Bacterial Endocarditis, Glomerulonephritis, and Amyloidosis in the Opossum *(Didelphis virginiana)*

B. F. Sherwood, D.V.M., D. T. Rowlands, Jr., M.D., D. B. Hackel, M.D., and J. C. LeMay, D.V.M.

THE SPONTANEOUS OCCURRENCE of bacterial endocarditis [1,2] in the opossum is of importance because of its similarity to the human disease. Fox [1] first recorded bacterial endocarditis in 10 opossums (*Didelphis virginiana*) dying in the Philadelphia Zoological Gardens. La Plante and Burrell [2] have described the condition more recently in 3 of 10 opossums which died in captivity. On the basis of these studies, the assumption was made that bacterial endocarditis is common among opossums in the wild.

A review of postmortem material obtained from opossums dying in our laboratory over a 2-year period confirmed the observation that there is an unusually high incidence of bacterial endocarditis in captive opossums. Further examination of these animals showed a significant number with glomerulonephritis and amyloidosis.

Since these observations were restricted to captive animals, it seemed pertinent to learn whether bacterial endocarditis truly occurred in the wild or whether it was a disease arising only during captivity. This paper describes the pathologic findings in opossums dying in captivity and compares them to opossums recently captured from the wild, and to opossums sacrificed while in captivity.

Materials and Methods

Animals

The 53 opossums used in this study were captured in the immediate vicinity of Durham, N.C. The animals were housed singly or in groups of two in 20- × 22- × 15-in. galvanized cages, and they were maintained on canned cat food and water ad libitum. The animals varied in weight from 1.1 to 3.6 kg., and they were judged to be mature on the basis of body weight, dentition, and development of their genitalia.

The animals used in this study comprised three experimental groups. Group A

From the Departments of Veterinary Medicine and Pathology, Duke University Medical Center, Durham, N.C.

Supported in part by Grant AM11797 from the U. S. Public Health Service.

Carried out during tenure of Career Research Award HE-K6-14,188 to Dr. Hackel from the U. S. Public Health Service.

Accepted for publication Apr. 4, 1968.

Address for reprint requests: Dr. Sherwood, Department of Veterinary Medicine, Duke University Medical Center, Durham, N.C. 27706.

Table 1. Pathologic Findings in Group A

	Opossum No.																														
	Subgroup with endocarditis and/or myocarditis																							Subgroup without endocarditis and/or myocarditis								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
Heart																															
Ao. valve	+	−	+	+	−	+	−	−	−	+	+	−	+	−	−	+	−	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−
M. valve	+	+	+	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−
T. valve	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Myocarditis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
Kidney																															
G-neph.	−	+	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−
Int. neph.	+	+	+	+	−	+	+	+	−	+	+	−	+	+	−	+	+	+	+	+	−	−	+	+	+	−	+	−	+	+	+	+	−
Infarct	+	−	+	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−
Lung																															
Pneumonia	+	−	−	+	−	+	+	−	−	−	+	−	+	−	−	−	−	−	+	−	+	−	+	−	+	−	+	−	−	+	−	−	−
Parasites	+	+	−	+	+	+	−	−	−	−	−	−	−	+	−	+	−	−	+	−	+	+	−	−	+	+	−	−	−	−	−	−	−
Edema	+	+	+	+	+	+	+	−	−	−	+	+	−	+	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	+	+	−
Liver necrosis	+	−	−	−	+	+	+	−	−	−	+	−	−	+	−	−	−	−	−	−	−	−	+	−	−	+	−	+	−	−	−	−	−
G.I. parasites																															
Stomach	+	+	+	+	+	+	−	−	+	−	−	+	−	+	+	−	−	+	+	−	+	+	−	−	+	+	+	+	+	+	+	−	+
Cecum	+	+	+	−	+	+	−	−	+	−	−	+	−	+	+	−	−	+	+	−	+	+	−	−	+	+	+	+	+	−	+	−	+
Brain inflam.	NE	+	NE	+	NE	+	NE	−	−	+	+	NE	+	+	+	NE	−	−	+	−	NE	−	−	−	−	NE	−	+	−	−	−	−	−
Amyloid																															
Spleen	−	−	−	−	−	−	+	+	−	−	+	−	+	−	−	+	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Liver	−	−	−	−	−	−	−	+	−	−	−	−	+	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

Plus indicates lesions present; minus, no lesions; NE, not examined; Ao, aortic; G.I., gastrointestinal; G-neph, glomerulonephritis; Int. neph., interstitial nephritis; M, mitral; T, tricuspid.

is composed of 33 opossums which died in captivity between January 1966 and December 1967. In most instances, these opossums showed no evidence of disease until they died suddenly 3 weeks to several months after capture. Group B consists of 10 opossums which were killed by the intraperitoneal injection of sodium pentobarbital on the day of capture. Group C is made up of 10 opossums which were housed in the same animal room as were those of Group A. They remained in apparent good health until they were sacrificed by the injection of sodium pentobarbital.

Pathologic Examinations

After gross examination, portions of heart, lungs, kidneys, spleen, lymph nodes, liver, and brain were removed and fixed in 10% buffered formalin. They were then embedded in paraffin, sectioned, and stained. The routine stain used for all tissues was hematoxylin and eosin. In addition, spleens and liver were studied using crystal violet, Congo red, and thioflavine-T. Routine sections were cut at 5 μ, but additional sections of the kidneys were cut at 3 μ. These thin sections were stained with periodic acid-Schiff (PAS) and periodic acid-methenamine silver stains. The heart valves were stained with hematoxylin and eosin and the Brown and Brenn stain for bacteria.

Bacteriology

Direct smears and bacteriologic cultures were obtained from valvular vegetations. The smears were stained by Gram's method. Blood cultures were obtained from each opossum in groups B and C.

Results

Group A

The pathologic findings in the 33 opossums dying in captivity are summarized in Table 1. The group is divided into two on the basis of the presence of myocarditis in 24 animals or its absence in 9 animals (Table 1).

Myocarditis usually appeared as focal aggregates of acute and chronic inflammatory cells, but in some instances the exudates were more diffuse. For the most part, myocardial necrosis was not evident and in no instance was there any lesion resembling an Aschoff body. In one animal, the pericardium was found to be thick, gray, and shaggy. Microscopically, this appeared as an acute fibrinous exudate.

The most striking gross lesions in these animals were valvular vegetations. In 7 animals these lesions were limited to the mitral valve, in 10 both mitral and aortic valves were affected, and in 1 the vegetations were found on tricuspid and mitral valves. An example of a small mitral valve lesion is shown in Fig. 1A with the soft, gray, friable vegetation being attached to the otherwise thin, normal mitral valve. In most cases, however, the vegetations were larger and bulkier, and were attached to swollen valve leaflets, on the atrial side of the atrioventricular valves

(Fig. 1) and on both sides of the aortic valve (Fig. 2A). When broken off, the vegetations left granular, eroded surfaces. In addition to the valvular lesions, there were associated swollen vegetations on the mural endocardium.

Microscopic examination of early valvular lesions characteristically showed swelling and edema of the valve leaflets, with erosion of the valvular surfaces. These changes were associated with lymphocytic and neutrophilic cellular exudates as well as with gram-positive cocci (Fig. 1C). Larger vegetations consisted of poorly organized masses of inflammatory cells, fibrin, and gram-positive cocci (Fig. 1D and 2A). In two cases, the vegetations appeared to be undergoing organization with proliferation of fibroblasts. Occasionally, mild chronic inflammation was evident near the base of the mitral valves.

Positive bacterial cultures were recorded in 7 cases of bacterial endocarditis (Table 2).

The kidneys of these 33 opossums were frequently the seat of glomerulonephritis, interstitial nephritis, or renal infarction. Five had glomerulonephritis. Each of these 5 opossums also had myocarditis and 4 had valvulitis. The glomerular lesions (Fig. 2C and 2D) were characterized by hypercellular, bloodless glomeruli, with adhesions between glomerular tufts and Bowman's capsule, and, in some instances, epithelial crescents. In some, the basement membranes were also thickened. Twenty-four of the 33 opossums had varying degrees of inflammatory exudate in the kidneys. In 11 instances this was a true acute pyelonephritis, with abscesses throughout the kidney. These cases involved only those animals that also had a vegetative valvulitis. In 13 other animals, the interstitial inflammation was composed mainly of lymphocytes and a few plasma cells. Seven of these animals had some myocarditis, but 6 were in the subgroup without myocarditis (Table 1). In addition, frank acute renal infarcts were present in 5 of the animals with valvular vegetations.

Table 2. Organisms Cultured from Heart Valve Vegetations

Opossum No.	Organisms
2	*Proteus mirabilis*
3	*Salmonella bern*
6	*Alpha streptococcus*
10	*Alpha streptococcus*
11	*E. coli*
19	*E. coli*
21	*Alpha and beta hemolytic streptococcus, Proteus spp., and Aerobacter spp.*

Inflammatory lesions were also frequent in the lungs. Twelve opossums had pneumonia with neutrophilic exudation in bronchi and in the surrounding peribronchial connective tissues and alveoli. In 12 cases, there were firm, discrete pulmonary nodules, which were grossly visible and contained helminths identifiable by their double opercular ova as *Capillaria* species. The cellular reaction to these parasites consisted of leukocytic exudation with surrounding zones of epithelioid cells and fibroblastic tissue. Although parasitic infestation and pneumonia were frequently found in the same lungs, there seemed to be no direct relationship between them.

Parasites were also present in the stomach and cecum of these opossums. Helminths (*Physaloptera turgida*) were attached to the gastric mucosa with the implantation sites being the seat of necrosis and mucosal ulceration. In one opossum, acute gastric and duodenal ulcers were identified which were not associated with parasitic infestation. The cecal parasites were helminths (*Cruzia tentaculata*). No pathologic lesion could be attributed to their presence.

In 9 cases, there were foci of necrosis in the liver, and in 8 of 25 animals in which the brain was examined, there were varying degrees of inflammation of the meninges or gray matter. In some animals, bacterial emboli could be identified in small vessels.

In 7 opossums, amyloid deposits were identified in the spleen (Fig. 2B). Four of these also had amyloid in the liver. The amyloid deposits appeared as hyaline eosinophilic material beneath the endothelium of vascular sinusoids in the liver and around the central arteries of the splenic lymphoid follicles. The amyloid stained with Congo red, crystal violet, and thioflavine-T.

Among those opossums dying in captivity which were free of myocardial lesions, no significant differences from the animals with myocarditis were seen in the incidence of other inflammatory lesions (Table 1). However, none of the opossums without myocarditis had amyloid in either the spleen or liver.

Group B

Endocarditis, glomerulonephritis, amyloidosis, and meningitis were absent in the 10 opossums sacrificed immediately after being captured from the wild. However, chronic inflammatory lesions of the kidney were common and in one instance there was focal hepatic necrosis. Parasitic infestations with *Physaloptera turgida* and *Cruzia tentaculata* were common. Two additional helminths were noted in this group: two hosts had a single acanthocephalan attached to the intestinal mu-

Table 3. Pathologic Findings in Group B

	Opossum No.									
	34	35	36	37	38	39	40	41	42	43
Heart—myocarditis	+	+	−	−	−	+	−	−	−	−
Kidney—int. neph.	+	+	−	−	−	+	−	−	+	−
Lungs										
Pneumonia	−	−	−	−	−	−	−	−	−	−
Parasites	+	+	−	+	−	+	−	−	−	+
Liver necrosis	−	+	−	−	−	−	−	−	−	−
G.I. parasites										
Stomach	+	+	+	+	+	+	+	+	+	+
Cecum	−	+	+	+	+	+	+	+	+	+
Brain	−	−	−	−	−	−	−	−	NE	NE

Abbreviations are the same as in Table 1.

cosa and lung flukes were seen in two other hosts. Table 3 summarizes the pathologic findings in this experimental group.

Group C

Table 4 summarizes the findings in the 10 opossums which were sacrificed while in captivity. Few lesions were identified on gross examination. However, small vegetations were found on the mitral valves of 2 opossums and there was myocarditis in 9 animals. Proliferative glomerulonephritis was present in 5 cases, and chronic renal interstitial inflammation was found in 7 instances. A mild meningitis was present in 8 of these opossums, and focal hepatic necrosis was identified in 2. No amyloid was identified in this group.

Table 4. Pathologic Findings in Group C

	Opossum No.									
	44	45	46	47	48	49	50	51	52	53
Heart										
M. valve	−	−	+	−	−	−	−	+	−	−
Myocarditis	+	+	+	+	+	+	+	+	+	−
Kidney										
G-neph.	+	−	−	+	−	+	+	−	+	−
Int. neph.	+	+	+	+	−	+	+	+	−	−
Lungs										
Pneumonia	−	+	+	+	+	+	−	−	−	−
Edema	−	−	−	−	−	−	−	−	+	+
Parasites	−	−	−	−	−	+	+	−	−	−
Liver necrosis	+	−	−	+	−	−	−	−	−	−
G.I. parasites										
Stomach	+	−	+	+	−	+	+	+	+	−
Cecum	−	−	−	−	−	−	−	−	−	−
Brain—meningitis	+	+	+	+	+	+	+	+	−	−

Abbreviations are the same as in Table 1.

Discussion

Bacterial endocarditis as seen in the opossum closely resembles its human counterpart with respect to the gross and microscopic appearance of the lesions, as well as their relatively high frequency on the mitral and aortic valves. The observations reported in this paper suggest that bacterial endocarditis in the opossum is a disease of captivity. There are several possible explanations for this phenomenon. It could be that the environment of the laboratory permits previously infected opossums to live long enough for valvular vegetations to occur, or it might be that captive opossums develop bacterial endocarditis because of intense exposure to microorganisms not ordinarily found in the wild. It seems unlikely that antibody deficiency could account for the high incidence of bacterial endocarditis since it has been demonstrated that these animals have ample immunoglobulins [3] and that they develop a good antibody response when challenged with *Salmonella typhosa* [4] or bacteriophage.[5] It could also be proposed that the stress of captivity and frequent handling leads to the development of some degree of cardiovascular damage. Ample experimental studies, such as the preparation of vascular shunts,[6] have indicated that stress can result in myocarditis. Diet is yet another factor to be considered. Our opossums are maintained on a diet of cat food. Although this seems adequate for maintenance of the animals, little is known about the dietary requirements of the opossum.[7]

Amyloidosis of the spleen and liver was a common finding in our opossums. Among the many associations of amyloidosis, that with chronic infection is probably of greatest interest with respect to the present study. Although most of our animals had evidence of chronic parasitism, little difference in this regard was evident between those animals with and those without amyloidosis, and in no case was death believed to be due to parasitism. It is, of course, tempting to relate the amyloidosis and the presence of severe and widespread infection. In this regard, all opossums with amyloidosis were in Group A; all had myocarditis, and all but one had bacterial endocarditis. Since it is apparent that with amyloidosis as with bacterial endocarditis, there is an association with captivity, it may be that diet or stress could be instrumental in the pathogenesis of amyloidosis as well as of bacterial endocarditis.

Several types of renal disease were also noted among the opossums studied. Pyelonephritis of varying degrees of severity was noted in many of the animals studied. It may be that this renal disease is merely

a concomitant of other bacterial infections in these animals, but it also seems possible that bacterial infections of the kidney serve as sources for the bacterial endocarditis already described. Proliferative glomerulonephritis was also a frequent finding. Since similar lesions in man have been associated with hypersensitivity to bacteria,[8] it is not out of place to suggest that a similar phenomenon may occur in the opossum.

Summary and Conclusions

Thirty-three opossums dying in captivity were studied in detail. The most impressive pathologic lesions identified in these animals were myocarditis and bacterial endocarditis. There was, in addition, evidence of inflammation in kidneys and lungs. Proliferative glomerulonephritis, and amyloidosis of liver and spleen were regularly identified in the animals included in this group.

Two control groups consisting of 10 opossums each were also examined. One group consisted of opossums sacrificed while in captivity and the second control group was made up of opossums sacrificed immediately after capture. Since endocarditis, amyloidosis, and glomerulonephritis were not found in opossums sacrificed immediately after capture, it is apparent that these disorders develop during captivity.

References

1. Fox, H. *Disease in Captive Wild Mammals and Birds; Incidence, Description, Comparison.* Lippincott, Philadelphia, 1924, pp. 52–53.
2. La Plante, E. S., and Burrell, R. G. Bacterial endocarditis in opossums. *Bull Wildlife Dis Ass* 2:10–12, 1966.
3. Rowlands, D. T., Jr., and Dudley, M. A. The isolation of immunoglobulins of the adult opossum (*Didelphys virginiana*) *J Immunol* 100:736–743, 1968.
4. Rowlands, D. T., Jr., La Via, M. F., and Block, M. H. The blood-forming tissues and blood of the newborn opossum (*Didelphys virginiana*). II. Ontogenesis of antibody formation to flagella of salmonella typhi. *J Immunol* 93:157–164, 1964.
5. Kalmutz, S. E. Antibody production in the opossum embryo. *Nature (London)* 193:851–853, 1962.
6. Lillehei, C. W., Bobb, J. R. R., Wargo, D., Visscher, M. B., Shaffer, J. M., and Bell, E. T. Experimental production of diffuse proliferative glomerulonephritis utilizing arteriovenous fistual stress with bacteremia. *Circulation* 4:587–603, 1951.
7. McCrady, E., Jr. *The Embryology of the Opossum.* Wistar Inst. Philadelphia, 1938.
8. Cluff, L. E., and Johnson, J. E. *Poststreptococcal disease in immunological Diseases.* Little, Boston, 1965.

The authors acknowledge the assistance of Reinard Harkema, Ph.D., Professor of Zoology, North Carolina State University, Raleigh, N.C. for parasite identification.

[*Illustrations follow*]

Legends for Figures

Except where indicated, sections were stained with hematoxylin and eosin.

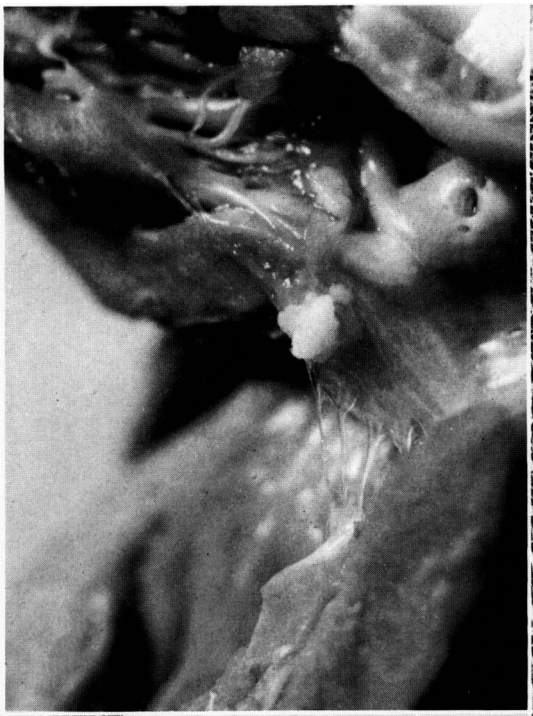
Fig. 1A. Small vegetation on atrial surface of mitral valve. Valve leaflet is edematous but shows no evidence of chronic lesion.

Fig. 1B. Normal mitral valve. × 25.

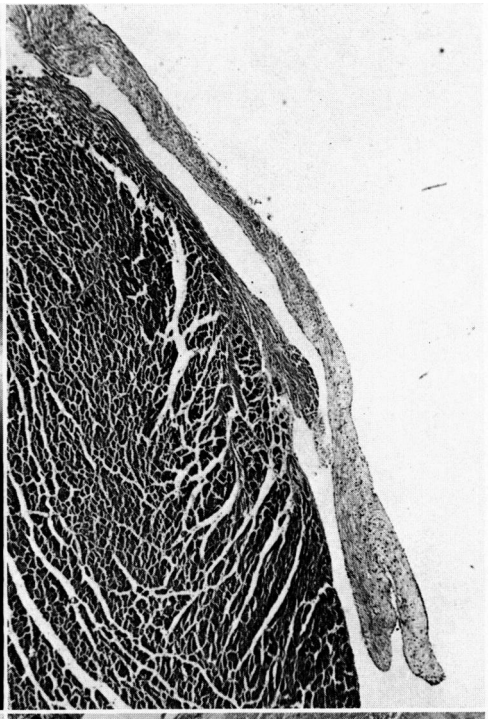
Fig. 1C. Small mitral valvular vegetation. Valve leaflet is edematous and is infiltrated by a moderate number of chronic inflammatory cells. Vegetation is on atrial side of mitral valve leaflet, and valve is eroded at site of vegetation. Vegetation consists of a mass of fibrin and acute inflammatory cells. Gram-positive cocci are included in the vegetation. × 25.

Fig. 1D. Large vegetation on atrial side of mitral valve. Valve is edematous and is infiltrated with polymorphonuclear leukocytes. Gram-positive cocci were found within valvular vegetation. × 25.

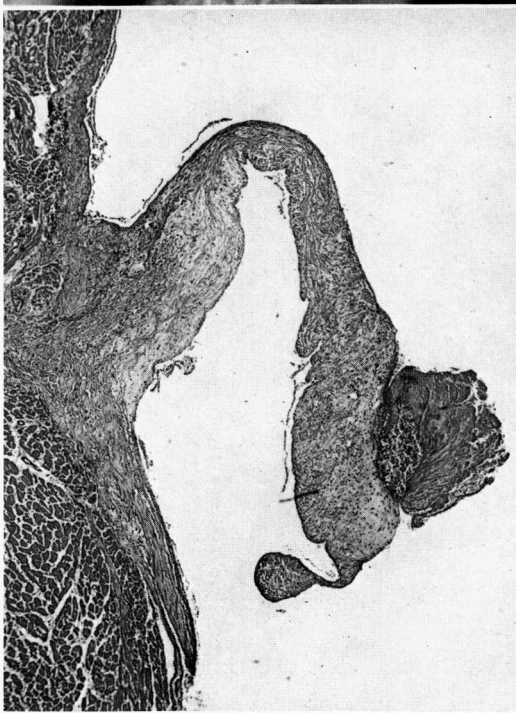
1A



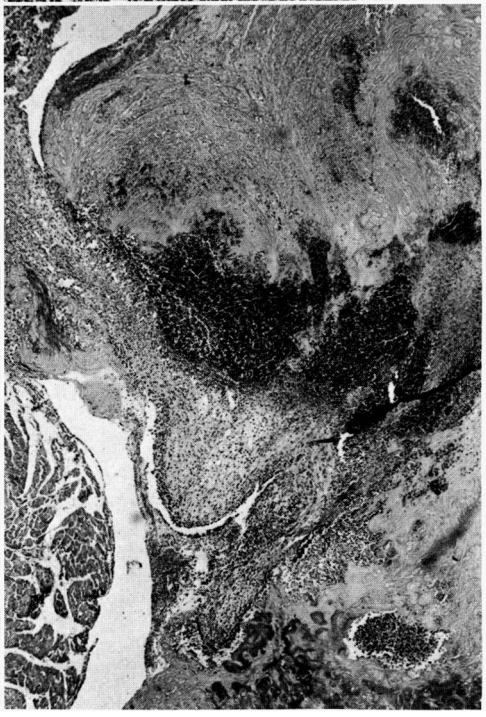
1B



1C



1D



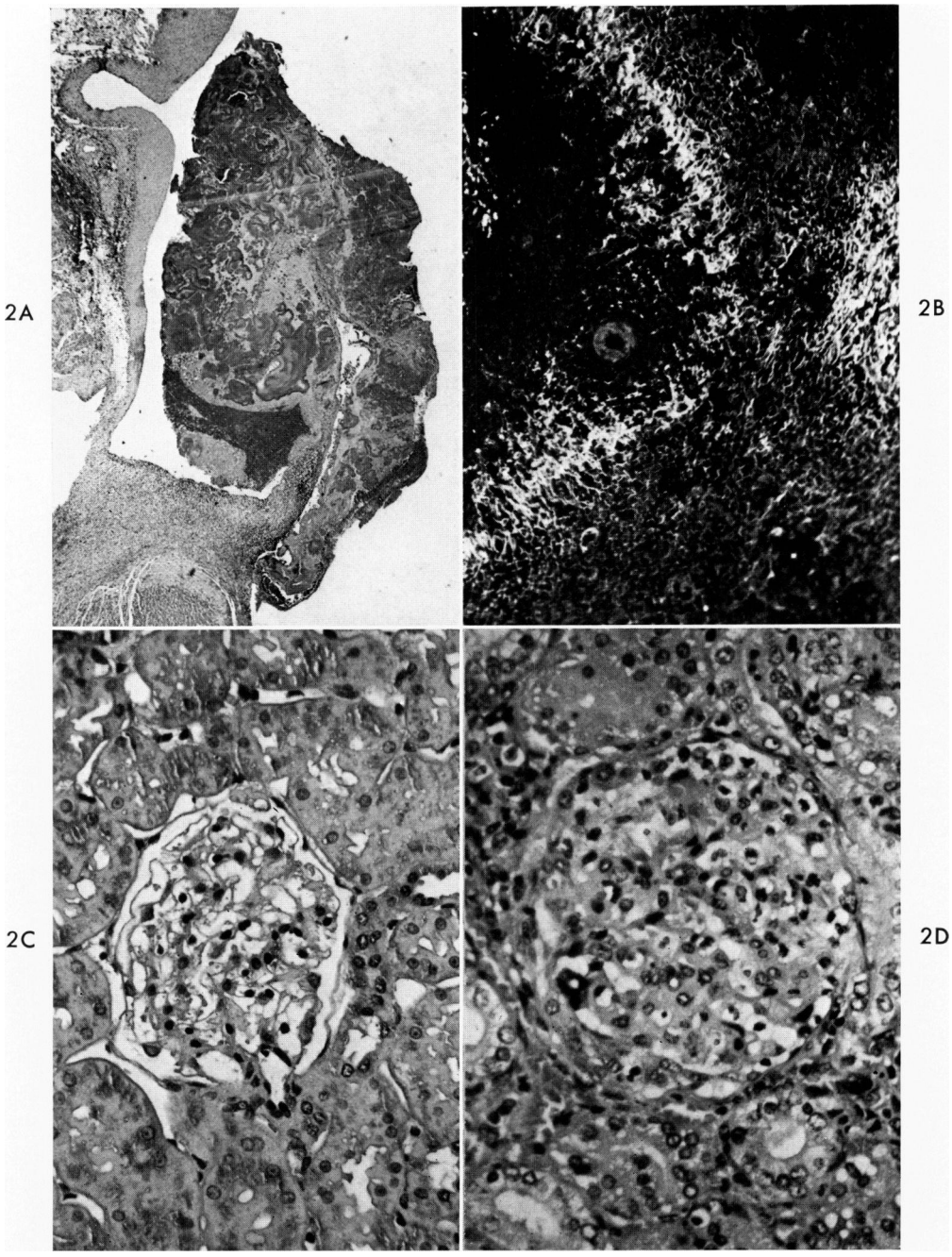


Fig. 2A. Large aortic valve vegetation is composed of masses of fibrin, neutrophils, and gram-positive bacteria. × 16. **Fig. 2B.** Spleen containing large amounts of brightly fluorescent amyloid. Thioflavine T. × 80. **Fig. 2C.** Normal glomerulus. × 250. **Fig. 2D.** Renal glomerulus displays marked cellular proliferation including formation of an epithelial crescent. × 250.