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Chronic Infection of Rodents by Machupo Virus

Abstract. *Machupo virus, the etiologic agent of human hemorrhagic fever in Bolivia, induced chronic asymptomatic infection in laboratory hamsters and colonized individuals of the peridomestic, wild, South American rodent,* Calomys callosus. *Viruria was detected for more than 500 and 150 days, respectively, in the two species. Chronic viremia was shown only for* Calomys. *Virus-neutralizing substances were present in parenterally infected adult animals, but not in animals born to, and in contact with, an infected female. Chronic infection in wild rodents may be an important mechanism in the natural history of Machupo and related virus infections.*

Machupo virus was first isolated in 1963 from the spleen of a patient from northeastern Bolivia who died of hemorrhagic fever (*1*). This agent was subsequently found to be serologically related to Junin virus, which has been associated with a similar hemorrhagic syndrome in Argentina (*2*), and to Tacaribe virus, originally recovered from bats on the island of Trinidad (*3*). Tacaribe virus, it had been previously demonstrated, shares antigens with Junin virus (*4*).

Table 1. Recovery of Machupo virus from parenterally infected adult hamsters at various times (in days) after inoculation. Symbols: +, virus isolated; −, virus not isolated; n, no test or unsatisfactory test.

3	6	8	9	10	12	14	22	509
				Urine				
−	−	+	+	+	+	+	+	+
				Feces				
−	n	−	+	−	+	−	n	n

Junin virus was isolated several times from wild rodents in Argentina (*5*), and it has been reported that arthropods might be important in transmission of infection (*6*). Machupo virus was recovered repeatedly from the small pastoral mouse *Calomys callosus* Rengger, 1830, captured in and near homes in the 1963 epidemic center of San Joaquin, Bolivia (*7*), but repeated attempts to detect the virus in many different hematophagous arthropods were unsuccessful.

In the course of laboratory studies with the Machupo virus in infant mice and hamsters, for which the agent had been shown to be pathogenic after parenteral inoculation, it was noted that uninoculated hamster dams frequently ate one or more of their offspring at about the time that virus-induced illness appeared in the litters. This interval was usually 6 to 10 days after administration of virus. Female white mice rarely cannibalized their young under similar circumstances. In two instances female hamsters became grossly ill with tremors and intermittent convulsions 19 and 21 days after they had eaten one or more infants of their demonstrably infected litters. Machupo virus was isolated from brain and spleen of both mothers by inoculation of infant hamsters. Complement-fixing (CF) antibodies to the virus also were detected in their serums. Fourteen asymptomatic hamster dams were bled 30 days after inoculation of their offspring with virus, and these serums were tested for CF antibodies. Each of these animals had eaten one or more of the infected infants. All serums contained CF antibodies for Machupo virus. These findings suggested that virus had been transmitted from infant to adult and that virus might be detectable for a considerable interval in infected adult animals.

To test this hypothesis, hamsters 5 to 6 weeks old were inoculated intraperitoneally (IP) with 10^4 $IHLD_{50}$ (infant-hamster lethal dose, 50 percent effective) of Machupo virus (strain Carvallo), that had been passed twice in hamsters. Urine and fecal specimens from asymptomatic individuals were obtained and assayed for virus in infant hamsters. Observations are summarized in Table 1. Fecal specimens were often toxic for infant hamsters; thus the results do not exclude the possibility of significant excretion of virus in feces. Urine samples obtained weekly from two animals uniformly continued to yield virus (509 days at this writing, 1 August 1965). Virus-neutralizing and CF antibodies were present 12 weeks after inoculation and were detected in similar titer in serums taken 46 weeks after infection. Systematic attempts to demonstrate viremia early in the course of infection were not made, but tests with whole blood were negative 32 and 47 weeks after inoculation.

In order to extend these observations a laboratory colony of *Calomys callosus* was established from 13 adult animals captured in San Joaquin, Bolivia. The original animals were subsequently killed, and their tissues were shown to be free of Machupo virus. Ten animals of the third laboratory generation, 5 to 7 weeks old and equally divided by sex, were inoculated intraperitoneally with $10^{5.0}$ $IHLD_{50}$ of the same virus pool used to infect the hamsters. All animals remained asymptomatic. Viruria was regularly demonstrable after the 15th day after inoculation. Titration of samples at intervals revealed continuous presence of 10^3 to 10^5 $IHLD_{50}$ of virus per milliliter of urine. Blood from seven of eight animals tested 6 weeks after virus inoculation contained virus, but neutralizing antibodies were detected in only one rodent at this time. At 20 weeks after inoculation six of seven animals had virus-neutralizing antibody titers from 1 : 4 to 1 : 256, and three of the positives had detectable viremia. Viruria was also present and has persisted through 153 days without end point.

Another pertinent observation was made as follows: in the course of an attempted virus titration experiment, a female *Calomys* delivered five infants 10 days after intraperitoneal injection of $10^{5.0}$ $IHLD_{50}$ of Machupo virus. These animals remained with the mother for 9 weeks; during this time

Table 2. Isolation of Machupo virus from *Calomys callosus* captured in San Joaquin, Bolivia, 20–27 May, 1964. Symbols: +, virus isolated; o, virus not isolated; n, no test or unsatisfactory test.

Block and house number	Virus isolation		
	Kidney	Spleen	Urine
	+	+	o
3–19	+	+	n
8–75	+	o	+
8–75	o	+	n
8–77	o	o	o
9–84	o	o	o
10–94	n	+	+
10–95A	+	+	+
10–95A	o	o	n
10–95A	+	+	n
10–98	+	+	o
10–98	+	+	n
13–117	+	+	n
21–234	+	+	+
28–307	o	o	o
28–308	+	+	o
28–309	+	+	+

virus was found in her urine. The offspring were then transferred to individual boxes, and each was shown to be viremic at 12 and 17 weeks of age. In contrast to adult animals inoculated parenterally, none of these *Calomys* had detectable virus-neutralizing antibodies 20 weeks after potential first exposure to infection.

The demonstration of chronic viruria in experimentally infected rodents stimulated a search for a similar pattern among wild *C. callosus* in San Joaquin, Boliva, where hemorrhagic fever among humans was then active. Seventeen animals were trapped alive from 20 to 27 May 1964, mostly from houses which were probable sources of recent human illness. Samples of voided urine were obtained from 11 of these grossly healthy animals, after which kidneys and spleens were removed. Results of isolation attempts (Table 2) are based on successful reisolation of virus. Machupo virus was recovered with nearly equal frequency from spleen and kidney, and quantitative determinations made in nine cases revealed from $10^{2.8}$ to $10^{5.2}$ $IHLD_{50}$ per gram of tissue. Virus was also detected from 5 of 11 urine specimens, and only from animals whose tissues were positive.

These results suggest certain biological parallels to a limited group of previously described viral agents, including the salivary gland viruses (*8*) and that of lymphocytic choriomeningitis (*9*). In the case of lymphocytic choriomeningitis, mice infected before or shortly after birth become chronically infected; and virus can be found in various tissues, in blood, in urine, and in saliva for long periods, probably for life, in the absence of easily detectable circulating antibodies (*10*). It seems possible that *C. callosus*, when infected prior to or shortly after birth, may also exhibit chronic infection without detectable immune response.

The data also provide a significant new concept that can be evaluated in the search for understanding of the natural history of infection with Machupo and related viruses. Neither Junin nor Tacaribe virus has been shown conclusively to be transmitted by arthropods. Efforts to incriminate such vectors in transmission of Machupo virus have also failed. Continuous contamination of the human environment by infectious urine shed by chronically infected peridomestic rodents could be an important mechanism leading to human disease (*11*).

The presence of viremia in addition to viruria in *C. callosus* preserves the possibility that hematophagous arthropods might play some role in virus transmission. Experimental arthropod transmission studies need to be done. However, if arthropods are not important, some means of rodent to rodent transmission is mandatory. It appears that the events comprising mating, gestation, and neonatal behavior provide the greatest number of potentially reliable mechanisms for maintenance of virus infection. Studies have been initiated to test these possibilities in colonized *C. callosus*.

A classical interpretation of the chronic asymptomatic infection induced by Machupo virus would strongly imply that this agent has had a long and successful history of parasitism in rodents of South America. If this hypothesis is correct, it is possible that human hemorrhagic fever on that continent is not a new disease, but, for a variety of reasons has been recognized as a disease only within the past 15 years.

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Sex Conversion Induced by Hydrostatic Pressure in the Marine Copepod Tigriopus californicus

Abstract. *High hydrostatic pressure applied to the naupliar larval stages of the marine copepod* Tigriopus californicus *converts some individuals that would have become males into females. The copepodid stages are not sensitive to pressure-induced conversion.*

Vacquier (*1*) has presented evidence that high hydrostatic pressures applied for 2 hours to the first naupliar larval stage of the marine copepod *Tigriopus californicus* produced an increased percentage of female survivors. Two hypotheses were advanced to account for this phenomenon: (i) The pressure effect was selective, killing larvae already determined to become males and either not affecting or with a reduced effect upon those destined to become females. (ii) The pressure was converting some potentially male larvae into females.

Norton (*2*) has presented statistical evidence for sex conversion based on Vacquier's (*1*) data. However, we be-