monary reaction observed macroscopically at autopsy. The pathogenicity and invasiveness of the neoplastic cells were evidently altered during their residence in inbred mice. How or when these changes occurred is a matter of speculation. On transmission to random-bred mice the altered cellular behavior was indicated by an increased toxic reaction in lymphoid tissue and by regular proliferation in the lungs. The latter change was so constant on both intraperitoneal and intranasal injection that the term pneumotropic line seemed appropriate.

In view of the present emphasis on viruses in the etiology of certain murine tumors including the leukemias the possible role of the respiratory tract in outward dissemination of these agents may be worthy of consideration.

There was no apparent difference between the 2 leukemic lines in regard to their effect on emergence of murine hepatitis virus in Princeton weanlings following intraperitoneal injection. Similar viruses were obtained with both lines. There was reason to believe, however, that nasal instillation of the high level leukemic cells provided a means of avoiding the liver lesions produced by these latent agents.

Summary. 1) Lymphocytic leukemia, of sporadic occurrence in random-bred Princeton mice (3.1% in 1050 adults), was transmitted to weanlings by intraperitoneal injection of lymphoid organ suspensions and maintained by serial passage. Spleens and regional lymph nodes were commonly enlarged but lungs were not involved. 2) A high level leukemia line from inbred Princeton mice, originally established by Dr. Clara Lynch, was similarly maintained in random-bred weanlings. Neoplastic cells also proliferated in lymphoid tissue, though with evidence of increased toxicity, and also invaded the lungs. Pulmonary lesions as well as generalized ones were regularly produced by intranasal injection of cells from high leukemia line but not from the low level one. 3) Necrotic lesions of liver appeared in one passage series with each of the 2 lines and murine hepatitis viruses of low virulence were recovered.

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Overwintering of Western Equine Encephalitis Virus.* (25955)

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The mechanism of maintenance of Western equine encephalitis virus in nature during non-epidemic periods and particularly during cold winter months has puzzled many investigators. This virus has been isolated from many species of birds(1,2,3) and a chronic viremia has been produced in experimentally infected wild birds from 1 to 10 months duration(4). This finding has suggested the possibility of an overwintering reservoir for the virus. In studies of 1503 birds, Kissling *et al.* (5) failed to isolate virus from wild birds either in winter or spring, but during the summer when mosquitoes were active virus infected birds were present. Although WEE virus has been isolated repeatedly from pools of mosquitoes collected during practically every month of the year in temperate cli-

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mates(6), there appears to be only one successful isolation from mosquitoes collected during winter months in an area of colder climate. Blackmore and Winn(7) found WEE virus in one pool of 50 female *C. tarsalis* mosquitoes taken from mine shafts in Colorado. The pool showing virus was one of 62 prepared from mosquitoes collected during Dec., Jan., and Feb. A total of 1361 females were obtained under conditions of cold weather in Colorado. Some authors(8,9) have discounted the importance of the surviving female *C. tarsalis* as a means of overwintering of WEE virus. Rosenbusch(10) was able to infect several species of snakes with WEE virus. He infected a largato snake (*Tupinambis teguixin*), a *Bothrops amnodytoides* snake and one *Bothrops alternata* maintained the virus in the brain for 37 days but not in the blood. Two *Phylodryas culebra* snakes injected with WEE virus failed to harbor virus in the brain or blood. Thomas *et al.*(11) were able to infect common garter snakes (*Thamnophis* spp.) by either direct injection of virus or by allowing infected *Culex tarsalis* mosquitoes to obtain a blood meal. Virus was maintained by one of these snakes for 36 days. Lennette *et al.*(12) has suggested that squirrels may play a role in maintenance of the virus, the virus having been found in the nervous tissue of wild squirrels caught during the summer. During 1958 the majority of the state of Utah experienced an unusually hot, dry spring, and early summer necessitating more irrigation than usual. *C. tarsalis* mosquitoes began to appear in increased numbers in May and into the summer months(13). Cases of Western equine encephalitis were first reported in horses in June and human infections were evident beginning in July. A total of 58 proven human cases with 6 deaths were reported. This paper reports results of a continuing study on the mechanism of overwintering of WEE virus in nature.

Materials and methods. Snakes (*Thamnophis* spp.) were collected, beginning in April and each month through July, 1959. In preliminary hibernating experiments 4 garter snakes (*Thamnophis* spp.) were injected intraperitoneally with 0.25 ml of Western equine encephalitis virus (LD_{50} $10^{-5.5}$ in suckling mice) diluted 10^{-1}. The snakes were kept at 4°C for 17 days (July, 1959) when they were then bled and sacrificed for nervous tissue. Twenty-one *Thamnophis* spp. snakes were injected intraperitoneally with 0.25 ml of WEE virus (LD_{50} $10^{-6.5}$ in suckling mice) Dec. 11, 1959, then placed in outside hibernating cages simulating natural hibernation conditions. The cages were buried 3 feet deep underground with burrows to the surface. Outside temperatures varied from +47°F to −1°F during the winter. Temperatures inside the underground cages varied from 15 to 50 F. Relative humidity varied from 31 to 83%. Five uninoculated controls were placed in the same outside cage which housed the inoculated animals. Bleedings were done Dec. 30, 1959, Feb. 13, Mar. 12, and Apr. 28, 1960. One-tenth ml of blood was added to 0.9 ml sterile skim milk and inoculated into suckling mice within 5 minutes after blood samples were obtained. The milk-blood mixtures were then held at −28°C and bloods showing positive virus content were then titrated in suckling mice.

Neutralization tests were carried out using known WEE positive immune serum and virus from snake bloods. The immune serum was held constant (1:10) and the virus was diluted with sterile skim milk. After 2 hour incubation at 37°C the mixtures were then injected intracerebrally into suckling mice in doses of 0.025 ml.

Results. Virus (WEE) was isolated from the blood of hibernating snakes 19, 64, 92, and 139 days after inoculation and then hibernating during the winter months. Virus decreased in quantity as the winter came to a close, but an increase of virus was present beginning with warm weather in April in not only a greater number of the snakes blood, but the titer increased. Results are presented in Table I.

Six inoculated snakes died during the hibernation period, 5 of which strangled in the underground wire cages. One developed a necrotic tail and was not bled in March nor April. Three of the uninoculated controls died during the hibernation period. No evidence of illness was noted in any snakes. No

TABLE I. Results of Overwintering of WEE Virus in Hibernating *Thamnophis* spp. Snakes. Injected Dec. 11, 1959 with 0.2 ml intraper., mouse brain WEE virus (LD_{50} $10^{-6.5}$).

Bleeding date and days after inj. of WEE virus		Snake No.	Virus isolated from blood of snakes, and titer	
12/30/59	19 days	1, 2, 3, 4, 5 Control 26	1, 5	(10^{-1})
			3	(10^{-5})
			4	(10^{-3})
2/13/60	64 days	2, 3, 4, 5, 7, 8, 9, 10	2, 3, 4, 5, 7	(10^{-1})
			10	(10^{-3})
3/12	92 days	3, 4, 5, 8, 9, 11, 13, 14, 15, 17, 18, 19, 20, 21 Controls 26, 27	13	(10^{-1})
4/28	139 days	2, 3, 4, 5, 7, 8, 9, 11, 15, 16, 17, 18, 19, 21	15, 21	(10^{-1})
			4	($10^{-1.5}$)
			11	(10^{-2})
			5	($10^{-2.2}$)
			2	(10^{-3})
			19	($10^{-3.2}$)
			3, 7	($10^{-3.5}$)

virus was isolated from the blood of the controls tested.

Of the 4 snakes inoculated with WEE virus and hibernated in the refrigerator (4°C) virus was isolated from the blood but not from the brain of 2; in one, virus was demonstrated in the brain tissue, but not in the blood. No virus was demonstrated in either the blood or nervous tissue of one snake.

Virus from the snake blood was proved to be WEE by neutralization tests, using specific immune serum. The neutralization index of this immune serum was over 10^3.

Discussion. In preliminary experiments common garter snakes (*Thamnophis* spp.) have been found to maintain WEE virus for at least 139 days during winter hibernation. The outside hibernation cages simulated the normal overwintering habitat of these snakes. The data have indicated that the titer of virus in the blood of these inoculated snakes decreases during the cold months of the year. With warmer temperatures in the spring, however, the viral content of the blood of these snakes increased. This carry over of virus suggests that snakes may become a source of an infective blood meal for spring and summer hatched mosquitoes.

For epidemic or endemic cases of Western equine encephalitis to develop, there must be a source of virus for mosquitoes to initiate such infections. For mosquitoes to obtain an infective blood meal, there must be a source of blood from an animal which exhibits a viremia at the time of the mosquito feeding. Such an animal must carry the virus during the winter months and exhibit a viremia in the spring of sufficient proportion to enable the vector mosquitoes to obtain an infective blood meal.

From results presented, the common garter snake appears to fulfill the above suggested requirements of overwintering WEE virus in nature and are capable of development of a viremia in the spring. From initiation of infection of the first few mosquitoes in the spring, secondary virus hosts such as birds, could expand the virus source for more mosquitoes to become infected, thus producing a high infection in the mosquito and bird population. With a high population of infected vector mosquitoes during the summer months, horses and man become an accidental host for the virus.

Summary. 1) Adult garter snakes (*Thamnophis* spp.) inoculated intraperitoneally, overwintered WEE virus for at least 139 days. There was a steady decline in virus, with only 1 of 14 showing a viremia after 92 days hibernation. With warm spring weather, (139 days after virus inoculation) 9 of 14 animals exhibited a viremia with blood titers varying from 10^{-1} to $10^{-3.5}$. 2) Two of 4 garter snakes inoculated with WEE virus and kept at 4°C, for 17 days harbored the virus in the blood, but not the brain. One snake harbored the virus in the brain but not in the blood. No virus was detected in either blood

or brain after 17 days at 4°C in one of the snakes.

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Erythropoietic Stimulating Action of Acetylcholinesterase. (25956)

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This author reported production of experimental hyperchromic anemia in dogs by daily injection of acetylcholine(1). Liver extract or folic acid injections caused remission of this anemia with a concomitant rise in serum cholinesterase activity which was postulated to be the cause of remission. Other experiments(2) indicated that cobalt injection or production of marked anemia by phenylhydrazine caused significant increases in serum cholinesterase activity. Both of these procedures increase plasma erythropoietic stimulating factor (ESF or erythropoietin)(3,4,5). These facts suggested the possibility that cholinesterase, or part of its molecule, might be concerned with erythropoiesis, and prompted the present investigation.

Procedures and methods. Injections of Acetylcholinesterase* (200 units daily) were given subcutaneously to each of 7 normal rats weighing about 180 g. Another group of 7 untreated rats served as controls. Blood samples were obtained by cutting off the tip of the tail, before and at various intervals after commencement of daily injections. Erythrocyte counts and reticulocyte percentages were determined by standard methods, and hematocrit percentage of packed cells was determined using capillary tubes and a Clay-Adams micro-hematocrit centrifuge.

Results. Table I shows results of daily subcutaneous injections of 200 units (*i.e.,* 4 mg of commercial preparation in 1 cc of water) of Acetylcholinesterase into 7 normal rats. After 5 to 7 daily injections, reticulocyte percentages were elevated to 6.1% (average), which represented a 5-fold increase over average pre-experimental value. Erythrocyte counts were not significantly increased at this time, but after 2 weeks of daily injections, they had increased by 18% (average) or 1.4 million cells per cmm of blood. Hematocrit percentages of packed cells did not increase significantly at any time. The control group of 7 untreated rats showed no significant changes in the blood throughout period of experiments. Observations performed on only 2 experimental animals indicated that Acetylcholinesterase injections increased hemoglobin values about 10%, but caused no significant changes in total leukocyte counts.

* Acetylcholinesterase from bovine erythrocytes contained 20,000 Ammon units in 400 mg of material. (Nutritional Biochemicals Corp.)