Experimental Lassa Virus Infection in the Squirrel Monkey

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Experimental Lassa virus infection was investigated in a nonhuman primate in order to elucidate the target organs of the viral infection and the course of pathologic events. Four squirrel monkeys (*Saimiri scirreus*) were inoculated intramuscularly with Lassa virus and sacrificed for organ titrations and histopathology, one each day, on Days 7, 12, 14, and 28 after inoculation. The animals showed a variable clinical course, with an incubation period of 8 to 18 days. The virus was demonstrated to be virtually pantropic; however, lymph node, liver, and kidney were key early targets. After the onset of overt disease, patterns of lymphoreticulotropism, hepatotropism, nephrotropism, adrenotropism, and persistent viremia were evident. Complement-fixing antibody failed to develop after 28 days of infection. Histopathologic findings included germinal center necrosis in spleen and lymph node; myocarditis; acute arteritis; renal tubular necrosis and regeneration; hepatocytic regeneration; chronic inflammation of choroid plexus, ependyma, and meninges; and cerebral perivascular cuffing. There is a relationship between many of these lesions and certain features of other arenavirus infections. The model offers the opportunity to pursue investigations of experimental pathogenesis, transmissibility, and efficacy of immunotherapy. (Am J Pathol 80:261–278, 1975)

LASSA FEVER was recognized first in 1969 during an outbreak of highly fatal febrile disease on the Jos plateau of Nigeria.[1] A virus was isolated from patients' specimens in this epidemic.[2] Subsequently, there have been two more epidemics in Nigeria, in 1970 [3-5] and 1974 [6]; one in Liberia in 1972 [7]; and one in Sierra Leone during 1970 to 1972.[8-10]

Lassa fever in humans may be subclinical; however, the typical clinical disease is devastating. It is characterized by an insidious onset of fever, chills, malaise, and myalgia; this is followed during the first week of illness by protean symptoms including pharyngitis, nausea, vomiting, diarrhea, cough, and abdominal and/or chest pain. Signs include low blood and pulse pressures, relative bradycardia, lymphadenopathy, and pulmonary rales. Survivors defervesce with disappearance of signs and symptoms between the second and fourth weeks of illness. In fatal cases, there is, characteristically, a development of serous effusions, facial edema, hemorrhagic diathesis, and shock.[1,4,11,12]

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Accepted for publication April 1, 1975

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Understanding of pathologic processes in human Lassa fever is based on limited clinicopathologic studies and a limited number of examinations of postmortem material.[1,13] Gross postmortem findings have included pleural effusions, ascites, visceral congestion and edema, and petechiae and hemorrhages principally in the gastrointestinal tract. Microscopic examination has revealed interstitial pneumonia, focal pulmonary edema, hepatocellular necrosis, focal renal tubular necrosis and tubular albuminous exudate, necrosis of the periarterial lymphocytic sheath of the spleen, and lymphoid depletion of the lymph nodes.

Such a small amount of tissue has been available from fatal cases in humans that non-end-stage pathologic changes remain unknown, and kinetic events in the development of lesions are only inferred from terminal conditions. Even the primary target organs of viral proliferation have not been identified systematically. In an attempt to explore these questions, the present study of pathogenesis of Lassa virus infection in the squirrel monkey (*Saimiri scirreus*) was undertaken. This model of infection was evaluated as an analog of the human disease.

Materials and Methods

Virus

Lassa virus, Bah strain, was isolated from the blood of a human case of Lassa fever in Sierra Leone. The isolate was passaged three times in VERO African green monkey kidney cells.[2] Stock virus was prepared in VERO cells, divided into aliquots to avoid multiple freeze-thaw cycles, and stored at −70 C. The infectivity titer of the virus stock was $10^{6.8}$ tissue culture median infectious doses $(TCID)_{50}$/ml when titrated in VERO cell cultures.

Animals

Four adult squirrel monkeys (*Saimiri scirreus*) were inoculated intramuscularly in the left thigh with 1 ml of undiluted virus stock. The inoculation, sacrifice, necropsy, and organ titrations were performed under Class III isolation conditions within the Maximum Security Laboratory at the Center for Disease Control. One of the 4 monkeys was sacrificed by cardiac puncture and exsanguination on Day 7, 12, 14, and 28, respectively.

Histology

Tissues consisting of 28 to 44 specimens were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned (6 to 8 μm), and stained with hematoxylin and eosin for histopathologic examination. Tissues which were examined microscopically from all animals were lymph nodes, adrenal, pancreas, stomach, skin, urinary bladder, salivary glands, kidney, liver, lung, heart, small and large intestine, esophagus, bone, spinal cord, synovium, brain, and skeletal muscle. The trachea, testis, inner ear, and spleen were examined from 3 animals. The prostate, seminal vesicle, gallbladder, and larynx of only 2 monkeys were studied. The thyroid, thymus, tongue, ovary, and uterus were obtained from only 1 monkey.

Viral Titration and Serology

Tissue was collected at necropsy from 20 to 22 separately dissected specimens from each animal. Organ infectivity titers were performed by titration in VERO cell cultures. In each organ, tissue, or fluid, the viral isolate was confirmed as Lassa virus by complement fixation test using the standard CDC method.[14,15]

Results

Clinical Observations

The clinical course of Lassa virus infection varied from animal to animal (Table 1). When the same route of inoculation and identical doses of virus inoculum were used, the disease course varied from a mild malaise to a fatal outcome after prolonged and progressive illness. Monkey A was well at the time of sacrifice on the seventh day after inoculation, having never developed any clinical signs of illness. Monkey B developed anorexia, lassitude, and polydipsia on the eighth day after inoculation. This animal's course was progressively downhill, with increasing prostration until it was sacrificed in a moribund state on the twelfth day after inoculation. Monkey C also developed signs of disease on the eighth day after inoculation. The following day this animal was severely depressed, sitting immobilized in a corner, drooling, and exhibiting episodic tremors. On the tenth day after inoculation, this animal appeared to have recovered and continued to appear healthy until it was sacrificed on the fourteenth day after inoculation. Monkey D did not exhibit any symptoms until the eighteenth day after inoculation, when it developed anorexia, polydipsia, and lassitude which persisted until the animal was sacrificed on the 28th day after inoculation, at which time the signs were beginning to abate.

Organ Titrations

The results of Lassa virus infectivity titrations of the organs and fluids (Table 2) revealed that in Monkeys B, C, and D the virus was virtually pantropic. Calculation of mean titers from positive tissues was made, with

Table 1—Clinical Course of Lassa Virus Infection in Squirrel Monkeys

	A	B	C	D
Day of sacrifice	7	12	14	28
Day of onset of illness	—	8	8	18
Clinical state at sacrifice	Well	Moribund	Recovered	III (improving)
Signs of illness	None	Anorexia, lassitude, depression, polydipsia, prostration	Anorexia, lassitude, polydipsia	Anorexia, lassitude, polydipsia

Table 2—Organ Titers of Lassa Virus in Squirrel Monkeys*

	A	B	C	D
Lymph node				
Femoral	2.8	4.5	2.8	3.5
Mesenteric	<1.8	5.8	5.2	2.2
Cervical	<1.8	3.5		2.5
Axillary				2.2
Spleen		≥6.5	4.5	1.8
Thymus	<1.8			<1.8
Bone marrow				<1.8
Heart	<1.8	4.5	3.5	2.2
Lung	<1.8	5.5	4.5	2.5
Liver	2.8	6.2	4.2	<1.8
Pancreas	<1.8	4.0	4.8	2.2
Parotid gland		4.5	C	<1.8
Submandibular gland	<1.8			2.5
Larynx	<1.8			
Esophagus		4.8		
Stomach			C	
Small intestine			C	C
Kidney	2.5	5.2	4.8	2.5
Urinary bladder	<1.8	4.5	4.0	<1.8
Prostate		5.5		
Uterus	<1.8			
Testis		3.5	3.8	<1.8
Ovary	<1.8			
Adrenal	<1.8	6.8	4.8	3.2
Cerebrum	<1.8	<1.8	C	2.5
Cerebellum	<1.8	<1.8	C	<1.8
Skeletal muscle		3.8	C	<1.8
Blood	1.8	4.5	3.8	2.2
Urine		5.5		
Throat swab				<1.8

* Values are given as $\log_{10}$ of tissue culture median infectious dose ($TCID_{50}$) per gram or milliliter of tissue or body fluid; when converted to per gram basis, the lowest significant positive titer was $10^{1.8}$ per gram. C = contaminated.

the possibility of bacterial contamination in some specimens taken into consideration. From Monkey B, 18 of 20 specimens contained Lassa virus, with a mean titer in positive tissues of $10^{5.0}$ $TCID_{50}$/ml. From Monkey C, 12 of 18 specimens yielded Lassa virus. The mean titer was $10^{4.2}$ $TCID_{50}$/ml. Thirteen of 23 specimens from Monkey D contained Lassa virus, with a mean titer of $10^{2.5}$ $TCID_{50}$/ml. Lassa virus was detected in only 4 of 18 specimens from Monkey A, which was asymptomatic when sacrificed on Day 7. The mean titer was $10^{2.5}$ $TCID_{50}$/ml.

Despite the large number of organs from these 4 animals which contained Lassa virus, a definite pattern of lymphoreticulotropism, hepatotropism, nephrotropism, adrenotropism, and persistent viremia was recognized. There was a close relationship between the severity of clinical illness and the distribution of infection and organ titers. Thus, the moribund animal (Monkey B) had the most organs and tissues that were positive and the highest organ and tissue mean titer. The other animals followed, in order: Monkey C (sacrificed 14 days after inoculation) carried the next greatest viral burden, Monkey D (28 days after inoculation) was next, and finally, Monkey A (7 days after inoculation) was the least infected.

Virus was detected in high titer in the urine of the single animal from which an adequate specimen was collected. Monkey A had been inadvertantly splenectomized during the conditioning period.

Despite the clinical improvement and prolonged course of infection (28 days), no complement-fixing antibody was detectable in the serum of Monkey D.

Histopathology

Lymph Nodes

Secondary follicles in the cortex of the lymph nodes from Monkey A were necrotic and contained multinucleate giant cells (Figure 1). There was moderate sinus histiocytosis. The lymph nodes from Monkey B also contained focally necrotic secondary follicles, sinus histiocytosis, and also a hyperplastic paracortical region with a starry sky appearance due to the presence of dendritic reticulum cells interspersed among small and medium sized lymphocytes. Marked sinus histiocytosis was seen in the lymph nodes of Monkey C in addition to hyperplasia of the paracortical region and moderate secondary follicle formation. In Monkey D, the lymph nodes showed remarkable reactive hyperplasia which partially effaced nodal architecture. Changes included paracortical hyperplasia, secondary follicle formation, and filling of sinuses with many large mononuclear cells and few multinucleate giant cells.

Spleen

The periarteriolar lymphocytic sheath of the spleen from Monkey B showed necrosis of the eccentrically located germinal centers with accumulation of eosinophilic amorphous debris and focal hemorrhage (Figure 2). In Monkey D, these germinal centers contained the same type of eosinophilic material; but rather than appearing depopulated, they contained a large number of lymphoblastoid cells (Figure 3).

Heart

Monkey B had a severe, irregularly distributed myocarditis with vacuolar degeneration and necrosis of myocardial fibers. Distribution of this lesion ranged from single isolated fibers to larger fields of contiguous fibers with adjacent interstitial accumulations of polymorphonuclear leukocytes, mononuclear cells, and occasional plasma cells (Figure 4). In the heart of Monkey D, there was a mild patchy pericarditis consisting principally of infiltrated mononuclear cells. There was a single focus of interstitial mononuclear infiltrate in the right ventricular myocardium and a focus of valvular subendocardial hemosiderin-laden macrophages.

Vessels

In a coronary artery from Monkey B, a severe transmural, segmental acute arteritis was noted (Figure 5). The endothelial cells had undergone proliferation and swelling, and there was an underlying fibrinoid degeneration with infiltration of the entire thickness of the media by polymorphonuclear (PMN) leukocytes.

A similar example of acute arteritis was seen in an artery at the junction of the pancreas and duodenum in Monkey D. In addition to a larger perivascular infiltrate of lymphocytes and macrophages, a PMN leukocytic infiltration of the arterial media was noted.

Kidney

Foci of tubules in the cortex of the kidneys from Monkey A contained degenerating multinucleate giant cells in their lumina (Figure 6). The nuclei of these giant cells were aggregated centrally and were surrounded by a moderate amount of cytoplasm. In Monkey B there was widespread but randomly distributed patchy necrosis of tubular cells. Entire individual tubular profiles were necrotic, although there was some asynchrony of degeneration and necrosis among adjacent cells. The tubular cells in the terminal stages of lysis had pale, vacuolated cytoplasm. Dense, amorphous, eosinophilic material completely filled the necrotic tubules (Figure 7). Other tubules with intact epithelium contained a paler, more homogeneous, eosinophilic proteinaceous material in their lumina. Foci of adjacent tubules in Monkey D were lined by flattened, elongated tubular epithelial cells; these tubules had dilated lumina. Some cross-sections of tubules were lined by epithelial cells with nuclei bulging into their lumina; the cytoplasm of these cells was extremely flattened. Occasional mitoses were observed in such tubules. These findings were interpreted as tubular regeneration. In addition, collecting ducts in the medulla and near the corticomedullary junction were focally filled with necrotic cellular debris.

Liver

In Monkeys A and B, widespread foci of inflammation were observed in liver parenchyma. These consisted of PMN leukocytes and mononuclear cells. In Monkey C, there were randomly distributed parenchymal foci of lymphocytes, plasma cells, and mononuclear cells. In Monkey B there were diffuse hepatocytic changes suggestive of a massive attempt at regeneration. These changes included many hepatocytic mitoses, binucleate hepatocytes, and large, irregularly sized and shaped hepatocytic nuclei (Figure 8). There was also a moderate degree of fatty metamorphosis.

The liver of Monkey D showed no parenchymal inflammation or necrosis. There was only nonspecific mild portal inflammation consisting of eosinophils and mononuclear cells.

Monkeys A, B, and C had been trapped in the wild and were infected with microfilaria; these were seen in liver sinusoids and, at times, in foci of hepatic necrosis and/or sites of acute inflammation. Their relation to the necrosis, whether causal, contributing, or unrelated, was impossible to determine. In addition, the bile ducts of Monkey C were infected by the fluke, *Athesmia foxi*. The degree of hepatic pathology directly associated with the fluke was minimal. Monkey D, which was born and raised in captivity, was not infected by microfilaria or *Athesmia foxi*.

Brain

No lesions were observed in the brains of Monkeys A, B, and C. Monkey D, however, exhibited significant neuropathology. Choroid plexuses in the third and fourth ventricles and lateral ventricles were infiltrated by large mononuclear cells, lymphocytes, and plasma cells (Figure 9). This infiltration extended into the fronds of the plexuses. The inflammatory infiltrate was located extravascularly between the central capillary and the choroidal epithelium, which was variably thinned. Focal calcification was observed in the inflamed choroid plexus of the fourth ventricle. The ependyma was infiltrated in multiple foci by mononuclear cells. Subependymal pathologic alterations included edema, microglial reaction, reactive astrocytes (Figure 10), and many perivascular cuffs containing mononuclear cells and lymphocytes (Figure 11). Similar perivascular cuffs were seen in smaller numbers deeper in the parenchyma of the cerebrum. A mild mononuclear infiltrate was present in the subarachnoid space of the leptomeninges.

Other Organs

Isolated instances of pathologic changes were observed in the adrenal, prostate, and bone marrow in Monkey B. The zona fasciculata of the

adrenal cortex contained multiple petechial hemorrhages which were not associated with any parenchymal adrenocortical cytopathology. In the prostate, glandular lumina were filled with exudate consisting of PMN leukocytes and amorphous, pale, eosinophilic material. No pathologic alteration of the glandular epithelium or stroma was associated with this evidence of prostatitis. Megakaryocytes from various samples of bone marrow exhibited striking degeneration characterized by pyknosis of nuclear lobules and pale vacuolation of cytoplasm.

Isolated examples of pathologic changes were observed in the lingual epithelium and pancreas of Monkey D. The lingual mucosa contained a focal subepithelial aggregation of lymphocytes which had ruptured the epithelial basement lamina and invaded the overlying stratified squamous epithelium. The epithelial cells associated with this lymphocytic infiltration were vacuolated and degenerated. However, the upper levels of epithelium, which were not invaded, were intact, and no ulceration was observed. The pancreas contained multiple foci of lymphocytic and mononuclear cell infiltrates in interacinar, periductal, and perineural interstitium.

In all animals, the lungs exhibited alveolar septal thickening; this lesion of unknown etiology occurs commonly and intercurrently in this species. This alveolar septal thickening was patchily distributed and was far more severe in the parasitically infected monkeys trapped in the wild than in the animal born and raised in captivity.

Discussion

The clinical disease course, the yield of virus from tissues and organs, and the character of lesions produced in the 4 squirrel monkeys infected with Lassa virus in this study suggest that this species provides a good model for study of human Lassa fever. Correlative pathologic findings, particularly in liver and spleen, suggest that there are fundamental mechanistic similarities between the simian and human disease. Further correlations are all the more probable. Thus, observations of organ tropisms and degenerative as well as regenerative phases of infection in the monkeys complement the limited observations of end-stage fatal human disease, observations which often have been complicated by attempted supportive therapy. The lymphoreticulotropism of Lassa virus in these monkeys, as indicated by lymph node and spleen infectivity titers and the necrosis of B-cell regions, may be a correlate of the delayed antibody response which is characteristic of several arenavirus infections of man and experimental animals.[5,9,16,17] Complement-fixing antibody develops slowly in human Lassa fever patients, and in the present study

the monkey which was sacrificed at 28 days was still negative. It is possible that the delay in anti-Lassa-virus antibody response could be due to viral infection and destruction of responding lymphoid cells. This delay in antibody response could also explain the prolonged clinical course of disease. The efficacy of administration of homologous immune serum to Lassa fever patients also correlates with this point.[11,18] Further experimental effort in this area depends on development of an assay for neutralizing antibody.

Another feature of the simian lymphoreticular histopathology was hyperplasia of the T-cell-dependent paracortical regions of lymph nodes and varying degrees of severity of sinus histiocytosis. This paracortical hyperplasia, as an indication of an active development of cell-mediated immune responsiveness, may have been acting for the good of the host, but because of the importance of cell-mediated immunopathologic processes in arenavirus infections,[19,20] further focus on this point is indicated. Although the precise role of cell-mediated immunopathology in man and nonhuman primates is not known, laboratory models of rodent infections with LCM and Tacaribe viruses have demonstrated T-cell-mediated immunopathology. A degree of sinus histiocytosis is, of course, a common finding in a lymph node draining an inflamed region. However, in these monkeys, the degree of sinus histiocytosis was so great in some nodes that it suggested the possibility of lymphatic obstruction. Obstruction to lymphatic flow may be the basis for the edema and effusions seen in human Lassa fever.

Myocarditis, a lesion which has not been seen at autopsy in human Lassa infections, was present in the infected monkeys. The coincident isolation of virus from myocardial tissue of 3 of the 4 monkeys suggests a direct viral cytopathic effect with inflammatory response to this insult. Some Lassa fever patients have manifested nonspecific EKG changes, relative bradycardia, low blood and pulse pressures, and pulmonary rales [11,18] which might reflect myocarditis and cardiac failure. Significantly, myocarditis has been described in humans with Argentine hemorrhagic fever,[21] and myocardial degeneration and necrosis have been observed in rhesus monkeys with experimental Bolivian hemorrhagic fever.[22] All of these hemorrhagic fevers are caused by arenaviruses.

Acute arteritis was observed in 2 of these infected monkeys. This lesion has not been described previously in Lassa virus infections. The coronary arteritis seen in 1 of the monkeys with myocarditis may have exacerbated the myocardial damage, but it cannot account for the amount, distribution, and nature of the myocardial degeneration and inflammation. The pathogenesis of this arteritis, whether due to immune complex deposition,

viral infection of the vessel wall or some other mechanism, could not be determined in the present study.

The kidneys of 3 of the 4 monkeys had tubular changes, and virus was isolated in moderate to high titer from all 4 animals' kidneys. It was possible to construct a hypothetical temporal sequence from early necrosis of tubular epithelium through late progressive regeneration. A patchy distribution and asynchrony of the degenerative phase of this sequence favors a direct viral cytopathic mechanism rather than tubular necrosis secondary to hypotension. The need for frozen-section immunofluorescence to unravel these mechanistic questions is apparent; use of such techniques are, however, precluded by the hazard involved. By analogy to the simian disease, the proteinuria commonly found in human Lassa fever could be explained as an impairment of protein resorption by damaged and necrotic tubular epithelium. The degree to which renal failure contributes to human disease is unclear; elevated blood urea nitrogen levels have been observed,[4] and facial edema is a common sign. Exacerbation of vascular permeability by renal failure could contribute to the formation of edema and cavitary effusions. In any case, the evidence of tubular regeneration seen in the monkey sacrificed at 28 days suggests that careful clinical management (fluid and electrolyte balance and possibly dialysis), while allowing tubular regeneration to ensue, would enhance chances for patient survival.

It has previously been shown that the liver was a target of human Lassa fever by the histopathologic finding of necrosis [1,13,23] and ultrastructural demonstration of necrosis and arenavirus virion production.[24] The hepatic changes in Monkey B were interpreted as a massive attempt at regeneration in response to a severe insult and emphasize the important regenerative potential of this organ. The demonstration of Lassa virus by organ infectivity titration in 3 of 4 monkeys illustrates by another technique, not previously reported, that the virus is present in significant amount in this organ. The presence of virus is circumstantial evidence of involvement in the pathogenesis of hepatic injury. However, whether the cytopathology is caused by a direct viral cytopathic effect or by antibody-mediated cytolysis is not apparent. The absence of detectable circulating antibody would mitigate the latter possibility; on the other hand, the large antigenic mass could be masking the presence of antibody by a sponge effect of antibody binding. The accumulated histopathology contains no evidence for hepatic cell-mediated immunopathology.

The neuropathologic findings were limited to the brain of the animal in which a virus was isolated from the same organ. It is not known whether this relationship was due to a restricted degree of neurotropism of this

virus, whether CNS infection requires a prolonged time for expression, or whether there is some other explanation. The fact that the brain lesions resemble those of murine lymphocytic choriomeningitis virus infection (the prototype arenavirus infection) raises the possibility of a similar cell-mediated immunopathologic mechanism in Lassa virus infection of the CNS.

In an attempt to generalize, it was noted that in lymph nodes, spleen, heart, kidney, and brain there seemed to be a correlation between the degree of pathology and the level of virus titer. However, there were many notable exceptions to this correlation: Minimal histopathology was associated with high infectivity titers found in the adrenals of Monkeys C and D ($10^{4.8}$ and $10^{3.2}$ $TCID_{50}$/g, respectively) the pancreas of Monkeys B and C ($10^{4.0}$ and $10^{4.8}$ $TCID_{50}$/g, respectively), and most of the other organs of Monkey C.

The incubation period of the 4 monkeys varied from 8 to 18 days. From epidemiologic data, it has been determined that most human cases occur 7 to 10 days after exposure, but a range of 3 to 17 days has also been postulated.

These observations of simian Lassa virus infection encourage use of this model for further studies. We need to know more about experimental pathogenesis, transmissibility, and efficacy of immunotherapy in order to rationally handle the emerging human disease.

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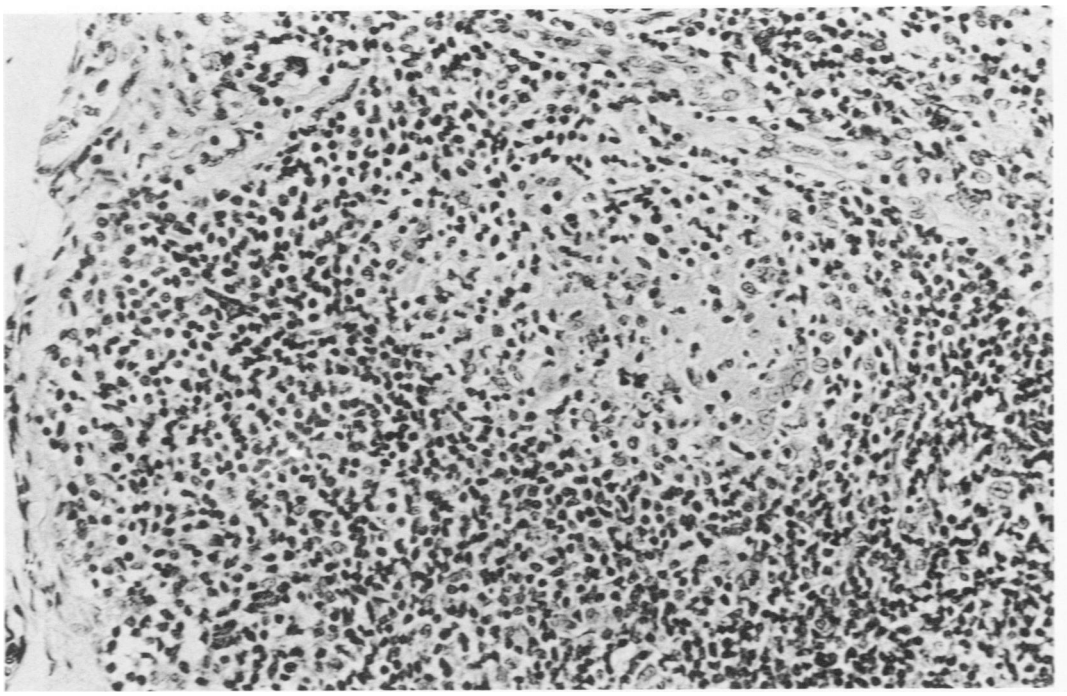
Acknowledgments

The authors would like to acknowledge the expert assistance of James V. Lange.



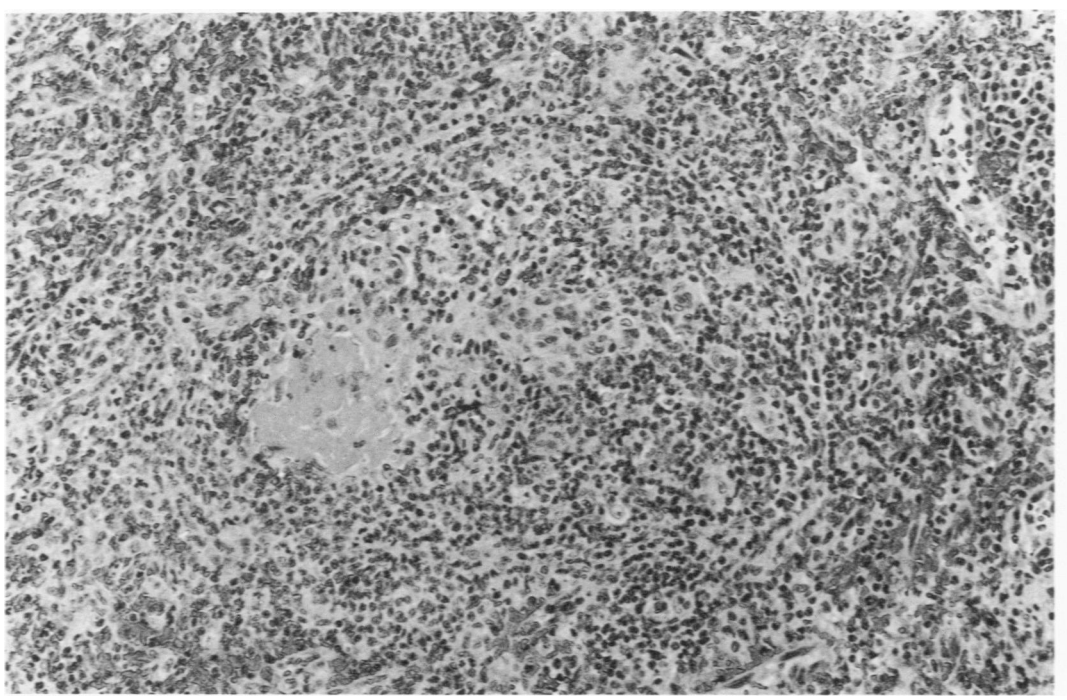


Figure 1—Lymph node from monkey 7 days after inoculation with Lassa virus. The subcapsular sinus is on the left margin. Necrosis of germinal center in cortex with syncytial giant cell formation is seen. (H & E, × 175) **Figure 2**—Spleen from monkey at Day 12. The periarteriolar lymphocytic sheath and its circumferential marginal zone occupy the center of the field. Necrosis of white pulp with accumulation of amorphous eosinophilic debris is seen. (H & E, × 225)

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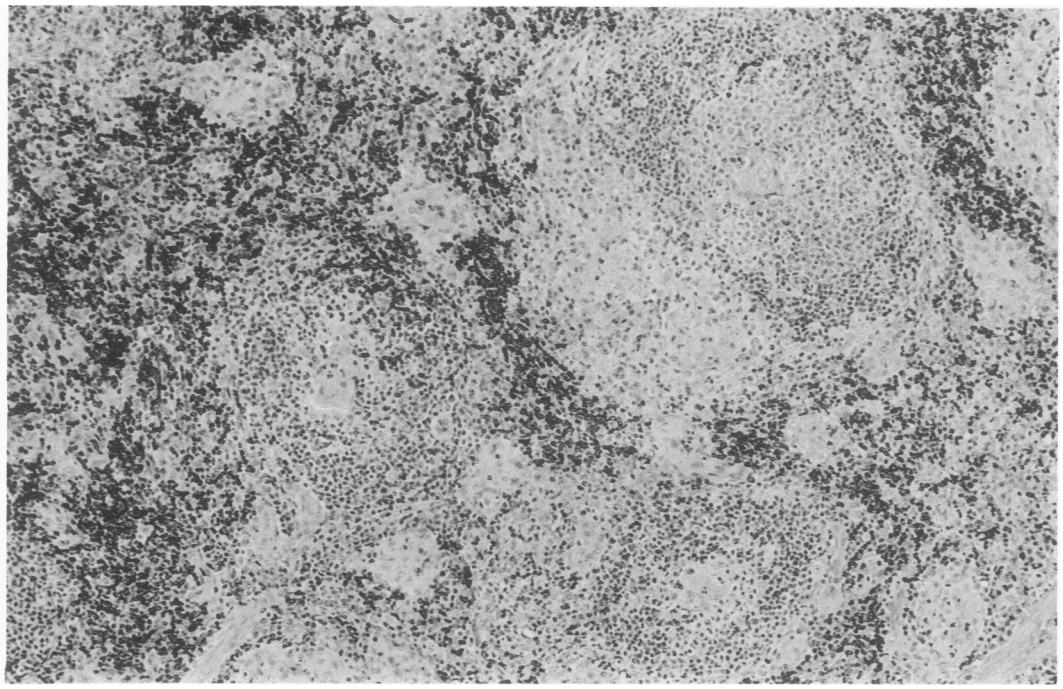

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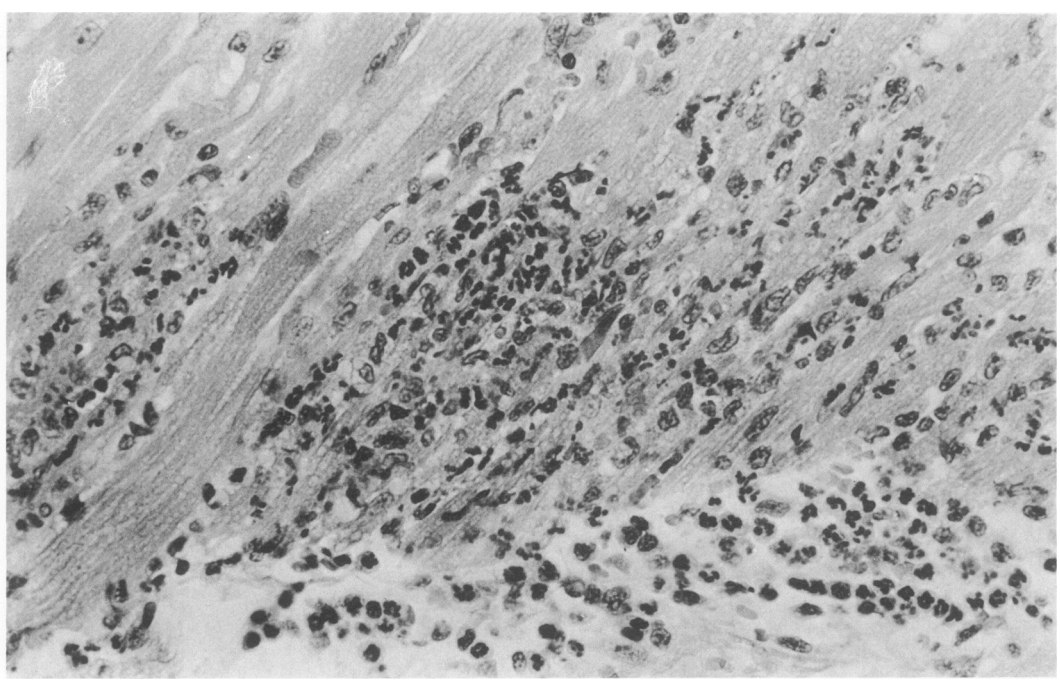
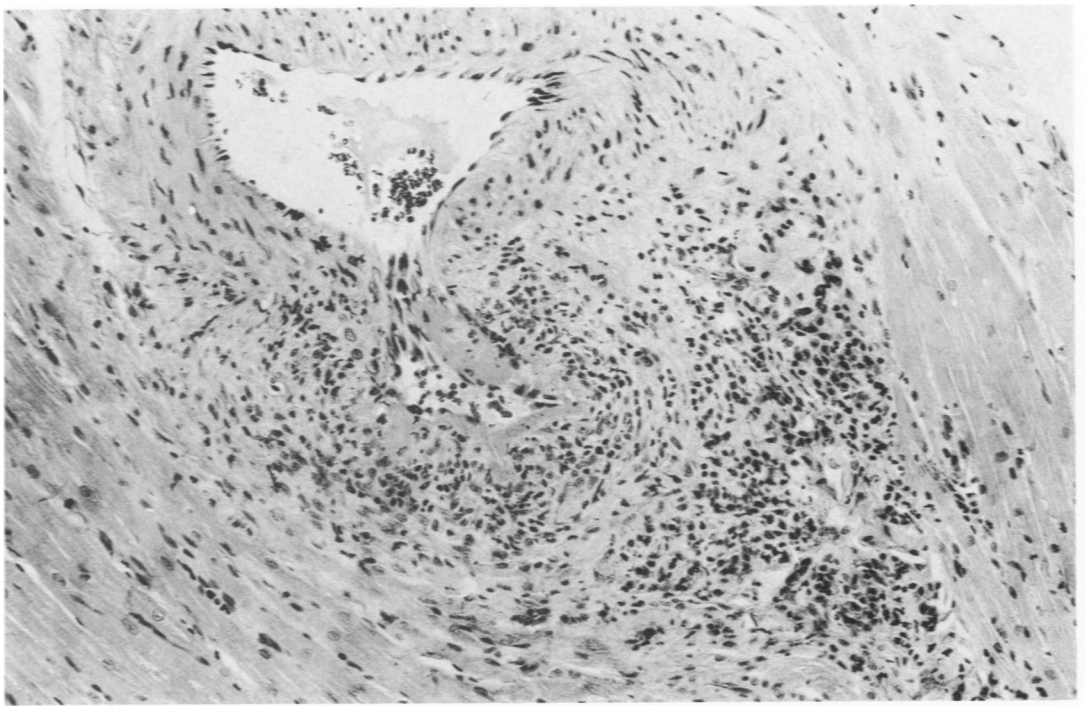


Figure 3—Spleen from monkey at Day 28. There are three periarterial lymphocytic sheaths with focal residual necrotic debris and adjacent increased numbers of lymphoblasts. (H & E, × 90)
Figure 4—Myocardium from monkey at Day 12 showing vacuolar degeneration and necrosis of myocardial fibers and inflammatory response consisting of mononuclear cells and PMN leukocytes (H & E, × 450).



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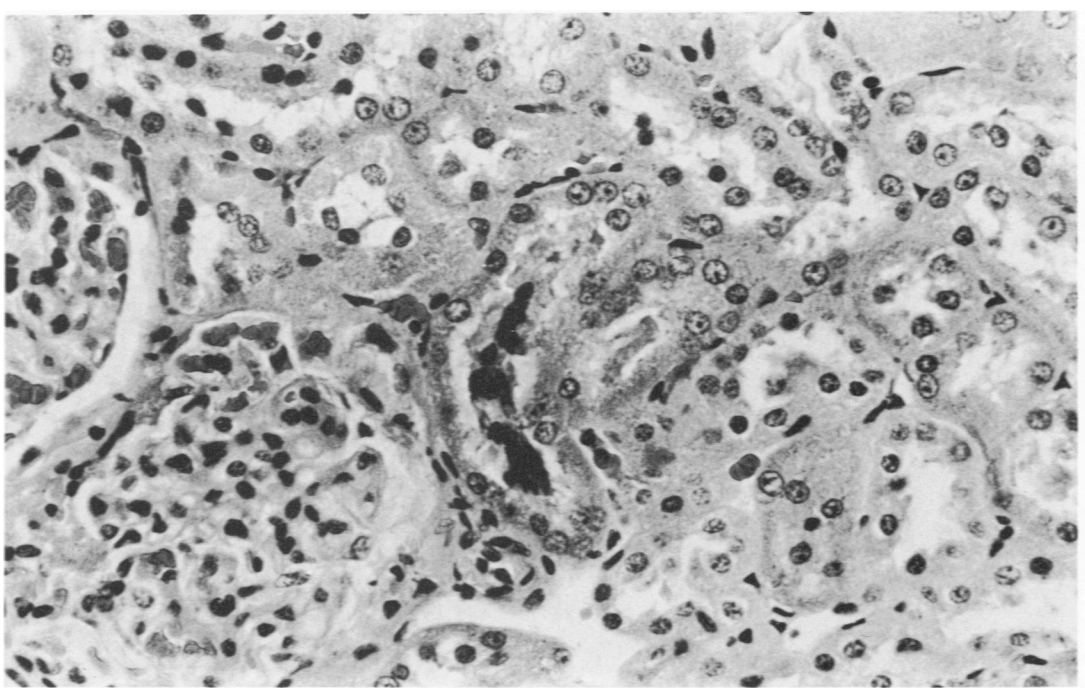

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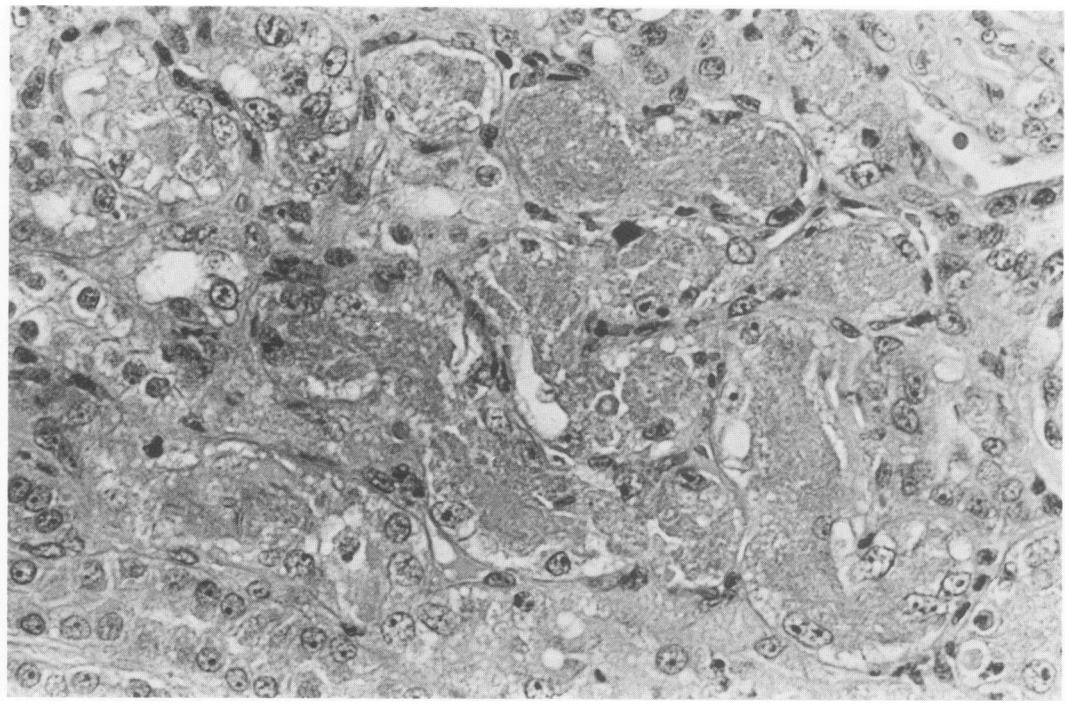
Figure 5—Coronary artery from monkey at Day 12 showing acute segmental arteritis with necrosis and PMN leukocyte and mononuclear cell infiltration (H & E, × 175). **Figure 6**—Kidney from monkey at Day 7. Degenerating nuclei are surrounded by cytoplasmic debris in tubular lumen. (H & E, × 450)



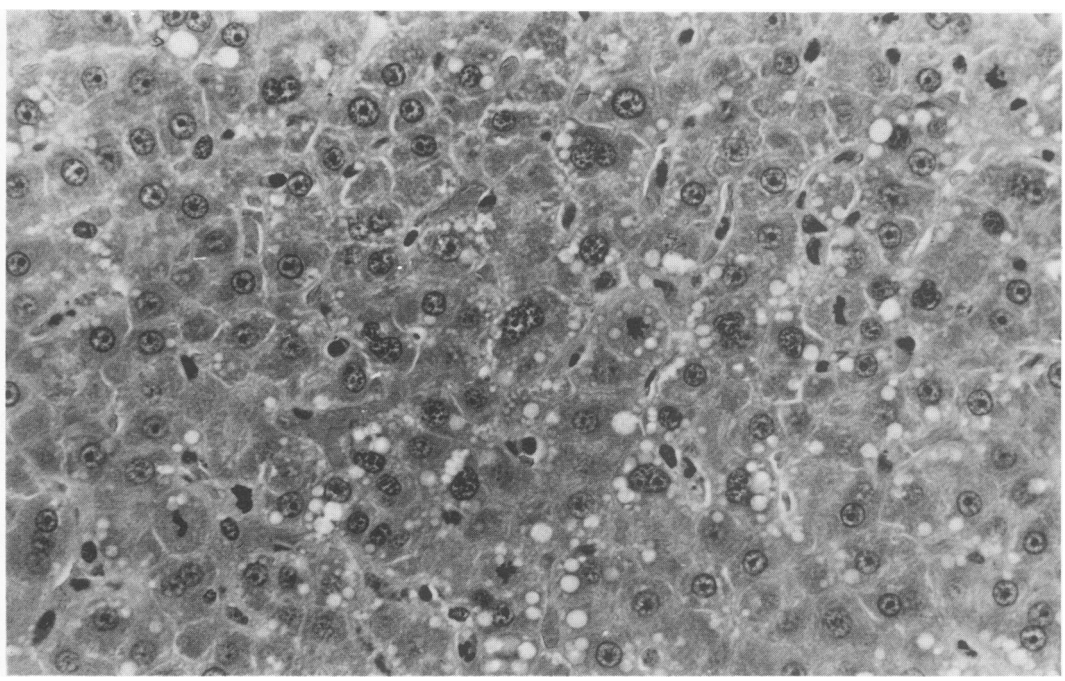
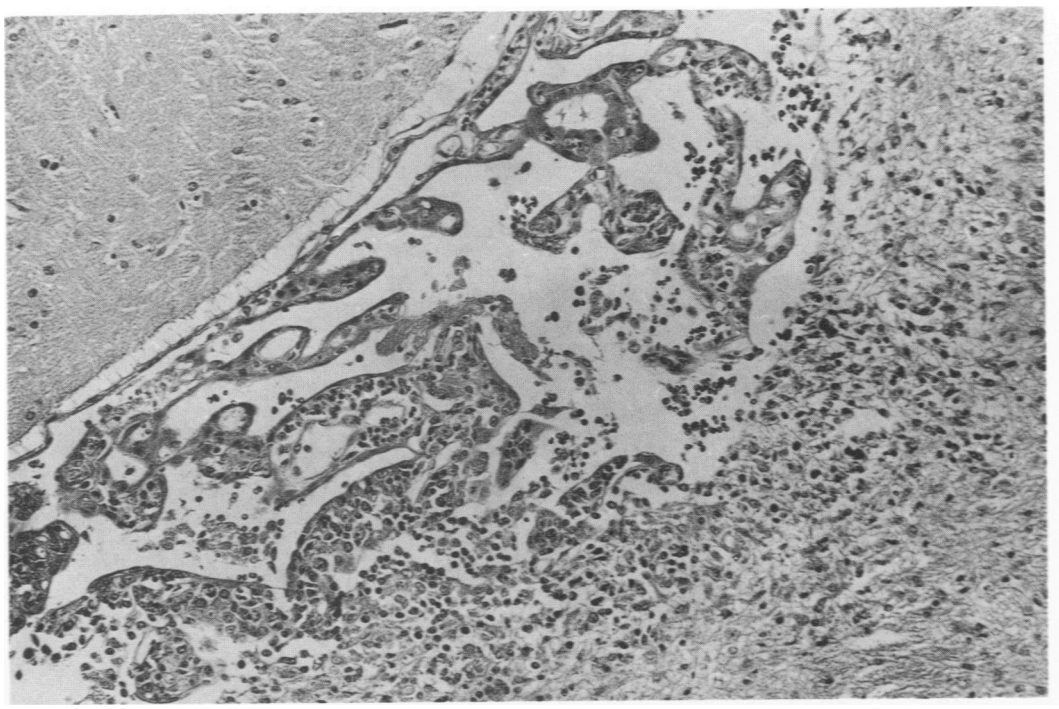


Figure 7—Kidney from monkey at Day 12. Degeneration and necrosis of tubular epithelium with accumulation of eosinophilic necrotic luminal debris are seen. (H & E, × 450) **Figure 8**—Liver from monkey at Day 12 showing numerous mitoses of hepatocytes, binucleate and large heteroploid hepatocytic nuclei, and mild fatty metamorphosis (H & E, × 450).



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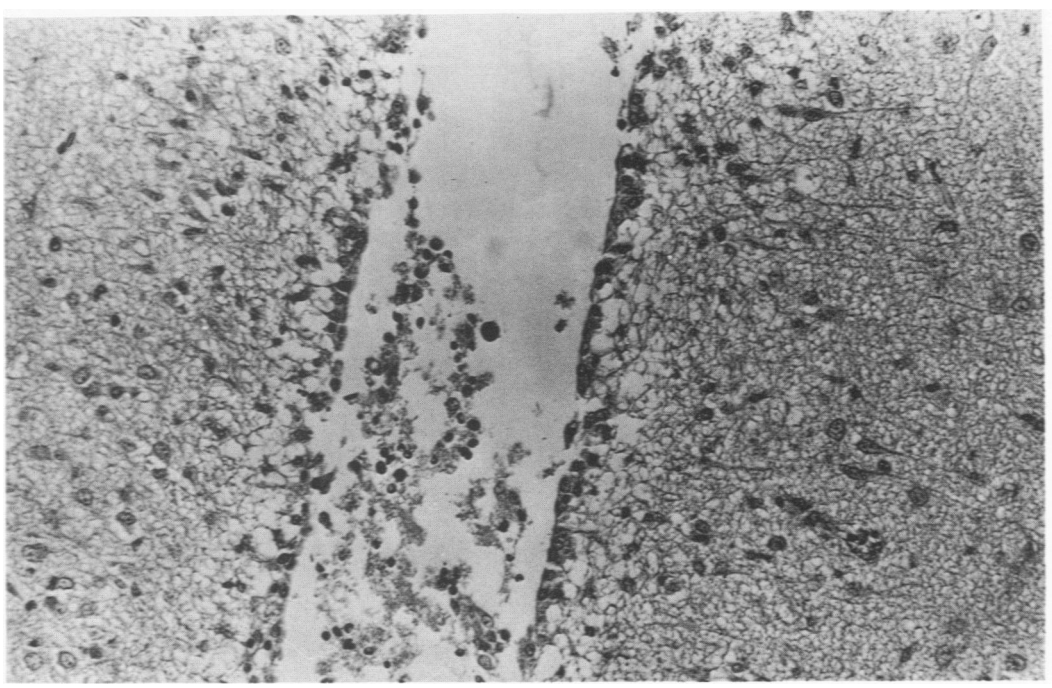

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Figure 9—Choroid plexus from monkey at Day 28. The adventitial space is infiltrated by mononuclear cells and plasma cells. (H & E, × 175) **Figure 10**—Brain from monkey at Day 28. Ependymal degeneration, subependymal edema and astrocytic reaction, and ventricular exudate are seen. (H & E, × 275)

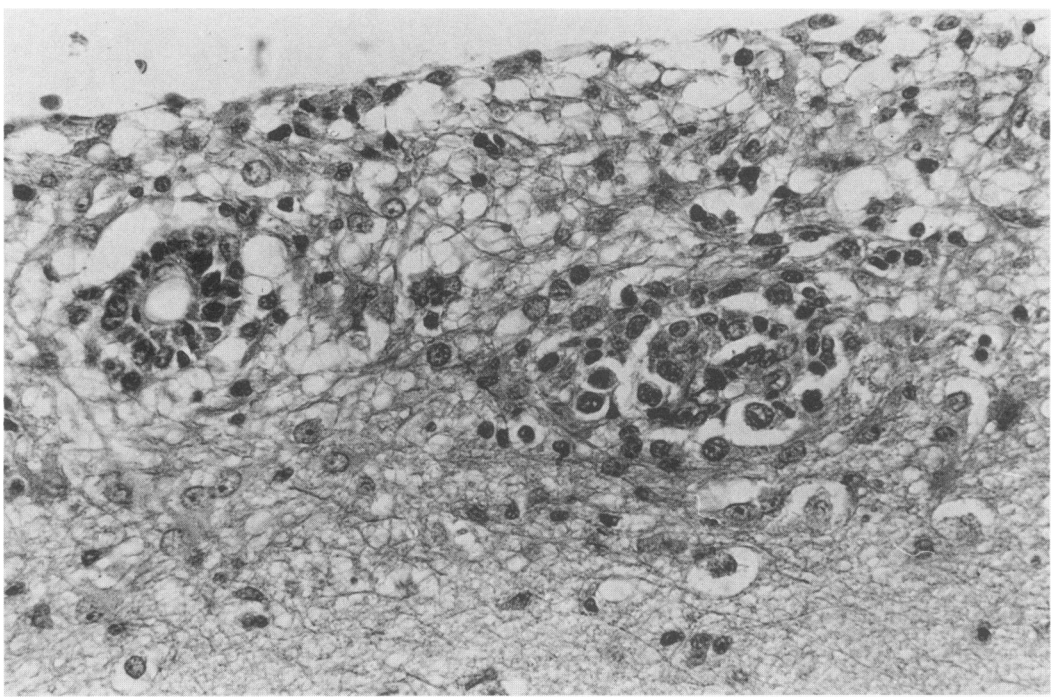

Figure 11—Brain (floor of fourth ventricle) from monkey at Day 28 showing subependymal perivascular cuffing by mononuclear cells (H & E, × 450).