BLOOD-SUCKING VECTORS OF ENCEPHALITIS: EXPERIMENTAL TRANSMISSION OF ST. LOUIS ENCEPHALITIS (HUBBARD STRAIN) TO WHITE SWISS MICE BY THE AMERICAN DOG TICK, DERMACENTOR VARIABILIS SAY

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Ever since the epidemic of acute encephalitis in the St. Louis area which occurred during the summer of 1933 and recurred in the summer of 1937, investigators have been interested in this disease entity, and considerable experimental work has been done with the virus isolated from brain tissue of fatal human cases. Of special interest has been the possible mode of transmission of St. Louis encephalitis. Until recently the general concensus had seemed to favor person-to-person transmission by droplet infection.

Certain features of the disease, however, suggested the possibility of an arthropod vector: (1) The virus has never been isolated from nasopharyngeal washings; (2) there is only meager evidence to suggest case-to-case transmission; (3) the disease is prevalent in summer and early autumn; (4) during the 1933 epidemic the first cases were reported from St. Louis County in areas adjacent to wooded districts in the watershed of a small stream, the River Des Peres. Only later in the epidemic were cases reported in the city proper, and these cases were most numerous in precincts adjoining the county line; (5) that an endemic focus exists in the St. Louis area is indicated by the occurrence of proved cases of St. Louis encephalitis in non-epidemic years: two during August, 1939, in Clayton, a suburb of St. Louis; another proved case during June, 1940, in Glendale, also a suburb; and two proved cases in the latter part of August, 1942, one a resident of Robertson, Missouri, in St. Louis County, and one, a resident of St. Louis who had been spending the summer at a children's camp in St. Louis County. It is pertinent to note that throughout the epidemic of 1933 there was a high per capita incidence of St. Louis encephalitis in these outlying communities, the second highest occurring in Glendale.

With these observations in mind we sought to ascertain, first, whether a blood-sucking vector is capable of being infected by the virus of St. Louis encephalitis, and second, if so infected, whether the disease can be transmitted to susceptible animals by bite. Preliminary studies (1, 2) indicated that both of the foregoing are true.
A survey of the literature emphasized the importance of blood-sucking vectors in other virus encephalitides. Syverton and Berry (3-6) conducted extensive studies of the wood tick, Dermacentor andersoni Stiles, demonstrating hereditary infection and experimental transmission of the Western type of equine encephalomyelitis. Gwatkin (7, 8) reported an incidental observation that the nymph of this species can acquire the virus from an infected animal and that the virus may be recovered from the body of the nymph following engorgement. Grundmann and his associates (17) were not able to demonstrate infection of the dog tick, Dermacentor variabilis Say with the virus of equine encephalomyelitis, Western strain, and had no evidence of hereditary transmission. Positive results were obtained by these investigators using the assassin bug, Triatoma sanguisuga Le Conte. Several genera of mosquitoes and assassin bugs are also thought to be involved in the epidemiology of equine encephalomyelitis (9-19). Similarly a number of species of Ixodes have been implicated in Russian encephalitis (20-25); two genera of mosquitoes in Japanese B (26, 27); the wood tick, the bed bug, and possibly a mosquito in lymphocytic choriomeningitis (28, 29). House and stable flies studied in connection with the epidemiology of poliomyelitis have given positive results (30-36). Recent work by Hammon, Howitt, and their associates has shown the natural occurrence of the virus of St. Louis encephalitis in the mosquito, Culex tarsalis, as well as the presence of type specific antibodies in the blood of numerous species of mammals and birds. These authors have demonstrated experimental transmission of the St. Louis virus in fowl by 9 species of mosquitoes from 3 genera. So far the fully incriminated species is Culex tarsalis (19, 37).

Life Cycle of Ticks and Care in the Laboratory

The common dog tick, Dermacentor variabilis, was selected as the arthropod vector for the experimental work reported here. Dermacentor variabilis must be differentiated from Dermacentor andersoni, well known as the chief vector of Rocky Mountain spotted fever. While some specimens of D. andersoni have been collected from the environs of St. Louis, the majority collected have been D. variabilis.

The following is a brief summary of the life cycle of variabilis under laboratory conditions (Fig. 1). Adult females feeding on a suitable host required on the average 4 to 6 days for complete engorgement. 4 days after complete engorgement eggs were laid. Within 15 days these developed into typical six-legged larvae, which, after a blood meal, metamorphosed within 10 days into eight-legged nymphs. These nymphs in turn became adults (8 legs) in 6 to 8 days following engorgement. It is of importance to note that a blood meal is required before metamorphosis from the larval to the nymphal stage and from the nymphal to the adult stage can occur. If such a blood meal is not available, the tick enters a diapause or latent stage. During diapause, which may extend for considerable time, the tick survives without food, with a minimum of moisture, and often under extremely adverse conditions. The egg also is resistant to drying and reasonably resistant to extremes of temperature. The life cycle of 45 to 50 days as observed in the laboratory under optimum conditions may be
extended in nature 2 years or longer. Apparently ticks can survive a winter in any stage of their development. Diapause may be induced in any stage, especially during inanition, by keeping ticks at low temperatures.

Ticks not being used in current experiments were kept in a refrigerator at a constant temperature of 12.5°C. The majority of the colony being used for experiments was kept at room temperature summer and winter. All ticks were housed in glass tubes stoppered with cotton and gauze and placed in screw top jars containing moist sand. As a precautionary measure against escape, these jars were kept at all times in shallow pans containing cresol solution. These conditions of temperature and mois-

Fig. 1. The life cycle of *Dermacentor variabilis* as observed in the laboratory. × 5.

Fig. 1. The life cycle of *Dermacentor variabilis* as observed in the laboratory. × 5.

EXPERIMENTAL

*Infection of Dermacentor variabilis by the Virus of St. Louis Encephalitis*

The primary phase of the experimental work concerned the question whether the tick, *Dermacentor variabilis*, could be infected by feeding on an animal previously inoculated with St. Louis encephalitis virus. The Hubbard strain of
St. Louis encephalitis virus used in this work has been maintained in consecutive mouse passage since 1937 when it was isolated from a fatal human case. Ticks in various stages of the life cycle, larvae, nymphs, adults, were allowed to feed on white Swiss mice or Syrian hamsters, *Cricetus auratus*, which had been inoculated with large doses of virus, usually intraperitoneally. In the case of mice, inoculation was often made intracerebrally as well as intraperitoneally, in the case of hamsters always intraperitoneally only. Since the virus is present in the blood stream for a relatively short period only (19, 20, 37, 39), ticks were given opportunity for attachment as soon as possible after inoculation. In our laboratory, tests on hamsters inoculated intraperitoneally with St. Louis encephalitis virus showed that the virus was present in the blood stream from 6 hours to 72 hours following inoculation. When engorgement was satisfactory, the ticks were removed carefully, triturated, and ground in an agate mortar with a small amount of broth. The resulting extract was inoculated intraperitoneally into white Swiss mice, 2 to 3 weeks of age. This age group was selected because it has been established (40) that young mice are extremely susceptible to small doses of virus even when injected intraperitoneally. Extracts of tick bodies obviously are not bacteriologically sterile. For this reason the intraperitoneal route of inoculation is of particular advantage in this work. In no instance were we able to demonstrate evidence of bacterial infection in animals so inoculated, and routine cultures of the brain, peritoneal sac, spleen, and lung were negative.

The young mice inoculated intraperitoneally with extracts of tick bodies were observed carefully. In 9 to 14 days definite evidence of illness, often with convulsions, was noted. Brains removed from such animals were injected intracerebrally for passage test into normal adults, which invariably developed typical symptoms of encephalitis in 3 to 4 days. The virus, which was recovered, was identified readily as the St. Louis type by appropriate mouse and egg protection tests, using the serum of rabbits hyperimmunized against St. Louis encephalitis virus, Hubbard strain.

The following is a summary of the experiments dealing with the infection of *Dermacentor variabilis* by the virus of St. Louis encephalitis:

1. **Adult Ticks.**—4 adult ticks were permitted to engorge for 3 days on a hamster inoculated intraperitoneally with the virus of St. Louis encephalitis, 1 ml. $10^{-1}$ suspension of mouse brain in broth. (The hamster died within 15 days, and the virus of St. Louis encephalitis was recovered from its brain.) An extract prepared by adding 2 ml. of tryptose phosphate broth to the triturated bodies of these 4 engorged ticks was inoculated intraperitoneally, 0.1 ml., into 16 white Swiss mice, 2 to 3 weeks of age. Within 9 to 14 days all 16 mice showed signs of illness: ruffled fur, hunching, loss of appetite, convulsions, and death, varying in severity with the individual. Secondary passage of the brains of these 16 mice was made to 60 adult mice, (0.03 ml. intracerebrally, $10^{-3}$) all of which developed convulsions within 3 to 5 days. The virus re-
covered from the brains of passage mice was identified readily by appropriate mouse
and egg protection tests. 6 other adult ticks allowed to engorge similarly on normal
animals, were ground and the extract injected, 0.1 ml. intraperitoneally, into 20 white
Swiss mice. In no instance did symptoms of encephalitis develop, and antibodies
were absent in the blood of several of these mice which were tested by protection
methods.

2. Larvae.—An extract was prepared by adding 1 ml. of broth to the triturated
bodies of 35 to 40 larvae, which had engorged for 3 days on mice, previously inoculated
intracerebrally (0.03 ml.) with $10^{-1}$ suspension of St. Louis encephalitis virus. 0.1
ml. of this extract was inoculated intraperitoneally into each of 34 mice, 2 to 3 weeks
of age. Within 7 to 10 days all mice receiving this larval extract showed signs of
illness as described above. The brains of these mice were passed, 0.03 ml. intracere-
braUy, $10^{-1}$, to a total of 134 adult mice, all of which developed convulsions within 3
to 4 days. As a control, 19 mice, 2 to 3 weeks of age, were inoculated in an identical
manner except that the larvae used in preparing the extract to be inoculated were
allowed to engorge for 3 days on normal mice. All 19 remained free of symptoms and
were discarded after a 2 months period of observation.

3. Nymphs.—Likewise nymphs could be infected with the virus of St. Louis
encephalitis and here also the virus recovered was identified as the St. Louis type.
Nymphs were allowed to engorge for 4 days on adult mice which had been inoculated
with St. Louis encephalitis virus, 0.03 ml. intracerebrally and 0.1 ml. intraperitone-
ally, $10^{-1}$. Extract of nymph bodies, 20 to 30 per ml. of broth, was injected intra-
peritoneally, 0.1 ml., into 24 mice, 2 weeks of age. 9 to 15 days later symptoms ap-
peared in all 24 mice receiving this extract. Secondary passage of their brains to 84
adult mice by intracerebral inoculation of 0.03 ml., $10^{-1}$, produced convulsions in all
84 within 3 days. By comparison, 16 2-weeks-old mice inoculated with 0.1 ml.
intraperitoneally of extract of nymphs (sister nymphs of the foregoing), prepared in
similar manner except that the nymphs were fed on normal mice, remained entirely
free of symptoms and no virus was recovered from the passage of their brains to adult
mice. The normal mice upon which these nymphs engorged also remained free of
symptoms and no antibodies for encephalitis could be demonstrated in the blood of
several tested.

Transmission of the Virus of St. Louis Encephalitis by Tick Bite

Having shown the dog tick capable of being infected by the virus of St.
Louis encephalitis, the next phase of the experiment concerned the question
whether encephalitis could be transmitted to other animals by the bite of such
infected ticks. Accordingly, ticks in all stages of development were given an
initial feeding on infected animals, after which they were transferred to normal
white Swiss mice, 2 to 3 weeks of age. Engorgement was continued on the
second host. In 9 to 14 days, signs of illness, often convulsions, appeared.
Here again the virus was recovered from the second host and in every instance
was identified as the St. Louis type. It is of interest that when older mice were
used as the second host, signs of illness were noted, such as listlessness, lack of
interest in food, hunched position, and ruffled fur. These older animals, however, showed only mild tremors and recovered. 8 to 12 weeks later we were able to demonstrate the presence of a high titre of antibody against St. Louis encephalitis virus in the blood of some of these recovered mice (Tables I and II). Others in this group showed a marked resistance to intracerebral inoculation of virus, withstanding as much as 10,000 minimal lethal doses. The following summary gives the detail of these experiments:

1. Larvae.—10 to 25 laboratory-bred larvae which had engorged partially for 2 days on infected adult mice (0.03 ml. intracerebrally and 0.1 ml. intraperitoneally, St. Louis encephalitis virus, $10^{-1}$) were transferred to each of 21 3-weeks-old mice and allowed to complete engorgement for 2 to 3 days. These young, second host mice showed characteristic signs of illness in 10 to 11 days. Brains from these 21 were passed to 62 adult mice in all, which developed convulsions in 3 days. Parallel

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**TABLE I**

*Serum-Virus Mouse Protection Test for the Presence of Antibodies to St. Louis Encephalitis (Hubbard Strain) in the Blood of Mice on Which Infected Ticks Were Fed, As Compared with the Blood of Mice upon Which Uninfected Ticks Were Fed*

Mouse protection test: mouse 1, heart blood 8 weeks following exposure by bite.

<table>
<thead>
<tr>
<th>Normal human serum + dilutions of virus as follows:</th>
<th>No. mice inoculated 0.03 ml. intracerebrally</th>
<th>Survived</th>
<th>Dead</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^{-4}$ (100,000 M.L.D.)</td>
<td>4 mice</td>
<td>None</td>
<td>All dead within 3 to 4 days</td>
</tr>
<tr>
<td>$10^{-4}$ (10,000 M.L.D.)</td>
<td>4 mice</td>
<td>None</td>
<td>All dead within 4 days</td>
</tr>
<tr>
<td>$10^{-4}$ (1,000 M.L.D.)</td>
<td>4 mice</td>
<td>None</td>
<td>All dead within 6 days</td>
</tr>
<tr>
<td>Hyperimmune rabbit serum + dilutions of virus as follows:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$10^{-4}$ (100,000 M.L.D.)</td>
<td>4 mice</td>
<td>All</td>
<td>None</td>
</tr>
<tr>
<td>$10^{-4}$ (10,000 M.L.D.)</td>
<td>4 mice</td>
<td>All</td>
<td>None</td>
</tr>
<tr>
<td>$10^{-4}$ (1,000 M.L.D.)</td>
<td>4 mice</td>
<td>All</td>
<td>None</td>
</tr>
<tr>
<td>Mouse 1 serum + dilutions of virus as follows:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$10^{-4}$ (100,000 M.L.D.)</td>
<td>4 mice</td>
<td>All</td>
<td>None</td>
</tr>
<tr>
<td>$10^{-4}$ (10,000 M.L.D.)</td>
<td>4 mice</td>
<td>All</td>
<td>None</td>
</tr>
<tr>
<td>$10^{-4}$ (1,000 M.L.D.)</td>
<td>4 mice</td>
<td>All</td>
<td>None</td>
</tr>
<tr>
<td>Control mouse serum + dilutions of virus as follows:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$10^{-4}$ (100,000 M.L.D.)</td>
<td>3 mice</td>
<td>None</td>
<td>All dead within 3 days</td>
</tr>
<tr>
<td>$10^{-4}$ (10,000 M.L.D.)</td>
<td>4 mice</td>
<td>None</td>
<td>All dead 4th day</td>
</tr>
<tr>
<td>$10^{-4}$ (1,000 M.L.D.)</td>
<td>4 mice</td>
<td>None</td>
<td>All dead within 4 to 5 days</td>
</tr>
</tbody>
</table>
experiments were carried as a control using larvae hatched from the same brood of eggs as above. 19 to 30 of these larvae were allowed to attach on each of 13 mice, 2 to 3 weeks of age, after previous partial engorgement on normal adult mice. Details of the experiment were identical with the foregoing. No signs of infection were observed over a period of 2 months.

2. Nymphs.—Nymphs metamorphosed from larvae hatched in the laboratory, were allowed to engorge partially (2 days) on adult white Swiss mice, inoculated with St. Louis encephalitis virus (0.03 ml. intracerebrally and 0.1 ml. intraperitoneally

\[
\begin{array}{|c|c|c|}
\hline
\text{Normal human serum} & \text{No. eggs inoculated} & \text{Growth of virus on chorioallantois} \\
& 0.1 \text{ ml. each} & \\
10^{-8} & 3 \text{ eggs} & ++++, +++++, +++++ \\
10^{-4} & 4 \text{ eggs} & +++, +++, +++, +++++ \\
10^{-4} & 4 \text{ eggs} & +++, +++, +++, +++++ \\
\hline
\text{Hyperimmune rabbit serum} & & \\
10^{-8} & 3 \text{ eggs} & 0, 0, 0 \\
10^{-4} & 4 \text{ eggs} & 0, 0, 0, 0 \\
10^{-4} & 4 \text{ eggs} & 0, 0, 0, 0 \\
\hline
\text{Mouse 1 serum} & & \\
10^{-8} & 3 \text{ eggs} & 0, 0, 0 \\
10^{-4} & 4 \text{ eggs} & 0, 0, 0, 0 \\
10^{-4} & 4 \text{ eggs} & 0, 0, 0, 0 \\
\hline
\text{Control mouse serum} & & \\
10^{-8} & 3 \text{ eggs} & +++, +++, +++++ \\
10^{-4} & 3 \text{ eggs} & +++, +++, +++++ \\
10^{-4} & 3 \text{ eggs} & +++, +++, +++++ \\
\hline
\end{array}
\]

mouse brain suspension, \(10^{-3}\)). 10 to 25 such partially engorged nymphs were transferred for completion of engorgement (2 days) to each of 8 3-weeks-old mice. In 12 to 15 days all 8 mice showed characteristic signs of encephalitis. Passage of the brains of these 8 to 22 adult mice showed the presence of virus which was identified as before by mouse and egg protection tests. In parallels carried as control, nymphs of the same original brood were used, and normal mice only were the source for engorgement. In none of these was there evidence of infection.

3. Adults.—6 adult ticks raised in the laboratory were allowed to engorge partially for 2 days on adult mice inoculated with St. Louis encephalitis virus as described above. After partial engorgement, each of the 6 was transferred to a normal white Swiss mouse, 2 weeks of age, and permitted to complete engorgement. All 6 of the
young mice thus exposed by bite showed illness with convulsions in 10 to 13 days. Secondary passage mice, 14 in number, developed severe convulsions in 2½ to 3 days. In parallels carried as control, adult ticks of the same original brood were used, and normal mice only were the source for engorgement. In none of these was there evidence of infection.

**Hereditary Transmission of Virus of St. Louis Encephalitis**

Another series of experiments was concerned with hereditary transmission of the virus. Adult female ticks were allowed to feed on an inoculated hamster. After complete engorgement, eggs were laid. In each case an extract of the eggs was injected intraperitoneally into young white Swiss mice. In 9 to 14 days evidence of infection was apparent. The virus of St. Louis encephalitis was recovered from the brain tissue of these young mice by intracerebral passage to older mice, and was identified by protection tests in the same manner as before. Likewise, larvae hatched from these eggs were studied for the presence of virus in their bodies. An extract prepared by grinding larval bodies was inoculated into young mice. Definite signs of infection were noted within 9 to 15 days. Further such larvae were allowed to engorge on normal young white Swiss mice, and in 9 to 15 days signs of infection appeared in these normal mice. Similar results were obtained for the nymphal and adult stages of development. Adult female ticks raised in the laboratory from infected eggs were allowed to engorge on normal hamsters. The virus was recovered from an extract of their eggs by inoculation of young mice and secondary passage to older mice. In this manner the virus was shown by extract to be present in the bodies of larvae, nymphs, and adults, themselves originating from the second generation eggs. All three of these stages were capable also of transmitting the virus by bite to susceptible young mice.

A parallel series of experiments was carried as control which was identical with the foregoing in all respects, except that in every step described above normal, in place of infected, mice or hamsters were used as the source of blood meals. No animals of this control series showed signs of illness, and all attempts to recover virus from their brains gave negative results.

The following gives the detail of the passages just described:

1. **Recovery of Virus from Eggs of Infected Females.**—4 female ticks were allowed to engorge for 5 days on a hamster inoculated 20 minutes previous to the beginning of the engorgement period, with 1 ml. intraperitoneally 10⁻¹ broth suspension of serial passage mouse brain, Hubbard strain. Eggs laid by each tick within a 4 day period following complete engorgement, were divided into half, one-half being kept for hatching. One-half the eggs of each of the 4 females was pooled and ground in 2 ml. of broth. The resulting extract was inoculated into 8 mice, 2 weeks of age, 0.1 ml. intraperitoneally. In 10 to 14 days characteristic symptoms were noted in these 8 mice: lassitude, loss of appetite, followed by mild convulsions. Secondary passage of
the brains of these 8 mice was made to 26 adult mice, 0.03 ml. intracerebrally, all of
which developed severe convulsions in 3 to 5 days. The virus was identified as be-
fore. As control, a broth extract was prepared from the total egg yield (in 1 ml. of
broth) of 2 female ticks which had engorged fully on a normal hamster. 6 mice, 2
weeks of age, received this extract, 0.1 ml. intraperitoneally, and showed no symp-
toms whatsoever.

2. Recovery of Virus from 1st Generation Larvae. Mother Tick Engorged on In-
fected Hamster.—One-half the egg yield of each of the 4 female ticks in 1 (above) was
allowed to hatch into larvae. An extract of some of these larvae, whose mother had
engorged on an inoculated hamster, was prepared by grinding their bodies in an agate
mortar, 24 to 30 larvae per ml. of broth. 19 mice (2 to 3 weeks old) were inoculated
intraperitoneally, 0.1 ml., with this extract. In 10 to 15 days, all 19 mice showed
typical illness. Secondary passage to 30 adult mice, 0.03 ml. intracerebrally, 10⁻¹,
perpetuated the virus, which was identified by mouse and egg protection tests as the
St. Louis type, Hubbard strain. Parallel controls carried as before showed no symp-
toms; larvae hatched from eggs laid by female ticks fed on a normal hamster were
used as control.

3. Recovery of Virus from 1st Generation Nymphs. Mother Tick Engorged on In-
fected Hamster.—A part of the larvae described in 2, whose mother had engorged on
an inoculated hamster, were allowed a blood meal on normal white Swiss mice, 3 weeks
of age, and permitted to metamorphose into nymphs. (The normal mice upon which
these larvae were allowed to engorge showed typical signs of illness in 11 to 14 days.
In each instance the virus was recovered from the brain by passage to adult mice and
was identified by the usual procedure as the St. Louis type.) An extract prepared
from these nymphs, 17 to 25 per ml. of broth, was inoculated into 8 2-weeks-old mice,
0.1 ml. intraperitoneally. 12 to 15 days later all 8 mice showed characteristic ill-
ness. Secondary passage was made in the usual manner to 19 adult mice which
developed convulsions in 2½ to 4 days. Virus was identified and controls were carried
as before.

4. Recovery of Virus from 1st Generation Adults, 1 Full Generation Removed from
Original Infected Females.—Sister larvae to those described in 3 were allowed to feed
on normal white Swiss mice, 3 weeks of age, and subsequently to metamorphose into
nymphs. Following another blood meal on normal mice the nymphs metamorphosed
into adults. (The normal mice upon which these nymphs engorged showed typical
symptoms of encephalitis within 12 to 16 days. The virus of St. Louis encephalitis
was recovered in the usual manner.) An extract prepared from 3 such adult ticks in
2 ml. of broth was inoculated into 14 mice, 3 weeks of age, 0.1 ml. intraperitoneally;
in 10 to 12 days illness was evident in these 14 mice, and the virus was recovered as
before by passage to 15 adult mice which developed convulsions in 2½ to 3 days.
Identification of the virus was made, as in previous experiments, by protection tests.

5. Recovery of Virus from Eggs of 1st Generation Adults.—3 female ticks metamor-
phosed from nymphs described in the series in 4 were allowed to engorge completely
(4 days) on a normal hamster. (The normal hamster upon which these 3 adult ticks en-
gorged died on the 28th day following the beginning of the engorgement period. Virus
was recovered from its brain by passage to adult mice and was identified as the St.
Louis type.) 5 days later the egg yield was removed from each tick, and the pooled
yield was separated into half. One-half of this egg yield was ground with 2 ml. of broth in an agate mortar, and the resulting extract was inoculated (0.1 ml. intraperitoneally) into 6 mice, 11 days of age. These young mice showed signs of illness on the 9th day following inoculation. The brain of each was passed to 3 adult mice, 0.03 ml. intracerebrally 10⁻¹. All 18 adult passage mice developed convulsions in 2 to 4 days.

6. Recovery of Virus from 2nd Generation Larvae, Offspring of Adults in 4 and 5.—
One-half the egg yield in 5 was allowed to hatch into larvae. An extract prepared by grinding some of these larvae, 28 to 35 per ml. of broth, was inoculated into 16 mice, 3 weeks of age, 0.1 ml. intraperitoneally. In 9 to 15 days these 16 mice became ill with characteristic symptoms. The brain of each of the 16 young mice was passed to 2 adult mice (32) which showed convulsions in 3 to 4 days. Identification of the virus was made in the usual manner.

7. Recovery of the Virus from 2nd Generation Nymphs Metamorphosed from Larvae in 6.—Some of the larvae described in 6 were allowed to engorge for 3 days on normal white Swiss mice, 2 to 3 weeks of age, and subsequently metamorphosed into nymphs. (The normal mice became infected as before; incubation period 12 to 15 days.) An extract prepared from such nymphs, 12 to 16 per ml. of broth, was inoculated into 10 mice, 12 days of age, 0.1 ml. intraperitoneally. In 13 to 14 days all of the 10 mice so inoculated were dead or ill. Passage to 30 adult mice resulted in convulsions in all 30 within 2 to 4 days.

8. Recovery of Virus from 2nd Generation Adults (2 Full Generations Since Females Were Infected).—Some of the group of nymphs in 7 were fed for 4 days on normal mice, 15 days of age, and allowed to transform into adults. (These normal mice developed symptoms in 13 to 18 days. Virus recovered as before.) 2 of these adults were extracted as before in 1 ml. of broth. The extract was injected intraperitoneally (0.1 ml.) into 10 mice, 17 days of age. In 13 to 14 days all 10 showed signs of illness, and the brain of each was passed to adult mice which developed convulsions within 2½ to 3 days. The virus was recovered from these secondary passage mice and identified by appropriate mouse and egg protection tests as that of St. Louis type, Hubbard strain, after passage through 2 full tick generations. Fig. 2 is a diagram which shows these 2 generations.

The above series shows that the virus of St. Louis encephalitis can be transmitted from an infected female through her eggs, through all stages of subsequent development, and is present in the eggs of her adult offspring, passing to the larvae in the course of development, in metamorphosis to their nymphs and to adults, 2 complete generations removed from the originally infected females which engorged on inoculated hamsters (Fig. 2). That the virus remained unchanged in essential character was shown by protection tests using the serum of rabbits hyperimmunized against St. Louis encephalitis stock virus, Hubbard strain. Presumably, the virus may be perpetuated indefinitely in the bodies of ticks provided environment for tick propagation is satisfactory.
Generation I

Mother tick infected by feeding on inoculated hamster.

Eggs → Extract of eggs injected intraperitoneally into normal mice, 2 weeks of age, produced encephalitis.*

Larvae

Extract of larval bodies intraperitoneally into normal mice, 2 to 3 weeks of age, produced encephalitis.

Transmission to normal mice, 2 weeks old, by bite.

Engorged on normal mice, 2 weeks of age, which developed encephalitis in 11 to 14 days.

Nymphs

Extract of nymphal bodies intraperitoneally into normal mice, 2 weeks of age, produced encephalitis.*

Transmission to normal mice, 3 weeks of age, by bite.

Engorged on normal mice, 3 weeks of age, which developed encephalitis in 12 to 17 days.

Adults

Extract of tick bodies intraperitoneally into normal mice, 3 weeks of age, produced encephalitis.

Transmission to normal mice, 2 to 3 weeks of age, by bite.

Engorged on normalhamster which died of encephalitis on the 28th day following the beginning of the engorgement period.

Generation II

Eggs → Extract of eggs injected intraperitoneally into normal mice, 11 days old produced encephalitis.*

Larvae

Extract of larval bodies intraperitoneally into normal mice, 3 weeks of age, produced encephalitis.

Transmission to normal mice, 2 to 3 weeks of age, by bite.

Engorged on normal mice, 2 weeks of age, which developed encephalitis in 11 to 14 days.

Nymphs

Extract of nymphal bodies intraperitoneally into normal mice, 12 days of age, produced encephalitis.

Transmission to normal mice, 2 to 3 weeks of age, by bite.

Engorged on normal mice, 2 weeks of age, which developed encephalitis in 14 to 16 days.

Adults

Extract of tick bodies intraperitoneally into normal mice, 15 days of age, produced encephalitis.

Transmission to normal mice, 3 weeks of age, by bite.

Fig. 2. Hereditary transmission of the virus of St. Louis encephalitis in the American dog tick, *Dermacentor variabilis.*

* Virus recovered in each instance was identified by appropriate protection tests as the Hubbard strain of St. Louis encephalitis virus.

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Duration of Infection in Ticks of Various Stages; Recovery of Virus from Dormant Ticks

In connection with the foregoing experimental work it was of interest to ascertain for what period of time the virus can remain viable in the body of the tick. To date the virus has been recovered from the bodies of ticks dormant for as long as 10 months. Adults, nymphs, and eggs were stored in a refrigerator, temperature constant at 12.5°C., where they remained for 10 months. During this period moisture was maintained as near optimum level as possible. Under these conditions eggs do not hatch and other stages of development enter a diapause, which may be terminated by return to higher temperatures.

The following summary describes the experimental procedure:

1. Recovery of the Virus from Adult Ticks Dormant for 4 Months, (September 17, 1942, through January 23, 1943).—4 adult ticks which had engorged partially (for 2 days) on a hamster inoculated intraperitoneally (10⁻¹, mouse brain suspension in broth, 1 ml.) with the virus of St. Louis encephalitis, were allowed to remain dormant at 12.5°C. for 4 months. Their bodies were triturated and ground in an agate mortar with 2 ml. of broth. This extract was inoculated, 0.1 ml. intraperitoneally, into 10 mice, 18 days of age. In 15 to 19 days all 10 mice gave evidence of illness. The brain of each of these 10 mice was passed to 4 adult mice and all such secondary passage mice developed convulsions within 4 to 6 days. The virus was recovered readily from the secondary passage mice and identified as the St. Louis type by mouse and egg protection tests.

Eight adult ticks engorged similarly on the same hamster as those just described were removed after 4 months of dormancy and allowed to feed for 4 days on 6 normal adult mice (2 months of age). Within 14 to 17 days following this exposure by bite all 6 mice were passed to 20 adult mice, (0.03 ml. intracerebrally 10⁻¹). All 20 secondary passage mice developed convulsions in 4 to 6 days. Identical parallel controls fed at all times on normal hamsters, gave negative results.

2. Recovery of Virus from Adult Ticks Dormant for 10 Months, (July 30, 1942, through June 2, 1943).—3 adult ticks, which had engorged partially (for 2 days) on a hamster inoculated intraperitoneally with the virus of St. Louis encephalitis and allowed to remain dormant at 12.5°C. for 10 months, were triturated and ground in an agate mortar with 1.5 ml. broth. The resulting extract was inoculated, 0.1 ml. intraperitoneally, into 6 mice, 13 days of age. In 17 to 18 days these 6 mice showed characteristic symptoms. They were sacrificed and the brain of each was passed to 4 adult mice (24), all of which developed convulsions in 3 to 4 days. The virus was recovered readily from the secondary passage mice and was identified as the St. Louis type in the usual manner.

Four adult ticks engorged similarly on the same hamster as those just described were removed after 10 months dormancy and allowed to feed for 4 days on 4 normal adult mice, 2 months of age. In 20 days following this exposure by bite these 4 mice were ill. The brains of all 4 were passed to 16 adult mice, 0.03 ml. intracerebrally, 10⁻¹, all of which developed convulsions in 4 to 6 days.

3. Recovery of Virus from Eggs Kept Dormant for 10 Months, (July 30, 1942, through
June 6, 1943).—3 female ticks were allowed to engorge for 4 days on a hamster which had been inoculated intraperitoneally with the virus of St. Louis encephalitis, 1 ml., $10^{-1}$, 15 minutes previous to the beginning of the engorgement period. The egg yields of these 3 ticks, kept separate, were placed in the refrigerator (12.5°C.) for 10 months. At the close of the dormant period the entire yield of 1 female was extracted in 2 ml. of broth by grinding in an agate mortar, and inoculated into 8 mice, 12 days of age, 0.1 ml. intraperitoneally. Within 15 to 17 days all 8 of these young mice showed signs of encephalitis, 2 of the 8 dying on the 17th day. The brains of all 8 were passed, 0.03 ml. intracerebrally, $10^{-1}$, to 3 mice each, and the 24 secondary passage mice developed convulsions in 3 to 5 days.

4. Recovery of Virus from Larvae Hatched from Eggs Kept Dormant for 10 Months, (July 30, 1942, through June 6, 1943).—The egg yield of each of the 2 remaining ticks described in 3 was removed from the refrigerator after 10 months and allowed to hatch into larvae. An extract prepared from these larvae, 25 to 30 per ml. of broth, was injected intraperitoneally (0.1 ml.) into 10 mice 17 days old. In 14 to 17 days these 10 mice were ill, 1 dying on the 15th day. The brains of the 10 were passed (0.03 ml. intracerebrally, $10^{-1}$) to 30 adult mice, which showed convulsions in 4 to 6 days. A number of these were dead by the 5th day. The virus recovered was identified as the St. Louis type by mouse and egg protection tests using as before hyperimmunized rabbit serum.

The foregoing experimental series shows that the virus of St. Louis encephalitis can remain viable in the bodies of adult ticks, partially engorged, and allowed to remain dormant for 10 months, in eggs kept at a temperature of 12.5°C. for a like period, and that the virus may be recovered from the bodies of larval ticks hatched from eggs which had remained dormant for 10 months. The virus recovered after 10 months was identified by means of egg and mouse protection tests using the serum of rabbits hyperimmunized against the virus of St. Louis encephalitis, Hubbard strain.

DISCUSSION

The present experiments give evidence that a blood-sucking arthropod vector may be infected readily with the virus of St. Louis encephalitis by feeding on inoculated animals and, so infected, can transmit the disease to susceptible animals by bite. A female tick, having engorged on an animal in whose blood stream the virus is present, passes the virus to her offspring through the egg, from which it is carried at hatching and metamorphosis to larvae, to nymphs, to adults. Eggs of these adults, whose mother acquired the virus from the initial feeding on an infected animal, again contain the virus which is perpetuated through metamorphosis as before. In this manner the virus has been followed experimentally into the 3rd generation. For these studies on hereditary transmission of the virus the parallel controls carried were always siblings of the same brood. None of the controls, fed only on normal animals, was found to harbor the virus.
The susceptibility of young mice to small doses of virus is of considerable importance. In our experience, adult mice are not consistent in their response to inoculation with infected tick extracts, and the signs of illness displayed by them may be overlooked easily. Such adult mice recover, showing later high antibody titres against the virus of St. Louis encephalitis and considerable resistance to intracerebral inoculation of virus.

Possible loss of virus by filtering out tick tissue was avoided by the use of unfiltered extracts inoculated into young mice intraperitoneally. Consistently sterile cultures of peritoneal sac and organs of mice thus inoculated showed that the mouse is able to withstand inocula not bacterially sterile when introduced intraperitoneally.

The importance of allowing ticks opportunity for attachment while the virus is at high concentration in the blood stream should be emphasized. Tests made of hamster heart blood at frequent intervals following inoculation by intraperitoneal route showed that the virus appears in the blood stream between 6 and 8 hours after introduction of virus and can be recovered from the blood at 8 through 72 hours. 3 adult ticks allowed to engorge on such a hamster during the period, 8 to 80 hours following inoculation, (removed at 80 hours), were shown to harbor the virus by preparation of extracts of their bodies and subsequent injection into young mice. Virus was absent from the heart blood after 72 hours following inoculation. Extracts of 2 adult ticks allowed to engorge on the same hamster beginning at 80 hours showed no virus present as tested by injection into mice. It may be added that it was not until the 14th day following intraperitoneal inoculation that symptoms of encephalitis appeared in this hamster, death occurring on the 15th day.

We do not believe that the dog tick is the vector responsible for the transmission of St. Louis encephalitis virus to man since no history of tick bite has been obtained in any case of St. Louis encephalitis observed. It is conceivable, however, that tick populations may play a part in the natural epidemiology of the disease by maintaining the virus. Possibly ticks, feeding on birds and mammals, create foci from which the virus may be carried to human beings by another blood-sucking vector such as the mosquito, as the work of Hammon and his associates suggests. Reservoir of infection in nature is indicated by the demonstration of type specific antibodies in the blood of a number of species of mammals and birds (19, 37).

**SUMMARY**

1. The common dog tick, *Dermacentor variabilis*, is capable of being infected with the virus of St. Louis encephalitis, Hubbard strain, by feeding on inoculated animals and, once infected, can transmit the virus to normal susceptible animals by bite. 2. A female can transmit the infection to her offspring, through all stages of metamorphosis of the 2nd generation into the
3rd generation. (3) Ticks infected under laboratory conditions and kept inactive at a temperature of 12.5°C., remained infective for at least a period of 10 months. Eggs laid by an infected female and stored in a refrigerator (12.5°C.) for 10 months retained infective virus, and larvae hatched from such eggs at the end of the 10 months of dormancy were also infective. (4) The present work, a preliminary account of which appeared in December, 1941 (1, 2), is of theoretical significance since in so far as we are aware, it represents the first successful transmission of St. Louis encephalitis to experimental animals by a blood-sucking vector.

BIBLIOGRAPHY

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