either more or less like the native protein. According to this interpretation, incomplete polypeptide chains produced by mutants YA 559 and NG 750 (areas 8 and 10), although presumably longer than that formed by NG 125 (area 6), would be folded less like the native. This implies further that these incomplete chains exist not in random configuration but in specific conformation. In addition, it is possible that the antibody itself may serve as a directing influence in folding (*13*).

Production of CRM by nonsense mutants is generally not observed in other systems. Since β-galactosidase is a very large protein, however, it is not surprising that many incomplete polypeptide chains retain antigenic determinants.

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Western Equine Encephalitis Virus in Saskatchewan Garter Snakes and Leopard Frogs

Abstract. *Western equine encephalitis virus was isolated from two naturally infected snakes on first bleeding and from seven others at subsequent bleedings, both with and without preliminary chilling. One snake, with neither detectable virus nor serum neutralizing antibodies when first bled, developed viremia later. Viremia in garter snakes has a cyclic rhythm independent of the temperature of the environment. Virus was isolated from 6 frogs, and 50 out of 179 had detectable serum neutralizing antibodies. Infections with this virus are widely distributed in garter snakes and leopard frogs in the agricultural area of Saskatchewan.*

It has been demonstrated (*1–3*) that common garter snakes (genus *Thamnophis*) are susceptible to Western equine encephalitis (WEE) virus by both inoculation and mosquito bite and that they may serve as possible overwintering hosts of this virus. From 1961 to 1963 Spalatin *et al.* (*4*) found three species of garter snakes in Saskatchewan with serum neutralizing (SN) antibodies to WEE virus. At about the same time the virus was isolated in Utah (*5*) from four species of naturally infected snakes, including garter snakes; in these snakes viremia recurred in response to a lowering of environmental temperature. Thus the snakes could qualify as overwintering hosts of WEE virus in a temperate climate.

In Saskatchewan, frogs and garter snakes occupy the same habitats during summer months, mosquitoes are known to feed on both (*2, 3, 5, 6*), and both can be infected by the oral route (*4*). Since frogs form a large part of the natural diet of garter snakes, it seemed reasonable to investigate further the possible role of frogs in the epidemiology of WEE. Spalatin *et al.* found evidence of natural infections in *Rana pipiens* by demonstrating SN antibodies in the blood, but viremia was not detected (*4*). This report is concerned with observations on three species of garter snakes: *Thamnophis radix haydeni*, the Western plains garter snake; *T. sirtalis parietalis*, the red-sided garter snake; and *T. ordinoides vagrans*, the Great Basin garter snake; and one species of frog, the American leopard frog *Rana pipiens*.

Snakes and frogs were collected by hand in the field, shipped to the laboratory, and bled as soon after their arrival as possible. Frogs were usually bled the same day, but some snakes were not bled until 42 days after capture, during which time they were held at room temperature. Snakes were bled by inserting a fine capillary pipette behind the eye; frogs were pithed and then bled from the heart with the use of a 1-ml hypodermic syringe and 25-gauge needle. From 0.2 to 1.5 ml of blood could be obtained at each bleeding, depending on the size of the snake or frog.

The same procedures for virus and antibody studies were used on both snake and frog blood. For attempting virus isolation, sufficient blood was added to the diluent to make a 1 : 5 or 1 : 10 dilution, and the test animals were chicks up to 12 hours old and 10-day chicken embryos in groups of five to ten. The virus diluent contained, per milliliter: penicillin, 500 units; streptomycin, 2.5 mg; and heparin, 20 units; normal rabbit serum was added to give a concentration of 10 to 15 percent. Chicks were inoculated intraperitoneally or subcutaneously, and embryos allantoically, with 0.1 ml of inoculum. Adult mice and mice 3 to 5 days old, used for confirming isolations, were injected intracerebrally and intraperitoneally, respectively, with 0.03 ml of inoculum. Blood samples were either examined at the time of bleeding or stored at −20°C until they were tested. Serum obtained from each bleeding was also stored and, at the time of testing, was diluted as required. Virus isolates from snakes and frogs were identified by means of immune serum from rabbits that had been inoculated with WEE virus 85 (Rocky Mountain Laboratory) and mosquito isolate 1540–63 identified as WEE. Ten-day chicken embryos were used for testing serum samples for SN antibodies against known strains of WEE virus. Varying amounts of virus, diluted with nutrient broth containing antibiotics and 10 percent normal rabbit serum, were added to constant amounts of test serum and then incubated at 37°C for 2 hours before the eggs were injected. Normal rabbit serum was also used as the control for calculating neutralizing indices by the Reed-Muench method (*7*).

Table 1. Western equine encephalitis (WEE) virus and serum neutralizing (SN) antibodies in the blood of garter snakes (genus *Thamnophis*) at first bleeding. T.s.p., *Thamnophis sirtalis parietalis*; T.r.h., *T. radix haydeni*; and T.o.v., *T. ordinoides vagrans*. Index of neutralization ($\log_{10}$) is expressed as follows: >1.99 was considered positive; 1.01–1.99, suspicious; and <1.01, negative.

Location	Date collected (1964)	Species	WEE virus/sample*	No. of snakes with SN antibodies		
				(>1.99)	(1.01–1.99)	(<1.01)
Beadle	28 April	T.s.p.	0/2	0	0	2
		T.r.h.	0/18	0	0	18
		T.o.v.	0/2	0	0	2
Pelly	15 May	T.s.p.	1/163†	40	35	85
Herschel Dam	1 June	T.r.h.	1/1	0	0	1
Beaver Creek	19 June	T.r.h.	0/1	1	0	0
Weyburn	26 June	T.r.h.	0/1	1	0	0
Beadle	31 July	T.r.h.	0/1	1	0	1
Colgate	15 Aug.	T.r.h.		1	0	0
Weyburn	15 Aug.	T.r.h.		1	1	0
Keeler	15 Sept.	T.r.h.	0/11‡	6	7	3
Maidstone	19 Sept.	T.s.p.		1	4	34
		T.r.h.		2	6	42

* Denominator denotes number tested; numerator, number positive. † Another snake from Pelly had viremia on second bleeding 1 September 1964, and a third snake from this area had viremia at the third, fifth, and sixth bleedings on 1 March, 5 April, and 21 April 1965, respectively. ‡ Five out of 13 Keeler snakes had viremia on second bleeding, 4 to 10 March 1966.

From 28 April to 19 September 1964, 295 garter snakes were collected from eight different localities in Saskatchewan—201 *Thamnophis sirtalis parietalis*, 92 *T. radix haydeni*, and 2 *T. ordinoides vagrans*. At first bleeding WEE virus was isolated from only 2 snakes but, as indicated in Table 1, 36.2 percent had detectable SN antibodies in their blood at this time. Hence, snakes arriving in the laboratory could be divided into three categories, based on serological reactions: (i) those (186) that gave no evidence of either WEE virus or SN antibodies in their blood; (ii) those (107) that gave no evidence of WEE virus but had detectable SN antibodies; and (iii) those (2) that had WEE virus but no evidence of SN antibodies in their blood. Snakes in the third category included a *T. sirtalis*, collected at Pelly on 15 May 1964 and bled 27 May, that had viremia but no SN antibodies; this snake died 21 July. The other was a *T. radix* caught at the Herschel Dam 1 June 1964 and bled the same day; it also had viremia but no SN antibodies. The latter snake was held at room temperature for 114 days and bled again; at this second bleeding it had a serum neutralizing index of $10^{2.24}$ chick embryo LD_{50}'s (lethal dose, 50 percent effective) but no viremia. It was placed in the cold room at 5°C for 203 days and then removed and held at room temperature. During this time the snake was removed from the cold room briefly on 27 March 1965 for bleeding; it was also bled at the end of this period on 14 April. Virus was present in blood from both bleedings, but no antibodies were detected. The snake was found dead on 12 June.

Six snakes included in the second category had viremia at a later bleeding. One of these, a *T. sirtalis* collected at Pelly on 15 May 1964, had SN antibodies but no virus when bled 27 May, but on 1 September, after 109 days at room temperature, it had viremia and no antibodies. Five other snakes in the same category, all *T. radix*, collected at Keeler on 11 September, had SN antibodies in their blood but no virus when bled on 23 September. They were held at room temperature for 162 to 168 days and bled a second time; viremia was demonstrated in each of these snakes, but SN tests were negative.

One snake in the first category, a *T. sirtalis* caught at Pelly on 15 May 1964, had neither WEE virus nor SN antibodies on 27 May. It was held at room temperature and bled again on 1 September. At this time the serum neutralizing index was $10^{1.20}$ chick embryo LD_{50}'s, but no virus was isolated. It remained at room temperature until 11 January 1965, was put in the cold room until 22 February, and kept again at room temperature. At the time of the third bleeding on 1 March WEE virus was present; the snake was found dead 28 June. In the intervening time it had been bled four times; WEE virus was isolated on 5 April and again on 21 April (325 and 341 days, respectively, after capture), but no virus was detected on either 22 March or 7 June. Because this snake was small, blood was not taken for antibody studies except on 7 June; the test was negative.

From 15 July to 17 September 1964, 179 frogs were collected from seven different localities in the province; 155 of these were tested for WEE virus, and all were tested for SN antibodies. Since the frogs were killed when they were bled, serological changes could not be followed in individual frogs. Virus was isolated from the blood of 6 frogs, and 50 had detectable SN antibodies (Table 2). During the second half of July, 105 frogs were taken and 28 of these had detectable antibodies; 3 out of 93 had virus in their blood. Of the 30 frogs taken during August, 8 had detectable SN antibodies, but none of 18 tested had viremia. In the first half of September 44 frogs were caught; of these, 14 had SN antibodies and 3 had WEE virus in their blood.

Frogs and snakes were taken together at only two localities—Herschel Dam and Weyburn. At Herschel Dam 1 garter snake and 44 frogs were collected. The snake was infected with WEE virus; 13 of the frogs had SN antibodies and WEE virus was found in the blood of 3. At Weyburn, only 3 snakes were taken but all had SN antibodies at first bleeding; of 11 frogs collected in that area, 4 had detectable

Table 2. Western equine encephalitis (WEE) virus and serum neutralizing (SN) antibodies in the blood of leopard frogs (*Rana pipiens*). Index of neutralization ($\log_{10}$) is expressed as follows: >1.99 was considered positive; 1.01–1.99, suspicious; and <1.01, negative.

Location	Date collected (1964)	WEE virus/sample*	No. of frogs with SN antibodies		
			(>1.99)	(1.01–1.99)	(<1.01)
Alsask Dam	15 July	0/52	13	1	38
Herschel Dam	22 July	3/41	11	2	31
Weyburn	28 July	0/0	0	1	8
Weyburn	15 Aug.	0/0	1	3	4
Weyburn	17 Aug.	0/1	0	0	1
Swift Current	17 Aug.	0/12	1	0	12
Battleford	28 Aug.	0/5	0	3	3
Weyburn	31 Aug.	0/0	0	0	2
Craven	11 Sept.	3/23	7	7	9
Colgate	17 Sept.	0/21	0	0	21

* Denominator denotes number tested; numerator, number positive.

SN antibodies. Based on the occurrence of viremia and demonstrable SN antibodies, the rate of infection with WEE virus in Saskatchewan garter snakes in 1964 was not very different from that in frogs—39.3 percent for garter snakes and 31.3 percent for frogs. This study and an earlier one (*4*) show that in parts of the province the infection rate in garter snakes is high (at Pelly, 75 out of 160; at Keeler, 13 out of 16).

Five isolations obtained 162 to 168 days after the first bleeding of the snakes, without preliminary chilling, suggest that recurrence of viremia has a cyclical rhythm independent of the temperature of the environment. Garter snakes may, therefore, be overwintering hosts of the virus regardless of the latitude at which they occur. Isolation of WEE virus from naturally infected *Rana pipiens* establishes that species as a potential reservoir host for this virus.

In contrast to the earlier study (*4*), in which natural SN antibodies were found in garter snakes and leopard frogs only in restricted geographical areas, evidence, in this study, of WEE virus infection was found in snakes and frogs from all 12 localities where they were collected. Accordingly, from 1961 to 1964, evidence of natural infection with WEE virus in leopard frogs or garter snakes has been found in 18 different localities in the province, ranging from Elmore (49°00′N) to Onion Lake (53°43′N). Thus WEE virus infections appear to be widely distributed in garter snakes and leopard frogs in the agricultural area of Saskatchewan (*8*).

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8. A more detailed account of our studies, with confirmatory observations made in 1965 and 1966, is in preparation.
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Respiration of a Forest Measured by Carbon Dioxide Accumulation during Temperature Inversions

Abstract. *Nocturnal accumulations of carbon dioxide during 40 temperature inversions in 1 year were used as an index of the metabolic activity of a forest. Rates of CO_2 production varied with temperature and with season. Spring and summer rates were 2 to 3 times higher than winter rates at the same temperature. Mean monthly temperatures, averaged over 15 years, were used with the curves of respiration on temperature to compute annual gross respiration of the Brookhaven oak-pine forest. The forest was estimated to have a yearly release of approximately 3400 grams of CO_2 per square meter, theoretically equivalent to 2104 grams of dry matter (carbohydrate).*

Although metabolism is one of the generalized functions of natural communities that has long been recognized to offer substantial diagnostic value, the difficulties of measuring it have limited its use, especially in terrestrial ecosystems. Those estimates of metabolism of terrestrial ecosystems that have been attempted have generally been based either on inference from detailed studies of structure (*1*), on measurements of CO_2 exchange rates of small enclosed segments (*2*), or on measurements of CO_2 flux (*3*).

Of these, measurement of CO_2 flux is an especially attractive technique because chambers are not required, and the problem of disturbing natural conditions is reduced to a minimum. The difficulties are substantial because elaborate instrumentation and a large number of measurements are necessary. Nevertheless, it has been applied successfully in various agricultural ecosystems (*3*). In forests where structure of the community is much more complex, the technique is even more difficult. We wish to report the successful measurement of nocturnal respiration of a forest by a simplification of the CO_2 flux method.

The forest is a relatively homogeneous, but diminutive and open, oak–pine stand on level glacial outwash sands of central Long Island, New York. The principal trees are white oak (*Quercus alba*), scarlet oak (*Q. coccinea*), and pitch pine (*Pinus rigida*). The larger oaks were 45 years old and 5 to 10 m tall at the time of this study. A few scattered pines were 100 years old and 12 to 15 m tall, but most were 10 m or less and the same age as the oaks. Blueberries (*Vaccinium angustifolium, V. vacillans*) and the black huckleberry (*Gaylussacia baccata*) form a shrub stratum. Soils are well drained, sandy, and podzolic.

An infrared gas analyzer was used to measure carbon dioxide concentrations at various heights in the forest during local, low-level, temperature inversions. Rates of increase of CO_2 concentration, integrated over that depth of the atmosphere influenced by the forest, gave a rate of CO_2 production which was correlated with temperature and was assumed to be a measure of the rate of respiration of the community. Temperature was measured to within less than $\pm$ 0.5°C with aspirated thermocouples. A complete set of measurements, including both CO_2 concentration and temperature, was recorded automatically at 15-minute intervals on punched tape. Sampling points were at eight heights on each of two towers in the forest (one 16 m tall, the other 21 m tall). Additional data taken at six heights on a 125 m tower about 2 km from the experimental forest clarified the gross structure of the CO_2 profile during these inversions, showing that the CO_2 build-up was restricted to heights less than 25 m.

It has been recognized for several years that carbon dioxide concentrations in the atmosphere follow a diurnal course related to the rates of photosynthesis and respiration of the plant cover. The CO_2 concentrations usually increase at night, especially close to the ground, and decline during the daytime. A diurnal course is recognizable, under certain conditions, as high as 100 m above the ground (*4*).