increase in the degree of negative superhelicity. In other words, DNA covalently closed in the presence of HMG_1 has a lower linking number than DNA covalently closed in the absence of this protein. Similar results were obtained with HMG_2 (Fig. 1, h and k).

The results were quantified as described for the unwinding of the DNA helix by *E. coli* RNA polymerase (*11*). For HMG_1, the average linking number of PM2 DNA molecule in samples covalently closed in the presence of 40, 80, 120, and 160 protein molecules per DNA molecule were reduced by 1.2, 2, 3.4, and 6.8 turns, respectively. For HMG_2, samples covalently closed in the presence of 50 and 100 protein molecules per DNA were found to have 3.2 and 8.5 turns, respectively. On the basis of the measurements of Shooter *et al.* (*5*), we believe that most of the protein molecules present during the ligase treatment were bound to the DNA.

A reduction in the linking number of the DNA by the presence of a protein during the covalent closure of the DNA could arise by a number of mechanisms. These include supercoiling of the DNA around a protein core, separation of DNA strands by cooperative or noncooperative binding of protein molecules to single-stranded DNA segments, and a change in DNA helix rotation by the binding of protein molecules. The lowering of the melting temperature of DNA by these proteins (*3*) suggests that the proteins probably uncoil the DNA double helix. How HMG_1 and HMG_2 affect the DNA structure remains to be determined.

HMG proteins are present in various tissues of a number of animals (*1*, *12*) and they have been found in nucleosomes (*13*). On the basis of the yield of these proteins, it has been estimated that the molar ratio of HMG_1 and HMG_2 to total histones is about 1 : 50. In other words, there is on the average approximately one molecule of either HMG_1 or HMG_2 per ten nucleosomes. Our observation that HMG_1 and HMG_2 can affect the helical twist or the tertiary coiling of the DNA points to the possibility that these proteins might play a structural role in the higher order coiling of the nucleosomes. The possibility also exists that by modifying the structure of the DNA helix, these proteins could affect processes such as transcription.

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26 October 1977

Isolation of St. Louis Encephalitis Virus from Overwintering *Culex pipiens* Mosquitoes

Abstract. *Two strains of St. Louis encephalitis virus were isolated from overwintering mosquitoes collected in Maryland and Pennsylvania during January and February 1977. These isolations from* Culex pipiens *constitute evidence that a mosquito-borne flavivirus can persist in a vector mosquito in temperate climates during the winter season.*

St. Louis encephalitis (SLE) virus leads all other arboviruses in causing human disease in the United States, and numerous epidemics have occurred since its initial detection in 1933. The virus is maintained in nature during the summer and fall by a mosquito-avian-mosquito cycle. At least three mosquito species, *Culex pipiens*, *Culex nigripalpus*, and *Culex tarsalis* have been incriminated as vectors. The seasonal distribution of human disease coincides with high infection rates in mosquitoes and birds during late summer and early fall, and human infections tend to be more prevalent in urban areas, especially in the eastern United States where the domestic mosquito *C. pipiens* is the principal vector.

Although details of the summer and fall mosquito-avian-mosquito cycle are relatively well known, the mechanism by which the virus persists during the winter season is obscure. Reeves (*1*) reviewed several hypotheses to explain the survival of arboviruses through these adverse periods, including one that hibernating vector mosquitoes served as overwintering hosts. In temperate regions, *C. pipiens* and *C. tarsalis* overwinter solely as inseminated females. The fact that these *Culex* mosquitoes are the primary vectors of SLE virus during the summer and fall has prompted several investigators to test the overwintering hypothesis. The SLE virus has been shown experimentally to persist for more than a month in *Culex* species (*2*, *3*). A single isolation of SLE virus was obtained from *C. tarsalis* females collected during March in the western United States (*4*). Other isolations of arboviruses during the winter include one strain of western equine encephalitis virus from *C. tarsalis* in Colorado (*5*) and two Japanese encephalitis virus strains from *C. pipiens* in Korea (*6*).

In spite of these findings, the hypothesis that hibernating mosquitoes harbor arboviruses over the winter has not gained wide acceptance. This is partly because of the belief that blood feeding in *Culex* mosquitoes is drastically reduced or suspended shortly before they hibernate so that their chance of taking a viremic blood meal before overwintering is exceedingly small or nonexistent (*2*). Furthermore, other investigations (*7*) have shown that parous females are seldom found among collections of overwintering mosquitoes, and those which are found are usually observed in early winter and are presumed not to survive until spring. However, ovarian diapause in laboratory-reared *C. pipiens* has been demonstrated (*8*). In these cases, females, experimentally induced to hibernate, took full blood meals but, in most instances, ovarian development did not follow. If this situation occurred in nature, such females would prove to be nulliparous upon dissection, in spite of having taken a blood meal. Nulliparity, then, would not be proof of the failure of the mosquito to obtain a blood meal shortly before hibernating.

Since the 1975 SLE epidemic, we have conducted investigations to reexamine

0036-8075/78/0324-1346$00.50/0

the hypothesis that SLE virus is maintained in hibernating *C. pipiens* females during the winter season. *Culex pipiens* were collected from overwintering sites in abandoned ammunition bunkers at several former U.S. Army forts. Collections were made in four mid-Atlantic states during January and February of 1976 and 1977 (Fig. 1). The bunkers consisted of a series of rooms 3 to 5 m wide, 5 to 10 m long, and 2 to 3 m high. Construction material was either steel-reinforced concrete or clay bricks. All rooms where mosquitoes were collected were covered with earth 2 to 3 m thick and had condensation on both walls and ceilings. The temperatures inside the bunkers were not recorded; however, subfreezing temperatures prevailed outside the bunkers much of the time, especially during 1977—one of the coldest winters on record (Table 1).

Mosquitoes were aspirated from the walls and ceilings and transported to the laboratory where they were retained in an insectary programmed for a daily photoperiod of 16 hours of daylight and 8 hours of darkness, and a constant temperature and relative humidity of 26°C and 80 percent, respectively. During the 1976 studies, 1116 overwintering mosquitoes were collected. After 1 day in the insectary, 715 were identified, pooled (10 mosquitoes per pool), and stored at −70°C for virus assay. The remaining 401 mosquitoes were held for 7 days in the insectary and then pooled. Each group of ten mosquitoes was separately triturated and injected as a suspension into suckling mice (Table 2). Virological procedures were the same for both 1976 and 1977 collections. No SLE virus was isolated from the 1976 material, but viruses that were lethal in suckling mice were isolated from mosquitoes collected in January at Fort Mifflin and in February at Fort Mott. These viruses do not appear related to any of the currently recognized alpha or flaviviruses of the eastern United States; however, the specific identity of these viruses remains to be determined.

Our failure to isolate SLE virus from overwintering *Culex* mosquitoes collected during 1976 prompted certain changes in experimental design for the 1977 studies. Experiments with Japanese encephalitis virus (*9*) have shown a marked reduction in virus multiplication in mosquitoes maintained at low temperature. These data suggested that any virus present would probably be in low concentration, possibly below detectable levels, and that incubation at high temperature might be necessary for detection of virus in mosquitoes from overwintering sites. We similarly speculated that if the mosquitoes took a blood meal immediately after hibernation, this might enhance the chance of our recovering the virus. All of the mosquitoes collected during 1977, therefore, were retained in an insectary as described above for various periods of time (Table 2). After the holding periods in the insectary, a chicken was placed in the mosquito cages overnight to serve as a blood-meal source. The blood-engorged mosquitoes were segregated from unfed mosquitoes and maintained for 10 days in separate cages until blood digestion was complete, then assayed for virus. All the chickens used to furnish blood meals for the mosquitoes were first tested to eliminate any possibility of their serving as a virus source. Blood from each chicken was diluted 1:10 in supplemented medium 199 with Hanks salts (see Table 2) and injected intracerebrally into suckling mice. The mice were held for 14 days during which time they showed no ill effects.

A summary of the *C. pipiens* collected

Table 1. Midwinter temperatures (°C) near the sites where overwintering *C. pipiens* were collected.

Site	December 1976		January 1977		February 1977	
	Average minimum	Departure from normal	Average minimum	Departure from normal	Average minimum	Departure from normal
Fort Washington*	−2.7	−1.0	−8.0	−5.6	−2.3	.8
Fort Mifflin†	−5.4	−2.7	−10.9	−6.8	−4.4	.2

*Recorded at Washington National Airport 16 km from Fort Washington. †Recorded at Philadelphia International Airport 1.6 km from Fort Mifflin.

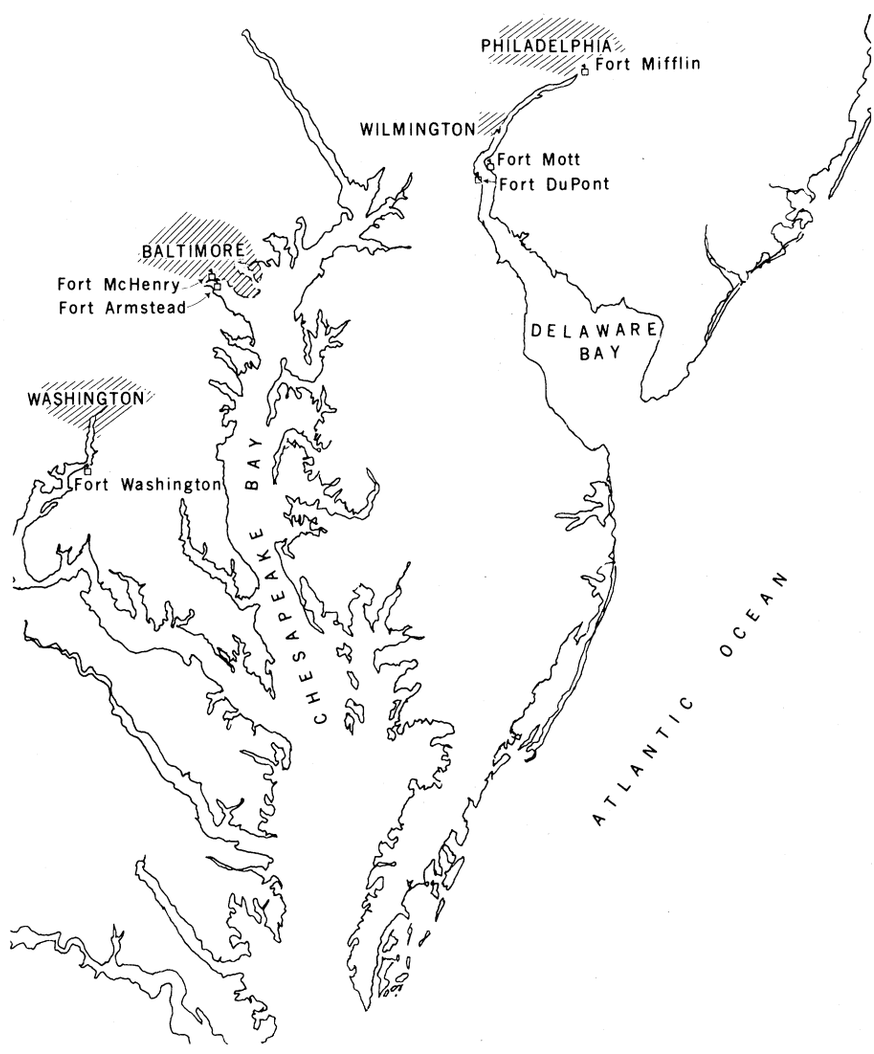

Fig. 1. Locations of former U.S. Army forts where overwintering mosquitoes were collected.

during the winter of 1977 is presented in Table 2. St. Louis encephalitis virus was isolated from mosquitoes that were collected (after the coldest periods of the winter) on 26 January and on 22 February at Fort Washington and Fort Mifflin, respectively. Suckling mice inoculated with mosquito suspension No. 7 (Fort Washington origin) developed signs of central nervous system (CNS) illness on day 7 after inoculation. Virus was readily reisolated from the original mosquito suspension even after two successive thaws. The LD_{50} (median lethal dose) in suckling mice injected intracerebrally gave a titer of 10^4. A titer of 10^5 plaque-forming units per milliliter was obtained on baby hamster kidney (BHK 21) cell monolayers. Two of ten suckling mice inoculated with mosquito suspension No. 34 (Fort Mifflin origin) developed CNS illness on day 8 after inoculation. Upon reisolation, only 5 of 20 mice inoculated with the original mosquito suspension developed CNS illness and died by day 7. A precise titer of the original mosquito pool suspension was not determined.

Virus isolates were identified as strains of SLE virus by means of a combination of complement fixation (CF) and plaque reduction neutralization tests (PRNT) for which hyperimmune mouse ascitic fluids were prepared for each virus. The PRNT was performed on continuous monkey kidney cell monolayers (LLC-MK_2). Prototype strains used as reference SLE virus included the Parton strain (*10*), and an isolate made from a light-trap collection (12 September 1975) of *Culex* sp. during the SLE outbreak of 1975 in Prince Georges County, Maryland (*11*). Presumptive identification based on CF results indicated that both overwintering isolates were similar to prototype SLE strains (Table 3) (*12*). Further testing by PRNT indicated only slight antigenic variation. Both isolates were clearly strains of SLE virus even though the virus preparation of the Fort Mifflin isolate No. 34 was more readily neutralized by all antiserums.

The data presented here indicate that SLE virus persisted through most of the winter in the primary eastern U.S. vector, *C. pipiens*. Previous studies (*7, 13*) on the hibernation of this species in the area from which these mosquitoes were collected indicate that the females, having survived the coldest part of the winter, were part of the overwintering population and would have survived until spring. They could well have reintroduced virus into the avian cycle when they took a blood meal after hibernation. Both virus isolates were obtained from mosquitoes given an avian blood meal after hibernation; however, the evidence is not conclusive that virus isolation was contingent upon these conditions. The absence of viremia and the negative blood serology in the chicken which pro-

Table 2. Hibernating *C. pipiens* collected at abandoned army forts located in Pennsylvania, New Jersey, and Maryland during the winter of 1977. Groups of mosquitoes (ten per group) were triturated in tissue grinders with 2 ml of medium 199 Hanks salts supplemented with 20 percent heated fetal bovine serum, $NaHCO_3$, penicillin (500 unit/ml), and streptomycin (500 μg/ml). After centrifugation for 30 minutes at 475*g* in a refrigerated centrifuge, each supernatent was inoculated intracerebrally into a litter of 3- to 5-day-old mice (0.02 ml per mouse). The remainder of each mosquito suspension was stored at −70°C to be used as necessary for further attempts to isolate virus. Moribund or dead mice observed during a 14-day period were frozen at −70°C. All such suspect isolates were passaged by injecting a 20 percent brain suspension, in the medium described, into a second litter of mice.

Mosquito collection			Laboratory handling				
			Days held in insectary before blood feeding	Blood fed		Non-blood fed	
Location	Date	Number collected		Number of pools tested	Number of isolations	Number of pools tested	Number of isolations
Fort Washington, Md.	4 January	97	21	3	0	7	0
Fort McHenry, Md.	5 January	9	7	0	0	1	0
Fort McHenry, Md.	11 January	3	0	0	0	1	0
Fort Armstead, Md.	12 January	33	20	2	0	1	0
Fort Washington, Md.	26 January	215	20	14	1	7	0
Fort Mifflin, Pa.	22 February	406	15	25	1	15	0
Fort Mott, N.J.	23 February	226	14	10	0	12	0
Fort Mott, N.J.	1 March	173	20	8	0	9	0
Total		1162		62	2	53	0

Table 3. Serological identification of SLE virus strains by means of complement fixation (CF) and plaque reduction neutralization tests (PRNT). The Parton strain of SLE was at passage 3 from the brain of a suckling mouse. The local 1975 isolate was obtained in the Washington, D.C., area from a light-trap collection of *C. pipiens* during the 1975 midsummer outbreak of SLE; antigen was prepared from mouse brain.

Virus strain	Antibody to hyperimmune mouse ascitic fluids*							
	Fort Washington No. 7		Fort Mifflin No. 34		Parton		Local 1975	
	CF	PRNT	CF	PRNT	CF	PRNT	CF	PRNT
Isolates from overwintering mosquitoes								
Fort Washington No. 7	256†	170‡	1024	120	1024	120	1024	285
Fort Mifflin No. 34	128	360	1024	840	1024	450	1024	760
Reference SLE virus strains								
Parton strain SLE	256	150	512	180	1024	200	1024	250
Local 1975 mosquito isolate	256	140	1024	200	1024	130	1024	420

*Prepared according to the methods of Chiewsilp and McCown (*15*). †Reciprocal of HMAF dilution exhibiting < 50 percent lysis at an optimal antigen dilution with 5 units of complement [J. F. Kent and E. H. Fife (*16*)]. ‡Reciprocal of dilution inhibiting 50 percent of a plaque dose of approximately 100 plaque-forming units as assayed on LLC-MK_2 cell monolayers.

vided the blood meals for the Fort Washington mosquitoes rule out the possibility that this chicken served as a virus source but similarly document the failure of infected mosquitoes to transmit virus by this feeding. The chicken used for feeding the Fort Mifflin mosquitoes died during exposure to the insects. The fact that both of the 1977 isolates came from mosquitoes which had been held in the insectary for at least 15 days may be significant.

There are two possible explanations for the presence of virus in these mosquitoes. One is that they became infected through transovarial transmission, even though work by Chamberlain *et al.* (*3*) fails to support this theory; the other is that these females took viremic blood meals prior to hibernation. Since the feasibility of the latter occurring has been demonstrated (*8*), we consider this to be the most likely explanation. In spite of several demonstrations of transovarial transmission of arboviruses by mosquitoes, all have involved bunyaviruses of the California encephalitis group and mosquitoes which overwinter in the egg stage; research by Tesh and Gubler (*14*) suggests that the phenomenon may be restricted to this combination. Our data support an overwintering mechanism in which female *C. pipiens* that are diapause-conditioned (that is, exposed to short day lengths and cool temperatures) take a viremic blood meal, overwinter, and emerge infected. Whether or not such overwintering mosquitoes can introduce SLE virus to the summer cycle upon emergence remains to be demonstrated.

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12 December 1977

Erythroid Progenitors Circulating in the Blood of Adult Individuals Produce Fetal Hemoglobin in Culture

Abstract. *Erythroid colonies, raised from erythroid stem cells circulating in the peripheral blood of normal adult individuals, synthesize considerable amounts of fetal hemoglobin. In cultures from persons with sickling disorders, amounts of hemoglobin F that are known to inhibit sickling in vivo are produced. The results provide evidence that primitive erythroid progenitors are able to express the hemoglobin F production program and that cultures of mononuclear cells of the adult blood can be used to investigate the mechanisms involved in regulation of γ-globin gene switching.*

We report that the erythroid progenitor cells found in the circulation of the normal adult individuals produce, in vitro, erythroid colonies that synthesize considerable amounts of fetal hemoglobin. These circulating erythroid progenitors are considered to be relatively primitive committed erythroid stem cells with proliferative potentials of the erythroid stem-cell class operationally defined as burst-forming unit erythroid (BFUe) (*1*). They are capable of producing clus-

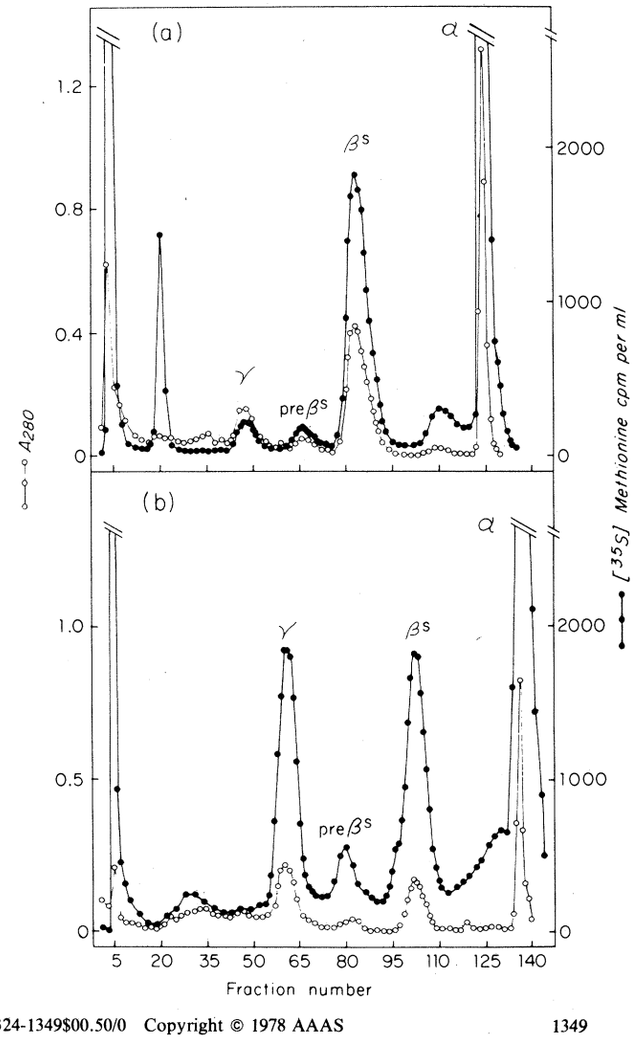

Fig. 1. Carboxymethylcellulose chromatography of globin chains from the hemoglobins produced by the cells of a subject with sickle cell/β-thalassemia. (a) Globin chain synthesis in blood reticulocytes. (b) Globin chains synthesized in blood BFUe cultures. There is a striking increase of γ chain radioactivity in (b).

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