EXPERIMENTS ON THE RÔLE OF THE CHICKEN MITE, DERMANYSSUS GALLINAE, AND THE MOSQUITO IN THE EPIDEMIOLOGY OF ST. LOUIS ENCEPHALITIS*

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Epidemics of acute encephalitis occurred in the St. Louis area in 1933 and 1937 (1, 2). The distribution of cases (3) and the summer incidence of the disease suggested the possibility of a blood-sucking vector. In 1933 two independent groups of investigators, one from the United States Public Health Service and the other from the United States Army, undertook studies concerning possible implication of mosquitoes. Results of their experiments were negative (1).

In 1935, however, Webster, Clow, and Bauer (4) showed that *Anopheles quadrimaculatus* could be infected with the virus of St. Louis encephalitis. Such mosquitoes shown to harbor the virus for from 21 to 42 days did not infect mice or monkeys by bite. In 1937, Fulton, Greutter, Muether, Hauss, and Broun (5) were successful in infecting *Culex pipiens* with the virus of St. Louis encephalitis by allowing them to feed on infected mice. The virus did not survive in the bodies of these mosquitoes for longer than 10 days, and normal mice could not be infected by their bite. Mitamura and his associates (6) reported in 1937 successful transmission of the virus of St. Louis encephalitis by *Culex pipiens* var. *pallens* Coq. and by *Aëdes logoi*.

In 1941, Blattner and Heys showed that an arachnid, the dog tick (*Dermacentor variabilis*), could be infected with the virus of St. Louis encephalitis and could transmit the virus to mice by bite (7). Subsequent investigations (8) revealed that under experimental conditions this tick is capable of transferring the virus to susceptible animals by bite in any stage of its life cycle and of passing the virus to its offspring through the egg. Ticks which had been kept for 10 months at a temperature of 12.5°C. retained virus during hibernation and were shown to transmit the infection to animals by bite. Likewise eggs laid by infected females retained virus over the winter, and larvae hatched from these eggs harbored virus and were capable of infecting susceptible animals. Thus for the first time it was demonstrated that transovarial or congenital passage of this virus in an arachnid occurs under experimental conditions.

In 1942, Reeves, Hammon, and Izumi (9) reported transmission of the virus of St. Louis encephalitis by *Culex pipiens* Linn. Subsequently Hammon and Reeves were able to show that mosquitoes belonging to a number of genera are capable of acquiring

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the virus and of transmitting the virus to animals (10). Hammon, Reeves, and their associates (11) isolated the virus of St. Louis encephalitis from culicine mosquitoes collected in nature during epidemic periods in the Yakima Valley in Washington.

The virus has been shown to remain in the body of the mosquito for 28 days (10) and for 42 days (4). Transovarial passage of the virus in the mosquito, however, has not been demonstrated, and to our knowledge the virus has not been detected in hibernating mosquitoes. In consequence it seems unlikely that the mosquito could be the sole agent concerned in the epidemiology of St. Louis encephalitis.

That an endemic focus exists in the St. Louis area since the epidemics of 1933 and 1937, is indicated by the fact that each summer cases of acute encephalitis have been proved by appropriate neutralization tests to be of the St. Louis type (12). A serum-neutralization survey was made in the St. Louis area in 1943 and 1944 (13). In St. Louis County where endemic cases of St. Louis encephalitis have been shown to occur antibody for the St. Louis virus was demonstrated in sera of chickens from certain flocks. Antibody of low titer was present in 11 to 33 per cent of chickens chosen from 5 flocks. Demonstration of antibody was equivocal in an additional 12 to 14 per cent of chickens in these same flocks. Of 97 people tested who had resided in St. Louis County through the epidemic years of 1933 and 1937, 37 per cent showed antibody to the St. Louis virus. In certain sections of St. Louis County the sera of approximately 50 per cent of residents tested contained antibody. On the other hand, only a few (3 of 56) people (children 3 to 7 years of age and adults who had come from states east of the Mississippi since 1937) who had resided in St. Louis County since 1937 showed antibody. In view of these results it seemed logical to conclude that some vector in the St. Louis area, which as a rule does not attack human beings, feeds upon chickens, and thereby introduces virus into their bodies. The chicken mite, *Dermanyssus gallinae*, an arachnid similar biologically to the dog tick, seemed a likely possibility. Accordingly, collections of this chicken mite were made in selected districts of St. Louis County. Chicken mites from 3 different sites, 6 to 10 miles distant, yielded an infectious agent which in each case was identified as the virus of St. Louis encephalitis (14).

In previous reports from this laboratory (14, 15) evidence was presented that the chicken mite (*Dermanyssus gallinae*) is capable of transferring the virus of St. Louis encephalitis congenitally to its offspring *ad infinitum*, and that a colony of mites once infected probably remains infected indefinitely. Uninfected mites derived from a single female and her nymph offspring and shown to be free of virus could be infected readily by feeding on animals inoculated with various strains of the virus of St. Louis encephalitis. Experimentally infected mites as well as those found infected in nature proved capable of transferring the virus through the egg to their offspring. Both naturally infected and experimentally infected mites transferred the virus to chickens by bite. Uninfected mites could acquire virus from chickens bitten by infected mites. Viremia in chickens bitten by such mites was demonstrated with regularity by the use of the chorioallantoic passage and subsequent intracerebral inoculation of mice (16). Isolation of the St. Louis encephalitis virus from mites in nature and

results of laboratory experiments indicating maintenance of the virus in mites suggest that the mite may serve as a reservoir in nature. Likewise the fact that virus is present in the blood of chickens following the bite of infected mites suggests the possibility that during the period of viremia such chickens might serve as a source of virus for other blood-sucking vectors, possibly the mosquito, of which several species are known to feed upon chickens (17).

The present report concerns experimental work which indicates that chickens acquiring the virus of St. Louis encephalitis from infected mites have sufficient virus in their blood during the period of viremia for mosquitoes to acquire the virus while feeding, and that such infected mosquitoes are capable of transmitting the virus to other animals.

Methods and Materials

Species of Mosquitoes.—Seven species of mosquitoes, belonging to 3 genera, used in the present investigations, were obtained from various sources: *Culex pipiens* Linn., collected as eggs, larvae, and pupae in St. Louis County and maintained as a breeding colony at Washington University; *Culex quinquefasciatus* Say, collected as eggs, larvae, and pupae in New Orleans and maintained as a breeding colony at Washington University; *Anopheles punctipennis* (Say), collected as larvae in St. Louis and vicinity; *Anopheles quadrimaculatus* Say, from eggs obtained from a colony at Tulane University (an Alabama strain) and also from eggs of females captured and from larvae collected in St. Louis and vicinity; *Aëdes aegypti* (Linn.), from eggs obtained from a colony at Tulane University (a New Orleans strain) and maintained as a breeding colony at Washington University; *Aëdes triseriatus* (Say), collected as larvae in the vicinity of St. Louis; and *Aëdes vexans* (Meig.), collected as larvae and pupae and also from eggs laid by females captured in St. Louis and vicinity.

Rearing of Mosquitoes.—All mosquitoes used in the experiments were reared in the laboratory from preadult stages. A mosquito-proof, air-conditioned room having a two doored vestibule and sealed windows was provided. A closed circuit of air was maintained by a specially designed humidifier communicating with the room. Air was drawn through excelsior-packed screens kept wet by dripping water. Manual adjustment of the flow and the temperature of the water controlled the rate of evaporation, providing the desired humidity and reducing temperature fluctuation. The temperature in the breeding room varied from 75° to 91°F. and the relative humidity from 85 to 92 per cent. This room was used only for the production of mosquitoes. No infected animals were permitted in the breeding room. Food for the adult mosquitoes in the breeding cages consisted of dextrose solution, cut prunes, and blood of uninfected host animals. For the species of *Culex*, young chickens were used as hosts and were kept in the breeding cages continuously. These chickens had been hatched in the laboratory and had been protected at all times from possible exposure to arthropods by fine wire screen and a surrounding moat of cresol. A guinea pig was used as host for *Aëdes aegypti* and a rabbit for *Anopheles quadrimaculatus*, the animals being placed in the cages for several hours each day. Eggs laid in a small bowl of water (*Culex*) or on wet filter paper (*Aëdes* and *Anopheles*) were transferred to larval breeding pans. Culicine larvae were fed with a suspension of brewer's yeast and dried beef blood albumen, added to the water once or twice during development; anopheline larvae received ground Purina dog chow daily. Pupae removed from breeding pans were placed in metal cups (50 to 150 in each cup) under lamp chimneys which were capped at the upper end with bobbinet mosquito netting. The rubber bands securing the netting also held a piece of filter paper suspended within the chim-

ney to provide additional resting space for the mosquitoes. When all adults had emerged from the pupae (2nd or 3rd day), the containers were removed from the cups, closed below by netting, and placed on wet cellucotton in Petri dishes. Pledgets of cotton saturated with dextrose solution were laid on the top netting to provide food. Mosquitoes were stored in this manner for at least 24 hours before exposure to experimental animals. Thus a constant supply of suitable mosquitoes was available for experimental use.

Infection of Mosquitoes.—A second mosquito-proof room in which the mosquitoes were infected was fitted with a double vestibule closed by three mosquito-proof doors and contained a large screened enclosure with its own two doored entry. This room was air-conditioned in the same manner as the breeding room. The temperature ranged from 72° to 94°F. and the relative humidity between 77 and 96 per cent, the usual range for temperature being 80° to 90°F. and that for relative humidity between 80 and 90 per cent. In the screened enclosure mosquitoes were allowed to feed upon infected animals, and the fed mosquitoes were transferred to clean lamp chimneys. Here the mosquitoes were stored for incubation of virus and later allowed to feed upon uninfected animals for possible transmission of the virus.

Mosquitoes were infected by allowing them to feed upon chickens having viremia or on a suspension of infected mouse brain tissue in broth and defibrinated rabbit blood.

The chickens used as a source of virus for mosquitoes were laboratory bred New Hampshire Reds, 10 to 20 days of age. Viremia was produced in these chickens by the subcutaneous inoculation of the Hubbard egg membrane strain of virus (0.2 ml. of a 1:100 dilution of infected egg membrane in broth) or by the bite of infected mites. Four colonies of infected mites were used for this purpose: the Rippy colony derived from mites found infected in nature and 3 colonies of experimentally infected mites. The latter were infected with 3 strains of St. Louis virus: (*a*) the Rippy strain (RN_6), a mouse brain virus isolated from mites found infected in nature, (*b*) the Hubbard egg membrane strain, an egg membrane strain of St. Louis virus (Hubbard) isolated in mice from human brain tissue in 1937 and maintained since 1938 on the chorioallantoic membrane of the developing hen's egg, (*c*) the Mullen strain isolated in mice in 1945 from the blood of a patient who recovered from encephalitis.

Chickens were used as potential sources of virus for mosquitoes between 48 and 92 hours following the inoculation of virus or following the exposure of chickens to infected mites in a manner previously described. This is the period during which viremia was shown to occur most commonly (15, 16). For verification of viremia in chickens, blood samples drawn before and, in most instances, after the mosquitoes had fed, were tested for virus in the following manner. Heparinized whole blood (0.08 to 0.1 ml.) was inoculated on the chorioallantoic membrane of the developing hen's egg. Four days after inoculation these membranes were harvested and ground with a small amount of tryptose phosphate broth. The resulting suspension was centrifuged at low speed for 2 minutes, and the supernatant fluid was passed to a second series of embryonated eggs. As before, the passage membranes were harvested after a 4 day period and were ground with broth. After centrifugation, the supernatant fluid was injected intracerebrally, in 0.03 ml. amounts, into white Swiss mice.

Mosquitoes to be infected by feeding upon chickens during viremia were allowed access to the unanesthetized chicken which was held by an oilcloth restrainer against the netting of the lamp chimney container. Before the chickens that had been infected by mites were used in this way for the infection of mosquitoes, any mites remaining on their bodies were destroyed by a 5 minute exposure to chloroform vapor. The body of the chicken was placed in a jar covered by a sheet of rubber through which the head protruded. As precaution against possible escape of mites remaining alive, a petrolatum barrier was placed around the base of the stand used to support the lamp chimney during the exposure of the chicken to mosquitoes. The exposure periods varied from 15 minutes to 12 hours, usually overnight, according to

need or convenience. At the close of the feeding period, mosquitoes which contained visible ingested blood were separated from the unfed mosquitoes and placed in other containers. Transfer of unanesthetized mosquitoes by means of an aspirator tube was carried out within a glass-topped box provided with armholes and sleeves of netting.

Mosquitoes to be infected by feeding on a suspension of virus were exposed to the suspension for periods up to 2½ hours. Lamp chimneys containing the mosquitoes were placed on pieces of cellucotton saturated with the virus suspension, the mosquitoes feeding readily through the netting. The virus suspension was prepared at the beginning of the feeding period by adding 1 part of a 20 per cent suspension of infected mouse brain tissue in broth to 6 parts of defibrinated rabbit blood. The brain tissue was that of mice infected with the RN_6 strain of St. Louis virus (isolated from mites collected in nature). The suspension in broth and defibrinated blood prepared at the beginning of the experiment was kept in the refrigerator, and fresh refrigerated suspension was added to the cellucotton at 30 minute intervals during the feeding period.

Mosquitoes that had ingested infective blood or virus suspension were stored in the lamp chimneys for varying periods to allow incubation of the virus. A piece of wire screening suspended in the container provided a resting surface for the mosquitoes. Water was provided through wicks in contact with the bottom netting, and raisins or cotton saturated with dextrose solution were placed on the top netting to supply food.

Isolation of Virus from Mosquitoes.—Attempts were made to isolate virus from suspensions of mosquitoes at periods varying from 8 to 29 days after the ingestion of infective blood. Samples of mosquitoes, after light chloroform anesthesia and removal of legs and wings, were triturated in tryptose phosphate broth using 0.1 to 0.2 ml. of broth per mosquito. In most instances 0.06 ml. of an uncentrifuged or a centrifuged suspension of mosquitoes was inoculated intraperitoneally into young Swiss mice 7 to 12 days of age. When the more concentrated or uncentrifuged suspensions were used, the mice sometimes died 1 to 2 days following inoculation, apparently as a result of heavy contamination of the inoculum. When convulsions or other signs of illness suggestive of encephalitis were observed, brain tissue from the young mice was passed intracerebrally to 4 to 6 adult Swiss mice. In a small number of experiments filtered suspensions of the mosquitoes were tested for virus by direct intracerebral inoculation of adult Swiss mice and/or by chorioallantoic passage on the developing hen's egg with subsequent intracerebral passage to adult mice.

Method for Demonstrating Transmission of Virus by Mosquitoes to Chickens.—Five to 55 days after an infective blood meal mosquitoes were allowed to feed on normal unanesthetized chickens in the same manner as that described for the initial feeding on infected chickens. These normal chickens ranged in age from 6 to 20 days, the majority being 10 to 12 days of age. Following the feeding period the mosquitoes containing visible ingested blood were counted in order to determine the approximate number of bites that the chicken had received. Since a mosquito may pierce the skin of the host without withdrawing a detectable amount of blood, a few exposed animals were tested for viremia even when no blood was visible in any of the mosquitoes. Most of the chickens exposed to the infected mosquitoes were bled only once for viremia test, on the 2nd or 3rd day (34 to 64 hours) after the feeding period. Eleven chickens were bled 3 times, at intervals between 1 and 4 days after the feeding period. Heparinized whole blood was tested for virus by chorioallantoic passage with subsequent intracerebral passage to adult mice, and in most instances serum from blood drawn simultaneously was inoculated intracerebrally into 4 to 6 adult mice.

Method for Demonstrating Transmission of Virus by Mosquitoes to Mice.—Six to 19 days after the infective meal, mosquitoes were allowed access to young Swiss mice, 6 to 14 days of age, which had been anesthetized by dial. The anesthetized mice were placed on the netting covering the lamp chimney containers and held in place by a second piece of netting. Mice

which received one or more bites as determined by counting the number of engorged mosquitoes after the feeding period, were observed for signs of encephalitis. Some of the young mice thus exposed were killed at 10 and 13 days after they had been bitten. Brain tissue from each of these was passed by intracerebral inoculation to 4 to 6 adult mice. The remaining young mice were observed for 21 days.

Method for Demonstrating Transmission of Virus by Mosquitoes to Hamsters.—Four to 42 days after an infective blood meal, mosquitoes were allowed to feed on young adult Syrian hamsters (*Cricetus auratus*) anesthetized with dial. Hamsters were exposed to infected mosquitoes for several hours or overnight by supporting a lamp chimney container over the animal in such a way that the netting covering one end of the container was in contact with the shaved abdomen. The number of mosquitoes biting a given hamster was determined by counting those which contained visible blood after the feeding period. For viremia test some of the hamsters were bled only once at 48 to 81 hours after the beginning of the period during which the mosquitoes fed; others were bled 2 or more times between 24 and 108 hours. Heparinized whole blood was tested for virus by chorioallantoic passage and subsequent intracerebral inoculation of adult mice. Several of the hamsters were inoculated intracerebrally with aleuronat at a time when viremia might be expected. It was thought that damage to the brain tissue produced in this manner might facilitate invasion of the brain by virus in the blood stream. The hamsters were observed for 2½ months.

Identification of Virus.—Strains of virus isolated from chickens and hamsters having viremia were compared with a known strain of St. Louis virus (Hubbard) and were identified as the St. Louis virus by neutralization in mouse protection tests. Immune rabbit sera used in mouse protection tests were prepared by repeated subcutaneous inoculation of normal rabbits with the Hubbard strain of St. Louis virus. Strains of virus isolated from each of 11 hamsters and 10 chickens having viremia as the result of the bite of infected mosquitoes were identified in this manner. These infected mosquitoes, the bite of which resulted in viremia in chickens and hamsters, had acquired the virus by feeding on chickens bitten by mites infected in nature or experimentally infected with 1 of 3 strains of St. Louis virus.

RESULTS

The primary purpose of the present investigation was to ascertain (1) whether or not mosquitoes can acquire the virus of St. Louis encephalitis by feeding on chickens infected by mites, and (2) whether or not mosquitoes thus infected can transmit the virus to chickens, mice, and hamsters.

Isolation of Virus from the Bodies of Mosquitoes

Before attempting transmission of virus by the mosquito it was advisable to show the presence of virus in the body of the mosquito. Accordingly, attempts were made to isolate virus from mosquitoes presumably infected in one of three ways: (1) mosquitoes fed on a suspension of infected mouse brain tissue in tryptose broth and defibrinated rabbit blood; (2) mosquitoes fed on chickens in which viremia had been produced by subcutaneous inoculation of the Hubbard egg membrane strain of virus; (3) mosquitoes fed on chickens having viremia as the result of the bite of infected mites.

The results of these isolation experiments can be summarized as follows:—

Five different species of mosquitoes (of 3 genera) were tested for virus after ingestion of infected mouse brain material. The bodies of the mosquitoes were triturated in tryptose

broth, 0.1 to 0.2 ml. per mosquito, and the resulting suspension was inoculated intraperitoneally into young Swiss mice. One of these 5 experiments, that with *Anopheles quadrimaculatus*, was unsatisfactory, presumably because the uncentrifuged suspension of 11 mosquitoes was contaminated heavily, and the test animals died within 1 or 2 days. Virus was obtained from each of the other 4 species of mosquitoes by the intraperitoneal inoculation of young Swiss mice. Uncentrifuged suspensions of mosquitoes were used in 3 instances and a centrifuged suspension in one instance These 4 species of mosquitoes were *Anopheles punctipennis* (7 mosquitoes), *Aëdes aegypti* (26 mosquitoes), *Aëdes triseriatus* (3 mosquitoes), and *Culex quinquefasciatus* (4 mosquitoes). The period allowed for incubation of the virus in the mosquitoes ranged from 10 to 15 days. Virus was isolated readily from each of the 4 samples, the young mice which were inoculated intraperitoneally developing definite signs of encephalitis in 4 to 9 days. Brain tissue from one or more young mice of each test group was passed to 4 adult Swiss mice by intracerebral inoculation. These inocula proved bacteriologically sterile on culture. The passage mice developed convulsions in 2 to 4 days following inoculations.

Mosquitoes of 3 species (*Aëdes aegypti*, *Culex quinquefasciatus*, and *Culex pipiens*) were tested for virus after being allowed to feed on chickens inoculated subcutaneously with the Hubbard egg membrane strain of virus. Only mosquitoes which had ingested blood were tested for virus. Each chicken used in this way as a source of virus for mosquitoes was shown to have viremia by the direct intracerebral inoculation of mice with serum, as well as by chorioallantoic passage with subsequent intracerebral inoculation of mice. Two samples of *Aëdes aegypti* consisting of 4 and 6 mosquitoes, one of *Culex quinquefasciatus*, 11 mosquitoes, and 1 sample of *Culex pipiens*, 13 mosquitoes, were tested for virus by intraperitoneal or intracerebral inoculation of suspensions into young mice. Owing to contamination 1 sample of *Aëdes aegypti* and 1 of *Culex pipiens* were not satisfactory. Consequently only 2 satisfactory tests were carried out using mosquitoes which had fed on chickens infected by subcutaneous inoculation of virus: 1 with *Aëdes aegypti* and 1 with *Culex quinquefasciatus*. The period allowed for incubation of the virus in mosquitoes in these two instances was 8 and 16 days respectively. A centrifuged suspension of the sample of *Aëdes aegypti* was inoculated intraperitoneally into young mice, and a filtered suspension of the sample of *Culex quinquefasciatus* intracerebrally into adult mice, both giving negative results.

Mosquitoes of 4 species, *Aëdes aegypti*, *Aëdes vexans*, *Culex quinquefasciatus*, and *Culex pipiens*, comprising 13 lots, were tested for virus after feeding on chickens bitten by infected mites. Nine of these 13 lots (3 of *Aëdes aegypti*, 1 of *Aëdes vexans*, 2 of *Culex quinquefasciatus*, and 3 of *Culex pipiens*), were fed on chickens infected by the bite of mites from the Rippy colony, a colony derived from mites found infected in nature. The number of mosquitoes tested varied from 1 to 60. The periods allowed for incubation of the virus in the mosquitoes varied from 13 to 29 days. One of the 9 samples of mosquitoes, an uncentrifuged suspension consisting of 4 *Aëdes aegypti*, was unsatisfactory because of contamination. No virus was isolated from the remaining 8 samples by intraperitoneal inoculation of young mice, none from centrifuged suspensions, and none from uncentrifuged suspensions. A portion of the centrifuged suspension from each of 2 samples, *Culex pipiens*, 60 and 11 mosquitoes respectively, was filtered by means of a Luer-Lok syringe with Swinny adapter. While no virus was isolated from these 2 filtrates by direct intracerebral inoculation of adult mice, the filtrate resulting from 60 mosquitoes which had fed 13 days before, was shown to contain virus by 2 chorioallantoic passages in the developing hen's egg. A suspension of chorioallantoic membrane on transfer intracerebrally to adult mice produced convulsions in 4 days.

One sample of each of 4 lots of *Culex pipiens* which had fed on chickens shown to have viremia as a result of the bite of experimentally infected mites, was tested for virus. The uncentrifuged suspension of 1 sample gave unsatisfactory results when inoculated intraperitoneally into young mice. The centrifuged suspensions of the other 3 samples consisting of

28, 31, and 55 mosquitoes, were filtered by means of the Swinny adapter and tested for virus by the direct intracerebral inoculation of adult mice and simultaneously by the chorioallantoic passage method. Virus was isolated by chorioallantoic passage but not by direct intracerebral inoculation of mice. In these 3 lots of infected mosquitoes, 3 strains of virus were involved: the RN_6, the Hubbard egg membrane, and the Mullen. The periods allowed for incubation of the virus in the mosquitoes ranged from 15 to 21 days.

As the foregoing has shown, virus was detected in suspensions of triturated mosquitoes which had fed on chickens infected by mites by the inoculation of filtered centrifuged suspensions on the chorioallantois of hens' eggs and subsequent intracerebral passage to mice. *Culex pipiens* was the only species of mosquitoes tested for virus in this manner. Virus was demonstrated in these mosquitoes 13 to 21 days following the infective meal. Virus was not isolated from mosquitoes which had fed on chickens infected by mites, either by direct intraperitoneal inoculation of young mice or by direct intracerebral inoculation of adult mice. The same was true for mosquitoes infected by feeding on chickens inoculated subcutaneously with virus suspensions of the Hubbard egg membrane strain. However, virus was isolated readily, by direct inoculation of mice, from mosquitoes of 4 species which had ingested infected mouse brain material 10 to 15 days previously. Where only small amounts of virus are present in the bodies of infected mosquitoes, as in those fed upon chickens infected by mites, chorioallantoic passage increased the amount of virus to an extent such that signs of encephalitis are produced in mice when egg membrane material is inoculated intracerebrally.

Transmission of the Virus to Chickens by Mosquitoes Infected by Feeding on Virus Suspension

Transmission of the St. Louis virus to normal chickens was attempted using mosquitoes of each of 5 species, (*Anopheles punctipennis*, *Anopheles quadrimaculatus*, *Aëdes aegypti*, *Aëdes triseriatus*, *Culex quinquefasciatus*), which had ingested a suspension of infective mouse brain tissue (RN_6 strain of virus) in broth and defibrinated blood. Following the ingestion of infective material, periods of 8 to 12 days were allowed for incubation of the virus in these mosquitoes. After the incubation period each of the 5 lots of mosquitoes was allowed access to one chicken. Three of the 5 chickens were bitten by 2 mosquitoes each and 2 chickens by only 1 mosquito each, as determined by counting the mosquitoes which had ingested blood. Three tests for viremia were made on each of the 5 chickens, blood being drawn at intervals between 1 and 4 days after exposure to the mosquitoes. Serum was tested for the presence of virus by direct intracerebral inoculation of adult mice. Simultaneously, heparinized whole blood was inoculated on the chorioallantoic membrane. In each of the 5 chickens viremia was detected by chorioallantoic passage and subsequent inoculation of adult mice. Virus was demonstrated in 3 samples of blood from

each of 4 chickens and in 1 sample (37 hour) from the fifth chicken. Results of direct serum inoculation were negative except in one instance in which the result was equivocal. In this instance 1 of 4 adult mice inoculated intracerebrally with serum (blood drawn at 89 hours) from 1 of the 5 chickens developed mild convulsions on the 6th day following inoculation. Later this mouse was found dead, but brain tissue was not tested for the presence of virus. Serum obtained from the blood of the other 4 chickens failed to produce signs of encephalitis in mice.

TABLE I

Transmission of Virus

Suspension of infective mouse brain tissue → mosquito → chicken

Strain of virus	Source of virus for mosquitoes	Mosquitoes		Time after infective meal when transmission was attempted	No. of bites	Blood tested for virus	
		Species	Lot No.			By hen eggs to mice	By mice only
				days			
RN_6	Suspension of infective mouse brain tissue	*Culex quinquefasciatus*	C1-3	12	1	+	−
"	" "	*Anopheles punctipennis*	C4-1	8	1	+	−
"	" "	" *quadrimaculatus*	C3-1	9	2	+	?
"	" "	*Aëdes triseriatus*	C6-1	9	2	+	−
"	" "	" *aegypti*	C5-1	8	2	+	−

Viremia resulted in chickens from the bite of each of 5 species of mosquitoes which had ingested virus suspension 8 to 12 days previously and was demonstrated by chorioallantoic passage in the developing hen's egg (Table I).

Transmission of the Virus to Normal Chickens by Mosquitoes Infected by Feeding on Chickens Inoculated Subcutaneously

Seven lots of mosquitoes of 4 different species, (*Anopheles punctipennis* and *Culex pipiens*, 1 lot each; *Aëdes aegypti*, 2 lots; *Culex quinquefasciatus*, 3 lots), were allowed to feed on chickens inoculated subcutaneously with the Hubbard egg membrane strain. Viremia in these chickens was demonstrated by chorioallantoic inoculation before and after the mosquitoes had fed. Ten to 33 days after the infective meal each of the 7 lots of mosquitoes was given opportunity to feed on a normal chicken. Blood was drawn from 5 of these 7 chickens 36 to 50 hours after exposure to the mosquitoes. Each of the other 2 chickens was bled 3 times, on the 1st, 2nd, and 3rd days after exposure. Blood samples

were tested for virus by chorioallantoic passage. Viremia was demonstrated in all of the 7 chickens. Since transmission occurred in 2 instances in which no ingested blood was seen in the mosquitoes, it is possible that mere probing by the mosquito may be sufficient to transmit the virus in some instances.

The 7 successful transmissions to chickens were accomplished with mosquitoes utilized 10 to 33 days after the infective meal (Table II).

TABLE II

Transmission of Virus

Chicken inoculated subcutaneously → mosquito → chicken

Strain of virus	Source of virus for mosquitoes	Mosquitoes		Time after infective meal when transmission was attempted	No. of bites	Blood tested for virus	
	No. of inoculated chicken	Species	Lot No.			By hen eggs to mice	By mice only
				days			
Hubbard egg membrane	1–83	*Culex pipiens*	C19-2a	11–13	6	+	−
" " "	1–77	*Culex quinquefasciatus*	C10-2a	10–11	3	+	−
" " "	1–77	" "	C10-2b	33	1	+	−
" " "	1–83	" "	C10-3	13	?	+	−
" " "	1–65	*Anopheles punctipennis*	C11-1	10–11	?	+	−
" " "	1–65	*Aëdes aegypti*	C8-1	10–11	6	+	−
" " "	1–77	" "	C8-2	11–12	2	+	−

Transmission of the Virus to Normal Chickens by Mosquitoes Infected by Feeding on Chickens Infected by Mites

Transmission of virus by mosquitoes to normal chickens from chickens infected by mites was attempted 30 times using 27 different lots of mosquitoes. These lots included 5 species: *Anopheles punctipennis*, *Aëdes aegypti*, *Aëdes vexans*, *Culex quinquefasciatus*, and *Culex pipiens*. In the greater number of these experiments (20) *Culex pipiens* was used. In 17 of the 30 trials chickens bitten by mites of the Rippy colony constituted the source of virus for the mosquitoes. In the other 13 trials the chickens which served as the source of virus for mosquitoes were infected by the bite of mites experimentally infected with 1 of 3 strains of virus, the RN_6 strain, the Hubbard egg membrane strain, and the Mullen strain. In all chickens serving as a source of virus for mosquitoes, viremia was demonstrated during the period when the mosquitoes were fed. The periods allowed for incubation of virus in mosquitoes ranged from 5 to 55 days. One sample of blood was drawn from each of 27 chickens, 34 to 64 hours

TABLE III

Transmission of Virus

Mite → chicken → mosquito → chicken

Strain of virus infecting mites	Source of virus for mosquitoes	Mosquitoes		Time after infective meal when transmission was attempted	No. of bites	Blood tested for virus	
	No. of chicken with viremia owing to bite of mite	Species	Lot No.			By hen eggs to mice	By mice only
				days			
Rippy*	1–82	*Culex pipiens*	C18-3	10	11	+	−
"	1–84	" "	C18-4	15	1	+	−
"	1–94	" "	C18-5	16, 24	1, 1	+, +	−, 0
"	2–02	" "	C18-10	5	2	+	−
"	2–10	" "	C18-12	5, 12	1, 4	+, +	−, −
"	2–73	" "	C18-22	55	3	−	0
RN_6‡	1–85	" "	C24-2	15–16	6	+	−
"	1–85	" "	C24-1	16	1	+	−
"	2–11	" "	C24-6	18	20	+	−
"	2–64	" "	C24-12	54	4	−	0
Mullen‡	2–01	" "	C26-1	12	9	+	−
"	2–01	" "	C26-2	18	10	+	0
"	2–21	" "	C26-4	8, 27	35, 12	−, −	−, 0
"	2–23	" "	C26-5	10	10	+	0
Hubbard egg membrane‡	2–03	" "	C29-1	14	9	+	0
" " "	2–13	" "	C29-4	14	4	+	0
" " "	2–22	" "	C29-5	12	3	+	0
Rippy*	1–64	*Culex quinquefasciatus*	C14-1a	11	1	+	−
"	1–76	" "	C14-2	12	8	+	−
"	1–88	" "	C14-3	9	1	+	−
"	1–82	" "	C14-4	12	0?	−	−
RN_6‡	1–79	" "	C21-1	8–9	6	+	−
Rippy*	1–64	*Anopheles punctipennis*	C12-1	10	1	+	−
"	1–64	*Aëdes aegypti*	C7-1	10–11	3	−	−
"	1–76	" "	C7-2	12	1	+	−
"	1–94	" *vexans*	C25-1, 2	20	1	+	0
"	2–17	" "	C25-4	11	12	+	−

+ = virus isolated.

− = no virus isolated.

0 = blood not tested.

* Mites found infected in nature.

‡ Mites experimentally infected.

after exposure to infected mosquitoes. Each of the other 3 chickens was bled 3 times, on the 1st, 2nd, and 3rd days after exposure.

In this series of 30 experiments transmission of the virus to chickens by the bite of mosquitoes occurred 24 times (Table III). Chorioallantoic passage was necessary for the demonstration of viremia. Transmission was successful in at least one instance with each of the 5 species of mosquitoes used, and all 4 strains of virus were transmitted. In those experiments where transmission was successful, the period of incubation in the mosquito varied from 5 to 24 days. The 6 unsuccessful trials occurred with 5 lots of mosquitoes: 1 of *Aëdes aegypti*, 1 of *Culex quinquefasciatus*, and 3 of *Culex pipiens*. One lot of *Culex pipiens* failed to transmit the Mullen strain of virus at 8 days and again at 27 days following infective meal. The other 2 lots of *Culex pipiens* which gave negative results had been held for 54 to 55 days after the infective meal before they were tested for transmission of virus. In the one instance of *Culex quinquefasciatus* it was not certain whether any of the mosquitoes had attempted to bite the chicken. In the one instance where *Aëdes aegypti* gave negative results, mosquitoes were used for transmission test 10 to 11 days following the infective meal.

Control Experiments

Two types of controls were used. Mosquitoes bred in the laboratory were allowed to feed on normal chickens, hatched and bred in the laboratory, and after a 12 day interval were allowed to feed on other normal chickens. Blood samples drawn from the latter approximately 40 hours after exposure to the mosquitoes, were tested for virus by the chorioallantoic passage method. In the second type of control, mosquitoes were allowed a blood meal from chickens fed upon 2 to 3 days before by mites from a colony shown to be free of virus. After intervals of 12 to 14 days these mosquitoes were allowed a blood meal from normal chickens never exposed to mites. Two blood samples were drawn from each of the first group of chickens between 49 and 97 hours following exposure to mites and 1 blood sample was drawn from each of the second group of chickens at approximately 40 hours following exposure to mosquitoes. The blood samples were tested for virus, as before, by chorioallantoic passage. In none of the control chickens was there any evidence of viremia. Two control experiments of the first type were carried out, 1 with *Culex pipiens* and 1 with *Aëdes aegypti* and 3 of the second type, each with *Culex pipiens*.

In summary, the mosquitoes used in successful transmission experiments were infected with the St. Louis virus in 3 ways: (1) by ingesting a suspension of infected mouse brain tissue (RN_6 strain) in broth and defibrinated rabbit blood; (2) by feeding on chickens inoculated subcutaneously with the Hubbard egg membrane strain of virus; and (3) by feeding on chickens in which viremia resulted from the bite of naturally infected mites and mites experimentally infected with 3 strains of St. Louis virus. Control experiments of 2 types gave negative results consistently. Seven species of mosquitoes of 3 genera were used in these transmission experiments.

Transmission of the Virus to Young Mice by Infected Mosquitoes

In attempts to transmit the virus to mice, young Swiss mice, 6 to 14 days of age, were anesthetized lightly with dial and exposed to the bite of infected mosquitoes. Difficulties were encountered in that the young mice were not always able to withstand the procedure and in that mosquitoes did not bite mice readily. Satisfactory results were obtained in 9 instances only.

Of these 9 mice (10 to 14 days of age), 5 received only 1 bite each, 2 received 2 bites each, and 2 mice received 5 and 16 bites respectively. The 9 lots of mosquitoes included 1 of *Anopheles quadrimaculatus*, 1 of *Anopheles punctipennis*, 5 of *Aëdes aegypti*, 1 of *Aëdes triseriatus*, and 1 of *Culex quinquefasciatus*. The experiments with *Culex pipiens* were among those which were considered inconclusive. The source of virus for 4 of the 9 lots of mosquitoes was a suspension of infective mouse brain material. Two lots of the mosquitoes used were infected by feeding on a chicken inoculated with virus and 3 lots by feeding on a chicken bitten by infected mites. Periods ranging from 6 to 19 days were allowed for incubation of the virus in mosquitoes. None of the 9 mice developed signs of encephalitis: 3 were observed for a period of 21 days and then discarded; 4 were killed 10 days following the bite of infected mosquitoes, and the brain of each was passed intracerebrally to 4 adult mice; 2 of the 9 mice were killed 13 days following the bite of infected mosquitoes, and the brain of each was passed intracerebrally to 4 adult mice. No signs of encephalitis were noted in any of these passage mice. Four lots of the mosquitoes used in these experiments were infected by ingesting a a suspension of infective mouse brain tissue. Virus was isolated from triturated bodies of mosquitoes from 3 of these 4 lots. Nevertheless transmission of virus to mice by bite was not accomplished.

Transmission of the Virus to Hamsters by Infected Mosquitoes (Culex pipiens)

When difficulties were encountered in the transmission of virus to mice by infected mosquitoes, experiments with the Syrian hamster were undertaken. Since the hamster is large enough for bleeding at intervals, viremia tests were possible in addition to observation for signs of encephalitis. Two series of experiments were carried out (Table IV).

In the first series 13 young adult hamsters under dial anesthesia were exposed to mosquitoes which had fed on chickens bitten by infected mites. Viremia was demonstrated in all chickens used as a source of virus for mosquitoes. Transmission of the virus was attempted with 6 different lots of *Culex pipiens*, 8 to 24 days after ingestion of infective chicken blood. In 2 of these lots the infective meal was obtained from chickens bitten by mites of the Rippy colony, and in 4 lots the infective meal was obtained from chickens bitten by mites experimentally infected with the Mullen strain of virus. Four of the 13 hamsters succumbed immediately after the exposure period, apparently as a result of anesthesia; 2 of the 13 hamsters were observed for signs of encephalitis but were not tested for viremia; 6 were tested for viremia by bleeding twice at intervals between 40 and 90 hours, and 1 was bled 4 times at intervals between 40 and 108 hours after the beginning of exposure to mosquitoes. Of the 7 hamsters tested, 2 were shown to have viremia. In 1 hamster, which had been bitten by 9 mosquitoes 13 days after the infective meal (Rippy strain of virus), viremia was demonstrated at 64 hours but not at 40, 87, or 108 hours. In the other hamster bitten by 18 mosquitoes 8 days after the infective meal (Mullen strain of virus), viremia was demonstrated at 40 and at 68 hours after exposure. In each instance virus was isolated from heparinized blood by the chorio-

TABLE IV

Transmission of Virus

Mite → chicken → mosquito (*Culex pipiens*) → hamster

Strain of virus infecting mites	Source of virus for mosquitoes: No. of chicken with viremia owing to bite of mite	Mosquitoes (*Culex pipiens*) Lot No.	Time after infective meal when transmission was attempted	No. of bites	Viremia in hamsters (blood tested by hen eggs to mice): +	Viremia in hamsters (blood tested by hen eggs to mice): −	Remarks
			days		*hrs.*	*hrs.*	
Series I							
Rippy*	1–94	C18-7	13	9	64	40, 87, 108	
"	2–02	C18-10‡	12	1		40, 90	
Mullen§	2–21	C26-3	8	18	40, 68		Died after second bleeding
"	2–21	C26-3	24	2		56, 80	
"	2–21	C26-4‖	19	25			Not bled
"	2–23	C26-6	17	2		40, 64	
"	2–23	C26-6	18	2		57, 79	Died 8 days after exposure to mosquitoes —virus not isolated from brain
"	2–23	C26-6	21	6		56, 80	
"	2–23	C26-5¶	18	5			Not bled
Series II							
Rippy*	2–57	C18-15	8	8		50	
"	2–57	C18-15	11	3	48		0.03 ml. aleuronat intracerebrally
"	2–59	C18-18	12	1	26, 53, 73		
"	2–73	C18-22**	6	8	64		
"	2–73	C18-22**	15	15	56		" " " "
"	2–86	C18-24	16	2		57	" " " "
RN_6§	2–60	C24-9	4	25	50, 74	24	Died after third bleeding
"	2–60	C24-9	8	5		50	
"	2–60	C24-9	14	2	59		0.03 ml. aleuronat intracerebrally
"	2–60	C24-9	27	4	56		" " " "
"	2–60	C24-9	43	5		50	" " " "
"	2–64	C24-11	5	4	48		Died after bleeding
"	2–64	C24-12‡‡	8	1	26, 53		0.03 ml. aleuronat intracerebrally
"	2–87	C24-16	15	7		81	" " " "

* Mites found infected in nature.

‡ Transmitted virus to chicken 5 days after feeding.

§ Mites experimentally infected.

‖ Failed to transmit virus to chickens 8 and 27 days after feeding.

¶ Transmitted virus to chicken 10 days after feeding.

** Failed to transmit virus to chicken 55 days after feeding.

‡‡ Failed to transmit virus to chicken 54 days after feeding.

allantoic passage method. The hamster in which viremia was demonstrated at 40 and at 68 hours died as a result of the second bleeding on the 3rd day after exposure to mosquitoes. The second of the 2 hamsters shown to have viremia, the 5 in which viremia tests were negative, and the 2 which were not tested for viremia, a total of 8, did not develop signs of encephalitis. One of the hamsters which was tested for viremia with negative results, died 8 days following exposure to mosquitoes. Brain tissue from this hamster was passed intracerebrally to mice with negative results. The 7 hamsters which were observed for approximately 2½ months showed no signs of encephalitis.

In the second series of experiments, 17 young hamsters under dial anesthesia, were exposed to mosquitoes (*Culex pipiens*) 4 to 43 days after the mosquitoes had fed on chickens infected by mites. The colonies of mites used to infect the chickens serving as source of virus for the mosquitoes, were derived from mites found infected in nature (Rippy colony) and from mites experimentally infected with the RN_6 strain of virus. Virus was isolated by chorioallantoic passage from the blood of all chickens used as a source of virus for mosquitoes. Two of the 17 hamsters exposed to mosquitoes died before they had been bled for the viremia test, either as a result of anesthesia or of trauma produced by intracerebral injection of aleuronat. Of the remaining 15 hamsters, 1 was not tested for viremia; 1 or more blood samples from each of 14 were tested for virus by chorioallantoic passage at periods of 26 to 81 hours after the hamsters had been bitten by mosquitoes. In nine hamsters viremia was demonstrated at periods from 48 to 74 hours after exposure to mosquitoes; 2 of the 9 were bled 3 times, and viremia was demonstrated in 1 at 50 hours and at 74 hours but not at 24 hours, in the other at 26, 53, and 73 hours. A third hamster which was bled at 26 and at 53 hours showed viremia at both bleedings. Viremia was not demonstrated in 5 hamsters each of which was bled once at 50 to 81 hours after exposure to mosquitoes.

Of the 15 hamsters tested for viremia, 1 died immediately after bleeding; 14, which were observed for 2½ months showed no signs of encephalitis. With the idea of facilitating invasion of the brain by virus present in the blood, a number of hamsters were injected intracerebrally with aleuronat after exposure to infected mosquitoes. While 5 hamsters which had received aleuronat proved to have viremia, there was no apparent effect of the aleuronat in breaking the blood-brain barrier since not one of the 5 developed signs of encephalitis.

Several lots of mosquitoes were used more than once in the transmission experiments. In one instance mosquitoes which were used on 5 different occasions, transmitted the virus to hamsters 4, 14, and 27 days after the infective meal but not at 8 and 43 days after the infective meal. In another instance 1 lot of mosquitoes which transmitted the virus to a hamster at 11 days after the infective meal had not done so at 8 days after the infective meal. A third lot of mosquitoes transmitted the virus at 6 days and again at 15 days; in two other instances, 1 lot of mosquitoes which had transmitted the virus to a hamster 7 days after the infective meal, failed to transmit the virus to a chicken at 54 days, and another lot which had transmitted the virus to hamsters 6 and 15 days after the infective meal, failed to transmit the virus to a chicken at 55 days.

As Table IV shows virus was isolated in two series of experiments from the blood of 11 of 21 hamsters tested for viremia, demonstrating the transmission of virus by mosquitoes infected through feeding on chickens infected by mites. Eight lots of mosquitoes transmitted virus at periods varying from 4 to 27 days after the infective meal. Viremia was demonstrated on two occasions when one mosquito only was known to have bitten the hamster. In the other 9 instances in which viremia was demonstrated, the number of known bites varied from 2 to 25.

DISCUSSION

Within recent years evidence has been accumulating from field and laboratory studies which indicates that St. Louis encephalitis is an arthropod-borne disease. Isolation of the virus of St. Louis encephalitis from culicine mosquitoes collected in nature during epidemics and the transmission of the virus to experimental animals by the bite of mosquitoes emphasize the importance of this blood-sucking vector in the epidemiology of St. Louis encephalitis. The mosquito probably transmits the infection to higher animals and man. However, certain facts suggest that it is not the sole vector involved in the epidemiology of the disease. The virus has not been shown to persist in hibernating mosquitoes, nor has transfer of the virus in mosquitoes by way of the egg been demonstrated. While humoral antibodies to the virus of St. Louis encephalitis are present under natural conditions in vertebrates, particularly birds, there is no evidence that these animals constitute more than a transient source of virus. Apparently, virus remains in their blood for a few days only. Thus the question where the virus of this seasonal disease persists from year to year cannot be answered on the basis of the mosquito hypothesis alone. Also, there has been no adequate explanation of why epidemics occur rarely in certain localities although a few endemic cases occur there from year to year.

The isolation of the virus of St. Louis encephalitis from chicken mites (*Dermanyssus gallinae*) collected under natural conditions during non-epidemic years has pointed to the possibility that this arachnid vector might be a reservoir of the St. Louis virus. It has been shown that under laboratory conditions the virus is transferred through all stages of metamorphosis in the chicken mite, and that, once infected, a colony of chicken mites, by reason of transovarial passage, may remain infected for an indefinite period. By actual test it was shown that the virus remained in mites housed in the laboratory for a period of 3 years. While these findings suggest that *Dermanyssus gallinae* is serving as a natural reservoir, the possibility must be considered that this arachnid may be merely an accidental host, and hence of no epidemiologic significance. In order to determine whether the chicken mite is concerned in the natural transmission of St. Louis encephalitis, it is essential to know whether infected chicken mites feeding upon normal chickens can produce viremia. During the course of the present work this was accomplished many times, the blood of chickens fed upon by infected chicken mites being positive for virus for periods of 1 to 3 days, and in some instances 4 days, after the feeding period. It was demonstrated that mosquitoes feeding upon chickens infected by mites can acquire virus from the blood during the period of viremia, and that mosquitoes thus infected can transmit the virus to other chickens and to hamsters. Thus it is possible that in the epidemiology of St. Louis encephalitis two blood-sucking vectors may be involved—one an arachnid, the mite,

maintaining the virus by transovarial passage and the other, an insect, the mosquito, which carries the infection from birds to other vertebrates including man.

In these studies demonstration of viremia in animals fed upon by infected vectors presented technical difficulties since virus is present in small amounts in the blood of such animals. Passage on the chorioallantoic membrane was necessary in order to increase the virus to a level sufficient to produce signs of encephalitis in white Swiss mice. The question of multiplication of the virus in the body of the blood-sucking vectors was considered. In the case of the chicken mite, the demonstration of congenital transfer of the virus and the comparative ease with which virus could be isolated from mites of succeeding generations constitute indirect evidence that multiplication of the virus occurs. However, the present results give no convincing evidence that the virus multiplies in the body of the mosquitoes which were used in this investigation. On the other hand the amount of virus present in the body of the mosquitoes appeared to have a direct relation to the amount of virus ingested, even when a 2 week period of incubation was allowed; that is, virus was demonstrated readily by direct inoculation of mice, with extracts from mosquitoes which had fed on a suspension of brain tissue containing high concentration of virus, whereas extracts from mosquitoes which had fed on chickens infected by mites contained small amounts of virus, which could be demonstrated only by means of chorioallantoic passage.

Whether the virus undergoes changes in its characteristics, perhaps becoming less infective for vertebrates while maintaining itself in the body of an arthropod vector, is a question. The results reported here give no evidence of such a change in the mite since the virus from the bodies of chicken mites procured under natural conditions was infective for white mice without requiring passage for adaptation, and since uninfected chicken mites were infected successfully with laboratory-adapted strains: the Hubbard egg membrane strain and a strain isolated from the blood of a patient.[1] However, no detailed studies concerning this problem were undertaken.

While it was somewhat disappointing that the bite of infected mosquitoes which had acquired the virus from chickens infected by mites did not result in objective signs of encephalitis in hamsters or mice, viremia was demonstrated in hamsters in a significant number of instances. Even when efforts were made to break down the blood-brain barrier by the injection of aleuronat no signs of encephalitis were observed in these animals.

These observations suggest that the epidemiology of St. Louis encephalitis is a complex one, involving two blood-sucking vectors. A diagrammatic representation of this concept is given in Fig. 1. The chicken mite seems to be an

[1] Strain F 103, isolated from the mosquito in California, was sent to us through the courtesy of W. McD. Hammon. Several attempts to infect *Dermanyssus gallinae* with this strain have been made but up to the present time without success.

important reservoir vector in the St. Louis area. In other localities some other vector, probably an arachnid, may be playing a similar rôle; some species of mite, or a hard bodied tick, or a soft bodied tick are likely possibilities. Previous experiments of Blattner and Heys with the tick, *Dermacentor variabilis*, demonstrating that the virus of St. Louis encephalitis can be passed through the egg and into the next generation through the various stages of metamorphosis, suggested the potentiality of this arachnid as a reservoir for virus. However, in so far as we are aware, the St. Louis virus has not been encountered under natural conditions in any arachnid other than the chicken mite. The

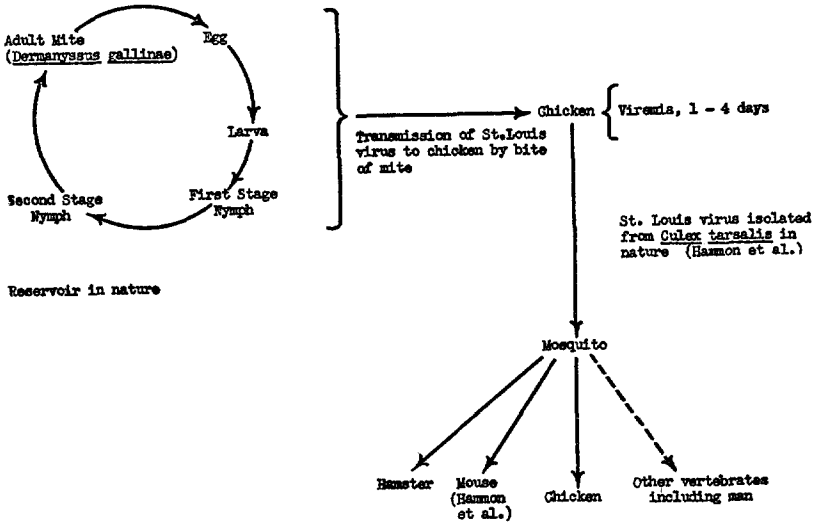

FIG. 1. The possible epidemiology of St. Louis encephalitis. ----- not yet proved; ——— proved experimentally.

concept of epidemiology outlined offers an explanation for the seasonal incidence of St. Louis encephalitis and for the persistence of the disease from year to year in a given community. Also it might explain adequately why so few major epidemics have been observed, since the requisite conditions appear to be exacting. Factors which probably would influence these conditions are temperature, relative humidity, the number of chickens in the community, the population of chicken mites, breeding places for mosquitoes, prevalence of certain species of mosquitoes, availability of susceptible vertebrates in the community, and the like. The few endemic cases which have been observed from year to year could be explained by assuming that in any given year relatively few mosquitoes might acquire the virus from chickens and that only a few individuals might be exposed to the bite of such infected mosquitoes.

Since the natural history of equine encephalomyelitis has many similarities to that of St. Louis encephalitis, it is conceivable that the same epidemiologic factors might be involved. The isolation by Sulkin (18) of the virus of equine encephalomyelitis from chicken mites collected in nature and the presence of the virus of equine encephalomyelitis in the bird mite, *Liponyssus sylviarum*, as shown by Reeves, Hammon, and their associates (19) lend support to this suggestion.

SUMMARY

The present experimental results concern primarily the question, whether or not mosquitoes feeding on chickens having viremia, as a result of the bite of infected mites, can acquire the virus of St. Louis encephalitis and whether or not mosquitoes thus infected, can transmit the virus to chickens and hamsters.

During the course of the investigation, 7 species of mosquitoes of 3 genera were infected with the virus in one or two or all of three ways: by feeding on a suspension of infected mouse brain tissue, by feeding on chickens in which viremia had been produced by subcutaneous inoculation of virus, and by feeding on chickens having viremia as a result of the bite of infected mites. These mosquitoes transmitted the virus to chickens at periods varying from 5 to 33 days after the infective meal.

The virus of St. Louis encephalitis was transmitted to hamsters by *Culex pipiens* at periods varying from 4 to 27 days after feeding on chickens having viremia as a result of the bite of infected mites. While viremia was demonstrated readily in hamsters, signs of encephalitis did not develop.

In all transmission experiments the method of chorioallantoic passage proved necessary for the demonstration of viremia.

A concept of the epidemiology of St. Louis encephalitis is presented: two blood-sucking vectors may be involved, one an arachnid, the mite, maintaining the virus in nature by transovarial passage, and the other, an insect, the mosquito, which carries the infection from birds to other vertebrates including man.

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