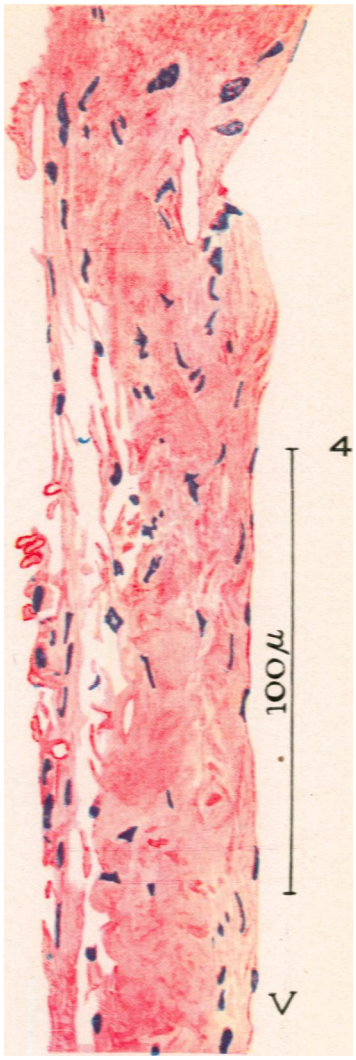
PLATE 88

FIG. 3. Rabbit 36. Section through part of the wall of the right ventricle stained by the method of Brown and Brenn [2] and drawn by camera lucida, objective 60 ×, N. A. 1.40, and ocular 4, at the magnification indicated by the included scale, which is standard for all colored drawings of this paper. The endocardial endothelial cells contain cocci and there are minute bits of clotted blood adherent to them in places. At about 0.1 mm. beneath the endocardium there is a thrombosed capillary containing many streptococci. Below the main drawing, this same capillary is shown in four successive serial sections of which the third in the series is the section represented in the larger drawing. It would appear that the thrombosis of the capillary has no local relation to the infection of the endocardial endothelium, which is quite general over the entire lining of the ventricle. The V is in the cavity of the right ventricle.

FIG. 4. Rabbit 34. Section through a mitral leaflet stained with hematoxylin and eosin and drawn by the same standard technic. The auricular face is roughened, and attached to it are bits of clot containing erythrocytes. The adjacent endothelial cells contain engulfed streptococci. The smooth endothelium on the ventricular face presents a sharp contrast. The V is in the cavity of the left ventricle.

FIG. 5. Rabbit 33. Drawing by camera lucida, objective 60 ×, N. A. 1.40, and ocular 4, at the standard magnification indicated by previous scales. This drawing shows a portion of the ventricular wall with streptococci in some of the endocardial endothelial cells at the upper border of the figure. A small capillary lying just beneath the endocardium is cut longitudinally for some distance and appears to be normal. The myocardial cells are fragmented.





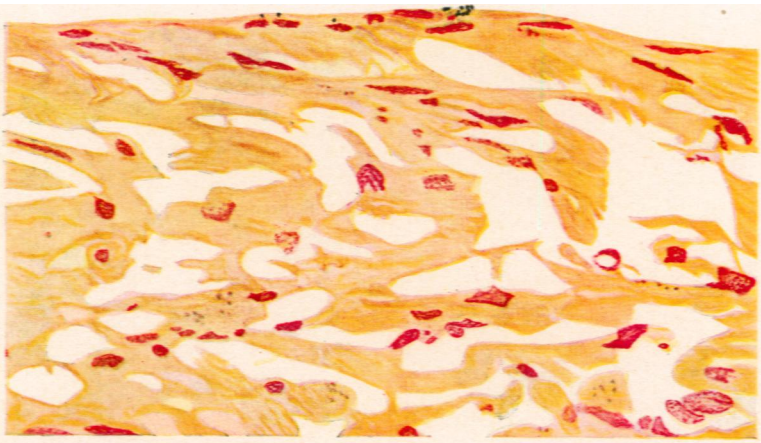


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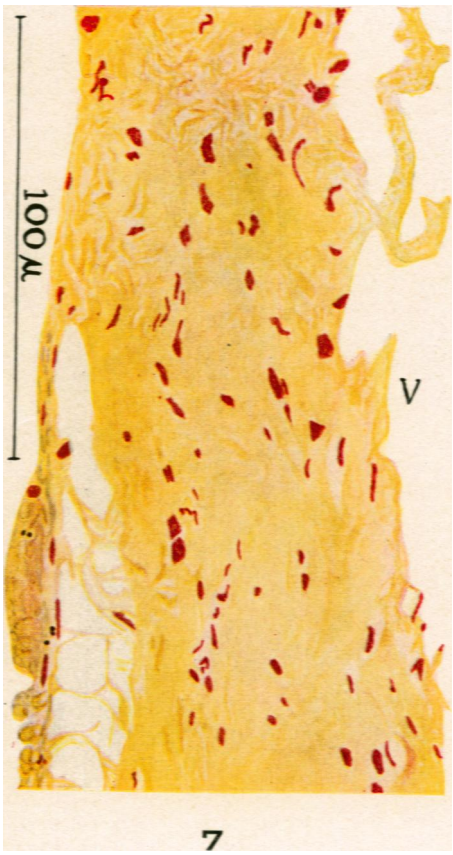
PLATE 89

FIG. 6. Rabbit 33. Drawing by the standard technic of part of the aortic leaflet in the same section as that shown in Figure 10. The endothelial cells contain cocci which are more abundant on the ventricular face (V) of the leaflet, where there is also evidence of more anatomic alteration. The location of this field is indicated in Figure 10 (V).

FIG. 7. Rabbit 34. Another section through a mitral leaflet stained by the method of Brown and Brenn [2] and drawn by the same standard technic. There are a few distinct bacteria in the endothelium on the auricular face of the leaflet and there is also an adherent clot nearly 0.1 mm. in extent, with erythrocytes and bacteria in it. The V is in the cavity of the left ventricle.

FIG. 8. Rabbit 33. Drawing by camera lucida, objective 60 ×, N. A. 1.40, and ocular 4, at the standard magnification for all colored drawings. This shows a small part of the wall of the ascending aorta. Some of the endothelial cells of the intima contain streptococci. In the upper half of the drawing the proliferation of the bacteria is associated with local necrosis.



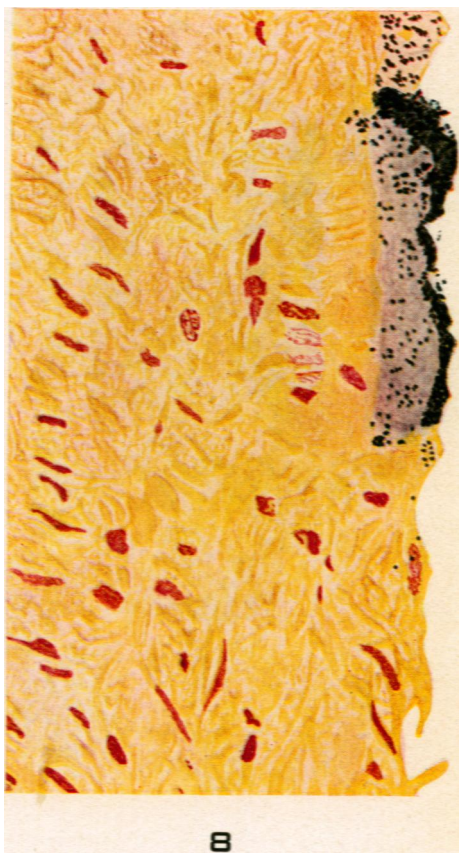


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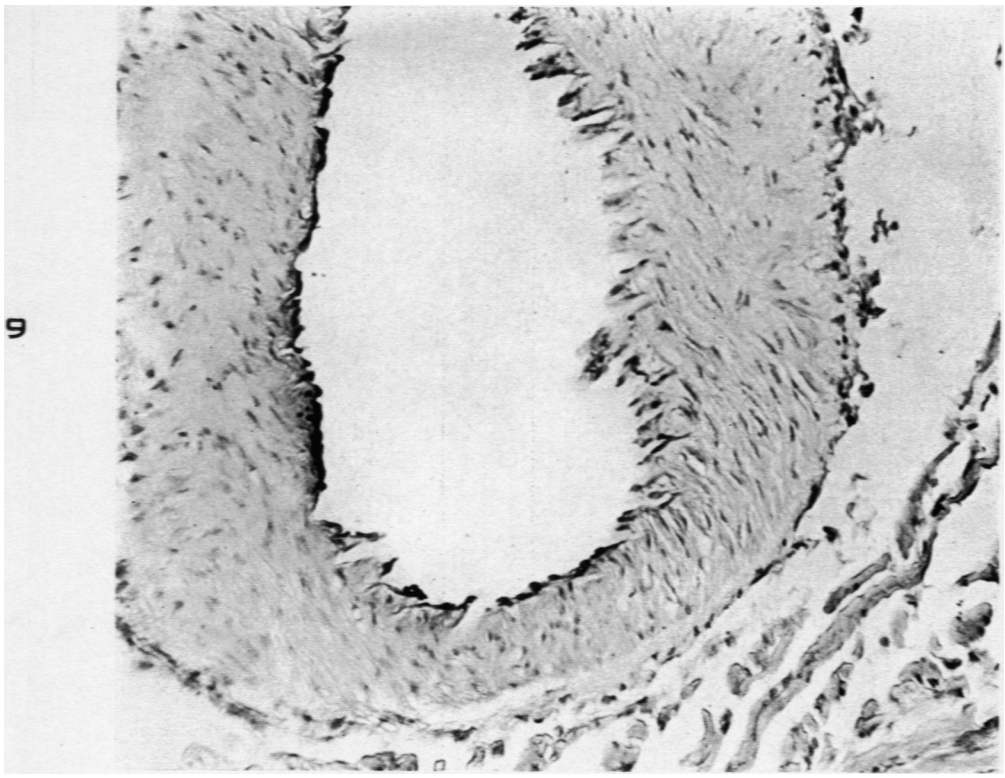
PLATE 90

FIG. 9. Rabbit 33. Photomicrograph of a coronary artery showing at one place on the intima (lower right) a small vegetation. The artery is about 0.4 mm. in diameter.

FIG. 10. Rabbit 33. Same section as that shown in Figure 2 but photographed at a higher magnification to show more detail of the valve leaflet. The small vegetation on the ventricular face of the cusp may be recognized, as well as the collections of bacteria in the endothelium.



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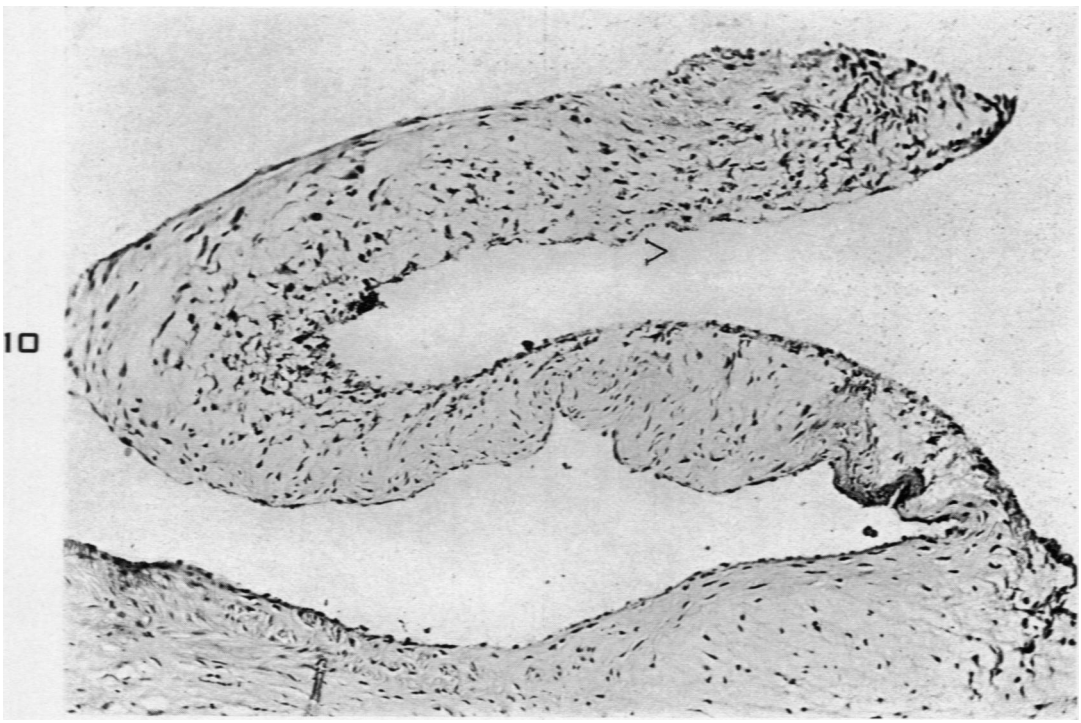

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PLATE 91

FIG. 11. Rabbit 33. Drawing by camera lucida by the standard technic. Here there is shown the small vegetation on the intima of the coronary artery seen in Figure 9. The intimal endothelium contains included cocci and clinging to it there is a deposit of fibrin in which there are erythrocytes and bacteria. The other arterial coats appear to be unaltered.

FIG. 12. Rabbit 33. Drawing by the standard technic. There is shown a thrombosed capillary in the ventricular myocardium. The thrombus is purulent, with abundant streptococci in the upper part of the longitudinal section. The lower part consists chiefly of fibrin and erythrocytes (aseptic extension of the thrombus). There is little reaction in the adjacent myocardium.

FIG. 13. Rabbit 33. Drawing by the standard technic of part of the aortic leaflet in the same section as that shown in Figure 6. Here the minute vegetation is included at the left. It has in it erythrocytes, fibrin and bacteria. At the right a polynuclear leukocyte with three included cocci adheres to an endothelial cell. The location of this field is indicated in Figure 10, in which the small vegetation serves as a landmark.

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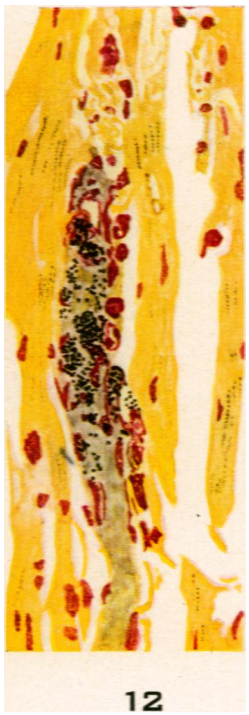

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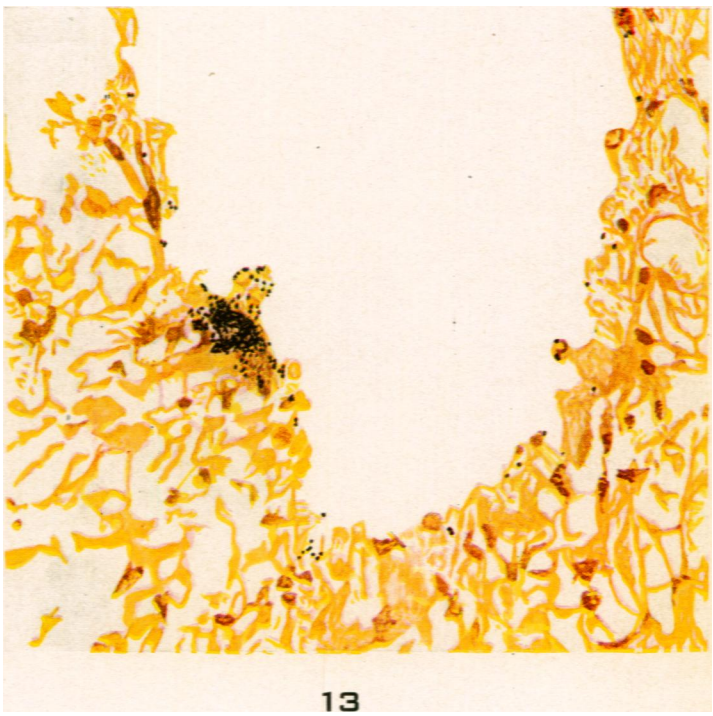

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