Comparative pathology of Lassa virus infection in monkeys, guinea-pigs, and *Mastomys natalensis* *

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Experimental Lassa virus infections of squirrel monkeys, guinea-pigs, and the African multimammate rat, Mastomys natalensis, *were studied virologically and pathologically. In the monkeys, early viral lymphoreticulotropism, hepatotropism, nephrotropism, and viraemia were noted. At the time of death, viral titres in nearly all target organs were associated with necrotic changes: splenic lymphoid necrosis, renal tubular necrosis, myocarditis, arteritis, and hepatocytic regeneration. In convalescent monkeys, organ titres diminished slowly, and viraemia persisted at 28 days. At this time, renal and splenic regeneration was occurring and a new lesion, choriomeningitis, was present.*

Guinea-pigs infected with Lassa virus developed respiratory insufficiency with pulmonary oedema, alveolar hyaline membranes, myocarditis, and focal calcification of myocardial fibres and hepatocytes. Dying animals contained Lassa virus in virtually every organ tested, whereas survivors at 56 days were free of virus and had high complement-fixing antibody titres.

Infection of neonatal Mastomys *did not cause any clinical disease or pathological lesions despite the presence of virus in the blood, lymph nodes, liver, spleen, lung, brain, urine, and throat secretions throughout the 74-day study. Infected adult* Mastomys *also remained normal but had virus in many organs. In one animal, virus persisted until the termination of the study at 103 days. Several animals developed a mild meningoencephalitis. The pattern of infection and virus shedding in* M. natalensis *is ideal for maintenance of the virus in nature; together with the epidemiological field data this emphasizes the incidental nature of the exposure and infection of man.*

INTRODUCTION

The pathogenesis of Lassa virus infection was investigated in this laboratory by experimental inoculation of 3 animal species: the squirrel monkey (*Saimiri scirreus*), the guinea-pig (*Cavia porcella*), and the African multimammate rat (*Mastomys natalensis*). Each species was chosen for different purposes. Infection in the squirrel monkey was studied as a model for pathogenetic correlation with human Lassa fever (1, 2). Guinea-pigs were studied because in the course of antibody production the live virus vaccine caused overt disease and the pattern of this disease appeared different from that in the other 2 animal species. *M. natalensis* was studied because it is the natural rodent reservoir host for Lassa in West Africa and thus the pathogenesis of the rodent infection is related to human exposure and human disease risk (3). The natural history of Lassa virus in this species is the key to understanding patterns of viral survival and perpetuation.

SQUIRREL MONKEY

Knowledge of the pathogenesis of Lassa fever in man is very scanty. The etiological agent has been isolated from human blood, urine, throat washings, and pleural, peritoneal, and pericardial fluids (4, 5);

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however, the primary target organs have not been identified by appropriate methods, such as organ titration or immunofluorescence. Electron microscopy has been used to examine only one liver specimen (6). Moreover, the course of development of histopathological changes from the prodromal period through convalescence is not known. In fact, there have been few descriptions of pathological alterations in humans, and these include only end-stage findings in fatal cases receiving hospital supportive care (7, 8, 9). Studies were therefore undertaken in the squirrel monkey to elucidate viral tropisms and the pathogenesis of infection.

Four monkeys were inoculated intramuscularly with Lassa virus and were sacrificed 7, 12, 14, and 28 days later; specimens of organs, tissues, and fluids were collected for infectivity titration and histopathology studies.

The 4 animals represented various stages in the infection course: Animal A was well at 7 days after inoculation and was considered to represent the incubation period of infection; animal B was moribund at 12 days; animal C was clinically recovered from a 2-day illness at 14 days; and animal D was convalescent from a 10-day illness at day 28. The organ infectivity titres are presented in Table 1. Many organs were infected, but signs of early lymphoreticulotropism, hepatotropism, and nephrotropism were prominent. In addition, the spleen, heart, and brain, which were infected at later times, showed pathological alterations. Viraemia was detected in all animals, even in the individual sacrificed at 28 days. The presence of virus in high titre in the single adequate urine sample, as well as the persistence of viraemia, paralleled findings in human Lassa fever. The greatest viral burden was seen in animal B, which also had the most severe cytopathological changes. Animal C, which had no significant lesions but was nearest in time of sacrifice to animal B (at 14 and 12 days, respectively), had the second highest yield of infectious virus from target organs. Even after 28 days of infection, virus in the tissues of animal D had not been reduced significantly, and many organs were still infected. This observation, along with the absence of a humoral immune response (as measured by complement-fixing (CF) antibody), indicated the difficulty involved in viral clearance and termination of infection.

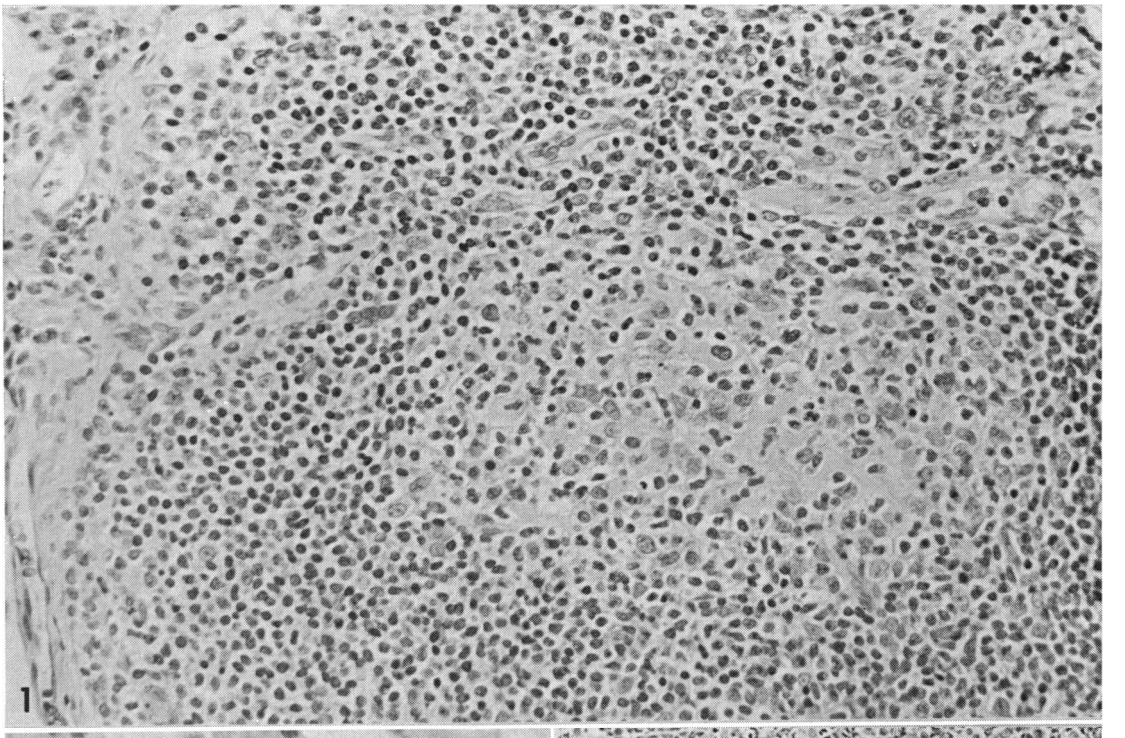
As has been noted in many clinically devastating viral diseases, there were no significant gross pathological lesions, but microscopic examination provided clear evidence of ongoing destructive processes. Lymph nodes from the monkey sacrificed on day 7 contained necrotic secondary follicles in their cortices, with multinucleated giant cell formation (Fig. 1). This animal's renal tubules were damaged and had foci of degenerating cells in their lumina (Fig. 2). Monkey B, which was sacrificed when moribund on day 12, had necrosis of the thymus-independent B-cell region of the splenic periarteriolar lymphocytic sheath (Fig. 3), transmural, segmental, acute arteritis (Fig. 4), and severe acute myocarditis (Fig. 5). Diffusely scattered hepatocytic mitoses, binucleation, and nuclear pleomorphism indicated a massive attempt at regeneration in response to a severe hepatic insult (Fig. 6). Renal function impairment was evidenced by widespread renal tubular necrosis (Fig. 7). Despite the high titres of virus in the organs of monkey C, there were no significant histopathological changes. Thus, the normal clinical appearance of this animal at the time of sacrifice correlated with the histology rather than the titration data. Monkey D exhibited evidence of recovery from the necrotizing effects of infection. The splenic white pulp contained lymphoblastic repopulation of the B-cell region, but it also still

Table 1. Organ titres of Lassa virus in squirrel monkeys.

Specimen	Titre[a]			
	A	B	C	D
Lymph node, femoral	2.8	4.5	2.8	3.5
Lymph node, mesenteric	neg	5.8	5.2	2.2
Lymph node, cervical	neg	3.5		2.5
Lymph node, axillary				2.2
Spleen		⩾6.5	4.5	1.8
Thymus	neg			neg
Bone marrow				neg
Heart	neg	4.5	3.5	2.2
Lung	neg	5.5	4.5	2.5
Liver	2.8	6.2	4.2	neg
Pancreas	neg	4.0	4.8	2.2
Parotid gland		4.5	<4.0	neg
Submandibular gland	neg			2.5
Oesophagus		4.8		
Kidney	2.5	5.2	4.8	2.5
Urinary bladder	neg	4.5	4.0	neg
Prostate		5.5		
Uterus	neg			
Testis		3.5	3.8	neg
Ovary	neg			
Adrenal	neg	6.8	4.8	3.2
Cerebrum	neg	neg	neg	neg
Cerebellum	neg	neg	neg	2.5
Skeletal muscle		3.8	neg	neg
Blood	1.8	4.5	3.8	2.2
Urine		5.5		
Throat				neg

[a] Log_{10} of tissue culture median infective dose ($TCID_{50}$) per gram or ml of tissue or body fluid.

Fig. 1. Lymph node from monkey at 7 days after infection with Lassa virus. Germinal centre necrosis. All photographs are of material stained with haematoxylin-eosin. × 275.



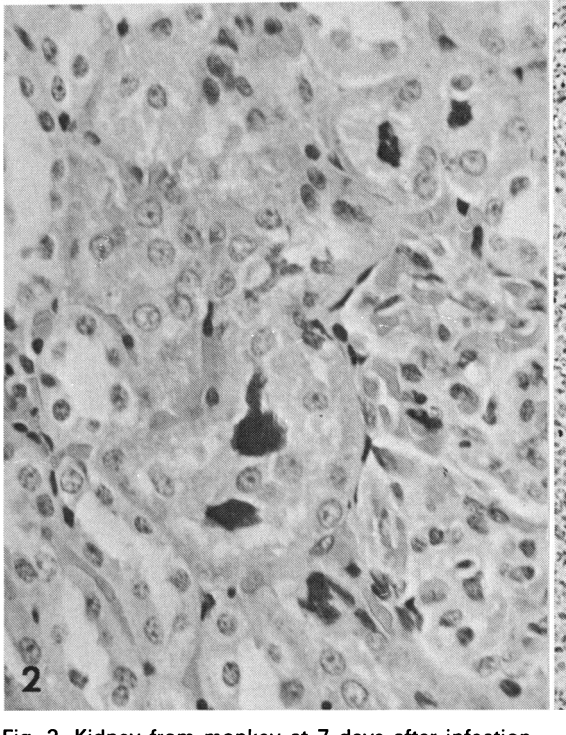


Fig. 2. Kidney from monkey at 7 days after infection with Lassa virus. Tubular lumina contain necrotic cells with aggregates of degenerating nuclei. × 450.

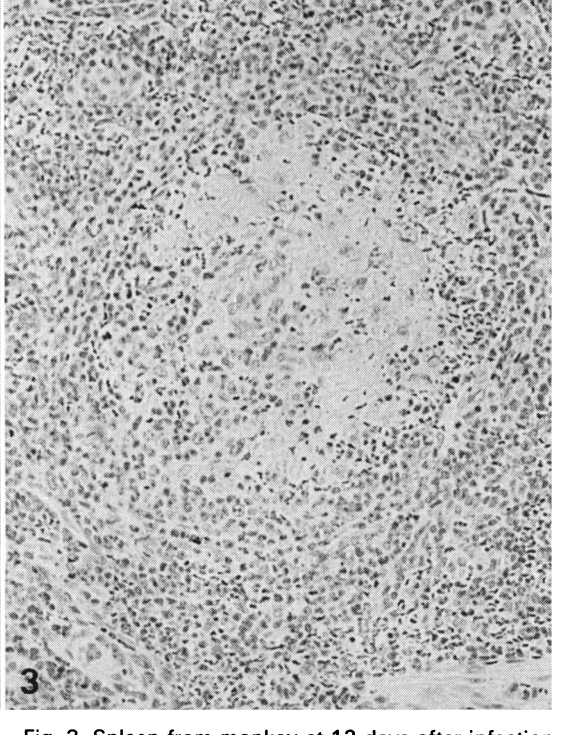


Fig. 3. Spleen from monkey at 12 days after infection with Lassa virus. Amorphous debris replacing cellular population of periarteriolar lymphocytic sheath. ×275.

Fig. 4. Coronary artery from monkey at 12 days after infection with Lassa virus. Acute segmental arteritis with vascular necrosis and polymorphonuclear leukocyte and mononuclear cell infiltration. × 175.

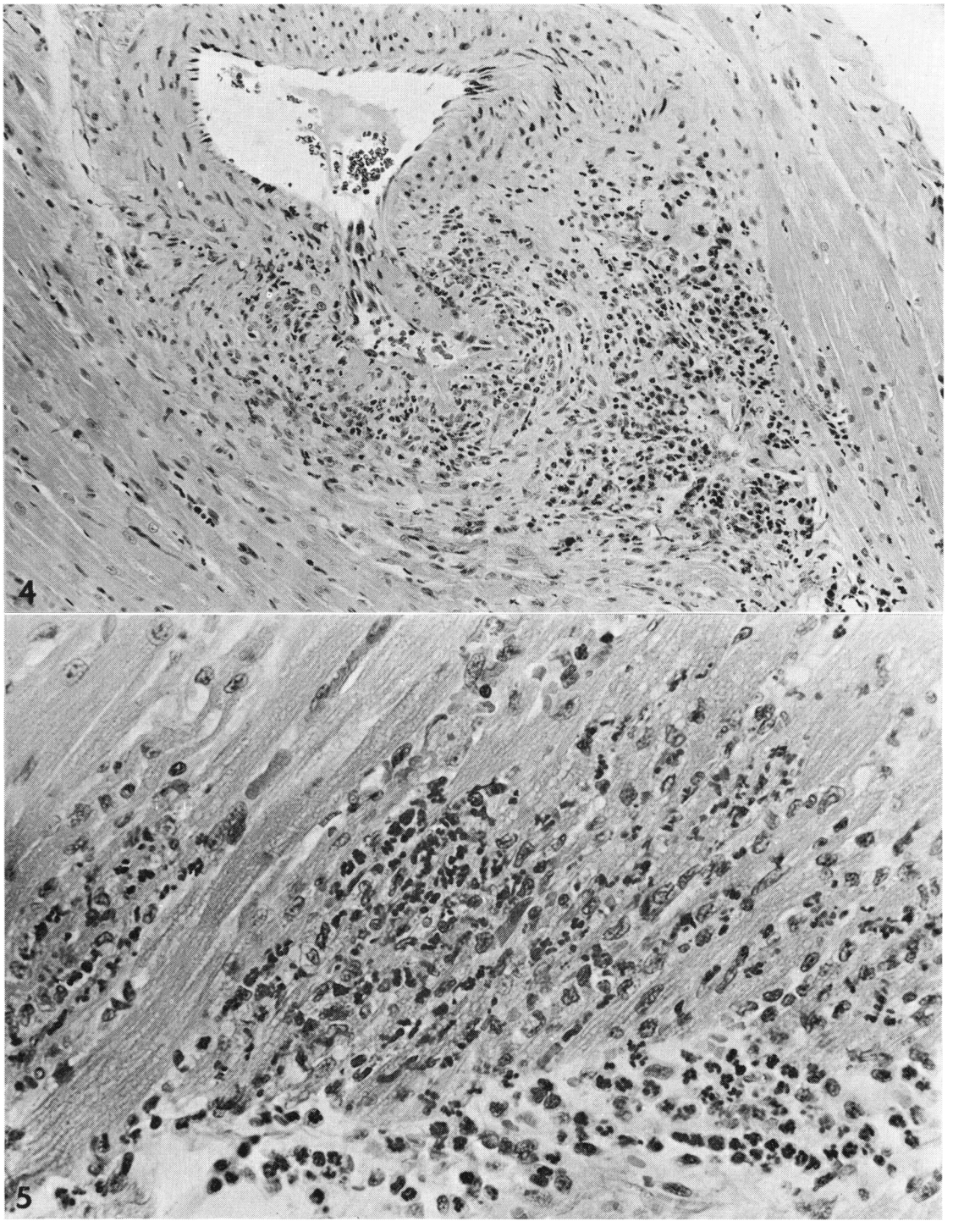


Fig. 5. Myocardium from monkey at 12 days after infection with Lassa virus. Acute myocarditis with vacuolar degeneration and necrosis of myocardial fibres and inflammatory response composed of polymorphonuclear leukocytes and mononuclear cells. × 450.

Fig. 6. Liver from monkey at 12 days after infection with Lassa virus. Hepatocytic regeneration with numerous mitoses, binucleate, and large heteroploid nuclei accompanied by mild fatty metamorphosis. × 450.

Fig. 7. Kidney from monkey at 12 days after infection with Lassa virus. Tubular degeneration and necrosis with accumulation of necrotic debris in lumina. × 450.

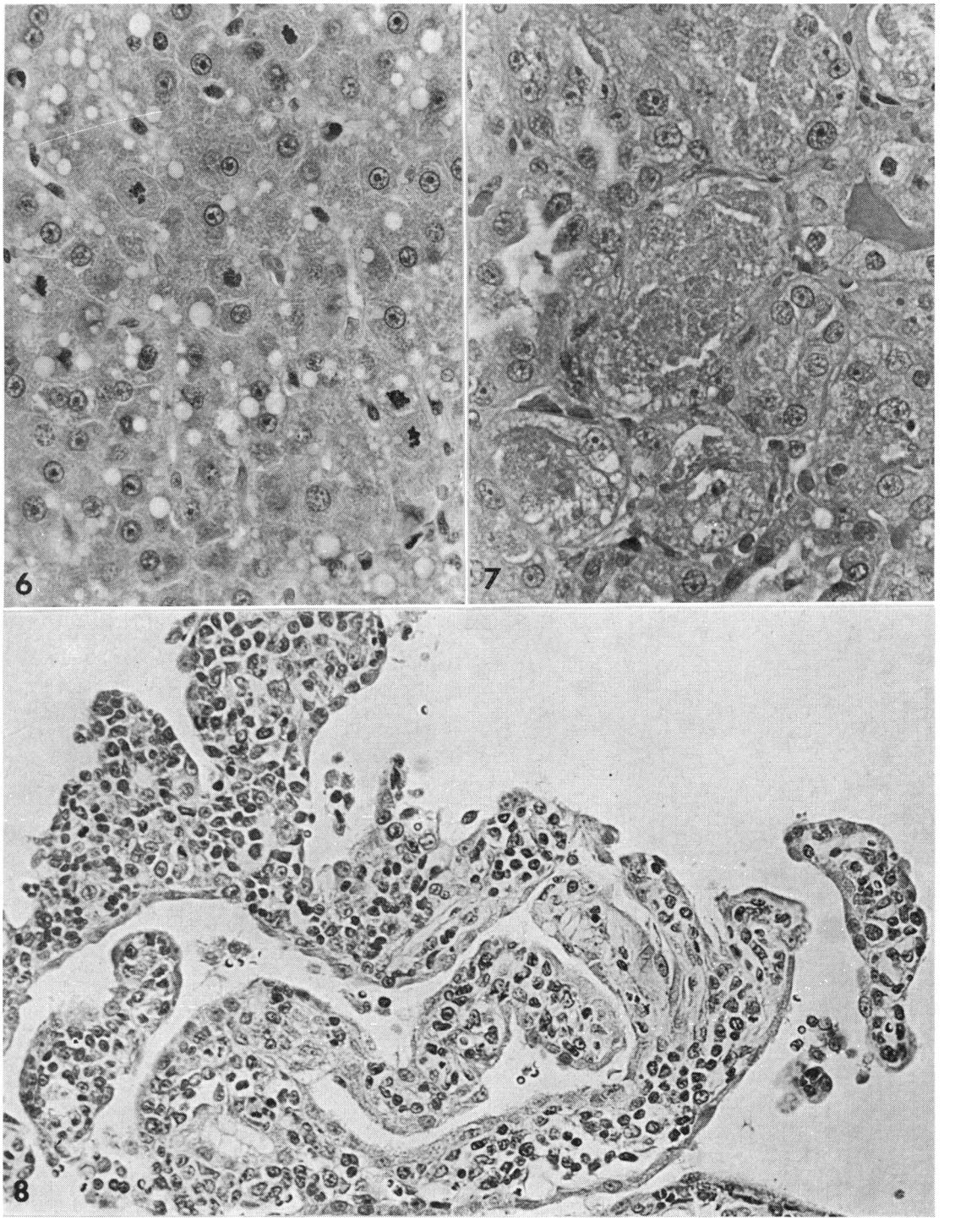
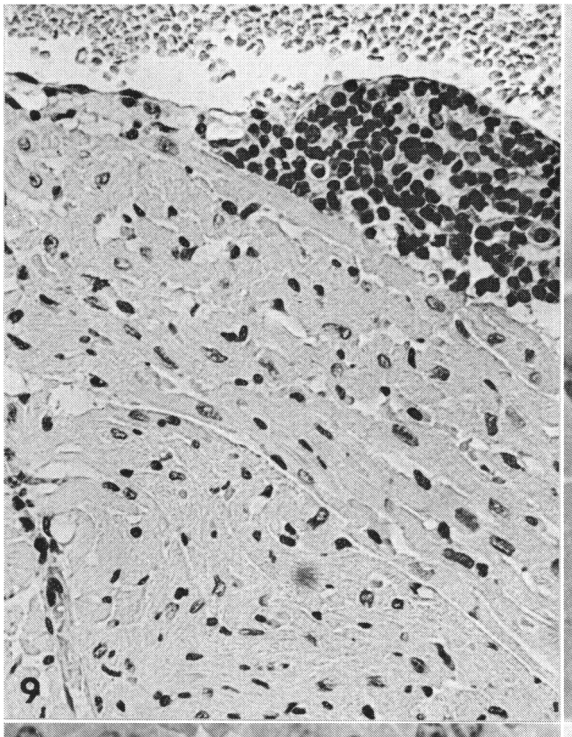
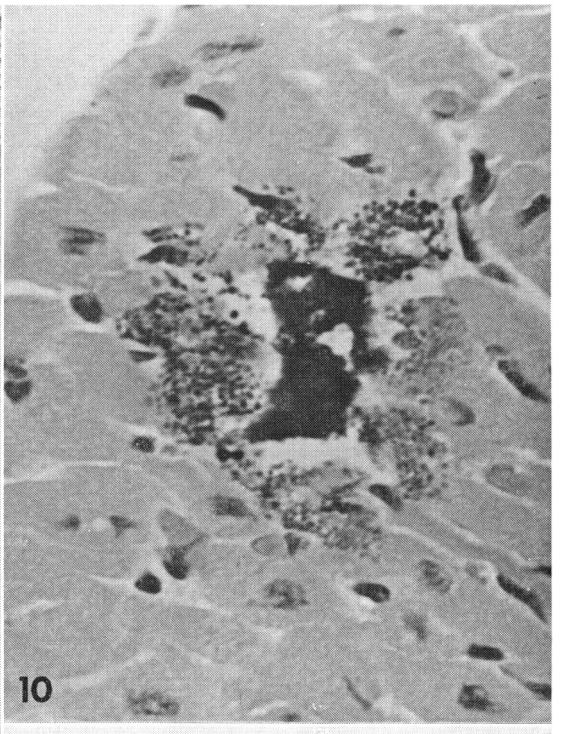


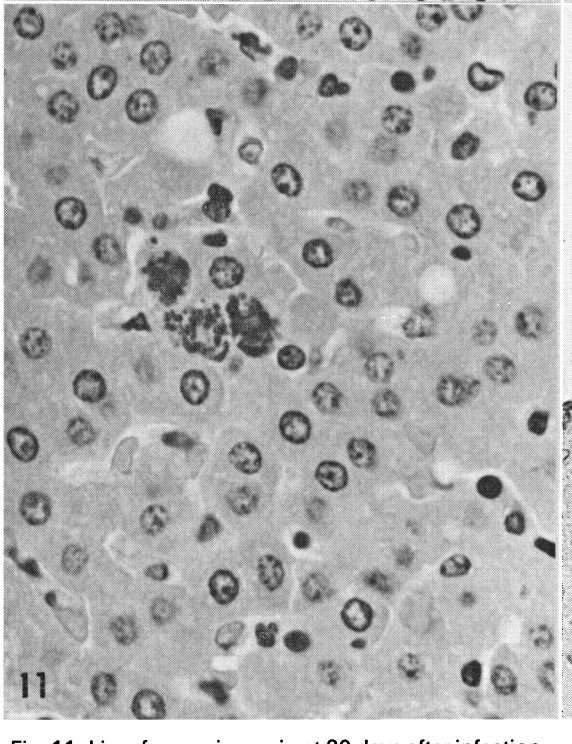
Fig. 8. Choroid plexus from monkey at 28 days after infection with Lassa virus. Adventitial space is infiltrated by mononuclear cells, lymphocytes, and plasma cells. × 175.

Fig. 9. Heart from guinea-pig at 24 days after infection with Lassa virus. Chronic myocarditis with prominent subendocardial mononuclear inflammation. × 450.

Fig. 10. Heart from guinea-pig at 21 days after infection with Lassa virus. A densely calcified myocardial fibre is surrounded by fibres with punctate oval cytoplasmic calcium deposits and normal nuclei. × 720.







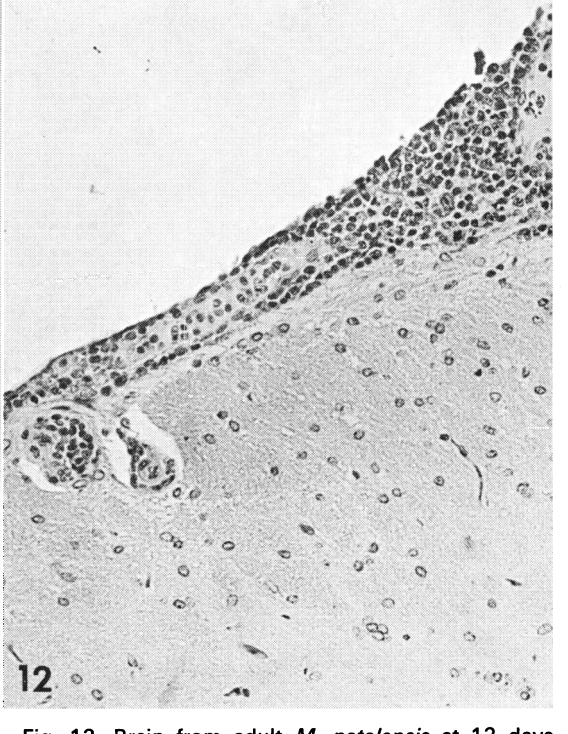


Fig. 11. Liver from guinea-pig at 20 days after infection with Lassa virus. Dense, oval, punctate, cytoplasmic calcifications surround intact nuclei in a focus of 3 affected hepatocytes. × 720.

Fig. 12. Brain from adult *M. natalensis* at 12 days after infection with Lassa virus. Leptomeninges and Virchow-Robin spaces are infiltrated by lymphocytes and mononuclear cells. × 175.

contained eosinophilic necrotic material. Renal tubular regeneration was characterized by elongated tubular cells and mitoses. This animal also had extensive choroiditis, ependymitis, focal subependymal perivascular mononuclear cuffs, and focal meningitis (Fig. 8) mimicking the neuropathology of lymphocytic choriomeningitis virus infection in mice.

The mechanisms active in parenchymal functional impairment in organs of Lassa fever patients remain unknown, but observations made in this study of infected monkeys may indicate the possibilities. Significant titres of virus in major target organs of these monkeys (liver, kidney, lymph node, and spleen) in the absence of evidence of a cellular inflammatory response suggested direct viral cytopathology. Likewise, the mixed cellular reponse in the myocardium of one monkey was most typical of response secondary to parenchymal cytonecrosis. On the other hand, arenaviruses are known to be minimally cytopathic in some instances and to evoke significant immunopathological processes in experimental animals (10). The acute segmental arteritis noted in one monkey is a lesion characteristically associated with immune complex disease. The mononuclear cell infiltration in the choroid plexus, meninges, and subependyma of one monkey was suggestive of an LCM-like cell-mediated immunopathological process. Similarly, the lymphoid necrosis in the spleens and lymph nodes and the indolence of the humoral immune response at 28 days favour consideration of a more complicated pathogenesis.

Extrapolation from this simian disease model would indicate that human patients should be investigated carefully for the possibilities of renal failure, myocarditis, arteritis, and choriomeningitis. Appropriate supportive therapy, such as dialysis, electrolyte management, arrhythmia monitoring, chemosuppression of overt arrhythmias, or anticonvulsant therapy, should be instituted if the corresponding complication should arise. Evidence of parenchymal cell regeneration in monkey liver and kidney and progressive decline in viral titres are reminders that prolonged supportive therapy may be the key to survival and recovery for many critically ill patients. The prominence of lymphoreticular lesions in these monkeys was probably related to the delay in immune responsiveness; a similar situation in man may be the reason that immune serum therapy has been efficacious, event late in infection (11).

This simian model offers an opportunity for further investigation of the mechanisms of Lassa fever. We must study viral immunopathology, the variation in susceptibility according to route of entry, and the variation in susceptibility in relation to virus dose. We should also be able to study immunoprophylaxis and immunotherapy.

GUINEA-PIG

When we attempted to produce antisera to Lassa virus in 12 guinea-pigs by intraperitoneal inoculation of live virus, the animals developed an illness characterized by respiratory insufficiency and a high mortality rate (67%). The associated pathology and organ tropisms were therefore investigated in an attempt to establish the general principles of Lassa virus infection.

Organ infectivity titrations showed high titres of Lassa virus in virtually all the organs tested between days 16 and 24 after inoculation (Table 2). Some of these organs may have contained Lassa virus because of viraemia, but a number of organs contained at least tenfold more virus than the blood. These organs were the spleen, lymph nodes, kidney, lung, heart, adrenal gland, liver, thymus, bladder, and pooled uterus and ovary. Pleural and ascites fluids also contained significant amounts of virus. Urine, which was never grossly contaminated with blood, also yielded virus in animals at 16 and 24 days after inoculation. This pattern of organ infection did not suggest any specific tropism, but resembled the " pantropism " seen in the squirrel monkeys. The prominence of lymphoreticulotropism, nephrotropism, myocardiotropism, hepatotropism, adrenotropism, and pneumonotropism indicates the primary target pattern of arenaviral disease.

Guinea-pigs that were sacrificed in a healthy state 56 days after inoculation contained no detectable Lassa virus in any of their organs, blood, or urine. This clearance of virus coincided with the development of high titres of CF antibody (1 : 1024).

Gross pathological lesions were observed only in animals that had died or were sacrificed in a moribund state between days 16 and 24. The lungs of these guinea-pigs were consolidated and red, and several had pleural effusions and ascites. Microscopic lesions were less extensive than in monkeys and man, being limited to the lungs, heart, and liver. All 8 animals examined between days 16 and 24 after inoculation had pulmonary oedema and 4 out of 8 had alveolar hyaline membranes. Underlying pulmonary pathology consisting of interstitial septal infiltration by mononuclear cells (a common spontaneous finding in this species) was present in all

Table 2. Organ titrations of guinea-pigs inoculated with Lassa virus

Animal number	1	2	3	4	5	6	7	8	9	10	11	12
Day of death/sacrifice	16	16	17	20	20	20	21	24	56	56	56	56
Specimen						*Titre*[a]						
Blood	5.5	5.2	5.5	4.2	3.5	5.8	—[b]	4.5	neg	neg	neg	neg
Spleen	6.8	6.5	6.5	3.2	4.8	5.5	4.5	6.2	neg	neg	neg	neg
Mesenteric lymph node	6.8	—	6.5	3.2	—	—	4.8	—	neg	neg	neg	neg
Cervical lymph node	6.2	—	6.2	—	—	—	—	—	neg	neg	neg	neg
Thymus	6.8	—	6.2	—	—	—	—	—	neg	neg	neg	neg
Liver	6.5	—	5.2	neg	neg	5.8	—	—	neg	neg	neg	neg
Heart	7.2	—	5.5	—	—	—	—	—	neg	neg	neg	neg
Lung	7.2	6.5	7.2	4.2	4.2	5.8	5.5	6.2	neg	neg	neg	neg
Kidney	6.8	—	6.5	—	4.8	—	—	—	neg	neg	neg	neg
Adrenal	6.8	—	5.2	—	—	—	—	5.8	neg	neg	neg	neg
Bladder	7.2	4.8	5.8	—	—	—	5.5	—	neg	neg	neg	neg
Uterus/ovary	6.5	—	5.8	—	—	—	—	—	—	—	—	—
Skeletal muscle	—	—	5.5	—	—	—	—	—	—	—	—	—
Cerebellum	4.5	—	4.5	—	—	—	—	—	neg	neg	neg	neg
Cerebral cortex	—	—	3.5	—	—	—	—	—	—	—	—	—
Pituitary	—	—	4.2	—	—	—	—	—	—	—	—	—
Pleural fluid	6.8	4.0	—	—	—	—	—	5.5	—	—	—	—
Ascites fluid	—	—	5.8	—	—	—	—	5.8	—	—	—	—
Urine	3.2	—	—	—	—	—	—	3.8	—	neg	—	—

[a] Log_{10} $TCID_{50}$
[b] — not available.

guinea-pigs, whether moribund, recovered, or uninfected. However, none of the controls or survivors had pulmonary oedema or hyaline membranes. Mild-to-moderate chronic myocarditis (Fig. 9) and focal calcification of myocardial fibres (Fig. 10) and hepatocytes (Fig. 11) were the only other histopathological lesions in infected guinea-pigs.

There was marked disparity between the lesions found in guinea-pigs infected with Lassa virus and those found in humans with Lassa fever or in nonhuman primates experimentally infected with Lassa virus. This was the case in spite of apparently similar patterns of viral tropisms in guinea-pigs and primates. Two examples of this disparity were seen in the liver and heart. Lassa virus is particularly hepatotropic; however, whereas man develops hepatocellular necrosis, the monkey shows increased regenerative activity and the guinea-pig only foci of calcified hepatocytes. On the other hand, Lassa virus is myocardiotropic and myocardiopathic in guinea-pigs and squirrel monkeys, but there have been no descriptions of human myocardial lesions. Human heart tissue has not been studied virologically despite the demonstration of changes in the electrocardiogram. The hepatocytic and myocardial injury was mild in guinea-pigs, and the cytoplasmic calcification observed was most likely a manifestation of sublethal injury to the plasma membrane of myocardial cells (12).

The pathogenesis of pulmonary oedema and hyaline membrane formation in guinea-pigs dying of Lassa virus infection is unclear. Interesting analogues to findings reported in human Lassa fever are pleural effusions, ascites, and respiratory insufficiency.

Although the guinea-pig disease differs too much from human Lassa fever to warrant further extensive investigation, the ease with which this model can be studied would make it a candidate for use in experiments on the effects of immunosuppression or immune serum therapy.

MASTOMYS NATALENSIS

M. natalensis is the only wild vertebrate species that has so far been shown to be infected with Lassa virus in nature (3). As with other pathogenic arenaviruses, human infection appears to result from contamination of man's environment with urine or other excreta from infected reservoir rodent species. Therefore, we undertook the experimental infection of adult and neonatal *M. natalensis* to study the pathogenesis of Lassa virus infection in its natural host. Our purpose was to relate pathogenetic patterns to the natural history of the virus and to evaluate further the role of *M. natalensis* in the transmission of Lassa virus infection to man. Twelve neonatal animals were inoculated intraperitoneally with Lassa virus and on days 7, 14, 21, 29, 50, and 74 were sacrificed in pairs with controls maintained in a separate cabinet of the Maximum Security Laboratory. Eight adult animals were inoculated intraperitoneally and sacrificed in pairs with their controls on days 12, 29, 58 , and 103 after inoculation. Eight 14-day-old animals were inoculated intraperitoneally with Lassa virus and were subsequently sacrificed on days 10, 18, 35, and 63 after inoculation. Specimens of lymph node, liver, spleen, lung, blood, brain, and urine, bladder, or kidney were collected for viral organ titration. All animals were subjected to complete histopathological examination. Impression smears of liver, spleen, lymph node, brain, and occasionally thymus were examined by indirect immunofluorescence for Lassa viral antigen. Sera from 5 adults, 6 neonatally infected animals, and 4 animals infected at 14 days of age were tested for Lassa CF antibody. All inoculated animals developed asymptomatic infection.

Organ titrations of neonatal *Mastomys* indicated significant viral titres in many organs, tissues and fluids, including lymph node, liver, spleen, lung, blood, and brain (Table 3). Animals examined on day 74 after inoculation continued to carry virus in the same organs. Moreover, Lassa virus was isolated from the urine of 5 out of 5 animals and from the throat swabs of 3 out of 5 animals examined. Immunofluorescent staining of Lassa antigen confirmed infection of hepatocytes, megakaryocytes, and small cells in the thymus. A few immunofluorescent cells were also observed in the lymph nodes, bladder epithelium, kidney, and brain. Although the impression smear technique was less sensitive than organ titration for demonstrating viral activity, it was clear that when facilities become available for frozen-section immunofluorescence with proper precautions to prevent the risk of biological contamination crucial information concerning viral pathogenesis will be forthcoming. Neonatal animals exhibited no significant histopathological alterations; they appeared to have developed a persistent tolerant infection such as has been described previously with other arenaviruses in their natural reservoir rodent hosts. Five of six animals infected neonatally had no demonstrable CF antibody (Table 4). One animal sacrificed 74 days after inoculation had a CF titre of 1 : 16, together with virus in all organs tested. The termination of this study at 74 days precluded

Table 3. Organ titrations of neonatal *M. natalensis* infected with Lassa virus

Animal number	Day of sacrifice	Titre[a]								
		Blood	Lymph node	Liver	Spleen	Lung	Brain	Kidney/ urine	Throat	Thymus
1	7	3.5		4.8	4.8	4.5	neg	4.2		4.5
2	7			3.8	2.5	3.5	neg	3.8		3.5
3–6[b]	14, 21									
7	29	4.5	3.2	3.5	5.5	4.5	3.5	2.8	neg	
8	29	3.8	neg	5.5	3.8	5.5	5.8	4.5	0.8	
9	50	neg	neg	neg	neg	neg	neg	2.5	2.5	
10	50	4.5	5.5	5.1	5.5	5.1	5.2	4.8	3.8	
11	74	3.5	5.5	3.8	6.5	4.2	3.5	3.5	neg	
12	74	3.5	3.8	5.2	3.5	4.2	—	3.5	2.5	

[a] Log_{10} $TCID_{50}$.
[b] Data not yet available.

Table 4. Complement-fixing antibody response to *Mastomys natalensis* infected with Lassa virus [a]

Infected as newborns						
Animal number	1	2	9	10	11	12
Days after inoculation	7	7	50	50	74	74
CF titre [b, c]	<8	<8	<4	4	<4	16
Infected as adults						
Animal number	5	6	7	8		
Days after inoculation	58	58	103	103		
CF titre [b, c]	16	<8	⩾32	<8		

[a] In addition, 4 animals infected at 14 days of age had no detectable CF antibody at 10 and 63 days after inoculation (2 each).
[b] Reciprocal of serum dilution endpoint in standardized micro-CF test.
[c] All uninoculated control animals had titres of <8.

detection of very slow viral clearance and immune reactivity, so that ultimate proof of immunological anergy and tolerance in the antibody-negative animals remains to be obtained. In any case, this host-virus relationship provides ample opportunity for long-term shedding and perpetuation of the virus in nature. The uniformity of this infection pattern and the ease with which it can be demonstrated by experimental inoculation further argue in favour of its role in nature.

In adult *Mastomys*, significant titres of Lassa virus were found in the blood, lymph nodes, liver, spleen, lung, kidney, bladder, urine, and brain (Table 5). Our preliminary data indicated a prominent lymphoreticulotropism (spleen and lymph node titres as high as $10^{4.2}TCID_{50}$/g of tissue). Some animals cleared virus from some organs, but there was persistence in other organs. Of the two adult *M. natalensis* tested for infectivity 103 days after inoculation, one was negative and the other still had virus in the axillary lymph nodes, brain, and urine. Only two animals developed CF antibody, one at 58 days and the other at 103 days. These two animals paradoxically contained more virus than the antibody-negative animals sacrificed at the same times. The only consistent histopathological finding was a moderate meningoencephalitis (Fig. 12) in adult *Mastomys*.

It is not clear from this preliminary study and from these incomplete data whether infection of adult *Mastomys* may result in real persistence of infection, but the observations at 103 days after infection support the premise made with neonatal infections that tolerance and persistence may be particularly easy to achieve in this virus-host pairing. Again, even if infection is cleared slowly (as in Tamiami virus infection of *Sigmodon hispidus* (13) or if some animals become tolerant while others become immune (as in Machupo virus infection of *Calomys callosus* (14, 15), there is adequate opportunity for virus seeding of a rodent's environment and massive opportunity for human exposure. Further experimental studies on Lassa virus infection of *M. natalensis*, including attempts to demon-

Table 5. Organ titrations of adult *Mastomys natalensis* infected with Lassa virus

Animal number	Day of sacrifice	Titre [a]							
		Blood	Lymph node	Liver	Spleen	Lung	Brain	Genito-urinary tract	Throat
1	12	1.8	4.2	1.8	4.2	3.5	2.2	3.2 [b]	neg
2	12	3.5	3.2	3.5	3.2	3.8	3.5	2.5 [b]	neg
3	29	neg	2.5		1.8	2.5	3.5	3.5 [c]	neg
4	29	neg	neg	neg	neg	2.5	neg	1.8 [c]	neg
5	58	neg	NA [d]	neg	2.2	neg	3.5	neg [b]	neg
6	58	neg	2.8	neg	neg	neg	neg	neg [e]	neg
7	103	neg	2.5	neg	neg	neg	2.5	1.5 [e]	neg
8	103	neg	neg	neg	neg	neg	neg	neg	neg

[a] Log_{10} $TCID_{50}$/ml.
[b] Urinary bladder.
[c] Kidney.
[d] NA = not available.
[e] Urine.

strate the role of vertical transmission and the limits of horizontal transmission, must be undertaken.

Finally, comparative studies are needed of Lassa virus infection of man, monkey, guinea-pig, and neonatal and adult *Mastomys* to look for similarities and to determine the range of differences. Similarities apparent from the available data are a wide organ tropism with prominent lymphoreticulotropism, prolonged viraemia, persistence of the virus in the body for long periods, delayed specific immune response, and a tendency for virus to be shed in the urine. There is a spectrum of clinical disease and pathological changes, which are most severe in man and are absent in neonatal *Mastomys* (with monkeys, guinea-pigs, and adult *Mastomys* in intermediate positions). The cellular mechanisms that determine these disparities in infection patterns and differences in pathogenicity must now be dissected.

RÉSUMÉ

PATHOLOGIE COMPARÉE DE L'INFECTION DUE AU VIRUS DE LASSA CHEZ LE SINGE, LE COBAYE ET LE *MASTOMYS NATALENSIS*

L'infection expérimentale par le virus de la fièvre de Lassa a été étudiée chez trois espèces, le saïmiri, le cobaye et le *Mastomys natalensis* (rat multimamelé africain qui constitue le réservoir naturel du virus). La pathogénie virale a été étudiée au moyen de titrages d'organes et selon des méthodes histopathologiques. Chez le saïmiri est apparue une maladie aux manifestations cliniques variables mais généralement graves marquées au stade initial par un lymphoréticulotropisme, un hépatotropisme et un néphrotropisme, ainsi que de la virémie. Ces troubles ont été suivis de l'infection de nombreux autres organes, avec présence du virus dans les urines et virémie pendant au moins 28 jours. On a observé chez ces singes de la myocardite, de l'artérite, une nécrose lymphoïde et une nécrose des tubes urinifères. La régénération du parenchyme hépatique a été suivie d'une régénération des tubes urinifères et d'une régénération lymphoïde. Vingt-huit jours après l'inoculation, on a décelé une chorioméningite. Les caractéristiques anatomo-pathologiques et les tropismes d'organes chez le modèle simien reflètent peut-être la pathogénie de la fièvre de Lassa chez l'homme. Chez le cobaye, l'infection s'est traduite par une forte mortalité (67%) due à une insuffisance respiratoire avec oedème du poumon, membranes hyalines alvéolaires, myocardite chronique et calcification focale des fibres myocardiques et des hépatocytes. La présence du virus a été constatée dans presque tous les organes chez les cobayes moribonds; toutefois, le virus avait disparu chez les animaux survivants sacrifiés 56 jours après l'inoculation, lesquels présentaient par ailleurs un titre élevé d'anticorps fixant le complément.

Des *Mastomys* nouveau-nés infectés par le virus de Lassa sont demeurés bien portants et n'ont jamais développé des lésions histopathologiques en dépit d'un titrage élevé de virus dans le sang, les ganglions lymphatiques, le foie, la rate, le cerveau, l'urine et les sécrétions pharyngées pendant les 74 jours qu'a duré l'étude. Ces animaux ont apparemment contracté une infection persistante bien tolérée qui, par l'élimination du virus dans les urines et les sécrétions pharyngées, semble convenir parfaitement au maintien de l'infection dans la nature. La contamination de l'environnement humain par des *Mastomys* infectés pourrait provoquer occasionnellement une infection humaine.

Les *Mastomys* infectés à l'âge adulte sont restés bien portants malgré une large diffusion de l'infection dans les organes. On a observé notamment un lymphoréticulotropisme et un neutropisme et le virus était présent dans les ganglions lymphatiques, le cerveau et l'urine d'un animal quand l'étude a pris fin après 103 jours. Les *Mastomys* adultes ont accusé une méningo-encéphalite modérée, mais sans autres lésions pathologiques.

En étudiant plus à fond l'infection par le virus de Lassa chez les singes, nous parviendrons à mieux comprendre le mécanisme de la maladie chez l'homme et, ainsi, pourrons envisager d'une manière rationnelle des moyens d'immunoprophylaxie et d'immunothérapie, ainsi que des soins complets. Une meilleure connaissance de l'histoire naturelle du virus de Lassa chez le *Mastomys* permettra d'établir le lien entre la transmission verticale et horizontale chez les rongeurs et la contamination de l'environnement. Nous aurons alors franchi une nouvelle étape en vue de réduire l'exposition humaine à cet agent pathogène virulent.

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DISCUSSION

GREGG: As an epidemiologist I am always interested in how a disease can be transmitted and I am extraordinarily impressed by the fact that you found this virus in every tissue you examined. Apparently you did not examine the skin. I wonder if the virus is in the skin or whether it can be found in the apocrine glands or in the sebaceous glands?

WALKER: This question could be answered by cryostat sectioning and immunofluorescent staining. Those facilities are not yet available in the CDC Maximum Security Laboratory but probably will be in the near future.

MIMS: In your tables the organ with the highest virus titre of all appeared to be the adrenal gland. This is possibly clinically important, and I wonder if you have any histological studies?

WALKER: We examined all the adrenal glands in detail; they were completely normal. We did not make functional measurements of steroid levels in the urine or blood, but grossly and histologically the glands were completely normal.

OLDSTONE: When looking at end-stage lesions, it should be remembered that perivascular round cell infiltration does not mean cell-mediated immunity. There are numerous examples of antibody transfer resulting in that type of lesion, so it is impossible to draw a pathogenetic conclusion.

MONATH: From clinical observations only, it appears that the human disease is characterized by what has been called " a diffuse capillary leak syndrome ", with the development of effusions, ascites, pulmonary oedema, etc., reminiscent of your findings in guinea-pigs. I know you have not done FA studies, which would really provide the answer, but does light microscopy tell you anything about the tropism of Lassa virus for vascular endothelium?

WALKER: I have been studying Rocky Mountain spotted fever for the last year and have found histological changes in the endothelium, but we have seen nothing of that nature in Lassa virus infections.

OLDSTONE: Has anybody looked for breakdown or split products of the complement system, many of which increase vascular permeability?

WALKER: Such studies have not been performed using the animal model, and I am not aware of them being done on clinical patients either.

CASALS: I would like to know whether in *Mastomys*, for example, an animal that has such high concentration of virus in the blood, you have examined blood smears by the FA technique? A similar method would certainly facilitate the early diagnosis in man.

WALKER: This has not been adequately investigated.

WOODRUFF: I think you said that in human cases there have been few observations of myocarditis, or myocardial damage, in Lassa fever. In this connexion it might be of interest to note that in our last patient there was marked evidence of electrocardiographic changes and material removed from the myocardium after death showed focal areas of necrosis.