TABLE III. Percent of Cells Parasitized by *B. melitensis* Revealed by Modified Machiavello Staining on Preparations from Sykes-Moore Chambers.

Time (hr)	Normal histiocytes	Immune histiocytes
0	(a) 21% (1-2 bact/cell)	25% (1-2 bact/cell)
	(b) 22% (*idem*)	24% (*idem*)
24	(a) 79% (49% 1-5 bact/cell; 30% 5-15 " ")	44% (never more than 10 bact/cell)
	(b) 78% (48% 1-5 bact/cell; 30% 5-15 " ")	52% (*idem*)
48	(a) 90% (all well above 15 bact/cell)	10% (1-5 bact/cell)
	(b) 98% (*idem*)	
72	(a) (b) 95% (many bact/cell)	11% (1-2 bact/cell)
		7% (*idem*)
144	(a) (b) All cells degenerated	100% infected

is nevertheless quite clearly demonstrated, both in terms of numbers of cells parasitized and in terms of numbers of brucellae per cell. This is shown in the data presented in Table III.

Unpublished data showed also that the peritoneal histiocyte induced by peritoneal inflammation is exceeded in its activity by immune alveolar histiocytes.

Fluorescein-labeled specific antiserum was employed along with the Machiavello stain to localize the intracellular brucellae. We could find no advantage between the two procedures in the early weeks of parasitization(3). In the later stages the value of the labeled serum clearly would be to detect antigenic material no longer present as intact cell susceptible to conventional staining.

Summary. Inflammatory-induced peritoneal histiocytes of rabbit, guinea pig and monkey, both normal and immunized, respond to the cytotoxic action of parasitizing brucella within 24 hours. Normal cells undergo about 30% loss whereas immune cells resist completely the cytotoxic action for at least 96 hours and retard intracellular multiplication for at least 144 hours. Cells of rabbits, guinea pigs, and monkeys react alike in these respects. The cellular immunity produced by the Rev I strain of *B. melitensis* was also operative against virulent *B. suis*.

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Propagation of Western Equine Encephalitis Virus in Mice Following Intramuscular and Intranasal Inoculation.* (29064)

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The hypothesis has been proposed that arthropod transmission may not be essential for maintenance of Western equine encephalitis (WEE) virus in the natural wildlife hosts(1). The purpose of this study was to determine the distribution of a non-neuro-adapted strain and a cloned variant of WEE virus in experimentally infected mice and to

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find out if infection of the respiratory tract could play a role in maintenance of the virus infection in rodents.

Materials and methods. The B628 strain of WEE virus used for this study was derived from a spleen-heart-lung-kidney pool of a 6-7 day old English sparrow nestling, *Passer domesticus*, collected in Kern County, California, July 29, 1957. This strain was isolated and maintained in hamster kidney tissue culture. Passage 3 (HK3) was used as an example of the natural non-neuro-adapted WEE virus. The stock HK3 virus had an LD_{50} titer of $10^{-6.7}$ when tested by intracerebral inoculation in infant mice. A variant of this strain (designated clone 15) was obtained by cloning on chick embryo cells(2,3). The stock clone 15 virus had an LD_{50} titer of 10^{-5} when tested by intracerebral inoculation in infant mice.

Twelve 3-week-old female mice were inoculated intramuscularly with 0.015 ml of the 10^{-1} dilution, 10 with the 10^{-2} dilution and 12 with the 10^{-3} dilution of the stock HK3 virus. Thirty-four 3-week-old female mice were inoculated intranasally with 0.015 ml of the 10^{-2} dilution of the stock clone 15 virus.

Throat swabs were obtained by wetting a cotton tipped wooden applicator stick in 1 ml of 0.75% bovine albumin in buffered saline (BAPS), and then swabbing the mouth. The cotton swab was replaced in the BAPS agitated, then discarded. The BAPS suspension was stored at —70°C until tested. Blood was collected by intracardiac puncture using a syringe wet with heparin solution (1000 U.S.P. units per ml). Whole blood was stored at —70° C until tested. Blood and throat swab specimens were collected from a single mouse but different mice were used on successive days. For tissue virus studies a mouse was bled out and then the brain, lungs, kidneys, pancreas and salivary glands were removed. Separate instruments were used for removing each organ and each specimen was cut with scissors and rinsed once or twice in saline to remove the remaining blood. The organs were stored at —70°C until tested. Each tissue specimen was ground in a mortar and diluted with 4 ml BAPS.

Blood and tissue specimens were tested for virus by intracerebral inoculation in 1-2-day-old infant mice. Throat swab specimens were tested for virus by intraperitoneal inoculation in 1-2-day-old infant mice. The blood specimens were tested without addition of antibiotics. Penicillin (500 U/ml) and streptomycin (1 mg/ml) were added to the other tissue specimens and the throat swab specimens. Titrations of blood specimens were done using log dilutions and BAPS as a diluent. The LD_{50} was calculated by the method of Reed and Muench(4). All positive specimens were confirmed by the fluorescent antibody test for WEE virus.

Results. A viremia was present in all mice tested on days 1 through 3 following intramuscular inoculation with the HK3 strain of WEE virus, and in one of two mice tested on day 4 (Table I). On day 7, 2 out of 7 mice had a viremia, both with titers less than $10^{-0.5}$. On day 3, one throat swab was positive for WEE virus, the remainder were negative. The distribution of virus in the organs tested was as follows: day 7, 7/7 brains, 7/7 kidneys, 3/7 lungs, 2/7 pancreases were positive; day 10, 6/7 brains, 1/7 kidneys were positive. The remainder of the organs tested were negative for WEE virus (Table I).

A viremia was demonstrated in all mice tested on days 1 through 4 following intranasal inoculation with clone 15 virus (Table II). The highest titer was $10^{-3.7}$ occurring on day 3. Three out of 6 brains harvested on day 7 and 1 out of 5 harvested on day 10 were positive for WEE virus. The throat swabs and the remainder of the organs tested were negative for WEE virus.

The morbidity rate is not given because mice were harvested and tested at intervals. The non-neuro-adapted HK3 WEE virus when given to young adult mice by intramuscular inoculation ordinarily produces a morbidity of approximately 20% and a mortality of 5 to 10 %. The clone 15 WEE virus does not produce obvious disease when given by intracerebral, intranasal or intramuscular inoculation to young adult mice.

TABLE I. Distribution of HK3 WEE Virus in Adult Mice Following Intramuscular Inoculation.

Specimen	Dilution–0.015 ml intramuscular	Days after inoculation 1	2	3	4	5	6	7	8	9	10	11	13	14	15
Throat swab	10^{-1}	0/1*	0/1	1/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
	10^{-3}	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Blood	10^{-1}	1/1 ($>10^{-3.5}$)	1/1 ($10^{-2.8}$)	1/1 ($10^{-2.8}$)	1/1 (10^{-4})	0/1	0/1	1/1†			0/1				0/1
	10^{-2}							0/5			0/5				
	10^{-3}	1/1 (10^{-4})	1/1 ($10^{-3.6}$)	1/1 ($10^{-2.6}$)	0/1	0/1	0/1	1/1†			0/1				0/1
Brain	10^{-1}							1/1			0/1				0/1
	10^{-2}							5/5			5/5				
	10^{-3}							1/1			1/1				0/1
Kidney	10^{-1}							1/1			0/1				0/1
	10^{-2}							5/5			1/5				
	10^{-3}							1/1			0/1				0/1
Lung	10^{-1}							0/1			0/1				0/1
	10^{-2}							2/5			0/5				
	10^{-3}							1/1			0/1				0/1
Salivary gland	10^{-1}							0/1			0/1				0/1
	10^{-2}							0/5			0/5				
	10^{-3}							0/1			0/1				0/1
Pancreas	10^{-1}							0/1			0/1				0/1
	10^{-2}							2/5			0/5				
	10^{-3}							0/1			0/1				0/1

* No. positive for WEE virus/No. tested. ( ) LD_{50} titer. † $<10^{-0.5}$

TABLE II. Distribution of WEE Virus in Adult Mice Following Intranasal Inoculation with Clone 15 Strain.

Specimen	Dilution–0.015 ml intranasal	Days after inoculation 1	2	3	4	5	6	7	8	9	10	11
Throat swab	10^{-2}	0/1*	0/1	0/1	0/1	0/1	0/1	0/1	0/10	0/1	0/1	0/10
Blood	10^{-2}	1/1 ($10^{-0.5}$)	1/1 ($10^{-2.8}$)	1/1 ($10^{-3.7}$)	1/1 ($10^{-1.4}$)	0/1	0/1	0/1			0/5	0/1
Brain	10^{-2}							3/6			1/5	0/1
Kidney	10^{-2}							0/6			0/5	
Lung	10^{-2}							0/6			0/5	0/1
Salivary gland	10^{-2}							0/6			0/5	0/1
Pancreas	10^{-2}							0/6			0/5	

* No. positive for WEE virus/No. tested. ( ) LD_{50} titer.

Discussion. The non-neuro-adapted HK3 strain of WEE virus was shown to be present in the lung tissue of 3 mice at 7 days after intramuscular inoculation with the virus. In one instance the virus was isolated from a throat swab specimen taken on the third day after intramuscular inoculation with the virus. This suggests that the virus could be spread *via* the respiratory secretions. Isolation of the virus from kidney and pancreas tissue specimens also suggests that the virus could be eliminated *via* the urinary or gastrointestinal systems. Although these experiments did not show a consistent infection of the respiratory tract of mice with the WEE virus used, it is possible that in nature the virus could be maintained by natural passage *via* this route, in view of the ease with which mice can be infected by intranasal inoculation. The frequent invasion of the brain without relation to symptomatology is of particular interest and deserves further study.

The attenuated strain of WEE virus consistently produced an asymptomatic infection in mice when inoculated intranasally. However, by this route of infection this variant of WEE virus did not localize in the lung, pancreas or kidney. After the period of viremia the virus was demonstrated only in the brain but less frequently than with the original virus strain.

Summary. A non-neuro-adapted WEE virus was demonstrated in the blood, brain, kidney, lung, pancreas and throat swab specimens of mice following intramuscular inoculation. The viremic phase ordinarily lasted from days 1 through 4. The virus was found in the brains of nearly all of the mice at 7 and 10 days after inoculation. The attenuated variant of the WEE virus infected mice when inoculated intranasally. The virus was demonstrated in the blood of all mice tested on days 1 through 4. At 7 and 10 days after inoculation the virus was occasionally found in the brain. The distribution of WEE virus in the organs of experimentally infected mice is consistent with the hypothesis that arthropod transmission may not be essential for the maintenance of the virus in rodent populations.

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