TABLE I. Results of Tests of Mosquitoes for Viruses, Weld County, Col., May-August, 1950.

Mosquito species	No. tested	Neurotropic viruses isolated WEE
Culex tarsalis	1141	0
Culiseta inornata	197	0
Aedes dorsalis	4492	2
vexans	1928	0
cataphylla	64	0
sticticus	57	0
trivittatus	6	0
Total	7885	2

while attempting to bite, anesthetized lightly with ether, separated by species into ampoules, the ampoules sealed in an acetylene flame, and the still living specimens frozen on dry ice. Ten to 50 mosquitoes were sealed in each ampoule and sent frozen to the CDC Virus and Rickettsial Section, Montgomery, Ala., for test. In the laboratory the mosquitoes were triturated in a sterile mortar with buffered saline containing 30% by volume of inactivated normal rabbit serum, diluted to a volume of 1 to 3 ml depending on the number present, and the suspension centrifuged in a cold room at 13,000 rpm in an angle centrifuge. The supernatant fluid was then inoculated both intracerebrally (0.02 ml) and intraperitoneally (0.04 ml) into 12- to 14-day-old white mice. The brains of mice which died or showed symptoms of encephalitis were removed, tested for bacteria, and suspensions of them inoculated intracerebrally into fresh mice. Finally, the isolated viruses were identified serologically by cross protection tests.

Results. The study in the summer of 1950 in Weld County, Colorado, resulted in the collection and testing of a total of 7,885 mosquitoes, comprising 7 species as shown in Table I. All the mosquitoes were captured between May 2nd and August 29th.

The viruses isolated from each of 2 ampoules of *A. dorsalis* had the following characteristics: Each strain passed through a Seitz filter pad; was free from bacteria; was lethal to mice in dilutions of $10^{-6.5}$ to $10^{-8.5}$; and was neutralized by an antiserum of western equine encephalomyelitis virus, but not by that of eastern equine encephalomyelitis or the St. Louis encephalitis viruses. The identification of the virus strains as those of western equine encephalomyelitis was completed by the challenge inoculation of guinea pigs previously immunized against this virus.

Among the mosquitoes identified throughout the summer as *A. dorsalis*, it was noted that considerable numbers of a dark form appeared which were readily distinguishable, morphologically, from the normal *A. dorsalis*. The dark form of *A. dorsalis* has also been noted in California(7) and one of the 2 lots found infected with WEE virus consisted entirely of the dark form. The dark form was routinely placed in ampoules separate from the normal *A. dorsalis*.

Summary. 7885 mosquitoes of seven species were collected in Weld County, Colo., in 1951 and tested for neurotropic viruses. Two strains of western equine encephalomyelitis virus were recovered from 2 lots of *Aedes dorsalis*.

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Encephalitis in Midwest VIII. Neutralizing Antibodies in Sera of Small Wild Mammals. Colorado 1950. (19050)

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A series of exploratory investigations were carried out by the Communicable Disease Center in Weld County, Colorado, in 1950, in an attempt to find a promising lead which might result in the clarification of the ecology of the viruses of western equine encephalo-

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myelitis (WEE) and St. Louis encephalitis (St. Louis) in nature. The results of one of these limited studies, which was undertaken to evaluate the part played by the small mammals, particularly those belonging to the order Rodentia, will be reported here. Studies carried out on this group of animals in the past 10 years have shown that although there is evidence of past infection, the percentage of animals affected is less than that found in birds or domestic animals. In California in 1941, Hammon and his colleagues(1) examined sera from 12 species of wild animals for neutralizing antibodies and they found that less than 10% were positive against either WEE or St. Louis viruses. At the same time, domestic birds gave evidence of almost 50% infection against each of the viruses, and wild birds were approximately 30% infected. Howitt and Herrick(2), in an endemic area, found that practically 100% of the domestic animals were positive against the western virus and 60% against the St. Louis virus, while with sera from the wild mammals in the same area the positives were again less than 10%. This was confirmed in another study by Hammon *et al.*(3), wild animals being again less than 10% positive against each of the viruses, while domestic animals and birds were approximately 35% and 50% positive, respectively. However, the virus itself had been recovered once from a ground squirrel(4), and possibly (again) on a second occasion; and there are indications that St. Louis virus can live in the brain of a field mouse (*Reithrodontomys megalotis*) for as long as 10 days without showing evidence of infection(5). In the Argentine, a disease causing high mortality in young nutria (*Myocaster coypus*) has been recognized since 1944, and recently a virus of the equine encephalomyelitis group has been recovered from these rodents(6). In addition, rodents have been suggested as the possible natural reservoir for the "encephalomyocarditis" group of viruses(7). On the other hand, WEE virus had been recovered only once from a bird in nature(8), and St. Louis virus had not been recovered at all.

In Weld County, Colorado, in 1949, during a small epidemic of WEE virus infection in humans and horses, the Center conducted a brief survey of the antibodies of WEE and St. Louis viruses in the bird population and found that out of 100 wild and domestic bird sera tested, approximately 30% gave positive reactions against WEE, but all were negative against the St. Louis virus(9). The present study consisted of testing sera from a wide range of small wild mammals in Weld County for neutralizing antibodies to these viruses to see if the percentage of positives would equal or exceed that of the birds. In such an event, it was planned to extend the studies and attempt to isolate the virus from the small mammals. However, if 10% or less were positive, as found in California, it would be concluded that the role of these mammals as a permanent reservoir of the viruses would be less than that of the birds, and further efforts would be concentrated on the birds rather than mammals.

Methods. Small mammals were trapped alive by all available methods, bled, and their sera separated. The specimens were sealed in tubes and stored in dry ice until shipment could be undertaken to the Center's virus and rickettsia laboratories in Montgomery, Ala. The specimens were packed in dry ice and shipped by air express. The technic of calculating the neutralization index was that

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3. Hammon, W. McD., Lundy, H. W., Gray, J. A., Evans, F. C., Bary, F., and Izumi, E. M., *J. Immun.*, 1942, v44, 75

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TABLE I. Weld County, Colo., 1950. Small Mammal Sera. Tested for neutralizing antibodies against the viruses of Western Equine Encephalomyelitis and St. Louis Encephalitis.

Species	No. tested WEE	No. WEE positive	No. tested St. Louis	No. positive St. Louis
Rodents				
Perognathus hispidus paradoxus (Kans. Pocket Mouse)	5		3	
Dipodomys ordii richardsoni (Richardson's Kangaroo Rat)	37	1	25	
Onychomys leucogaster arcticeps (Great Plains Grasshopper Mouse)	2		1	
Reithrodontomys megalotis dychei (Prairie Harvest Mouse)	1		1	
Peromyscus maniculatus osgoodi (Black-eared Deer Mouse)	6		4	
Microtus pennsylvanicus modestus (Sawatch Meadow Mouse)	5		3	
Rattus norvegicus (Norway Rat)	18		7	
Spermophilus tridecemlineatus pallidus (Pale-striped Ground Squirrel)	2		1	
Spermophilus spilosoma obsoletus (Northern Spotted Ground Squirrel)	4		1	
Cynomys ludovicianus (Black-tailed Prairie Dog)	26		15	
Scirus niger rufiventer (Western Fox Squirrel)	6	1	5	1
Other mammals				
Myotis volans interior (Interior Long-legged Bat)	2			
Procyon lotor (Raccoon)	5		3	
Mephitis mephitis varians (Long-tailed Texas Skunk)	3		3	
Taxidea taxus taxus (Badger)	1		1	
Lepus townsendii campanius (White-tailed Jack Rabbit)	6	1	5	
Lepus californicus melanotis (Great Plains Jack Rabbit)	24	2	14	
Lepus americanus bairdii (Rocky Mt. Snowshoe Rabbit)	1		1	
Sylvilagus sp. (Cottontail)	37		16	
Total	191	5	109	1

given in the U. S. Army laboratory manual. All sera were inactivated at 56°C for 30 minutes. The serum virus mixtures were prepared in the late afternoon and held overnight in the refrigerator before the inoculation of the mice. Indices of 50 or more were regarded as positive. Results of the survey are given in Table I.

Discussion. It has been pointed out frequently by workers on yellow fever that results of serum surveys in wild birds and mammals are most difficult to evaluate. Certain of the sera from these animals possess non-specific viricidal substances which can give false positives in the neutralization tests. Moreover, neutralizing antibodies develop in some of the animals, following a viremia so transient as to prevent these animals from taking any prominent part in the permanent maintenance of the infection in nature. In other animals the antibodies tend to disappear very rapidly, so that a negative result may not necessarily mean that the animal in question has never been infected.

With these points in mind, it may be concluded from our figures that the percentage of positives found in the survey of mammals is well below the percentage for birds. These findings give little encouragement for the continuance of further work on this type of animal life.

Summary. (1) A serum survey of small mammals for neutralizing antibodies showed that 2.6% were positive against WEE virus and less than 1% against St. Louis virus. (2) In view of the finding some months ear-

lier that wild and domestic birds gave 30% positives, it was concluded that further study of the small mammals was less likely to be productive of major results than the study of birds.

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Intraperitoneal Susceptibility of Suckling Mice to MEF1 Poliomyelitis Virus.* (19051)

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Casals and Olitsky have recently adapted the MEF1 strain (Lansing type) of poliomyelitis virus to suckling mice, and have demonstrated that a specific complement fixing antigen may be produced from the CNS of these animals(1-3). The present communication reports the results of experiments designed to determine the susceptibility of suckling mice to the intraperitoneal inoculation of this adapted MEF1 strain. The positive results obtained suggest the possibility that this may provide a useful and sensitive method of determining the antibody content of various agents used to confer passive protection. Preliminary experiments employing gamma globulin tend to bear this out.

Materials and methods. The suckling mouse adapted strain of MEF1 virus (54th passage) was made available to us through the kindness of Dr. Casals and was maintained in our laboratory by intracerebral passage in 3-day-old mice which were sacrificed when moribund or paralyzed. The brains and spinal cords were harvested and pooled; the titer of the resulting suspensions varied from $10^{-3.5}$ to $10^{-4.4}$ when titrated in 3-4-week-old mice

TABLE I. Mortality of Mice of Various Ages Inoculated with .05 ml or .1 ml of 10^{-1} Virus Dilution by the Intraperitoneal Route.

Age, days	Mortality ratio of mice: Exp. 1	2	3	4	5	Total	Total % mortality
1	2/16*					2/16	13
2	1/7	2/12				3/19	16
3	1/5	9/22				10/27	37
5	8/11	5/20	22/38	20/30		80/160	50
6	6/15	19/46					
7	7/12	3/5				10/17	59
8	3/4					3/4	—
9		4/9				4/9	44
13					5/9	5/9	56
15					3/3	3/3	—
16					6/19	6/19	32
19					5/20	5/20	25
21–28	1/32	0/16				1/48	2
Mouse passage	60	54, 56, 60	59	59	60		
I.P. inoculum (ml)	.1†	.05	.1	.1	.1		
I.C. titer		$10^{-4.4}$	$10^{-3.7}$	$10^{-3.5}$	10^{-4}		

* Numerator/denominator = No. deaths/No. inoculated.
† Mice 1 and 2 days old given only .05 ml of virus suspension in Exp. 1.

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