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Fetal Infection of the Baboon *(Papio cynocephalus)* With Lymphocytic Choriomeningitis Virus

By

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With 8 Figures

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Summary

Recent observations of LCM-induced fetal damage in humans suggested attempts to develop an animal model for studies on viral congenital malformations. We report herein viral studies on three pregnant baboons *(Papio cynocephalus)* inoculated subcutaneously with LCM virus strain WE3. The first animal, inoculated in the 9th week of pregnancy, aborted 9 days after a high virus dose. Inoculation of the second baboon during a later stage (23rd week) of pregnancy with a moderate virus dose, resulted in the demonstration of virus in the placenta, amniotic fluid, and cord blood. The infant showed only a slight pleocytosis of the spinal fluid, but no virus shedding and no late sequelae. The third baboon inoculated with a high virus dose during the 21st week of pregnancy delivered an underweight, icteric infant that succumbed on the 6th day. All organs of this animal that were tested contained virus. Microscopic examination of these tissues revealed multifocal necrosis, cerebral glial nodules, meningitis, and bilateral choriovasculitis. These results illustrate that fetal damage observed in the LCM-inoculated baboon resembles that seen in humans following infection with LCM virus.

Introduction

A number of observations indicate that lymphocytic choriomeningitis (LCM) virus may damage the human fetus (1, 2, 6, 13). Infection during early pregnancy has been reported to cause abortion (2, 6); during late pregnancy, however, hydrocephalus, chorioretinitis with vision impairment, bilirubinemia, and mental and physical retardation occur (1).

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Because disease of the mother may be extremely protean, there are generally long intervals prior to the discovery of the fetal damage. As a consequence, the specific etiologic diagnosis is frequently not demonstrated. Four human cases (2, 6) recently were detected only because of the uncommon source of infection, namely pet hamsters. Recent serological findings, however, indicate that LCM virus plays a greater role among the known agents responsible for fetal damages than heretofore suspected. Of 28 children with a congenital hydrocephalus, Sheinbergas reported nine had antibody to LCM virus as determined by immunofluorescence (20).

Since fetal morphogenesis of the baboon *(P. cynocephalus)* is very similar to that of man, this animal was selected in an attempt to determine the pathogenesis of fetal damage caused by LCM virus. Previous studies with vaccinia (10) and other viruses (unpublished data) in this host also suggested the suitability of this selection. This report describes the effect of infection during early and late gestation. Additional studies with the baboon model will be reported separately.

Materials and Methods

Animals

Three pregnant baboons *(P. cynocephalus)* are described herein; one inoculated in the first and two in the third trimester. All animals were inoculated subcutaneously (s.c.) in the abdominal area. Pre-inoculation blood samples were obtained in all instances and the animals were observed for evidence of clinical disease. Infants were separated from the mother either by cesarean section or immediately after birth and maintained in incubators.

Mice

ICR Swiss and NMRI Han mice, 3 to 4 weeks old, were used for virus isolation and neutralization experiments. These specific pathogen free mice were either obtained from commercial sources (ICR) or from the Staatliche Versuchtstieranstalt Hannover (NMRI Han) and maintained in a colony at these institutions. Every 6 months any NMRI mice remaining were sacrificed and new stocks obtained. Only sufficient ICR mice for immediate use were obtained at any time. Control mice were regularly tested for LCM virus by blind passages in mice and tissue cultures.

Virus

LCM virus strain WE 3 was used after 33 continuous mouse brain passages. Brains were prepared into 10 per cent suspensions in Tris-HCl buffer solution (pH 7.4) containing 10 per cent heat inactivated (20 minutes at 60° C), calf serum, and antibiotics (100 units penicillin, 50 micrograms streptomycin per ml). To determine virus titer, six mice were inoculated intracerebrally (i.c.) with each tenfold dilution and observed for 3 weeks. Deaths after 5 days were considered to be specific. The LD_{50} titer was calculated according to the method of KÄRBER (11). Titers of 10^6 or greater were considered to be "high" virus doses, those between 10^3 and 10^5 as "moderate" and titers less than 10^3 as "low" doses.

Virus Isolation and Typing

For virus isolation, sterile blood or the blood clot was triturated and diluted 1:5 in physiologic saline. Antibiotics (100 units penicillin, 50 micrograms streptomycin per ml) were added to all urine (undiluted) specimens. Tears collected on filter paper discs (9) were diluted in 3.0 ml Tris buffer and centrifuged 15 minutes at 5000 rpm. All specimens were inoculated i.c. into six mice. Brains were harvested from mice dying after

the 5th day and either stored at −80° C or made into 10 percent suspensions with Tris buffer. Frozen brains were subsequently thawed and similarly treated. After one additional passage in mouse brain, 10 per cent suspensions (1.0 ml) were inoculated (in duplicate) onto monolayers of BHK (baby hamster kidney) or GMK (green monkey kidney) cell cultures in 100 ml glass bottles. After ½ hour, 9 ml MEM containing 10 per cent fetal calf serum was added to all bottles. After 5 days' incubation, the medium was removed, 2.0 ml veronal buffer added, and the cell cultures frozen and thawed several times. The material from the duplicate bottles was pooled, lightly centrifuged, and the supernatant fluid tested in the complement fixation (CF) test for the presence of LCM antigen. In each test control antigens from uninoculated cell cultures and mice were included.

Neutralization Test

The method used in this laboratory has been previously described (1, 14). All virus dilutions were made in Tris buffer. Known human convalescent serum served for virus typing and controls. Briefly, the test consisted of using 10-fold dilutions of virus against a constant amount (undiluted) serum (not inactivated). After 2 hours at 37° C, the virus-serum mixture was inoculated i.c. into six mice per dilution. Mice dying from day 6 until day 21 were considered as dying of LCM. All tests included positive and negative control sera as well as virus titrations.

Complement Fixation Test

CF tests were performed according to the method of SEVER (19). LCM virus antigen was prepared from guinea pig lungs as described by SMADEL *et al.* (21). Guinea pig LCM hyperimmune serum was used as a control.

Immuno-Globulin Determinations

A modified MANCINI *et al.* (16) radial immunodiffusion procedure was used on Hyland's Immuno-Plates. Levels lower than 10 mg were repeated on Behring LC Partigen low level immunodiffusion plates according to the manufacturer's directions.

Histologic Staining

Representative pieces of tissues, including the brain of the dead infant, were fixed in a modified Millonig's buffered formalin; the eyes in Bouin's solution. Hematoxylin-eosin and Masson's trichrome stains were applied to 5 μ sections.

Results

Experiment 1

One baboon in the 9th week of pregnancy was inoculated S.C. with 3×10^8 LD_{50} (mice). This animal appeared normal until the 9th day post infection (p.i.), when it developed genital bleeding and appeared less active. No other abnormal clinical changes could be detected. Rectal examination indicated that the animal had apparently aborted during the night, but the fetus was not recovered.

By the 9th day, IgM increased slightly (5.2 mg/ml), continuing to increase from the 4th to the 7th week. A lymphocytosis of approximately 60 percent was noted in the peripheral blood between the 2nd and 7th week. Neutralizing antibodies increased to an index of 4.0 within 5 weeks. No change in CF antibody was ever detected, nor was virus ever isolated from the mother throughout the study period (Figure 1).

Experiment 2

One baboon was inoculated s.c. during the 23rd week of pregnancy (i.e., 4 weeks before the calculated date of birth) with 2×10^4 LD_{50} (mice). No overt clinical manifestations other than malaise and loss of appetite were ever observed on this animal during the test period. LCM virus was isolated from maternal blood between the 12th and 17th day p.i. A lymphocytosis of approximately 60 per cent developed between the 3rd and the 7th week, and 7 weeks p.i. the cisternal cerebrospinal fluid (CSF) contained 7.4/mm³ mononuclear cells. Normal values for the baboon have been previously reported (8). Three days before the calculated date of birth, the baby was delivered by cesarean section. Macroscopic appearance of the placenta was normal, and several areas of the placenta, when examined microscopically, failed to demonstrate lesions. LCM virus, however, was isolated from the placenta and from the amniotic fluid.

Eight weeks p.i., maternal serum had 4.0 logs neutralizing antibodies which persisted with little loss in titer for several months (Fig. 2). Low level CF antibodies could be similarly detected during this period. Throughout this study period, this baboon remained asymptomatic.

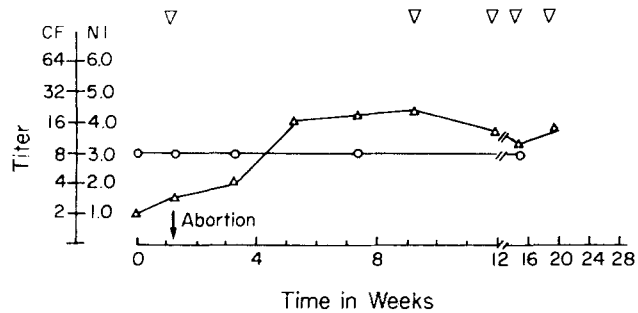

Fig. 1. Complement fixation (○) and neutralization (△) results on sera from a baboon inoculated during 9th week of pregnancy. Virus was not isolated at indicated test samplings (▽). 3×10^8 virus given s.c. at day 0

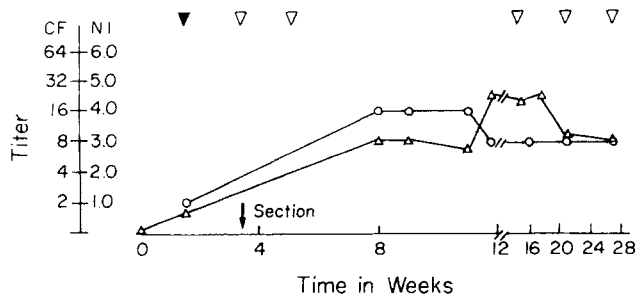

Fig. 2. Serologic results on a baboon inoculated during 23rd week of pregnancy. Virus was isolated (▼) from the peripheral blood on the 11th day p.i., but not at other indicated test sampling (▽). 2×10^4 virus given at day 0

The newborn baboon appeared normal, with normal values of: weight, 841.5 g; measurement from crown to rump, 18.7 cm; and a head circumference of 22.0 cm. The animal appeared to be in good condition, normal in behavior, and had a temperature of 96.8° F and no detectable malformations. No differences could be seen in the hematological, behavioral, and growth patterns of this animal which compared favorably with those of noninfected newborn baboons. LCM, however, was isolated from the cord blood and identified in the CF test.

Twenty-two days after birth, the cisternal CSF contained 16.2 cells/mm^3. IgM could not be detected. No LCM virus was isolated from the blood on the 2nd, 8th, 17th, or 23rd day, nor during the following 5 months. Virus was not recovered from urine on the 8th, 11th, or 17th day, nor from tears on the 8th day or from CSF on the 22nd day. Neutralizing and CF antibodies developed within the first 6 weeks, as indicated in Figure 3. The animal matured normally and showed no clinical symptoms or abnormal behavior during the following 24 months.

Experiment 3

A baboon in the 21st week of pregnancy, approximately 33 days before parturition, was inoculated s.c. with 1×10^7 LD_{50} (mice). Toward the 8th day p.i., the animal appeared less aggressive and demonstrated a slight temperature increase. This general appearance of decreased activity persisted for approximately

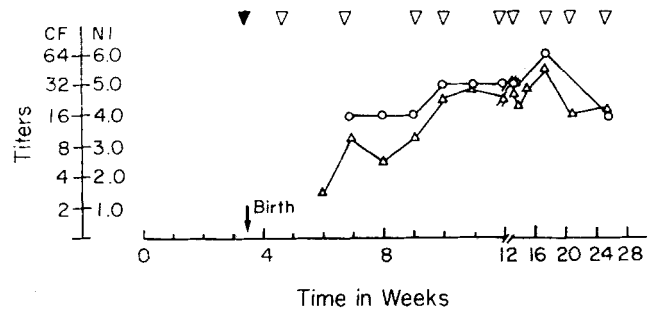

Fig. 3. Serologic results on infant derived from baboon inoculated in 23rd week of pregnancy. Virus was not isolated from peripheral blood (nor urine) at indicated collection times. See Figures 1 and 2 for legend. 2×10^4 virus given mother at day 0

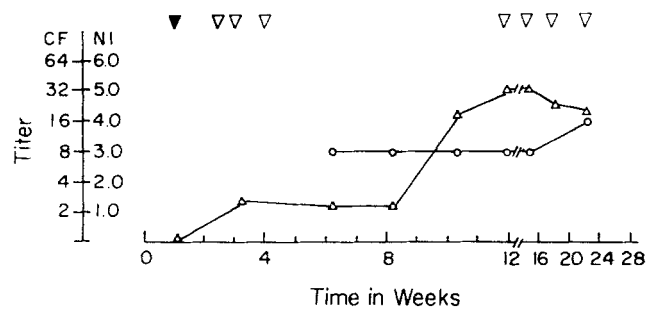

Fig. 4. Serologic and virus isolation results on a baboon inoculated during 21st week of pregnancy. See Figures 1 and 2 for legend. 1×10^7 virus given at day 0

1 week. After the 20th day, the animal was asymptomatic and delivered naturally on the 23rd day p.i. The placenta was cannibalized by the mother, but the infant was recovered. The mother was unusually lethargic for a few days post delivery, but recovered quickly thereafter. No other clinical symptoms were noted during the following 6 months.

The maternal blood picture 2 weeks p.i. showed a lymphocytosis and, following delivery, a granulocytosis for approximately 6 weeks. At the 30th day p.i. the CSF contained 17.3 cells/mm^3, mostly mononuclear. LCM virus could be isolated from the blood 8 days p.i. but not 23 days p.i. or during the following months. No virus was isolated from the CSF days p.i. Between the 8th and 10th week p.i., neutralizing antibodies increased markedly; however, CF antibodies increased only moderately (Fig. 4).

The female newborn baboon weighed 610 g, with a crown to rump length of 16 cm and a head circumference of 18 cm, and was fully developed but appeared weak and icteric. The blood picture was within the normal range.

The infant was maintained in an isolator, but from the beginning its food intake was very poor. Milk was limited to 3 to 5 ml per meal and baby food to 30 to 40 ml per day. A weight loss of 100 g occurred in the first 4 days, with

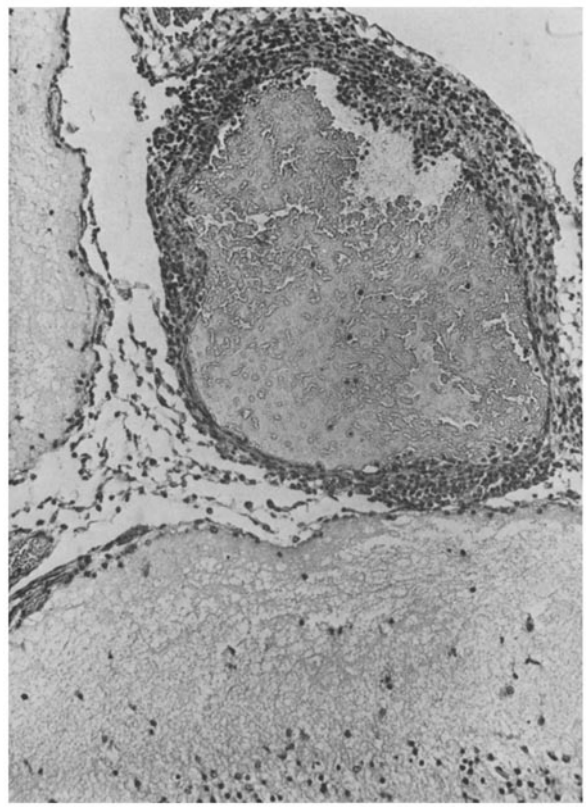

Fig. 5. Mononuclear cell infiltration in wall of meningeal vein overlying the cerebral cortex. A few inflammatory cells are present in the meningeal spaces. Hematoxylin and eosin. × 160

an associated decrease in activity. On the 5th day, head deflection was noted, but without stiffness of the neck. Amino-acid-supplemented fluids were given, but the infant died during the night of the 6th day.

At necropsy the lungs were mottled dark red with petechiae and failed to collapse when removed from the chest. A small amount of amber fluid was present in the pleural and pericardial spaces. The thymus was congested, but it was normal in size and consistency. No other abnormal gross findings were noted.

Histopathologic examination revealed serous and purulent exudation within the lungs, and the majority of the alveolar septae were thickened by cellular infiltration. In some areas the alveolar spaces were obliterated by hemorrhage and inflammatory exudate, while other alveoli were filled with serous fluid containing few cells. The blood vessels were markedly congested. Clusters of neutrophils were scattered throughout the inflamed regions. Alveolar macrophages were present in increased numbers. Some bronchioles were engulfed by the inflammatory cells. Bronchi were unaffected except for small amounts of purulent exudate within their lumens.

The cerebral cortex and cerebellum showed marked congestion of all vessels. Modest amounts of hemorrhage were present within the meninges. A marked vasculitis, particularly affecting small muscular arteries, was also present (Fig. 5).

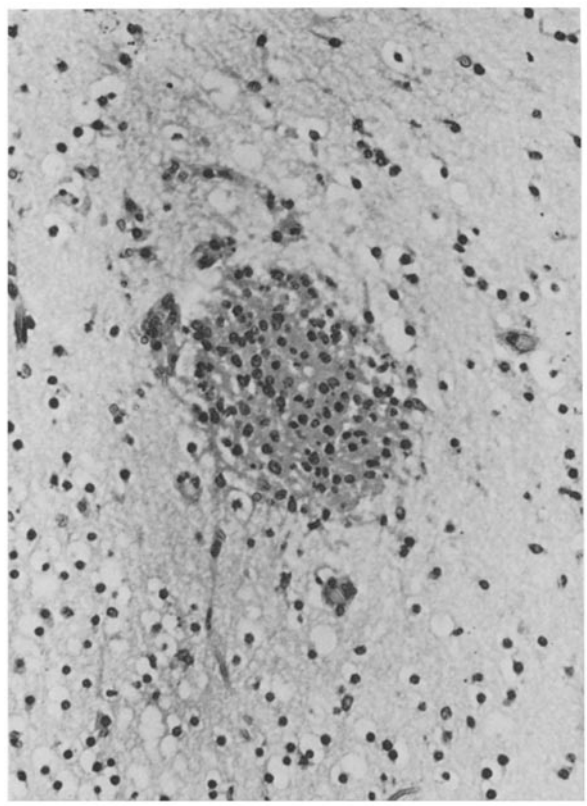

Fig. 6. Glial nodule in gray matter of the cerebral cortex. Hematoxylin and eosin. × 475

Prominent glial nodules were observed within the grey matter along with hemorrhage (Fig. 6). Both eyes showed foci of choriovasculitis (Fig. 7); the right eye had a focal retinal gliosis.

A few scattered foci of interstitial cell necrosis involving only a few cells per focus were seen in the kidney. In addition, multifocal interstitial inflammation was noted. Focal endocarditis and myocarditis were demonstrated in the interventricular septum of the heart.

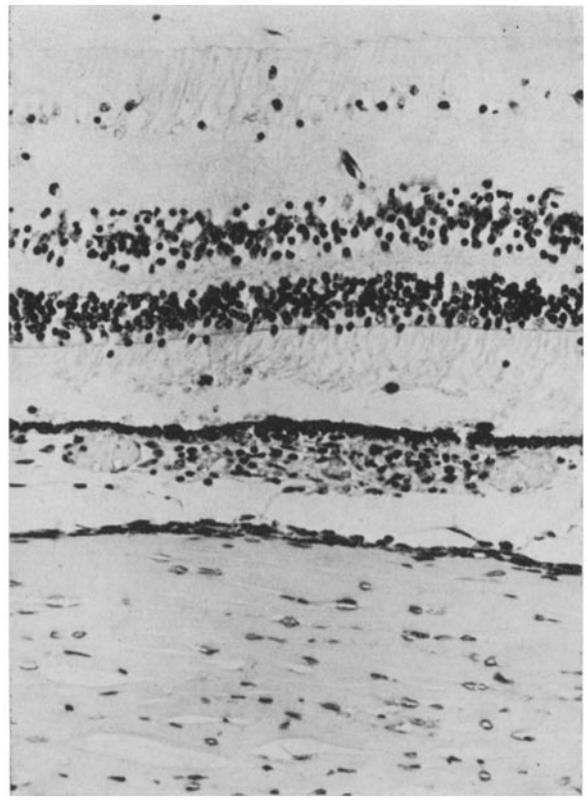

Fig. 7. Sequential mononuclear cell inflammation in chorioidal vein. Tissue disruption in the overlying retina is artefactual. Hematoxylin and eosin. ×475

The lobules of the thymus (0.4 g) were without a corticomedullary pattern. They were composed of congested vessels, reticulum cells, lymphocytes, eosinophils, and a few neutrophils. Necrotic cells were frequent. Epithelial cells and Hassall's corpuscles were present but few in number. One lobule contained a large cyst lined by endothelium or squamous epithelium and held erythrocytes, epithelial cells, lymphocytes and amorphous eosinophilic debris. Inflammatory cells protruded through the cyst wall in several places. The thymic lobules were surrounded by considerable hemorrhage.

The axillary lymph nodes lacked a corticomedullary pattern and were densely populated with reticulum cells. Lymphocytes were sparse, with a few small foci

being located in the cortical region. Numerous macrophages were present within the medullary sinuses. The medullary cords contained a limited number of necrotic foci.

The white pulp of the spleen was sparsely populated, the principal cell type being reticulocytic. Few lymphocytes were present and germinal centers had not developed. Necrosis was infrequent. The red pulp was congested and small foci of necrosis involved a few to several cells. A modest number of plasma cells were present.

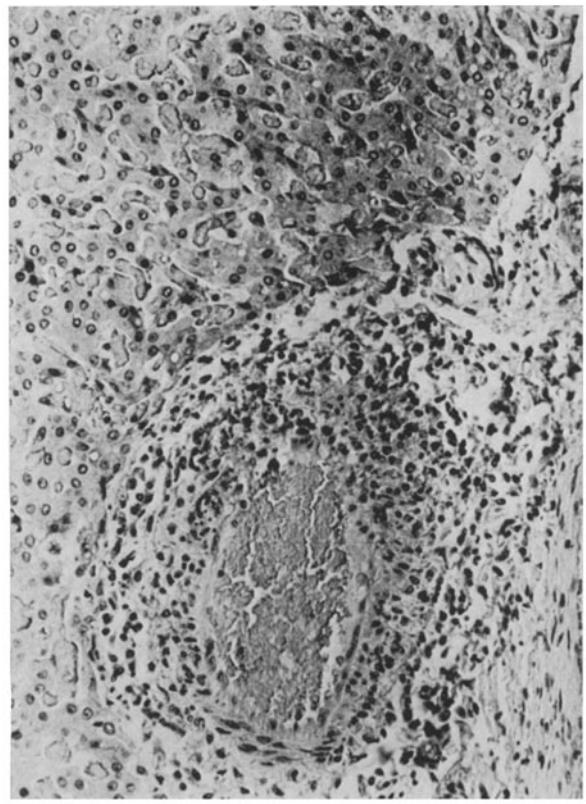

Fig. 8. Mixed cell inflammation in wall of portal vein. Adjacent hepatic artery and hepatic parenchyma is unaffected. Hematoxylin and eosin. × 400

Foci of hepatocyte necrosis affecting only a few cells were associated with accumulations of histiocytes. In addition, histiocytic and lymphocytic infiltrations were present around many of the central veins and periportal structures. Necrosis was minimal in these lesions. In one lesion, numerous eosinophils were present. The vessels and sinusoids throughout the section were uniformly congested (Fig. 8).

The right adrenal gland showed numerous scattered foci of necrosis within the capsule, zona fascicularis, and medulla. Several cells were involved in each focus of necrosis, and a moderate histiocytic response had been made to the necrosis. The vessels were markedly congested. Numerous solitary cells within

the zona fascicularis were undergoing degenerative changes—i.e., swollen, poorly staining cytoplasm and shrunken hyperchromatic nuclei.

The mucosa of duodenum, ileum, and colon, and the pancreas were autolysed, but no obvious lesions were observed. The intestinal lymphoid structures were poorly cellular.

LCM virus, identified in the CF test, could be isolated from all investigated organs, with titers in individual tissues of $10^{3.49}$ to $10^{7.02}$ LD_{50} (mice) (Table 1). Bacteriologic testing failed to demonstrate any abnormal findings. All tissues (Table 1), with the exception of the ileum, were bacteriologically sterile.

Table 1. *Titers of LCM virus in the organs of a 6-day-old baboon whose mother was infected 23 days before delivery*

	log LD_{50} (mice)/ml
Brain	5.35
Cerebellum	4.99
Thyroid	3.49
Heart	7.02
Thymus	6.02
Lung	5.69
Liver	6.35
Spleen	7.02
Pancreas	5.02
Ileum	5.02
Kidney	6.35
Adrenal	6.33
Uterus	5.02
Breast muscle	6.99
Axillary lymph node	6.65
Mesenteric lymph node	6.99

Discussion

These experiments demonstrate that the baboon is susceptible to LCM virus as are humans and other primates such as rhesus, cynomolgus, and cebus monkeys (3, 4, 5, 7, 8, 12, 15, 17, 19, 21). All three baboons, when inoculated s.c. with a moderate to high dose of LCM virus (strain WE3), developed slight malaise and fever lasting for a few days. In two cases, a pleocytosis (7.4 and 17.3/mm^3) was found in the spinal fluid, without clinical signs of a meningoencephalitis. Similarly, the surviving newborn baboon which appeared normal, but from which LCM virus was isolated (cord blood), contained 16.2 cells/mm^3 in the CSF. The blood showed a minor, transitory lymphocytosis and, in two instances, LCM virus could be isolated from the blood during the 2nd week p.i. Antibodies, as detected by serum neutralization, developed similar to those observed in humans (18), CF antibody titers were low, but the titers of neutralizing antibodies were relatively high, reaching peak titers within 8 to 10 weeks. Questions may be raised regarding the preferred use of the serum neutralization test rather than various other available procedures. Most important is the singular, inherent specificity of this test, developed over years of experience by this investigative group. Further, in spite of several recognized limitations with the neutralization test, the results

obtained were directly applicable to this study and provided an acceptable assay system.

The baboon infected in early pregnancy with a high LCM virus dose aborted 9 days later. Associating this abortion with the LCM infection in this animal is difficult, particularly since the fetus and the placenta were unavailable. Nevertheless, we could speculate that the infection may have been generalized, resulting in abortion following involvement of the uterus. LCM infection of baboons under natural conditions has been reported (8). Abortions of presumably normal animals are also recognized. However, abortion rates occurring in the colony under study herein are very low, i.e., approximately 5 percent. Further, no attempt has ever been made to correlate natural LCM infections and natural abortions. It is also of some relevance to indicate that LCM virus has never been isolated from hundreds of normal baboons. In the two reported human cases, abortions occurred within 4 weeks after the onset of the disease (2, 6). In these human cases, both women probably had been infected with low virus doses from pet hamsters.

The two animals inoculated during the 3rd trimester of pregnancy demonstrated that transmission of LCM virus to the fetus occurred. Inoculation of a low virus dose induced only mild fetal alterations. Virus could, however, be isolated 26 days p.i. (at time of C-section) from the placenta, amniotic fluid, and cord blood. A slight pleocytosis of the CSF indicated involvement of the nervous system. There were no clinical signs of a meningoencephalitis or a hydrocephalus. In spite of the short infection, no persistent viremia or virus shedding in urine or tears could be demonstrated. The baboon appeared to develop normally.

When a pregnant baboon was inoculated with a high virus dose in late pregnancy, severe damage of the fetus occurred. The infant was underweight and obviously incapable of surviving. Death could be attributed to insufficient intake of food and widespread necrosis, hemorrhage, and inflammation, particularly affecting the lungs. LCM virus was demonstrated in all of the investigated organs including the lungs from this animal. The observed lesions were compatible with a virus infection and similar to those described from adult monkeys (3). Isolation of LCM virus from the lungs along with the failure to recover any other microorganism strongly supports the induction of pneumonia by this virus. Hypoplasia of the lymphoid structures may have been due to the shortened gestation period and a lowered rate of mitosis resulting from the virus infection and associated stress. A meningoencephalitis with glial nodules similar to those described by Lillie (15) in LCM-infected rhesus monkeys was also observed. In addition, both eyes demonstrated choriovasculities similar to that observed in two of the human cases (2). Alterations of the liver parenchyma may explain the icterus noted at birth, which was also seen in a more severe form in one of the human cases (2).

These experiments in the baboon resulted in damages similar to those occurring in humans as observed by the limited recorded observations. Infection in early pregnancy appeared to induce abortion. In late pregnancy, infection of the mother with a moderate virus dose caused only a slight meningitis of the baby, without obvious sequelae. Contrasting with this was the generalized infection and death of the newborn following use of a high virus dose. Meningoencephalitis, choriovasculitis, and involvement of the liver resemble very closely the disease of the human newborn after infection during pregnancy with LCM virus.

These data suggest that the pregnant baboon may be suitable as an experimental model for studies on fetal infection with LCM virus. The adult female baboon is susceptible to the LCM virus and develops a very mild disease, but transmits the virus to the fetus.

These findings confirm the observations made in humans of the fetopathogenic effects of LCM virus. Infection of the fetus may occur without any sequelae. Furthermore, the results suggested that damage to the fetus may be dose dependent. Additional baboon studies to be reported in detail separately support these findings of congenital infection.

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