HEREDITARY TRANSMISSION OF THE WESTERN TYPE OF EQUINE ENCEPHALOMYELITIS VIRUS IN THE WOOD TICK, DERMACENTOR ANDERSONI STILES

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PLATES 27 AND 28

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The natural mode of transmission of equine encephalomyelitis virus is not understood, although epidemiological data point strongly to an arthropod vector. Accordingly, we have studied the wood tick as a potential agent for disseminating the disease. Our findings have been briefly presented in preliminary communications (1, 2). It is the purpose of the present paper to describe in detail those of our experiments that demonstrate the hereditary transmission of the Western type of equine encephalomyelitis virus in the wood tick, Dermacentor andersoni Stiles.

Equine encephalomyelitis, of the type caused by the virus recovered first from affected horses by Meyer, Haring, and Howitt (3), is a disease which has shown an increasing incidence and a widening distribution in the United States (4–6). The disease has been found in nature only in members of the equine (3–5), human (6–13), and avian (14, 15) groups. Experimentally, however, the susceptibility of a wide variety of mammals and birds has been demonstrated (3, 4, 14–25). Nevertheless, no natural reservoir has been found among the domestic or wild animals native to North America. In the absence of mechanical interference or trauma, there has been no evidence to suggest infection through contact (4). The disease has appeared, furthermore, only during the summer and fall months. Finally, sporadic and apparently unrelated outbreaks have characterized the disease. These factors—the limited seasonal occurrence, the sporadic nature of the outbreaks, and the absence of infection through contact—have suggested that an insect or arachnid vector is probably responsible for the natural transmission of the virus.

When the possibility of the arthropodal transmission of a disease is under consideration, a point of prime importance is whether the infectious agent is present in the blood during the course of that disease. There is no question about this point in the case of equine encephalomyelitis, for the blood invariably contains the virus at some period of the illness. After infection has occurred, the virus may appear in the blood within a few hours, or it may not appear for several days. Thereafter, it increases in amount rapidly, attains a maximum, and then decreases gradually. By the time that the encephalomyelitic manifestations have developed, it is rarely detectable. Thus, the total
period during which the virus is recoverable from the blood may vary in different infected animals from a few hours to several days.

Theoretically, the chances for an arthropod to acquire equine encephalomyelitis virus while feeding on a sick host are increased proportionately as the period of feeding is prolonged. Of the blood-sucking arthropods, the feeding habits of the ixodid ticks ought to fit them exceptionally well to acquire the virus. Their period of engorgement requires several days. Since they are apt to get on an animal while it is actively moving about, furthermore, their period of engorgement is almost certain to include the pre-encephalomyelitic phase of the disease, a time when the virus attains high levels in the blood.

While it has been shown that mosquitoes of the genus *Aedes* can harbor and transmit the virus under experimental conditions, the virus is limited in this vector to the adult female. Adults of the genus *Aedes*, furthermore, do not survive the rigorous winters which are typical in the regions where the disease is most prevalent. It does not seem probable, therefore, that this genus of mosquito can be the sole, perhaps even the principal, vector of the disease. Finally, it is noteworthy that the attempted experimental transmission of the virus by other genera of mosquito has not succeeded.

**Materials and Methods**

**Virus.**—A strain of the Western type of equine encephalomyelitis virus, which was recovered in South Dakota (26), was used. It was kindly supplied to us in September, 1935, by Dr. W. S. Gouchenour of the Bureau of Animal Industry. The virus has since been maintained by successive passages in guinea pigs.

**Animal Hosts.**—The ground squirrel, *Citellus richardsonii* (Sabine) (20), commonly called the gopher, and the guinea pig were selected as animal hosts in which to carry the virus by serial passage and in which to make test and control observations of immunity. They were also used to provide the meals of blood needed by the successive stages of the ticks. The guinea pig, being uniformly and highly susceptible to the virus, has long been established as a most satisfactory animal in which to study equine encephalomyelitis. The ground squirrel was found to be equally susceptible (20). In contrast to the guinea pig, furthermore, it was not greatly disturbed by the discomfort associated with the feeding of the ticks. It was selected for use in the present study, moreover, because of its possible rôle as an intermediary host for the virus of equine encephalomyelitis.

The ground squirrels and guinea pigs were housed in metal cages in a special room which was as insect-proof as we could make it. In order to lessen their activity, not more than three animals were kept in a single cage. They were fed a diet of alfalfa, cabbage, and oats. Each animal was observed twice a day and its temperature was recorded daily. Many of the animals, on which the ticks were fed, died. When an animal survived, it was observed for at least 30 days, its temperature being recorded daily for 3 weeks.

**Vector.**—The wood tick, *Dermacentor andersoni* Stiles, which was employed as an experimental vector, was made available to us in March, 1936, through the courtesy of Dr. C. B. Phillip of the United States Public Health Service. We chose this tick largely because it belongs to the genus *Dermacentor* (27), a genus which has essentially the same geographical distribution (Fig. 1) and seasonal occurrence as equine encephalomyelitis.
This tick is most prevalent during May, June, and July, although its period of activity may include several months before or after this time, as determined by local climatic conditions.

Our selection of a tick belonging to the genus *Dermacentor*, furthermore, was influenced by certain of its characteristics that make it theoretically an almost ideal intermediary or reservoir host for a virus. Of these characteristics, the most important are its habits of securing blood from several different hosts, its varied requirements during estivation and hibernation, and its prolonged and complicated life cycle.

The life cycle of *Dermacentor andersoni* Stiles (Fig. 2) consists of four successive stages—egg, larva, nymph, and adult (male and female). Each female deposits from 4000 to 7000 eggs on the ground, usually under an accumulation of leaves or other débris. The larva, which hatches from the egg, climbs up on vegetation to await a passing host upon which to attach itself. If successful, the larva feeds to repletion on the blood of the host and falls to the ground to molt. After being dormant for several weeks, the nymph climbs up on vegetation to await a host. If successful, it engorges, drops to the ground, and transforms into an adult. The adult attaches itself to a third and final host to mate and to obtain a meal of blood. The male does not concern us further. The pregnant female engorges until it is many times its former size, falls to the ground, oviposits, and dies. Thus, to complete its four-stage life cycle, this tick must feed thrice on blood, once as a larva, once as a nymph, and once as an adult. Following each meal of blood, the tick drops off its host to molt, if it is in the larval or nymphal stages, or to deposit eggs, if it is an adult female.

Under natural conditions, the completion of the life cycle of *Dermacentor andersoni* Stiles usually requires two years, but it may require three or four. The hexapod larvae infest rodents during June, July, or August of one year; the octopod nymphs usually do not feed on rodents until April, May, or June of the next year, and the adults do not ordinarily feed until the following spring. These characteristics provide many opportunities whereby the tick may spread an infectious agent to different species of animal hosts, for the larvae and nymphs usually feed on small rodents and birds, whereas the adults feed on the larger mammals, such as rabbits, man, and the members of the equine family. If the infectious agent be hereditary in the tick, furthermore, it is clear that this arachnid can serve as a reservoir, carrying the virus from year to year.

**Husbandry of Ticks.**—The ticks were kept in tubes of pyrex glass (12.5 × 2.5 cm.) during the periods between feeding. Each tube contained a strip of gauze and was stoppered with non-absorbent cotton enclosed in gauze. The segregation of the ticks in such tubes simplified the making of accurate records. The tubes of ticks were kept at room temperature in a tight metal box, in which the humidity was maintained at a high level by means of open vessels of water and a layer of wet sand (2 inches deep) in the bottom. Extraneous bacterial or mycotic infections were held to a minimum by sterilizing all of these materials just before use.

The technique employed for feeding the ticks was essentially that which was devised by Jellison and Philip (28). Their method, with the minor modifications described below, proved to be most convenient, for the ticks could be readily observed while they were feeding and easily removed from the host when repletion had taken place.

1 Zobec filmated bandage, Johnson and Johnson, New Brunswick, New Jersey.
EQUINE ENCEPHALOMYELITIS IN TICKS

At the beginning of our work, the hosts frequently gnawed through the adhesive tape which held in place the container (Fig. 3) in which the ticks were confined during feeding, thus permitting unattached ticks to escape. This difficulty was avoided (Fig. 4) by placing a tightly fitting collar made of flexible metal over the threaded basal portion of the container. This collar, shaped like a saddle blanket, had a central perforation which was only slightly larger in diameter than the basal portion of the container. The container and collar were attached to a guinea pig or gopher as follows: The flanged base was first covered with adhesive tape. Then the threaded basal portion of the container was affixed to the animal by a girdle of adhesive tape. Next, the metal collar was put on and finally the collar was secured with a second girdle of adhesive tape (Fig. 4).

Infection of Ticks.—Ticks were experimentally infected with the virus of equine encephalomyelitis by being permitted to feed on guinea pigs which had been inoculated with the virus. These guinea pigs had received, by the intracerebral route, 0.1 ml. of a Berkefeld V filtrate of the supernatant of a lightly centrifuged 10 per cent suspension in Locke's solution of brain tissue from a guinea pig which was moribund 96 hours after the intranasal instillation of a viral suspension. The ticks were permitted to feed on infected guinea pigs either as nymphs or adults. After a single feeding on an infected host, the progeny of the infected ticks were thereafter fed only on normal animals, which were carefully protected from accidental contact with the virus. Therefore, when such normal hosts developed equine encephalomyelitis after the ticks had fed, it proved that the ticks had transmitted the disease.

Tests for the Presence of Virus.—Each guinea pig or gopher, that had provided a meal of blood for the ticks, was carefully tested to determine whether it had acquired the virus of equine encephalomyelitis. These hosts fell into two groups: those which died while the ticks were feeding or died subsequently (group A), and those which survived the feeding (group B). From the animals comprising group A, an attempt was made to recover virus from the brain tissue of each animal. When a virus was recovered, it was identified as the Western type of equine encephalomyelitis virus by neutralization tests.

The procedure employed in the attempted recovery of virus was as follows: After removal of the brain from the skull, one-half was triturated in Locke's solution to yield a 10 per cent suspension and the other half was placed in 50 per cent glycerol. The suspension was centrifuged horizontally for 15 minutes and the supernatant filtered through a Berkefeld V or N candle. Finally, the filtrate was inoculated into normal animals and into animals known to be immune to the Western type of equine encephalomyelitis virus. Each animal received from 100 to 1,000,000 minimal lethal doses. (The route of inoculation used is indicated in the protocols.) When these normal animals died from characteristic equine encephalomyelitis and the animals known to be immune survived without having shown evidence of illness, it was considered to be good presumptive evidence that the original host animals on which the ticks had fed had died from the Western type of equine encephalomyelitis. Final evidence was secured by the use of the neutralization test.

The neutralization test was done by mixing 0.5 ml. amounts of decimal dilutions of a Berkefeld V filtrate, prepared from a 20 per cent suspension of brain tissue, with equal amounts of undiluted immune serum. After the mixtures had been kept for about an

2 We are indebted to Dr. H. R. Cox for samples of serum from rabbits immune to the virus of equine encephalomyelitis, Western type.
hour at room temperature, 0.1 ml. of each mixture was injected by the intracerebral route into each of two guinea pigs. The least amount of virus that resulted in the death of both guinea pigs was designated as the end point.

Each of the animals comprising group B—animals that survived the feeding of the ticks—was tested for active immunity to the Western type of equine encephalomyelitis virus by injecting, intracerebrally or subcutaneously, from 100 to 1,000,000 minimal lethal doses. Normal animals were always included as controls. Those of the tested animals that failed to develop equine encephalomyelitis were accepted as having been previously immunized by the virus that they had received during the feeding of the ticks. The results of no immunity test were accepted as conclusive unless all of the normal animals used as controls died of equine encephalomyelitis of Western type. Detailed reference is made in the text to each control animal that survived. There were only a few such instances.

The immunity tests, all of which were essentially alike, were numbered consecutively in order that each test could be indicated in Charts 2 and 3. A typical immunity test is also illustrated in Table I (immunity test 1, Experiment 2)—in order to save space the details of the other tests are not tabulated.

A sample of each viral suspension was cultured in broth and on blood agar plates under both aerobic and anaerobic conditions. No suspensions contaminated with bacteria were used in the experiments.

EXPERIMENTAL

Two series of experiments were carried out to determine whether *Dermacentor andersoni* Stiles could acquire the Western type of equine encephalomyelitis virus by feeding on experimentally infected guinea pigs, and whether it could pass the virus to subsequent developmental stages in its life cycle and to its progeny. This ability was measured by permitting ticks in later developmental stages to feed on normal guinea pigs and gophers and determining whether the ticks had transmitted the virus to these hosts.

**Series A. First Generation**

*Experiment 1.*—On May 20, 1936, a guinea pig (GP 8-80) was infected intracerebrally by the injection of 0.1 ml. of a 10 per cent suspension of brain tissue derived from an animal dead of equine encephalomyelitis. Immediately thereafter, 30 nymphs were permitted to feed on this guinea pig. Having fed to repletion, the nymphs were removed from the guinea pig, when it died 3 days later, and placed in tubes to molt.

In Experiment 1, normal ticks in the nymphal stage were fed on an animal that died of equine encephalomyelitis. This feeding constituted the sole contact which the ticks used in the experiments of series A had with an infected host. Since the elapsed time between the inoculation and the death of the host was essentially the time required by the nymphs for their repletion, and since the virus is almost always present in the blood during the acute phase of the disease, it seemed probable that the virus had also been ingested.
EQUINE ENCEPHALOMYELITIS IN TICKS

Experiment 2 was planned to establish three points: whether the nymphs used in Experiment 1 had acquired the virus during feeding; if so, whether the virus could survive the process of molting into the adult stage; and, if so, whether the adult ticks could infect normal hosts by feeding on them.

Source of virus: GP 8-80, injected intracerebrally on May 20, 1936, and died on May 23, on which Nymphs engorged for 3 days, from May 20 to 23.

These molted into Adults, which engorged for 5 days, from June 26 to July 1, on

G 5-1  G 5-2  G 5-3
these gophers survived and were found to be immune (Table I)

GP 9-61  GP 9-62
unfiltered brain suspension passed to

died 3rd day  died 3rd day  died 4th day  died 4th day  died 3rd day  died 2nd day  died 3rd day

* Virus recovered from the brain of GP 9-72 was proved by a neutralization test to be the virus of equine encephalomyelitis, Western type.

Chart 1. Results of Experiment 2. The ability of adult ticks (Dermacentor andersoni Stiles) to transmit equine encephalomyelitis virus of Western type to the normal hosts on which they were permitted to engorge. These ticks had acquired the virus 37 days before while feeding as nymphs on an experimentally infected guinea pig.

Experiment 2.—Experiment 2 was begun on June 26, 37 days after the nymphs had engorged on the infected guinea pig. During this period they had molted and become adults. These adults were permitted to feed on three normal gophers (G 5-1, G 5-2, and G 5-3) in lots of four males and four females per gopher. The ticks engorged rapidly. When replete, the females were placed in individual tubes to oviposit, the males were eliminated from the experiment.

One of the gophers (G 5-1) died 5 days later after an illness that was typical of equine
encephalomyelitis. Its brain was removed; one half was placed in 50 per cent glycerol, the other half was used to initiate a series of brain-to-brain passages in guinea pigs. These passages were made by means of Berkefeld filtrates. Neutralization tests were carried out after the second passage to establish the identity of the virus.

Two gophers (G 5-2 and G 5-3) survived. Their responses were apparently limited to febrile reactions. Each was tested for active immunity to the Western type of equine encephalomyelitis virus.

### TABLE I

**Results of Immunity Test 1, Experiment 2**

**Proof That the Gophers (G 5-2 and G 5-3), Responding with a Febrile Reaction Only to the Feeding of Ticks, Had Nevertheless Acquired Sufficient Equine Encephalomyelitis Virus of Western Type to Be Immunized**

<table>
<thead>
<tr>
<th>Host animals</th>
<th>Results of challenge inoculation</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First day of fever</td>
<td>Day of death</td>
</tr>
<tr>
<td>Test animals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Series A</td>
<td>G 5-2 3</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>G 5-3 3</td>
<td>40</td>
</tr>
<tr>
<td>Series B**</td>
<td>G 4-9 6</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>G 5-0 7</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>GP 9-56 3</td>
<td>30</td>
</tr>
<tr>
<td>Control animals</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G 5-8 7</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>G 5-9 7</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>G 6-0 6</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>GP 9-91 3</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>GP 9-92 5</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>GP 9-93 2</td>
<td>9</td>
</tr>
<tr>
<td>Immune</td>
<td>GP 8-79 3</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>GP 8-89 3</td>
<td>30</td>
</tr>
</tbody>
</table>

* The challenge inoculation consisted in the subcutaneous injection of 1.0 ml. of a Berkefeld V filtrate prepared from a 10 per cent suspension of the pooled brains of three guinea pigs that had died of experimental equine encephalomyelitis.

** The results of Experiment 10 in series B, to be described later, are included in Table I, because the same set of control animals served for this experiment as for Experiment 2 of series A.

The findings of Experiment 2 are summarized in Chart 1. From the data presented, it may be concluded that the gopher (G 5-1) dying 5 days after the ticks had been placed on it, and the gophers (G 5-2 and G 5-3) surviving after febrile reactions, had all been infected while the ticks engorged. The evidence for this conclusion is, first, that an infectious agent, which had been obtained from the brain of the first gopher (G 5-1) and successfully carried by Berkefeld filtrates through two brain-to-brain passages in guinea pigs, was proved by a neutralization test to be the Western type of equine encephalomyelitis virus, and, second, that neither surviving gopher (G 5-2 and G 5-3)
reacted to the injection of a test dose of the virus which killed the controls, thereby showing that both gophers had been effectively immunized. The data that established their immunity are presented in Table I.

The results of Experiment 2 established three points: that nymphs can acquire equine encephalomyelitis virus of Western type by engorging on an infected guinea pig; that the virus, so acquired, can persist through the process of molting into the adult stage; and that the infected adults can transmit the virus to the normal hosts on which they are permitted to feed. These findings are presented in Table II, in which all of the data for the first generation of series A are also summarized.

**TABLE II**

Results of Experiment 2 and Summary of the Data for the First Generation in Series A

Passage of Equine Encephalomyelitis Virus of Western Type from the Nymphal to the Adult Stage of Dermacentor andersoni Stiles

<table>
<thead>
<tr>
<th>Stage in development cycle</th>
<th>Lot number</th>
<th>Number fed</th>
<th>Host on which preceding stage engorged</th>
<th>Date placed on host</th>
<th>Date removed from host</th>
<th>Identification number</th>
<th>Effect of tick engorgement</th>
<th>Immunity</th>
<th>Proof that virus was transmitted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nymphal</td>
<td>D1-1</td>
<td>30</td>
<td>GP 8-80</td>
<td>1936</td>
<td>May 20</td>
<td>GP 8-80</td>
<td>Died</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult</td>
<td>D2-1</td>
<td>4♀, ♀♀</td>
<td>G 5-1</td>
<td>June 26</td>
<td>July 1</td>
<td>G 5-1</td>
<td>Died</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>D2-2-3</td>
<td>“”</td>
<td>G 5-3</td>
<td>“”</td>
<td>“”</td>
<td>G 5-3</td>
<td>Survived</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

* Infected by the intracerebral route with 0.1 ml. of equine encephalomyelitis virus of Western type. This animal was inoculated to initiate the experiments of series A.

**Series A. Second Generation**

The purpose of Experiment 3 was to find out whether equine encephalomyelitis virus of Western type could pass from the infected female ticks, described in Experiment 2, through the egg stage to the larvae of the next generation.

**Experiment 3.**—The larvae hatching from the eggs deposited by the infected ticks that had been used in Experiment 2 were permitted to feed, between Aug. 28 and Sept. 21 (from 57 to 79 days after their mothers had fed), on six gophers and six guinea pigs in lots of from 75 to 100 larvae per animal. After repletion, the larvae were put into tubes to molt. The hosts, none of which died, were tested from 20 to 45 days later for active immunity to equine encephalomyelitis virus of Western type.

The findings of Experiment 3 are presented in Table III. Each of the 12 hosts survived the feeding of the larvae, and each was tested for active immunity to the Western
TABLE III
Results of Experiments 3, 4, and 5 and Summary of the Data for the Second Generation in Series A
Passage of Equine Encephalomyelitis Virus of Western Type through the Egg, Larval, Nymphal, and Adult Stages of Dermacentor andersoni Stiles

<table>
<thead>
<tr>
<th>Stage in developmental cycle</th>
<th>Lot number</th>
<th>Number fed</th>
<th>Host on which preceding stage engorged</th>
<th>Date placed on host</th>
<th>Date removed from host</th>
<th>Identification number</th>
<th>Effect of tick engorgement</th>
<th>Immunity</th>
<th>Proof that virus was transmitted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg</td>
<td>D3-1</td>
<td>75-100</td>
<td>G 5-1</td>
<td>Aug. 28</td>
<td>Sept. 3</td>
<td>G 5-6</td>
<td>Survived</td>
<td>+ (    )</td>
<td>No</td>
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<tr>
<td></td>
<td>D3-2</td>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
<td>GP 9-86</td>
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<tr>
<td></td>
<td>D3-3</td>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
<td>Sept. 9</td>
<td>&quot;&quot;</td>
<td>GP 9-87</td>
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<td></td>
<td>D3-4</td>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
<td>G 6-4</td>
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<td>D3-5</td>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
<td>G 6-5</td>
<td>+</td>
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<tr>
<td></td>
<td>D3-6</td>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
<td>GP 10-00</td>
<td>+</td>
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<td>D3-7</td>
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<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
<td>GP S1</td>
<td>+</td>
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<td>D3-8</td>
<td>&quot;&quot;</td>
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<td>GP S2-3</td>
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<td>GP S2-4</td>
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<td>D3-10</td>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
<td>G 6-7</td>
<td>+</td>
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<tr>
<td>Larval</td>
<td>D4-21</td>
<td>30</td>
<td>G 6-5</td>
<td>Oct. 23</td>
<td>Nov. 1</td>
<td>G 9-2</td>
<td>Survived</td>
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<td>D4-22</td>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
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<td>G 9-4</td>
<td>+</td>
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<td>D4-23</td>
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<td>G 9-5</td>
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<td>D4-24</td>
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<td>&quot;&quot;</td>
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<td>G 9-6</td>
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<td>No</td>
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<td>D4-25</td>
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<td>G 9-7</td>
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<td>July 12</td>
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<td>GP S7-30</td>
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</table>

Type of equine encephalomyelitis virus in immunity tests 2, 3, and 4. It was found that four (G 5-7, G 6-4, GP 9-86, and GP 9-87) gave no evidence of immunity, six (G 6-5, G 6-6, G 6-7, GP 10-00, GP S1, and GP S2-3) were sufficiently immune to withstand the
challenge inoculation, and one (GP S2-4) died of an intercurrent infection. The immunity of one gopher (G 5-6) was not established because controls were lacking in immunity test 2. Its failure to react to a test dose of the virus that killed the other animals in the same test, however, provides some evidence that it really was immune.

The results of Experiment 3 established the fact that equine encephalomyelitis virus of Western type can pass from infected female ticks through their eggs to the larvae of the next generation, and that such larvae can transmit the virus to the normal hosts on which they are permitted to feed.

The purpose of Experiment 4 was to learn whether the nymphs deriving from the larvae that had been used in Experiment 3 also harbored the virus, and, if so, whether they could transmit it to the normal hosts on which they were permitted to engorge. These nymphs fell into two groups: nymphs known to have carried virus in the larval stage, and nymphs derived from larvae that had failed to transmit virus when they were tested in Experiment 3. This latter group was included because of the possibility that the virus might be present in them even though it had not been demonstrated in the larvae from which they had come (these larvae, be it noted, hatched from eggs laid by female ticks that had transmitted the virus to normal hosts).

Experiment 4.—The nymphs were derived from the larvae that had been shown (Experiment 3) from 45 to 300 days previously to have transmitted the virus to normal hosts. In addition, a special group of nymphs was used in Experiment 4. This group (lot D4-20, 26) had been derived from larvae (lot D3-3) that had engorged on a gopher (G 6-4) without transmitting the virus to it (Table III).

The nymphs were permitted to feed, between Oct. 23 and July 7, on 13 gophers in lots of 30 nymphs per gopher. After repletion, they were placed in tubes to molt. The gophers, all of which survived, were tested from 20 to 45 days later for active immunity to the Western type of equine encephalomyelitis virus.

The findings of Experiment 4 are presented in Table III. The only clinical evidence of infection observed was a transitory fever in six of the 13 gophers. But when these 13 gophers were tested for active immunity (immunity tests 5 and 6), three (G 9-4, G 9-8, and G 1-61) survived the challenge inoculation, while ten (G 9-2, G 9-3, G 9-5, G 9-6, G 9-7, G 9-9, G 1-00, G 1-59, G 1-60, and G 1-68) succumbed. Of the controls, on the other hand, all ten of the normal animals died, whereas 14 of the 16 animals known to be immune survived the same dose of the virus. One of the immune controls, a guinea pig (GP 8-79), died from a staphylococcal septicemia following an incomplete abortion. The death of the other immune control, a gopher (G 6-6), was apparently due to the challenge inoculation. The degree of immunity in this particular animal was inadequate to withstand the massive dose of virus used.

The results of Experiment 4 demonstrated that second generation nymphs deriving from larvae known to have transmitted the virus (Experiment 3)
can also transmit the virus to normal hosts. Not all of these nymphs, however, were shown to have transmitted the virus. Only three of the 13 animals on which the nymphs engorged became sufficiently immune to survive the challenge inoculation, the other ten died. It should be noted, though, that this challenge dose of the virus was large—probably sufficiently large to have broken through a low grade of immunity. Some of the ten animals that died, therefore, may actually have acquired the virus from the ticks.

No evidence that any virus was transmitted, on the other hand, was obtained in the tests made with the special lot of second generation nymphs that had been derived from larvae that, in Experiment 3, had failed to transmit the virus. It appears, therefore, that the ability to maintain and transmit the virus is not uniformly shared by all of the progeny of a given developmental stage.

In Experiment 5, the purpose was to find out whether the adult ticks, deriving from the nymphs that had been shown to have transmitted the virus (Experiment 4), also harbored the virus, and, if so, whether they could transmit it to the normal hosts on which they were permitted to engorge. These adults were the final developmental stage in the second generation of series A. Two groups of adults were used: those coming from nymphs shown to have transmitted the virus, and those coming from nymphs not shown to have done so.

Experiment 5.—The adult ticks were derived from the nymphs that had been used in Experiment 4. These adults were permitted to engorge, between June 17 and Sept. 8 (from 56 to 238 days after the nymphs had fed), on five gophers and eight guinea pigs in lots of four male and four female ticks per animal. Of the 13 lots of ticks used, nine lots originated from the nymphs shown to have been infected in Experiment 4, and four lots from the nymphs that had yielded negative results. After repletion, the females were put into tubes to oviposit and the males were discarded. Ten of the hosts survived, three died after developing a flaccid paraplegia.

The brains of the three animals that died (G 1-56, G 2-05, and GP S5-31) were removed for attempted recovery of the virus. The ten survivors were tested, between 30 and 45 days later, for active immunity to equine encephalomyelitis virus of Western type.

The findings of Experiment 5 are presented in Table III. Of the three animals that died, the death of one, a guinea pig (GP S5-31), resulted from equine encephalomyelitis, and that of the other two, both gophers (G 1-56 and G 2-05), from what was possibly tick paralysis. These diagnoses were based on the recovery of a virus from the guinea pig and its identification as the Western type of equine encephalomyelitis virus by a neutralization test, and on the failure to demonstrate an infectious agent from either of the gophers. When the ten animals that survived were examined for active immunity (immunity tests 6, 7, and 8), one gopher (G 1-57) was found to be immune to the Western type of equine encephalomyelitis virus. Of the nine animals (G 1-62, G 1-77, GP S5-30,
EQUINE ENCEPHALOMYELITIS IN Ticks

GP S5-73, GP S5-74, GP S6-79, GP S7-54, GP S7-55, and GP S7-80) that succumbed to the challenge dose of virus, four (G 1-77, GP S5-73, GP S5-74, and GP S6-79) had provided meals of blood for the adult ticks (D5-6, 7, 10, 11, 19, 34, 38) that as nympha (D4-21, 22) had yielded no evidence for the transmission of virus (Experiment 4).

The results of Experiment 5 proved that a lethal dose of equine encephalomyelitis virus of Western type can be transmitted by ticks in the final developmental (adult) stage of the second generation. They provided further evidence that the virus, once it has disappeared, will not appear again in a later developmental stage.

Series A. Third Generation

The primary object of Experiment 6 was to learn whether the virus could pass through the egg stage a second time to appear thereby in the larvae of the third generation. This point was determined by permitting the larvae that hatched from the adult female ticks used in Experiment 5 to engorge on normal hosts and by examining these hosts for evidence of infection. A further object was to find out whether the larvae hatching from the eggs laid by females not shown to have transmitted the virus (Experiment 5) might nevertheless harbor it.

Experiment 6.—The larvae that hatched from eggs deposited by the adult females used in Experiment 5 were permitted to feed, between July 30 and Sept. 14 (from 33 to 76 days after the mothers had fed), on nine gophers and seven guinea pigs in lots of from 100 to 150 larvae per gopher. Of these 16 lots of larvae, 12 were derived from the infected females and four from the non-infected females described in Experiment 5. After repletion, the larvae were put into tubes to molt. One animal host, a guinea pig (GP S7-81), died, the other 15 survived. The brain of the dead guinea pig was examined for the virus; the surviving hosts were tested from 30 to 45 days later for active immunity to the Western type of equine encephalomyelitis virus.

The findings of Experiment 6 are presented in Table IV. What led to the death of the guinea pig (GP S7-81) was not established. The absence of an infectious agent in its brain, spleen, and heart's blood suggested that tick paralysis had caused its death. When each of the 15 hosts that survived the feeding of the larvae was tested (immunity tests 7 and 8) for active immunity to the Western type of equine encephalomyelitis virus, the immunity of only one animal, a gopher (G 1-82), was possibly established (immunity test 7). This animal remained entirely asymptomatic after inoculation. Of the other animals that were included in this test, three test animals and two of three normal controls died. The single normal control that survived did so only after a prolonged illness that left it permanently paralyzed. It becomes apparent, therefore, that incontrovertible evidence for the immunity of gopher 1-82 was not obtained. Of the remaining 14 animals, 11 (G 2-06, G 2-12, G 2-13, G 2-14, G 2-17, G 2-20, GP S6-77, GP S7-56, GP S7-74, GP S7-55, and GP S7-79) succumbed and three (G 2-15, G 2-16, and GP S7-78) survived. These three were not regarded as being immune, however, for they were part of an immunity test (immunity test 8) that gave inconclusive results, probably because the challenge inoculation was too weak (three of five normal controls, four of
four immune controls, and ten of 17 test animals survived). The hosts for the larvae derived from the non-infected females used in Experiment 5 gave no evidence of immunity.

**TABLE IV**

Results of Experiments 6, 7, and 8 (Third Generation of Series A)

*Passage of Equine Encephalomyelitis Virus of Western Type for the Second Time through the Egg and Larval Stages of Dermacentor andersoni Stiles*

<table>
<thead>
<tr>
<th>Stage in developmental cycle</th>
<th>Tick vector</th>
<th>Animal host</th>
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<tbody>
<tr>
<td>Lot number</td>
<td>Host on which preceding stage engorged</td>
<td>Date placed on host</td>
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<td>Egg</td>
<td>D6-4</td>
<td>GP S5-31</td>
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The results of Experiment 6 indicated the probable passage of the virus into the larvae of the third generation (twice through the egg stage). There was no evidence that the larvae derived from the non-infectious females used in Experiment 5 had transmitted any virus to the normal animals on which they engorged. This finding agrees with earlier observations.
The purpose of Experiment 7 was to find out whether the virus was present in the nymphs that originated from the larvae used in Experiment 6. All of the nymphs coming from the larvae that had fed on the only host animal, a gopher (G 1-82), with suggestive evidence of having acquired the virus had unfortunately died during the winter. Thus, the only nymphs available were those derived from the larvae from which we had failed to obtain evidence for the transmission of the virus.

Experiment 7.—The nymphs came from the larvae tested in Experiment 6—larvae that had not been shown to be infectious when used from 256 to 295 days previously. These nymphs were permitted to feed on seven gophers and three guinea pigs in lots of 30 nymphs per animal. Having fed to repletion in from 5 to 7 days, they were placed in tubes to molt. Each of the ten hosts, none of which died or showed any clinical evidence of infection, was tested between 14 and 40 days later for active immunity to equine encephalomyelitis virus of Western type.

The findings of Experiment 7 are presented in Table IV. Each of the ten hosts survived the feeding of the nymphs. When these animals were tested for active immunity (immunity test 9) none manifested sufficient immunity to withstand the challenge dose of the virus (approximately 90,000 M.L.D.). This immunity test was not entirely satisfactory; for no immune controls were available. Nevertheless, the results had some significance, since earlier tests had shown that host animals becoming immune after infected ticks had engorged on them could survive greater challenge doses.

It is clear that Experiment 7 failed to establish the transmission of the virus from the larval to the nymphal stage of the third generation. However, since the only nymphs available for study had been derived from larvae not shown to be infectious, it is equally clear that the principal point at issue could not be tested. The only significant finding was the further evidence that virus which disappears from one stage of the tick cycle does not become manifest in succeeding stages.

From the results of Experiment 7, it was evident that the passage of the virus through ticks had been halted by the unfortunate death of all of the infected nymphs, after the series had embraced seven successive developmental stages involving three generations. In an effort to reestablish this series, Experiment 8 was undertaken. Adult ticks, one generation removed from adults that were known to have been infected, were tested for their ability to transmit virus, which it was hoped they might still be carrying.

Experiment 8.—The adult ticks used were the direct descendants of the adults (D5-8, 27) that had been shown in Experiment 5 to be harboring the virus. They were permitted to feed, between June 16 and 29, 1938, (from 237 to 280 days after they had fed as nymphs), on six gophers in lots of two male and two female ticks per gopher. After repletion, the females were put into tubes to oviposit and the males were discarded.
Chart 2. Diagrammatic representation of the findings in passage series A (Experiments 1 to 8). The virus of equine encephalomyelitis, Western type, having been acquired by nymphs of *Dermacentor andersoni* Stiles, as the result of feeding on experimentally infected guinea pigs, passed through seven successive developmental stages of the tick to the larvae of the third generation.
The hosts, all six of which survived, were tested from 30 to 45 days later for active immunity.

The findings of Experiment 8 are presented in Table IV. When each of the six gophers (G 2-36, G 2-45, G 2-46, G 2-47, G 2-53, and G 2-55) surviving the feeding of the adult ticks (D8-3, 9, 17, 21, 22) was tested for immunity to the virus (immunity test 9), none gave evidence of having acquired any. Although no immune controls were available when the animals were tested for immunity, the infectious titer of the viral suspension employed was not so great but that evidence for a slight degree of immunity should have been elicited. (The dose of virus injected into the test animals and into the normal controls was uniformly lethal, but a tenfold dilution of the same preparation was non-lethal.) These findings were interpreted as showing that the ticks had not transmitted any virus to the normal hosts.

Experiment 8 failed to reestablish the passage of the virus through ticks. Series A, which is graphically summarized in Chart 2, demonstrated that the virus of equine encephalomyelitis, Western type, having been acquired by nymphs of *Dermacentor andersoni* Stiles by feeding on an experimentally infected guinea pig, can pass through seven successive developmental stages of the tick cycle with probable involvement of larvae of the third generation. This necessitates two successful passages through the egg stage. The passage series was unfortunately terminated at this point by the death of all of the infectious ticks.

**Series B. First Generation**

In order to confirm the findings which had been obtained in series A, a second passage series, series B, was undertaken. Since the plan of procedure was the same as that described fully for series A, the experiments will be presented more briefly.

*Experiment 9.*—On May 11, 1936, six adult ticks, three males and three females, were placed in a feeding container on a guinea pig (GP 9-24). 48 hours later, this guinea pig was infected subcutaneously by the injection of 1 ml. of a 10 per cent suspension of brain tissue derived from an animal dead of experimental equine encephalomyelitis. Having engorged, the female ticks were removed from the guinea pig, when it died 5 days later, and placed in tubes to oviposit. The males were discarded.

In Experiment 9, normal adult ticks were fed on an animal that died of experimental equine encephalomyelitis. This feeding, which extended over a period of 7 days, theoretically insured the ingestion of the virus, for it included the 5 days between the injection of the virus and the death of the host. This single feeding was the only contact which the ticks that were used in the experiments of series B had with an infected host.
Series B. Second Generation

Experiment 10 was planned to demonstrate whether the adults used in Experiment 9 had acquired the virus, and, if so, whether any virus would appear in the larvae hatching from the eggs which they deposited.

Experiment 10.—The larvae hatching from eggs deposited by the adult females used in Experiment 9 were permitted to feed, between June 24 and 27, 1936, (from 33 to 37 days after the mothers had fed), on two gophers (G 4-9 and G 5-0) and one guinea pig (GP 9-56) in lots of from 50 to 75 larvae per animal. When replete 3 days later, the larvae were placed in tubes to molt. The three hosts, all of which survived, were tested 25 days later for active immunity.

The findings of Experiment 10 are presented in Table V. When the three hosts were tested for immunity (immunity test 1, Table I), one gopher and one guinea pig (G 5-0 and GP 9-56) reacted with only a transitory fever to a challenge dose of the virus which killed the third test animal, a gopher (G 4-9), and all of the six normal controls, but which was without apparent effect on the two immune controls.

The results of Experiment 10 established three points: that female ticks can acquire the virus by engorging on an experimentally infected guinea pig (GP 9-56) in lots of from 50 to 75 larvae per animal. When replete 3 days later, the larvae were placed in tubes to molt. The three hosts, all of which survived, were tested 25 days later for active immunity.

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pig; that the virus, so acquired, can be passed through the egg stage to the larvae of the next generation; and that these larvae can transmit the virus to normal hosts.

Experiment 11 was planned to determine whether the virus would pass through the infected larvae used in Experiment 10 to the next developmental stage, the nymphal.

Experiment 11.—The nymphs came from the larvae which had been shown (Experiment 10) from 80 to 95 days previously to have transmitted the virus. These nymphs were permitted to feed, between Sept. 15 and 28, on three gophers (G 6-2, G 6-8, and G 6-9) in lots of 10, 20, and 30 nymphs, respectively. After repletion, they were placed in tubes to molt. The gophers, all three of which survived the feeding, were tested for active immunity 21 days after the ticks were removed.

The findings of Experiment 11 are presented in Table V. When the three gophers (G 6-2, G 6-8, and G 6-9) that survived the feeding of the larvae were tested for active immunity (immunity test 4), each proved to be immune.

The results of Experiment 11 showed that the virus can pass from infected larvae to nymphs.

Experiment 12 was planned to determine whether the virus could be transmitted to normal animals by the adult ticks that came from the infected nymphs used in Experiment 11.

Experiment 12.—Adult ticks coming from the infected nymphs that had been used from 266 to 289 days previously (Experiment 11) were fed on two gophers in lots of three male and three female ticks per gopher. When replete, the adult females were placed in tubes to oviposit, the adult males were discarded. Both gophers survived the feeding and were tested for active immunity 21 days later.

The findings of Experiment 12 are presented in Table V. When the two gophers (G 1-55 and G 1-69) were tested for immunity (immunity test 6), both succumbed to a challenge dose of the virus which also killed the normal controls, but which was without apparent effect on the single immune control. If any virus was transmitted to the gophers, therefore, the amount was insufficient to immunize them effectively.

It could only be concluded that the virus had not passed from the infected nymphs used in Experiment 11 to the adult females. The death of the larval progeny of these females of the second generation, furthermore, forced the termination of series B at this point.

Series B, which is graphically summarized in Chart 3, confirmed a number of points brought out in series A. Thus, by obtaining single meals of blood from an animal experimentally infected with the Western type of equine encephalomyelitis virus, adult ticks can acquire the virus and pass it to their progeny, which in turn can transmit it to susceptible animals. This
confirms the passage of the virus through the egg stage from one generation to the next. Passage from the larvae to the succeeding nymphal stage was also confirmed.

<table>
<thead>
<tr>
<th>EXPERIMENT NUMBER</th>
<th>HOST ANIMALS ON WHICH THE TICKS WERE FED</th>
<th>HOST ANIMALS</th>
<th>NORMAL ANIMALS USED FOR CONTROLS</th>
<th>IMMUNE ANIMALS USED FOR CONTROLS</th>
<th>SUMMARY - PROPORTION OF HOST ANIMALS THAT WERE INFECTED BY THE FEEDING TICKS</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>A</td>
<td></td>
<td></td>
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<td>2/3</td>
</tr>
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<td>10</td>
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<td></td>
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<td></td>
<td>3/3</td>
</tr>
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<td>11</td>
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<td>0/3</td>
</tr>
</tbody>
</table>

**KEY**
- **A** Original source of the virus (guinea pig died of equine encephalomyelitis)
- **●** Type of host animal
- **○** Death from confirmed equine encephalomyelitis
- **□** Immunity to equine encephalomyelitis established
- Numbers within the symbols (□) are those of the immunity tests concerned - see text
- ▲ Death unrelated to equine encephalomyelitis

**Chart 3.** Diagrammatic representation of the findings in passage series B (Experiments 9 to 12). The virus of equine encephalomyelitis, Western type, having been acquired by adults of *Dermacentor andersoni* Stiles, as the result of feeding on an experimentally infected guinea pig, passed through three successive developmental stages of the tick to the nymphs of the second generation.

**DISCUSSION**

Under the experimental conditions described in the present paper, it has been found that the wood tick, *Dermacentor andersoni* Stiles, can function in two important roles with respect to the virus of equine encephalomyelitis, Western type, viz., that of a transmitting agent, and that of a reservoir host. When infected ticks are permitted to feed on guinea pigs or gophers, these
animals can acquire the infection. This is true irrespective of the stage of tick which is allowed to feed—larvae, nymphs, and adults can all transmit the virus. Previously, no tick of the genus *Dermacentor* has been implicated in the transmission of a viral disease, although other ticks belonging to the same family (Ixodidae) can serve as vectors. Once *Dermacentor andersoni* Stiles has acquired equine encephalomyelitis virus, furthermore, the tick may become a reservoir host, for virus can pass from generation to generation, *i.e.*, the infection can become hereditary. It should be noted that the virus passes through the egg stage as well as through the stages from larva to nymph and from nymph to adult. While it is common knowledge that some infectious agents, *e.g.*, certain of the rickettsiae and spirochetes, may become hereditary in ticks, the present demonstration is the first that a virus can be carried as a hereditary infection in a tick belonging to the family Ixodidae. The reasons why this family of tick was employed in the present work were stated in the introduction.

In an infectious disease, which is characterized by sporadic outbreaks, a limited seasonal occurrence, the absence of infection by contact, and the presence of the infectious agent in the blood, it is necessary to postulate a vector and a reservoir host in order to account for the natural occurrence of the disease. Equine encephalomyelitis has each of these characteristics. There are indications that the ground squirrel, *Citellus richardsonii* (Sabine), may be a reservoir host (20, 21, 36). There are probably other animals which can act in this capacity, for the susceptibility of a considerable variety has already been established (3, 4, 16–25).

3 The ability of ticks to serve as vectors has been demonstrated for four viruses: loping ill virus, transmitted by *Ixodes ricinus* (Linnaeus) (29) and by *Rhipicephalus appendiculatus* Neumann (30); the virus of tick-borne fever (sheep), by *Ixodes ricinus* (Linnaeus) (31); the virus of Nairobi sheep disease, by *Rhipicephalus appendiculatus* Neumann (32, 33) and by *Amblyomma variegatum* (Fabricius) (34); and the virus of rabbit papilloma (Shope) by *Haemaphysalis leporis-palustris* (Packard) (35). In the first two instances it has been shown that the virus can successfully pass from one stage in the life cycle to the next, but cannot pass the egg stage barrier to the next generation. In the case of Nairobi sheep disease, on the other hand, the virus has been found capable of passing the egg stage barrier. Nevertheless, passage of the virus in ticks is self-limited, unless reinfection takes place at each feeding. Thus, the virus of Nairobi sheep disease is not hereditary in the tick. The transmission of the virus of rabbit papilloma (Shope) by *Haemaphysalis leporis-palustris* (Packard) was demonstrated for only a single stage, the nymphal.

4 The following animals and birds have been shown to be susceptible to experimental infection by the virus of equine encephalomyelitis. (They are arranged alphabetically for convenience of reference.) Mammals—cat, dog, guinea pig, hedgehog, monkey, mouse, cottontail rabbit, domestic rabbit, jack rabbit, rat, ground squirrel, field vole,
The ticks that had acquired equine encephalomyelitis virus engorged, molted, and were ready to feed again much more rapidly than normal ticks. As explained above, *Dermacentor andersoni* Stiles usually requires at least two years to complete a single life cycle. We have found that the infected ticks completed a single life cycle in the unprecedentedly brief period of 156 days. Since this acceleration may be the direct result of stimulation by the virus, this interesting observation deserves further study.

No vector has been found to date that harbors or transmits the virus of equine encephalomyelitis under natural conditions, although theoretical considerations suggest strongly that such a vector must exist. Experimentally, however, two arthropodal vectors, the mosquito and the tick, have been shown to be capable of transmitting the virus. The mosquito has received the most consideration as a possible vector. While it has been shown that mosquitoes of the genus *Aedes* can harbor and transmit the virus under experimental conditions, the virus is limited in this vector to the adult female. Furthermore, adults of the genus *Aedes* do not survive the rigorous winters that are typical in regions where the disease is most prevalent. Thus, it does not seem likely that this genus of mosquito can be the sole, perhaps even the principal, vector. It is noteworthy, finally, that attempts to demonstrate that other genera of mosquito can transmit the virus have not succeeded.

In contrast to the mosquito, the tick has been shown in the present investigation to be capable of passing the Western type of equine encephalomyelitis virus to its progeny, whereby the virus is carried from one generation to the next. It is not surprising that the tick can acquire the virus during its long period of feeding on an infected host, for this period is almost certain to include the phase of the disease during which the virus is present in the blood. Neither is it unexpected that the tick can transmit the virus, for its ability to transmit a variety of other infectious agents is common knowledge.

The possible epidemiological implications of the present findings are

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and woodchuck. Birds—blackbird, chicken, cowbird, dove, duck, goose, grackle, guinea fowl, hawk, Western burrowing owl, ring-necked pheasant, pigeon, quail, sparrow, stork, turkey, and tawny vulture.

6 Gwatkin, in 1939 (36), reported a single observation which also demonstrated that the nymphal stage of *Dermacentor andersoni* Stiles can acquire the virus from an animal infected with equine encephalomyelitis of Western type. One of the ground squirrels that was used in his study was carrying three partially engorged nymphs when it was captured. Following the inoculation of this gopher with the virus of equine encephalomyelitis, Western type, the virus was recovered from the triturated ticks.
several. Although *Dermacentor andersoni* Stiles is limited in occurrence to the Rocky Mountain region, other species of the genus *Dermacentor* are widely distributed throughout the United States. Possibly some of these species can also transmit and harbor equine encephalomyelitis virus. If ticks are found to possess this ability under natural conditions, it will go a long way towards accounting for the epidemiology of equine encephalomyelitis. Furthermore, it is possible that ticks may play an important rôle in other viral diseases, as indicated by the findings of Shaughnessy and Milzer (37). These workers reported that *Dermacentor andersoni* Stiles can acquire the virus of lymphocytic choriomeningitis and pass it through successive stages from one generation to the next. Evidence for the successful transfer of this virus by the ticks when they were permitted to engorge on the host animals, however, was limited to a single stage, the nymphal, which had fed as larvae on an infected guinea pig.

In view of the findings reported in the present paper, it would seem to be worth while for those working in the field to investigate the possible rôle of *Dermacentor andersoni* Stiles in the epidemiology of equine encephalomyelitis. We wish to emphasize that the work reported herewith is experimental in character only. Nevertheless, it suggests that ticks may be responsible for the maintenance of viruses from year to year and for their transmission to a variety of hosts, especially during the early spring. Other vectors would be more likely in the distribution of disease during the summer and fall (at least in the United States). The heightened incidence of equine encephalomyelitis during this period can best be explained, perhaps, by the assumption that the mosquito is the responsible transmitting agent.

**SUMMARY**

The Western type of equine encephalomyelitis virus can be passed as an hereditary infection in a tick of the family Ixodidae, *Dermacentor andersoni* Stiles. Under experimental conditions, this virus has been carried in this tick for two successive generations, possibly for a third, passing certainly once, and possibly twice, from the female through the eggs to the larvae. The virus-carrying larval, nymphal, and adult stages of this tick, furthermore, are capable of infecting susceptible hosts when they are permitted to feed on them.

**BIBLIOGRAPHY**

EXPLANATION OF PLATES

PLATE 27

Fig. 1. Distribution in the United States of the two most prevalent species in the genus *Dermacentor*, *Dermacentor andersoni* Stiles and *Dermacentor variabilis* (Say).

Fig. 2. Photographs of *Dermacentor andersoni* Stiles showing the four successive stages in its life cycle. All photographs were made by Mr. M. C. Orser.
REGIONAL OCCURRENCE OF TWO TICKS OF GENUS DERMACENTOR

1. DERMACENTOR ANDERSONI  Stiles
2. DERMACENTOR VARIABILIS  (Say)

Developmental cycle of ticks of the genus Dermacentor

Adult → Egg → Larva → Nymph

♂ X 2.5   ♀ with eggs X 1

♀ X 2.5   Enorged ♀ X 1

♂ X 2.5   Egg X 25

♀ X 25   Enorged larva X 25

Nymph X 25

(Syverton and Berry: Equine encephalomyelitis in ticks)
PLATE 28

FIG. 3. Photograph of the ground squirrel, *Citellus richardsonii* (Sabine), wearing the container in which the ticks were fed. \( \times \frac{1}{2} \).

FIG. 4. Photographs to show the parts of the feeding container with the steps in its assembly. \( \times \frac{1}{4} \).

*A*, cardboard mailing tube (15 \( \times \) 3.75 cm.). After the top of the tube has been immersed in hot water for a few minutes, the metal top is readily separated from the cardboard.

*B*, *C*, *D*, successive stages in making the screw top—*B*, the unaltered screw top; *C*, the top after a circular perforation has been made; *D*, the top after the copper screen (60 mesh per inch) has been soldered in place.

*E*, *F*, *G*, successive stages in making the threaded base—*E*, the unaltered threaded base; *F*, the base after the teeth (3 to 4 mm. in width) have been cut below the threaded portion and bent outward at right angles; *G*, the base showing the teeth covered with adhesive to prevent injury to the host.

*H*, the flexible metal collar, shaped like a saddle blanket. The circular perforation is just large enough to fit tightly over the threaded basal portion of the container.

*J*, the first girdle that is affixed to the host. This band of adhesive plaster (7 to 8 cm. in width) is shown with the adhesive surface and the flanged basal portion of the container uppermost.

*K*, the second girdle. The band is shown inverted with the saddle blanket collar attached to the adhesive surface. Fig. 3 shows both of the girdles attached.