In comparing the immunogenic properties, the attenuated virus seems to be superior to UV Col SK virus in dosage and number of injections to immunize. From the fact that a single injection of 10^{-4} dilution of the attenuated virus was enough to immunize mice, it is considered possible that the virus may have propagated subclinically. Similarly, Sabin(9,10) reported that attenuated poliovirus which had been cultured in rhesus monkey cells lost its virulence by intraspinal injection in the chimpanzee but still retained it for rhesus and cynomolgus monkeys. Berge, Banks and Tigertt(11) used guinea pig heart cells to attenuate Venezuelan encephalomyelitis virus in mice. Findlay and Mac Callum(12) passed a pantropic yellow fever virus in mouse carcinoma 63 and found that the passed virus lost pathogenicity for the monkey but retained virulence for mice. From these reports it seems that attenuation of the viruses was induced by use of the heterologous hosts. On the other hand, in our case, attenuation of Col SK virus was obtained during cultivation in homologous mouse tumor cells and in a homologous ascites medium. It is of interest that the virus, now adapted to growing in tumor cells *in vitro*, is unable to grow in normal cells and in the same tumor cells in the homologous animal.

Summary. Attenuation of Columbia SK virus by *in vitro* cultivation in 30 serial passages in Sarcoma 180 ascites cells is described, during which the virus lost neurotropic virulence and the HA property. Nonspecific HA inhibitor in the culture medium has no connection with the changed virus nature. The attenuated virus could be used as a live vaccine for prophylaxis of Columbia SK mouse infection.

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Overwintering of Western Equine Encephalomyelitis Virus in Garter Snakes Experimentally Infected by *Culex tarsalis*. (27225)

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Previous studies have shown that garter snakes are readily infected with western equine encephalomyelitis (WEE) virus either by bites of infected mosquitoes or by the intraperitoneal (IP) injection of infected mouse brain tissue(1). Snakes inoculated by the IP route with 1 million suckling mouse LD_{50} of WEE virus have been shown to hibernate overwinter under simulated natural conditions and to circulate virus as long as 70 days the following spring and summer(2). *Culex tarsalis* became infected by feeding upon these post-hibernating snakes and transmitted the virus to young chicks.

Although garter snakes inoculated IP were shown to be capable of serving as an overwintering reservoir of WEE virus, a natural means of introducing the virus into snakes would have perhaps altered the picture. The present study was undertaken to determine

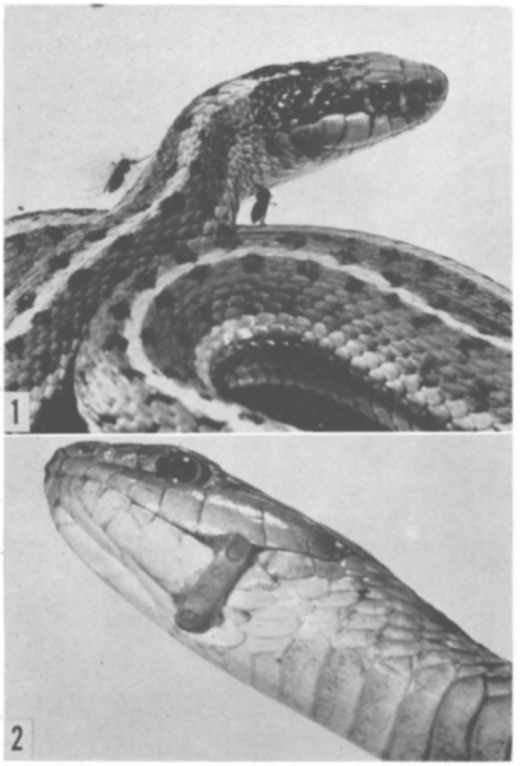

FIG. 1 and 2.

whether garter snakes infected by the bites of infected mosquitoes could serve as an overwintering reservoir for WEE virus.

Materials and methods. The hibernation cage has been described previously(2). Temperatures for the area were as follows:

Month	Minimum	Maximum	Median
Oct.	21°F	82°F	48°F
Nov.	3	59	36
Dec.	0	48	23
Jan.	−2	52	30
Feb.	21	56	38
Mar.	15	67	39
Apr.	19	68	43
May	29	84	52
June	38	98	66
July	40	96	69

The strain of virus used was originally isolated from *C. tarsalis* from the State of Washington in 1957 and had undergone one chick passage and three suckling mouse passages.

The strain of *C. tarsalis* used to infect garter snakes was received from the Communicable Disease Center, Greeley, Colorado, through the courtesy of the late John S. Blackmore. Two blood-engorged mosquitoes are shown resting on a garter snake (Fig. 1).

Wild-caught Great Basin garter snakes (*Thamnophis ordinoides vagrans* Baird and Girard) were supplied through the courtesy of Dr. Wayne Jensen, Director of the Bear River Refuge, Brigham City, Utah. Fish tags (style 1005, size 1, obtained from National Band and Tag Co., Newport, Kentucky) were applied through the lower jaw to assure correct identification of snake by number (Fig. 2).

Technics of infecting mosquitoes, bleeding snakes, isolating, identifying, and titrating virus have been described(2).

Laboratory-reared *C. tarsalis* became infected after feeding on young chicks experimentally infected with WEE virus. After an extrinsic incubation period of 6-14 days, the "infected" mosquitoes fed on garter snakes and then suspensions of these mosquitoes were tested for virus. In some instances snakes were checked for viremia before they were placed in the cage for hibernation overwinter. Six "normal" garter snakes, not tagged, were placed in the cage along with the "infected" snakes as controls. In an attempt to determine the influence of temperature on appearance of viremia in post-hibernating snakes, a few were caught and held several days at room temperature before they were returned to the hibernation cage. In general, snakes were returned to the hibernation cage immediately after each bleeding.

Results. The method of tagging snakes proved satisfactory. Numerals on tags of post-hibernating snakes were distinct as shown in Fig. 2. Snakes were not adversely affected by the tag.

Twenty of the 23 tagged snakes put into the hibernation cage (Table I) were recovered and tested for circulating virus. Snakes are listed in the order of their capture date. WEE virus was isolated and identified at various times from 9 of the 20, questionable isolations* were made from 2, and no virus was detected in the remaining snakes. Virus was not detected in control snakes bled at

* Questionable isolation: Deaths in mice attributable to malnutrition, cannibalism, or possibly infection with WEE virus.

TABLE I. WEE Virus Isolations from Post-Hibernating Snakes Infected by Mosquitoes.
(Hibernation study 1960-61)

Snake No.	Mosq. fed on WEE-chick (date)	Mosq. fed on snake			Viremia in snakes (days after mosq. fed)	Snake put in cage	Snake recovered from cage	Days after snake recovered on which blood tested for presence of virus
		No.	Date	Virus†				
5035	9/20	4	9/26	+		9/26	3/13	[*0,*2*,4,7] 8,15,22,36,(51)(57) [(63)(64)(65)(66)(67)(70)(72)(80)]
5045	10/19	19	10/27	+	4	10/31	3/13	[0,(2),*4*,7] 8,15,24,36,51
5031	9/20	2	9/27	+		9/27	3/14	[(0)(1),*3*,6] (14)(21)(35)(50)(56) [(63)(64),*65,*66,*69*,*71,(79)]
5029	9/20	4	9/28	+		9/28	3/19	(1)
5036	9/15	3	9/27	+		9/27	3/19	[*1*,3] 16,(30)
5043	10/ 5	4	10/12	+	(5)(9)(12)(14)	10/26	3/30	(0)(19)(34)(40)(47)(55)
5027	9/15	4	9/27	—		9/27	4/17	(1)(16)(22)(37)(45)(51)(60)(66)(72)(79)(85)
5028	9/15	3	9/28	*		9/28	4/17	(1)(16)(22)(29)(37)(45)(51)(60)(66)(72)(79)(85)
5032	9/27	1	10/ 5	—		10/ 5	4/17	(1)(16)(22)(29)(37)(45)(51)(60)(66)(72)
5037	10/11	73	10/19	+	*2,(5)(7)	10/26	4/17	(1)(16)(22)(29)(37)
5040	10/11	30	10/18	+	(3),8	10/26	4/17	(1)(16)(22)(29),37,*45*,51,(60)(66)(72)(85)
5041	10/19	3	10/28	+	(3)	10/31	4/17	(1),16
5042	10/11	3	10/21	+	5	10/26	4/17	(1)(16)(22)(29),*37*,(51)(60)(66)(72)(79)(85)
5044	10/11	11	10/19	+	(2),7	10/26	4/17	(0)(16)(22)(29)(37)(45)(51)(60)(66)(72)(79)(85)
5026	9/14	1	9/28	+		9/28	5/ 3	(0)(6)(13),*21*,29,35,44,(50)(56)(63)(69)
5030	9/15	1	9/27	+		9/27	5/ 3	(0)(6)(13)(21)(29)(35)(44)
5033	10/11	56	10/18	*	(3)(8)	10/26	5/ 3	(0)(6)(13)(21)(29)(35)(44)(50)(56)(63)(70)
5046	10/11	21	10/18	+	3,(8)	10/26	5/ 3	(0)(6),*13
5047	10/11	5	10/20	+	6	10/26	5/ 3	*0,*6
5049	10/19	26	10/26	+	(5)	10/31	5/ 3	(0)(6)(13)(21),*29*,35,*44,(50)(56)(63)(69)
5034	9/20	33	9/27	+		9/27	—	
5039	10/25	6	11/ 2	+	*5	11/ 7	—	
5048	10/11	2	10/21	—	(3)(5)	10/26	—	

* Questionable isolation.
† Mosq. tested for virus in suckling mice.

[ ] Snakes held at laboratory temperatures = ± 22°C. ( ) No virus detected. Italicized figures indicate day virus identified.

various intervals throughout the study.

Virus was detected in one snake (No. 5045) on the initial day (day 0) of recovery from hibernation cage, while in another snake (No. 5040) it was not detected until after the 29th day. Snake No. 5030 developed a viremia within the first week after it had been held at room temperature. After it was returned to the hibernation cage, blood samples obtained at weekly intervals up to the 56th day remained free of virus, however when the snake was again held at room temperature a short period of viremia was demonstrated. WEE virus was identified from blood samples of this snake collected on days 3 and 69.

On day 7, following capture of snakes No. 5035 and 5045, virus was detected in blood dilutions of $10^{2.8}$ and $10^{5.0}$, respectively.

Discussion. In previous studies, means of satisfactorily identifying individual snakes was a problem. Adhesive tape tags applied to the tail have been used with moderate success, however, the tags had to be replaced at various intervals because of molting of skin and poor legibility of numerals. Tags became lost in several instances. Other methods of marking snakes, such as dyeing of skin, tattooing numerals on skin, and the notching of scutes in coded patterns have been used with varying degrees of success. The use of fish tags has proven most satisfactory for tagging reptiles used in studies of long-term duration.

In attempting to explain the failure to demonstrate circulating virus in certain snakes it is noted that virus was not detected in mosquitoes that fed on snakes Nos. 5027 and 5032, questionable isolations were made from mosquitoes that fed on snakes Nos. 5028 and 5033, and the intervals between bleedings of the remaining 5 snakes (Nos. 5030, 5037, 5041, 5043, and 5044) may not have been spaced properly to have detected viremia of short duration.

Effect of temperature appears to be of great importance in the appearance of viremia. A larger percentage of snakes would have perhaps developed detectable viremia if all snakes had been maintained at room temperature after recovery from the hibernation cage. It appears that, in northern climates, cold-blooded animals should be maintained at room temperature several days before final attempts are made to isolate virus from the blood.

In the general area where the present studies were conducted, the earliest emergence of *C. tarsalis* and garter snakes occurs at essentially the same time. In this study, virus was shown to circulate in snakes as early as March 13 (Snake No. 5045) and as late as June 14 (Snake No. 5026), which is more than sufficient time to permit infection of overwintering mosquitoes and their progeny. The delay in appearance of and persistence of viremia should afford excellent opportunity for a mosquito-snake virus transmission cycle to take place. It has been shown previously(2) that garter snakes develop a viremia of sufficient titer to infect *C. tarsalis*.

Although the isolation of WEE virus from snakes collected in nature has not been reported, further evidence is presented here to indicate the possible role that these cold-blooded vertebrates may play in the epidemiology of this important virus disease.

Summary. Garter snakes were infected with western equine encephalomyelitis (WEE) virus by the bites of experimentally infected *Culex tarsalis*. The snakes hibernated overwinter under simulated natural conditions and viremia was detected up to 69 days after emergence the following spring. Snakes which were maintained at room temperature after emergence developed viremia within a few days, while those maintained under simulated natural conditions required up to approximately 5 weeks. As shown in previous studies, circulating virus was detected in concentrations sufficient to infect *C. tarsalis*. The use of fish tags proved satisfactory in assuring positive identification of individual snakes.

The technical assistance of Jack Cory and Edward Patzer is gratefully acknowledged.

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