Pathology of Bolivian Hemorrhagic Fever in the Rhesus Monkey

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Gross and microscopic lesions associated with Bolivan hemorrhagic fever virus infection in the rhesus monkey were studied in 10 animals which died following inoculation. Gross lesions included skin rash, lymphadenopathy, splenomegaly, meningeal edema, hydropericardium and enlarged friable livers. Hemorrhagic manifestations of the infection were not consistently observed, but hemorrhages were present in the skin, heart, brain and nares in some monkeys. Histopathologic lesions were fairly consistent. Hepatic necrosis with the presence of acidophilic hyaline bodies, necrotizing enteritis, epithelial necrosis and adrenal cortical necrosis were present in all monkeys. Those monkeys which died after the seventeenth day of infection had nonsupurative meningoencephalitis; lymphoid necrosis was present in 3 monkeys that died after day 18. Other microscopic lesions included myocardial degeneration, lymphoid and reticuloendothelial cell hyperplasia and lymphoid depletion. Most of the histopathologic lesions described in human autopsy material were reproduced; however, the necrosis in the skin and oral mucosa, mucosa of the gastrointestinal tract and the adrenal cortex have not been described in man. Despite these apparent discrepancies the results of this investigation indicate that the rhesus monkey is a good experimental model for the study of Bolivian hemorrhagic fever infection (Am J Pathol 73:477–494, 1973).

THE RHESUS MONKEY (*Macaca mulatta*) has recently been shown to be susceptible to experimental infection with Machupo virus, the etiologic agent of Bolivian hemorrhagic fever.[1] Clinical manifestations in this monkey are similar to those observed in man,[2–4] suggesting that the rhesus monkey may serve as an excellent primate model in which to investigate the disease.

The present investigation was initiated to characterize the salient gross and microscopic lesions of Machupo virus infection in the rhesus monkey and perhaps to give some insight into the pathogenic process in man.

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In conducting the research described herein, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences—National Research Council. The facilities are fully accredited by the American Association of Accreditation of Laboratory Animal Care.

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Materials and Methods

Virus

A suckling hamster brain pool of the Carvallo strain of Machupo virus was used in the study. The virus titer in the stock solution was 2×10^6 plaque-forming units (PFU) of virus. Dilutions were prepared in cell culture medium at 10^{-1}, 10^{-3} and 10^{-5}. Each monkey was inoculated with 0.5 ml of a dilution; a virus plaque assay in Vero cell culture at the time of injection indicated that the inocula contained 10^5, 10^3 and 10^1PFU of virus, respectively.

Animals and Inoculation

Twelve fully conditioned, young, adult female rhesus monkeys were placed in standard primate restraint chairs and rectal thermocouples were inserted to monitor body temperatures. The monkeys were allowed 2 weeks to adjust to the chairs before virus inoculation. Monkeys were divided into three groups of 4 monkeys each; those in one group were inoculated with 10^5 PFU of virus subcutaneously, and those in the other two groups were inoculated with 10^3 or 10^1 PFU of virus, respectively. The infection was allowed to follow its natural course in all of the monkeys; necropsies were performed shortly after death on those animals that died.

Histology

Tissues from all organ systems were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 6 to 8 μ and stained with hematoxylin and eosin for routine histopathologic examination. Selected sections were stained with Giemsa and McCallum-Goodpasture stains for the demonstration of microorganisms, Mallory phosphotungstic acid hematoxylin for fibrin thrombi, Perl's method for iron to demonstrate hemosiderin and von Kossa's stain for mineral.

Virus Assay and Serology

Approximately 1 ml of blood was collected daily by femoral venipuncture for virus assay and hematologic determination and 5 ml each third day for serologic analysis. In addition, tissue sections were collected from the liver, brain, spleen, lymph nodes, kidneys and small intestine at necropsy and frozen for virus assay. The results of these determinations are to be reported elsewhere.[1]

Results

Clinical Observations

The correlation of deaths with the different dose levels of virus inoculum is summarized in Table 1. Ten of the 12 inoculated monkeys died. The two survivors, monkeys that received the low dose (10^1 PFU), did not develop clinical or serologic evidence of infection. One monkey (No. 10) died of acute gastric dilatation on the second day postinoculation and did not exhibit any of the gross or microscopic lesions that were typical of Machupo virus infection. The other low dose monkey (No. 9) developed evidence of clinical infection later and died on the 37th day postinoculation.

Table 1—Clinical Course of Infection in Rhesus Monkeys Inoculated with Three Different Doses of Machupo Virus

Animal No.	Dose (PFU)	Clinical course (days)
1	10^5	13
2	10^5	14
3	10^5	14
4	10^5	17
5	10^3	17
6	10^3	18
7	10^3	18
8	10^3	25
9	10^1	37
10*	10^1	2
11†	10^1	—
12†	10^1	—

* Cause of death was acute gastric dilatation.
† No clinical infection.

All of the monkeys inoculated with either the high (10^5 PFU) or median (10^3 PFU) dose level of virus died. The development of lesions was similar in both groups, but those that received the higher dose died earlier.

Gross Pathology

Table 2 summarizes the gross necropsy findings. Hemorrhagic manifestations of the disease were present, but they were not consistent and were generally not severe enough to cause death. Petechiae were observed in the skin covering the face, thorax, abdomen and medial surfaces of the thigh and forelimbs in 1 monkey at the time of death.

Table 2—Gross Lesions of Bolivian Hemorrhagic Fever in Rhesus Monkeys*

Monkey No.†	Skin petechiae or rash	Yellow liver	Meningeal edema	Lymph-adenopathy	Myocardial hemorrhages	Pericardial effusion
1	+	+	+	—	+	+
2	+	+	—	+	+	+
3	—	+	+	+	+	+
4	+	+	—	+	—	—
5	—	+	+	+	—	—
6	—	0	+	+	—	—
7	+	0	+	—	—	—
8	+	0	+	—	—	—
9	+	+	—	—	+	+

* + = present; — = not present; 0 = not recorded.
† Monkey 10, which died of acute gastric dilatation, is not included.

An exudative rash was present in 4 monkeys and had the same anatomical distribution as the petechiae. The rash was dry and scaly in the 2 monkeys with protracted illness (No. 8 and 9). Hemorrhage from the nares was observed in 2 infected monkeys (Figure 1). Myocardial hemorrhages were observed in 4 monkeys which also had serosanguinous pericardial effusions. One monkey (No. 4) had intracranial hemorrhage from the basilar artery (Figure 2), and meningeal edema was present in 7 monkeys. The livers of most of the monkeys were yellow, mottled, enlarged and friable. Generalized lymphadenopathy was present in 5 monkeys.

Microscopic Pathology

Microscopic findings are summarized in Table 3.

Liver

Hepatic lesions were present in all monkeys which died of virus infection. The parenchyma contained many areas of acute coagulation necrosis of hepatocytes (Figure 3). The necrotic areas were widespread and randomly distributed in the hepatic lobule. Spherical hyaline acidophilic bodies that resembled the Councilman bodies of yellow fever were present in the livers of all monkeys with hepatic necrosis (Figure 4). They varied in size from 5 to 20 μ and were present intracellularly in the cytoplasm of hepatocytes and Kupffer cells and extracellularly in the hepatic sinusoids. The acidophilic bodies were often, but not exclusively, associated with areas of necrosis. Those that were adjacent to necrotic areas were usually free in the sinusoids, and some of these bodies contained basophilic structures resembling degenerated pyknotic nuclei. The acidophilic bodies in hepatocytes were generally smaller and surrounded by a clear space.

Mitotic figures were present in hepatocytes; multinucleated hepatocytes were common. A diffuse infiltration of leukocytes was also present in many of the liver sections, and the amount of cellular infiltration appeared to be proportional to the severity of the hepatic necrosis. Most of the monkeys which survived beyond the 17th day postinoculaion had minimal infiltration of lymphocytes and plasma cells in the periportal triads. The reticuloendothelial (RE) cells of the liver were also more prominent in those animals that survived longer.

Lungs

Two monkeys (No. 1 and 3) developed secondary bacterial bronchopneumonia, apparently associated with the inhalation of regurgitated

Table 3—Incidence and Severity of Significant Microscopic Lesions of BHF in Rhesus Monkeys*

Lesion	Monkey No.†								
	1	2	3	4	5	6	7	8	9
Hepatic necrosis	++	+++	++	+	+	+++	+++	+	+
Acidophilic bodies	++	+++	++	++	+	++	+	++	+
Myocardial degeneration	0	+++	+	0	0	0	++	+++	+++
Myocardial hemorrhage	0	+++	+	0	0	0	0	0	+
Necrotizing enteritis	++++	+++	++++	++++	+++	++	++++	+	+++
Epithelial necrosis	++	++	+++	+++	++	+	++	+	+++
Adrenal necrosis	+++	+	++	+	+	++	++++	+++	+
Lymphoid or RE cell hyperplasia	0	0	++	+++	+	0	++	0	+
Lymphoid depletion	+	++++	+++	+++	+++	+++	0	0	0
Lymphoid necrosis	0	0	0	0	0	0	++++	++++	+++
Central nervous system									
Nonsuppurative vasculitis	0	0	0	+	0	0	+	+++	++
Gliosis	0	0	0	+	+	0	+	+++	++

* ++++ = severe, +++ = moderate, ++ = mild, + = minimal, 0 = absent.
† Monkey 10 which died of acute gastric dilatation is not included.

ingesta. Focal alveolar hemorrhages were present in 2 monkeys, and pulmonary edema was observed in 3. In 4 of the monkeys, foci of subacute or chronic interstitial pneumonia were present. It was difficult to determine if the lesions were produced by the infection or by lung mites (*Pneumonyssus simicola*) which were present in all monkeys.

Kidneys

One monkey (No. 2) had acute fibrin thrombi in glomerular capillaries. This monkey also had fibrin thrombi in small vessels in the dermis of the skin. The only other lesions observed in the kidneys were interstitial foci of lymphocytic and plasmacytic infiltration in the 2 monkeys that survived until days 25 and 37 postinoculation.

Adrenals

The only consistent lesions in the adrenal glands involved the cells of the zona fasciculata. They consisted of swelling with resulting loss of cytoplasmic density of the cells and focal areas of acute necrosis (Figure 5). These lesions varied markedly in severity from minimal to severe but were present in all monkeys examined. One monkey (No. 9) had diffuse infiltration of lymphocytes and plasma cells in the adrenal.

Spleen

Varying degrees of lymphoid depletion were evident in 5 of the 10 monkeys that died. The depletion was evidenced by a decrease in the density of the white pulp forming the periarterial sheaths. In many areas, an amorphous eosinophilic ground substance was present in areas normally populated with lymphocytes (Figure 6). Lymphoid depletion was more common and severe in the monkeys which died between days 13 and 18 postinoculation.

Acute lymphoid necrosis in germinal centers of the spleen was observed in 3 of 4 monkeys which died on or after day 18; it was not observed earlier (Figure 7).

Lymph Nodes

Hyperplasia of lymphoid and/or RE elements was present in the lymph nodes of 5 monkeys, 4 of which died between days 14 and 18. In several monkeys the subcapsular and medullary sinuses were filled with histiocytes with abundant, foamy cytoplasm.

Lymphoid depletion was observed in the nodes of 1 monkey (No. 5) which also had depletion of the lymphoid elements of the spleen.

Lymphoid necrosis was present in the nodes of the 3 monkeys (No. 7, 8 and 9) that exhibited lymphoid necrosis in the spleen.

Bone Marrow

Lesions in bone marrow were variable. Necrosis was present in 2 monkeys (No. 2 and 3). Minimal hypoplasia of the marrow was also present in 2 monkeys (No. 3 and 4).

Heart

Myocardial hemorrhage and degeneration and/or Zenker's necrosis was present in 5 monkeys, including the 4 which had gross evidence of myocardial hemorrhage and hydropericardium. The degenerative lesions involved the subpericardial muscle fibers, and inflammatory cell infiltration was absent or minimal.

Brain and Meninges

Mild congestion of the meningeal vessels was present in several monkeys. Evidence of inflammation was observed in the central nervous system of 5 of the 6 monkeys that died on or after day 17 postinoculation. The lesions consisted of a lymphocytic vasculitis and perivascular cuffing (Figure 8) with multifocal areas of gliosis in the neuropil (Figure 9). Lymphocytic meningitis was also present, but involved only the meninges of the cerebellum. Vasculitis and gliosis were most common and severe in the medulla oblongata.

Two monkeys (No. 2 and 3) had focal areas of mineralization in the brain.

Gastrointestinal Tract

All of the monkeys that died of the virus infection had microscopic evidence of necrotizing enteritis (Figure 10). The basic lesion was coagulation necrosis of the actively dividing epithelial cells lining the mucosal crypts (Figure 11). There was blunting of the villi in the small intestine and a decrease in the number of mucosal crypts in the small and large intestines. Leukocytic infiltrations were not marked in most monkeys, but the monkey that survived until day 37 postinoculation had diffuse lymphocytic infiltration into the lamina propria of the small and large intestines.

The necrotizing lesions were more severe in the lower jejunum and ileum than in the duodenum. The cecum and large intestine were diffusely affected. Lesions were not observed in the stomach.

Epithelium

All monkeys developed necrotic lesions in the epithelium of the tongue, esophagus, buccal mucosa and skin at about the same time as the enteric lesions. The early lesion consisted of multifocal areas of acute coagulation necrosis of the basal epithelial cells and did not involve the superficial layers of the epithelium (Figure 12). Acute lesions were present in monkeys that died on days 13 and 14 postinoculation; those that survived longer had larger more severe lesions, consisting of erosions and ulcers (Figure 13).

Discussion

The pathologic changes associated with BHF infection in man have been described by Child *et al.*[5] The disease has not been extensively studied in the laboratory, possibly because of the lack of a good laboratory model of infection. The Central American marmoset (*Saguinus geoffroyi*) can be fatally infected with Machupo virus.[6,7] The only other adult animal proven to be susceptible to infection is the guinea pig (*Cavia porcellus*), the strain 13 guinea pig being the most consistent in its response to infection.[8] The rhesus monkey appears to be an excellent primate model. Infections were produced in all monkeys inoculated with either 10^5 or 10^3 PFU of virus, and 1 of 4 monkeys inoculated with 10^1 PFU of virus. The 1 monkey that received the low dose of virus exhibited clinical manifestations of the infection 10 to 13 days later than the monkeys that received higher doses. The possibility that this monkey was secondarily infected by aerosol or contact with contaminated fomites or food rather than the initial inoculum cannot be ruled out. The other low dose monkey which died had spontaneous acute gastric dilatation.[9] This syndrome has been observed in several monkeys at this Institute and is unrelated to the experimental procedure. The 2 monkeys that received the low dose of virus and survived showed no signs and were fatally infected with Machupo virus 2 months later.

Many aspects of the pathologic changes associated with Machupo virus infection in the rhesus monkey are similar to those reported in man, but there are several striking differences (Table 4). Hepatic necrosis was observed more frequently in monkeys than in man. The hyaline acidophilic bodies present in liver sections from monkeys were similar to those described in the human infection. These bodies have been characterized morphologically and histochemically;[10] they are probably degenerated hepatic cells. Interstitial pneumonia was described in human infections; such lesions were present in the lungs of several

Table 4—Comparative Pathology of BHF in Man and the Rhesus Monkey

Lesion	Incidence	
	Man*	Monkey†
Liver		
Necrosis	2/7	9/9
Acidophilic bodies	7/7	9/9
Lung		
Interstitial pneumonia	6/6	4/9
Pulmonary edema	NR	2/9
Kidneys		
Hyaline tubular casts	6/8	0/9
Central nervous system		
Vasculitis and/or lymphocytic cells	1/6‡	4/9
Gliosis	5/6	5/9
Lymphoreticular system		
Lymphoid and RE hyperplasia	4/8	5/9
Lymphoid depletion, spleen	NR	5/9
Lymphoid necrosis, spleen and lymph nodes	NR	3/8
Heart		
Myocardial hemorrhage	NR	4/9
Myocardial degeneration	NR	5/9
Adrenal		
Cortical necrosis	NR	9/9
Gastrointestinal tract		
Enteritis	NR	9/9
Epithelial necrosis	NR	9/9

* Child et al[5]

† Does not include monkey 10, which died of acute gastric dilatation on the second day postinoculation.

‡ Doubtful in 2 others.

NR = not reported.

monkeys, but were similar to those caused by pulmonary acariasis. It was impossible to determine if they were produced by infection or by the presence of the parasite. Hyaline tubular casts were also present in the kidneys of most of the human cases of BHF reported, but were not observed in the monkeys.

Some of the most severe and consistent lesions of the infection in the rhesus were not described in humans.[5] These include enteritis, epithelial necrosis, adrenal cortical necrosis and lymphoid necrosis. Reference was made to gastrointestinal symptoms in humans,[2–4] but the only lesions described were hemorrhages into the intestinal tract. The nature of the lesions in the intestinal tract and epithelium of the monkey suggest a tropism of the virus for the dividing epithelial cells. The intestinal lesions may well be the cause of the diarrhea and dehydration observed clinically in the monkeys.[1] Damage to the in-

testinal mucosa was uniformly severe with a loss of a large amount of the surface area. It is possible that similar lesions occur in humans but are obscured by rapid postmortem autolysis which occurs in the intestinal tract. Enteritis does not occur in experimental BHF infections in marmosets.[8] The adrenal cortical lesions were unique in that they involved only the cells of the zona fasciculata. Similar lesions have not been reported in humans, nor have we observed them in monkeys that died of other causes. The necrosis was minimal in some monkeys; in others it was definitely severe enough to contribute to the death of the animals.

The importance of the mechanisms of disseminated intravascular coagulation (DIC) in the pathogenesis of the hemorrhagic fevers including BHF, Argentine hemorrhagic fever (AHF), Korean and Thai hemorrhagic fevers, and Kyasanur Forest disease has been discussed by McKay and Margaretten.[11] Clinical evidence for DIC and intravascular fibrin thrombi have been reported in humans infected with Junin virus, the etiologic agent of AHF.[12] Junin virus is antigenically related to Machupo virus, and both are serologically classified in the Tacaribe group of the arenaviruses. Microscopic evidence for DIC and intravascular fibrin thrombi were specifically looked for in the tissues from the infected monkeys. One monkey did have fibrin thrombi in the glomerular capillaries of the kidney and in small vessels in the dermis of the skin, but a diagnosis of DIC based on morphology could not be justified. There were no specific vascular lesions observed in any monkeys. Clinical biochemical tests were not performed to determine if there were abnormalities in the clotting mechanism, and it is possible that DIC did occur in the infected monkeys but was undetected on histopathologic examination because of lysis of the thrombi prior to death. DIC was not present in the human cases.[5]

Machupo virus infection produces a chronic virus carrier state in the *Calomys callosus,* a peridomestic rodent native to the endemic area of infection.[13] This carrier state is similar to that observed with Junin virus and lymphocytic choriomeningitis (LCM) virus, both of which are arenaviruses.[14,15] In LCM, the carrier state is related to viral lymphotropism; [16] a similar mechanism has been suggested with Machupo virus in *C callosus.*[17] The role of the lymphoreticular system in the pathogenesis of BHF in man and the rhesus monkey awaits further elucidation; however, it does appear to be involved. Histopathologic changes involving the lymphoreticular system were nonspecific and variable. Hyperplasia of the lymphoid and RE systems in 5 monkeys was similar to that described in human cases of BHF.

Lymphoid necrosis was observed in monkeys which died after the seventeenth day of infection. Mononuclear cell infiltration into various organs, notably the liver, kidneys and adrenals, was observed at the same time. CNS lesions also developed at about the same stage of infection. Interestingly, circulating complement-fixing antibodies were first observed on day 18 of infection.[18] These temporal relationships suggest a possible immunologic basis for the lesions.

The present findings suggest that the rhesus monkey is a suitable laboratory model in which to study BHF. The basic pathologic change of the disease in man were reproduced in the monkey model. The development of some of the lesions not described in human infections may add some understanding to the pathogenesis of infection, and further studies into the mechanisms of the disease are indicated.

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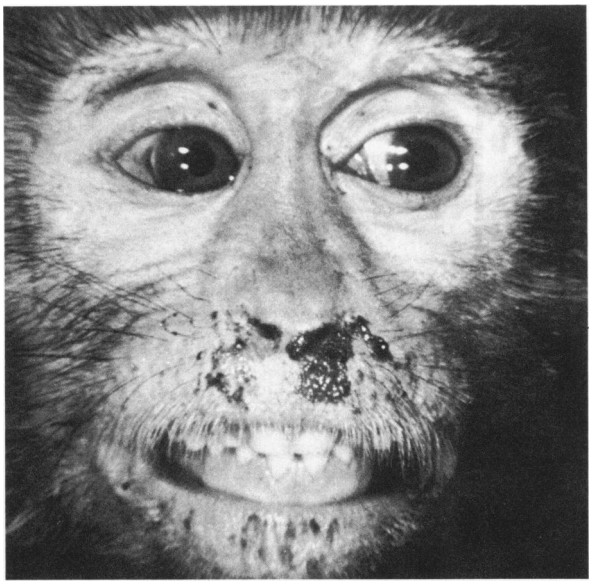

Fig 1—Hemorrhage from the nares of monkey 5 which died on day 17 postinoculation.

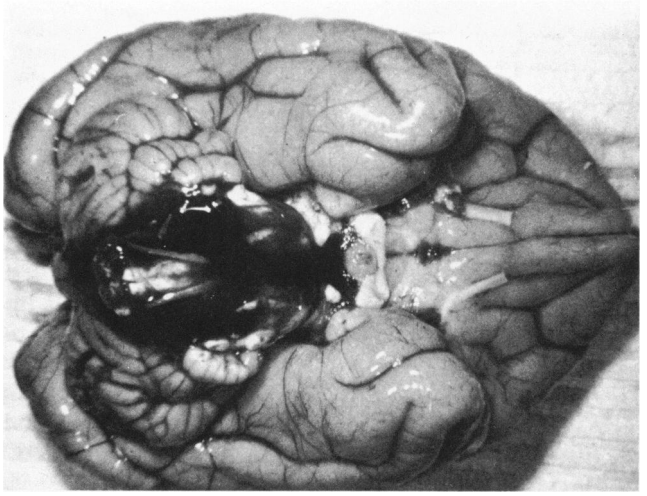

Fig 2—Basilar hemorrhage in the brain.

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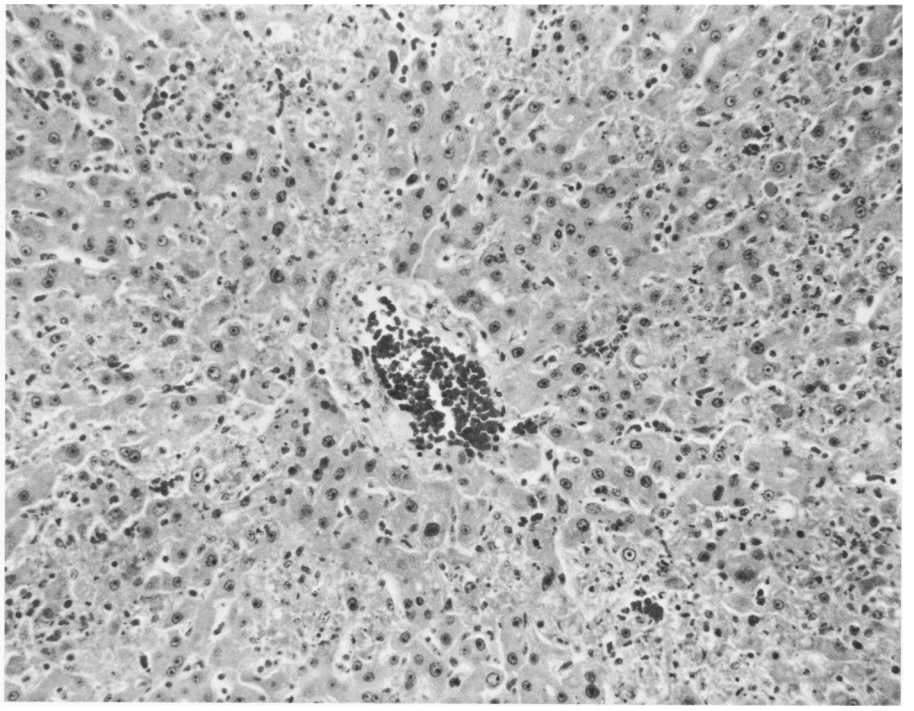

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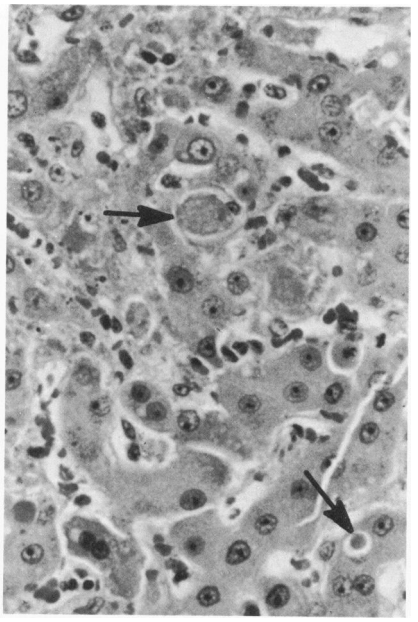
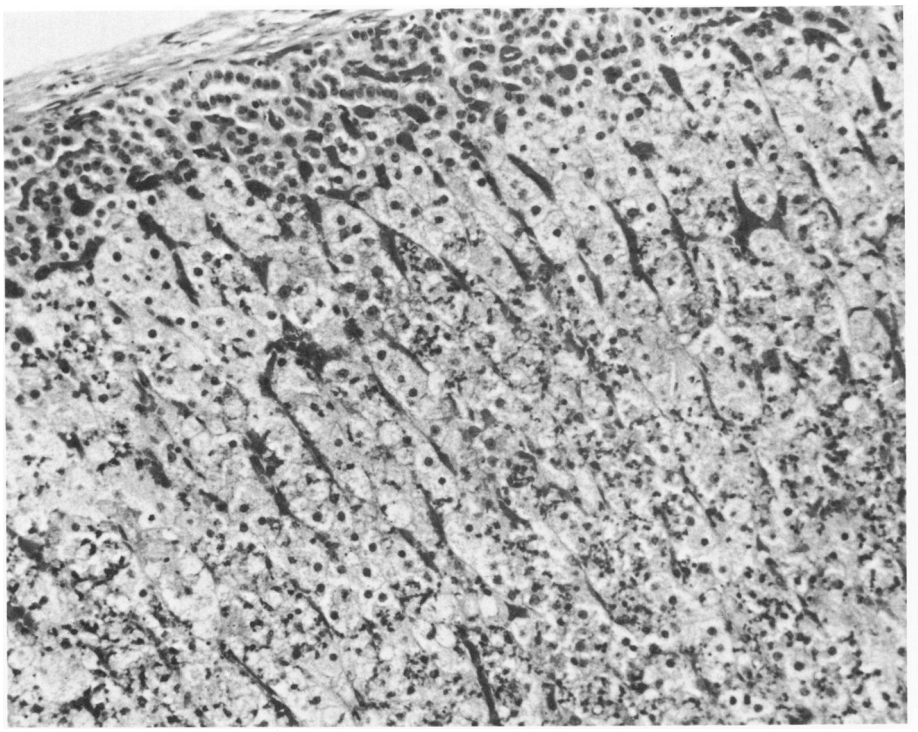


Fig 3—Liver of monkey which died on day 14 postinoculation. There are multiple randomly distributed areas of acute coagulation necrosis of hepatocytes in the parenchyma around the central vein. Inflammatory cell infiltration is minimal (H & E, × 128). **Fig 4**—An area of necrosis in the hepatic parenchyma. Two acidophilic, hyaline bodies resembling the Councilman bodies of yellow fever are present (*arrows*). The larger body near the center of the field is contained within a Kupffer cell. The smaller body in the cytoplasm of a hepatocyte in the upper right corner of the field is surrounded by a clear space. Note binucleated hepatocytes and a mitotic figure within hepatocyte (H & E, × 256).



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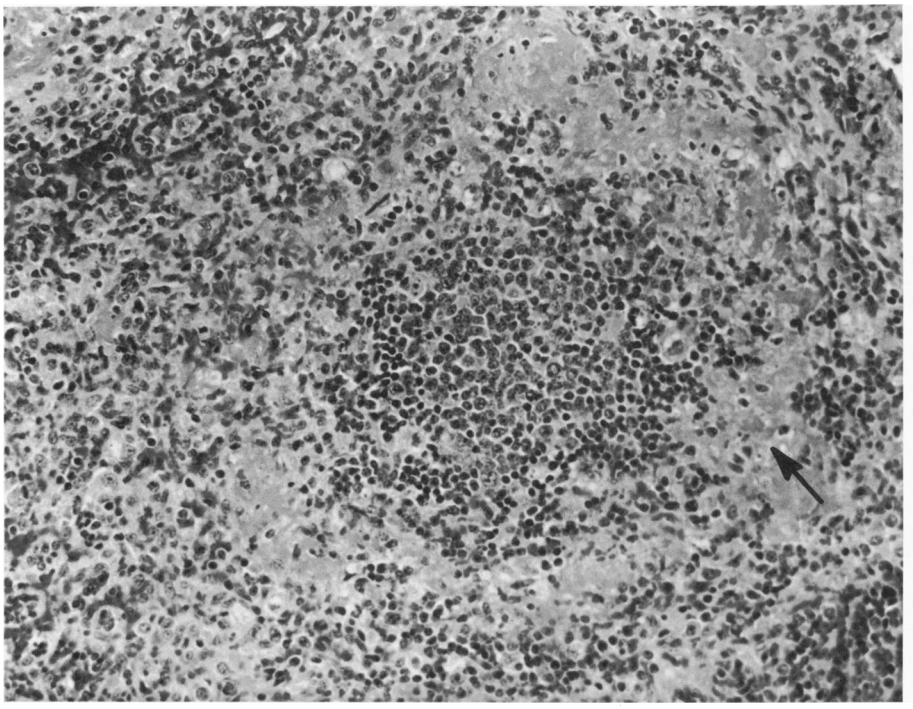

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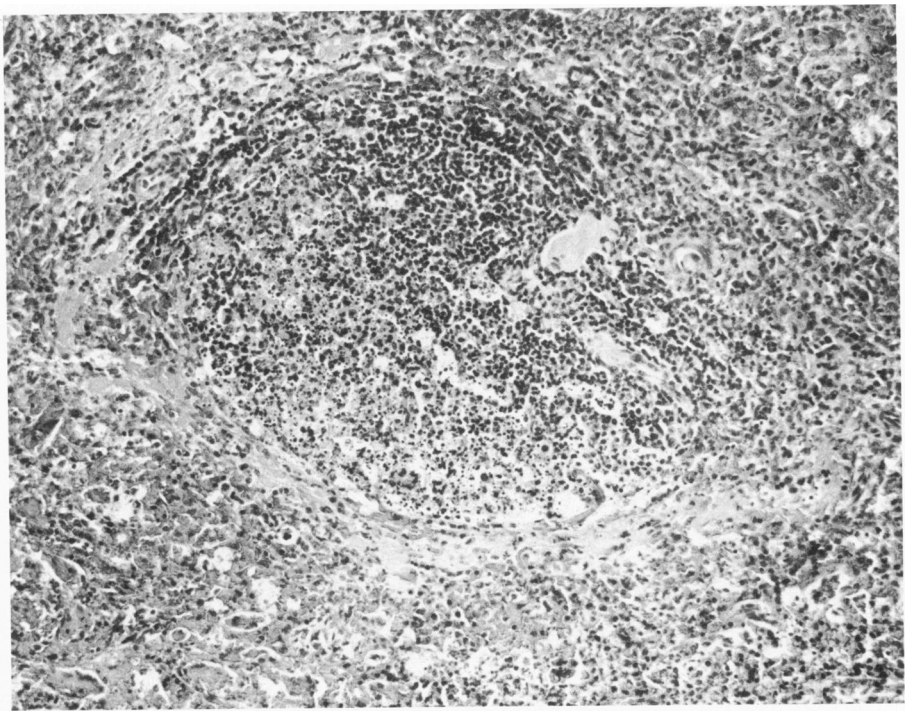
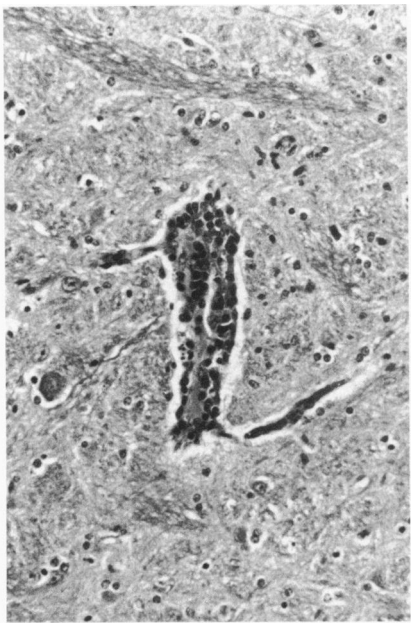
Fig 5—Adrenal cortex from monkey which died on day 18 postinoculation. The cells of the zona fasciculata are swollen with resulting loss of cytoplasmic density. In addition, there are areas of acute necrosis. The cells of the zona glomerulosa and zona reticularis are not affected (H & E, × 128). **Fig 6**—Depletion of lymphocytes around a Malpighian corpuscle in the spleen. There is an amorphous eosinophilic ground substance occupying the space normally occupied by small lymphocytes (*arrow*) (H & E, × 160).





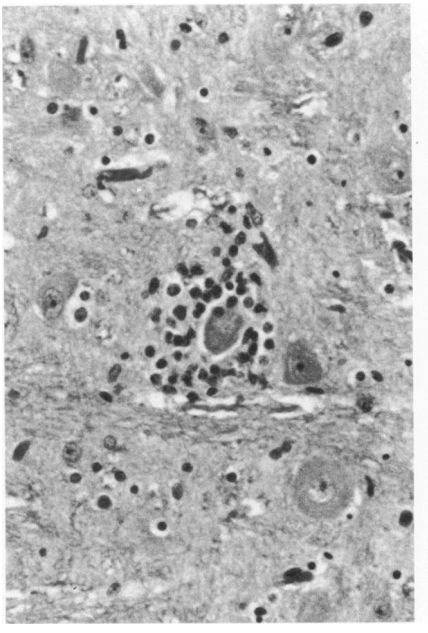
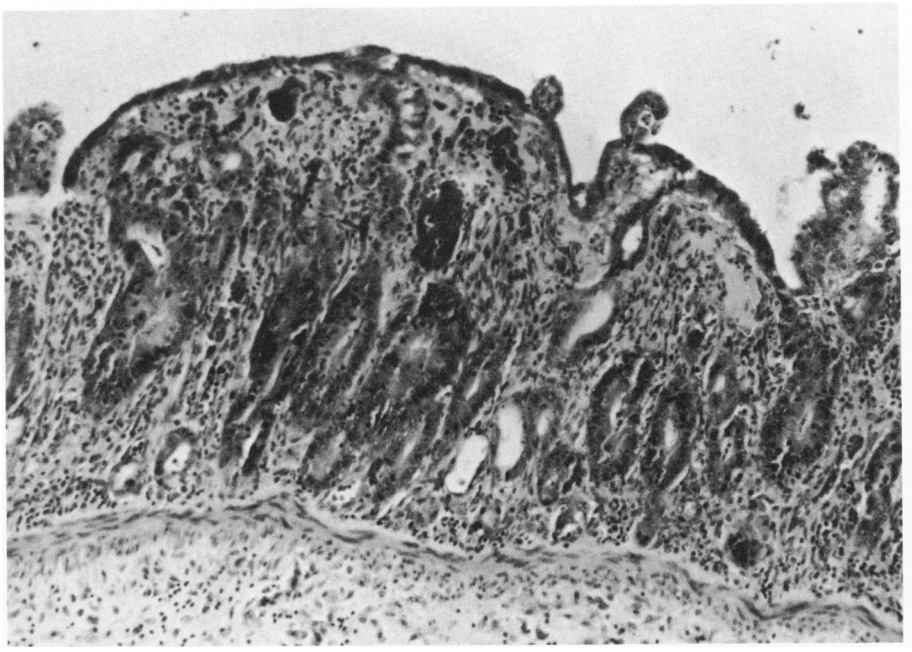


Fig 7—Acute lymphoid necrosis in a germinal center in the spleen of monkey 8 which died 25 days postinoculation (H & E, × 128). **Fig 8**—Blood vessel within the medulla oblongata of monkey 8. There is an infiltration of lymphocytes into the wall of the vessel and into the surrounding Virchow-Robin space (H & E, × 160). **Fig 9**—Focal area of satellitosis in the brain from monkey 8 (H & E, × 224).



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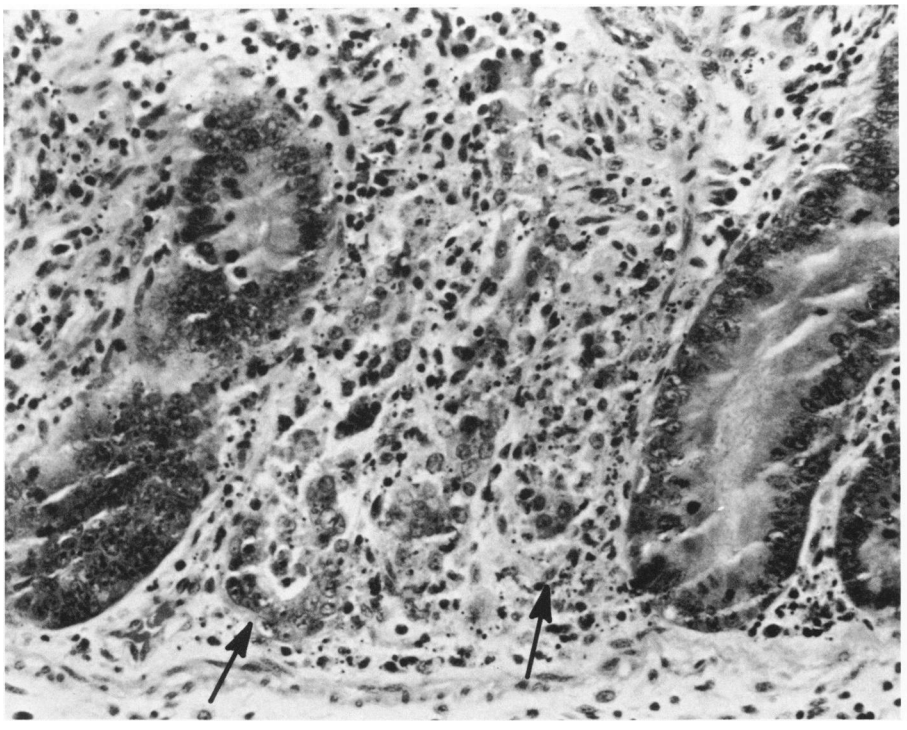

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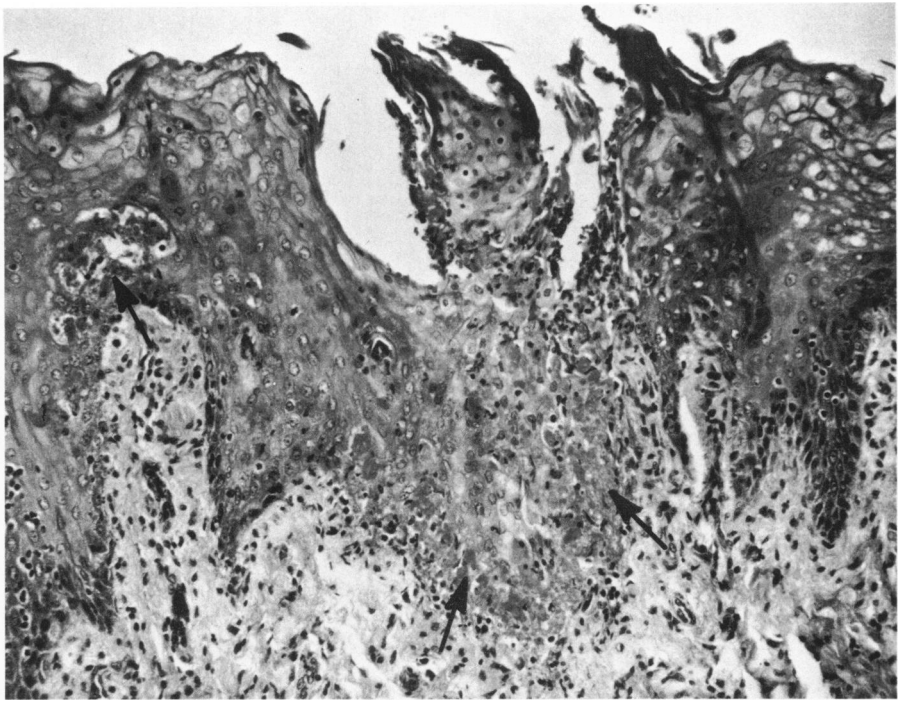
Fig 10—Section of jejunum. There is destruction of the epithelium resulting in a loss of villi and capping of the surface epithelium. Necrotic cellular debris is present in the lamina propria (H & E, × 90). **Fig 11**—Section of small intestine illustrating acute coagulation necrosis of the epithelium in two crypts of Lieberkuhn (*arrows*) in an infected monkey. There are nonaffected crypts on each side of the necrotic ones (H & E, × 220).

12



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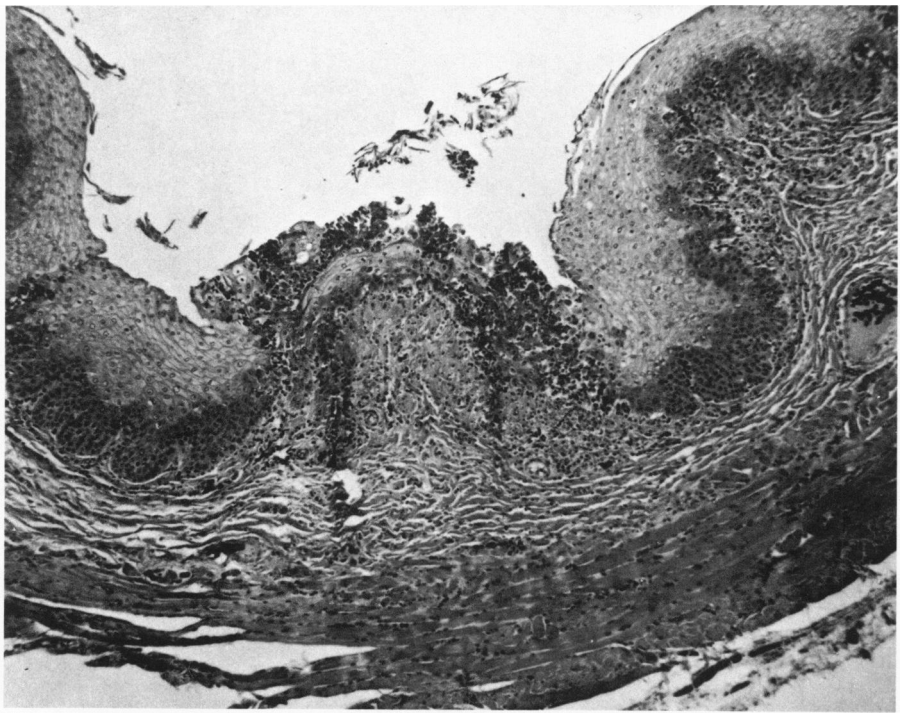

Fig 12—An acute lesion on the surface of the tongue from an infected monkey. There are areas of coagulation necrosis in the basal epithelial layer (*arrows*), but the surface epithelium is intact (H & E, × 128). **Fig 13**—Focal erosion in the mucosa of the esophagus from monkey 7 (H & E, × 50).