STUDIES ON TYPHUS FEVER

VIII. Ticks as a Possible Vector of the Disease from Animals to Man

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In his study of the epidemiology of typhus in the southern United States, published in 1926, Maxcy (1) came to a number of important conclusions, the most significant of which was that the epidemiological characteristics observed by him were at variance with the idea that the disease is kept alive by man to man transfer through lice. He suggested at that time, on the basis of case studies, that there might be an animal reservoir of infection, possibly in rodents—rats or mice—from which the disease is occasionally transmitted to man.

The most likely manner of such transmission, of course, would be by means of an insect, and in a previous study by the writers of this paper (2) experiments were reported on the infection of bedbugs with typhus virus, an insect which was investigated as the most likely possibility suggested by Maxcy's results. It was found at that time that bedbugs experimentally infected per rectum or permitted to feed on benzol-treated typhus rats could harbor the virus in potent form for from 5 to 9 days (the limits of the actual experiment). The viscera of such bedbugs injected into guinea pigs produced typical typhus infection with Rickettsiae, subsequent immunity to both European and Mexican typhus and characteristic brain lesions. We did not succeed in transmitting the disease to guinea pigs by the natural process of allowing the infected bedbugs to feed upon them. But the guinea pig is more resistant than man, and our cycle was in other respects complete in that we proved the possibility of natural infection of the insects and the viability of the potent virus within them for at least 9 days. These facts we took to be of considerable epidemiological importance.
in view of the wide distribution of bedbugs and their plebeian catholicity in the choice of a large variety of hosts.

In the communication referred to above, Maxcy cites an observation made by Hone in Australia in which typhus fever occurred in individuals occupied in the handling of wheat and other food stuffs. He mentions another report, by Wheatland, in which typhus seemed related to a migration of mice and was spoken of as “mouse fever.” Maxcy in his discussion concludes that rodents—rats and mice—constitute the most likely animal reservoir and that, as parasitic intermediaries, the first suppositions should fall upon “fleas, mites or, possibly ticks.” The recent work of Dyer (3) has confirmed at least one of Maxcy’s theories by the demonstration that rat fleas collected from rats trapped in a typhus focus contained a virus apparently identical with the “Wilmington” typhus strain of Maxcy.

It has thus been shown already that typhus virus can remain alive in at least three common, blood-sucking insects—the louse, the flea and the bedbug.

Our own success with bedbugs induced us to extend our studies to other insects before we knew of the work of Dyer and, for a number of reasons—the most potent of which was an extremely helpful letter from Dr. Halliday of the Maryland Department of Health—we chose ticks as our next line of attack.

Are Dogs Susceptible to Typhus?

Before beginning to work with the ticks themselves, we thought it important to determine whether or not dogs could harbor the typhus virus, since this might throw some light upon the type of tick with which it would be best to work. Dogs have no doubt been injected with typhus fever, but we could find no record of their having been injected with tunica rich in Rickettsiae from guinea pigs. We therefore injected two half grown mongrel puppies with a considerable dose of tunic suspension rich in Rickettsiae, a suspension which produced a rapid and typical disease in a control guinea pig, administering the virus intraperitoneally in one dog and intravenously in the other. One of these dogs, the intraperitoneally injected one, ran a temperature touching 104°+ on the 8th, 9th and 10th days, and the dog was for this reason killed and brain material injected into a guinea pig.
other dog was observed for 21 days, during which he developed no febrile reaction, but brain from this dog was also injected into a guinea pig. Neither of these guinea pigs showed any symptoms or temperature simulating typhus fever, one of them running between 103° and 104°C. for about 2 or 3 days, but coming down again promptly. Both guinea pigs were subsequently tested for immunity and found susceptible.

We concluded, in consequence, that even large doses of infectious material did not produce either apparent or inapparent typhus in dogs, and that the dog is probably not, therefore, the animal reservoir for the disease. Further experiments on dogs of course should be done, but on the principle of following the most likely clue first, we abandoned work on dogs and turned our attention directly to the investigation of ticks that might be obtained from a variety of sources.

Experiments with Ticks

Experiment 1.—On Dec. 24, 1930, we obtained some ticks through the courtesy of Dr. Bustamente from Vera Cruz, which were later identified for us by Dr. Parker of the U. S. Public Health Service as belonging to the genus *Amblyomma*. On Jan. 12, 1931, eleven of these ticks were injected *per rectum* with the tunic emulsion of a guinea pig, material which was very rich in *Rickettsiae* and produced typhus fever promptly. The injection of the ticks was not easy. Some of them could be injected directly into the intestinal tract, but in the others the fluid passed into the celom. The injected ticks were incubated at 30°C. On Jan. 14th ten ticks which remained alive were put on a guinea pig for 24 hours’ feeding. This guinea pig did not later develop typhus fever. We had in this first experiment an unfortunate mortality among the ticks, and on Jan. 21st only one of the injected insects remained alive. This tick was washed in alcohol, the viscera dissected out and injected into guinea pig Experiment 1, T 1. As seen in Chart 1 this guinea pig, after a preliminary rise of temperature, returned to normal, but on the 4th, 5th, 6th and 7th days ran a high temperature with early swelling of the scrotum. It was castrated on the 5th day and typical *Rickettsiae* found. The tunic of this guinea pig, T 1, was injected into T 2 and T 3 intraperitoneally with typical results, namely, temperature, swelling and *Rickettsiae* in both cases. It was carried into a third generation by injection of brain into Guinea Pig T 3 and tunic, and thence into a third generation, T 4 and T 5.

The accompanying chart of Guinea Pig T 1 and the protocol of subsequent manipulations of the typhus strain passed through the tick serve to describe this experiment.
### Chart 1. Record of guinea pig inoculated with viscera of *Amblyomma*, as described in Experiment 1.

**Tick Experiment 1 (Protocol)**

**Guinea Pig 1**

Typical Mexican typhus. *Rickettsia* material from tunica injected rectally into adult *Amblyomma* on Jan. 12, 1931.

Jan. 21, 1931. Viscera of one surviving tick injected into Guinea Pig T1 (see Chart 1). Emulsions of tunica of this guinea pig injected into Guinea Pig T2: Swelling, 5th day. Temperature 105.6° on 6th day. Castrated, 6th day. *Rickettsiae* +.

Guinea Pig T3: Temperature 105° and swelling, 6th day. *Rickettsiae* +. Immune to European typhus fever.

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Sections of brain of Guinea Pig T2 showed no lesions, but T3 and T4, reinoculated with European typhus virus, simultaneously controlled on normal animals, proved to be immune.

**Experiment 2.—*Dermacentor andersoni* adult ticks obtained through the kindness of Dr. Parker of the Hamilton, Montana, laboratory of the U. S. Public Health Service**
Health Service were rectally injected on Feb. 26th by the capillary pipette method with tunica material from a Mexican typhus guinea pig, containing numerous *Rickettsiae*. These ticks stood the injections much better than those of the other species we had used, and very few died. Sixteen were injected and classified into those in which the rectal injection had been wholly successful and those in which success, as observed under the binocular, was questionable.

![Chart 1](image1.png)

**Chart 1.** Reaction of guinea pig injected with *Dermacentor andersoni* viscera, as described in Experiment 2.

![Chart 2](image2.png)

**Chart 2.** Control guinea pig injected simultaneously with Chart 2 guinea pig with material from normal ticks of the same lot.

On Mar. 6th, 8 days after the rectal injection, three of these ticks were washed in alcohol, eviscerated as usual and the viscera injected into guinea pig Experiment 2, T 1.

At the same time, four ticks of the same lot, but uninfected, were similarly treated, and their viscera injected into a control guinea pig. The purpose of this
was twofold: First of all, to provide the control necessary to insure against error
from unknown causes, but also to make sure that the ticks as received did not
naturally harbor either Rocky Mountain spotted fever or tularemia. Dr. Parker
had called our attention to these possibilities, stating that he thought them un-
likely, but advised caution.

The guinea pig injected with infected ticks developed a temperature of 104°F.,
with scrotal swelling, on the 8th day. By the 11th day the swelling was much
increased and the temperature had risen to 105°. At this point one testicle was
removed and plentiful and typical *Rickettsiae* found in the tunica. On the 13th
day the animal was killed and *Rickettsiae* again found in smaller numbers. The
brain was removed, sectioned and a few small lesions found which Dr. Wolbach was
kind enough to examine for us and which he regarded as consistent with early typhus
brain lesions. In view of the fact that the animal was killed early, and that Mexi-
can typhus animals do not show brain lesions with the same regularity and number
as do those infected with European virus, these lesions, though small and few, have
considerable importance.

The control guinea pig injected with the viscera of the uninfected ticks of the
same lot remained normal.

These results are shown in Chart 2, under the headings of Experiment 2, T 1 and Guinea Pig Control 1. The subsequent course of the experiment is discussed in the protocol.

**Tick Experiment 2 (Protocol)**

Feb. 26, 1931, *Dermacentor andersoni* rectally injected with Mexican
typhus virus. Mar. 6th, into

<table>
<thead>
<tr>
<th>Guinea Pig T 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature and swelling, 8th to 12th day. <em>Rickettsiae</em> +.</td>
</tr>
</tbody>
</table>
| Killed 13th day—brain lesions, few and small but suggest-
  tive. March 13th—tunica material of 8th day into |

<table>
<thead>
<tr>
<th>Guinea Pig T 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swelling and temperature 105° 5th day.</td>
</tr>
</tbody>
</table>
| *Rickettsiae* +. Reinoculated on 13th
day and found immune |

<table>
<thead>
<tr>
<th>Guinea Pig T 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swelling and temperature 105.6° on 4th day. Castrated. <em>Rickettsiae</em> +. Tunica material into</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Guinea Pig T 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typical temperature and swelling, 4th and 5th days</td>
</tr>
</tbody>
</table>

*Experiment 3.*—Two of the same ticks used in Experiment 2 and injected inter-
rectally on Feb. 26th were allowed to feed on a normal guinea pig from Mar. 6th
to Mar. 9th by the method formerly used by Dr. Wolbach, namely, attaching
them in an open pill box with a bandage to the shaved abdomen of a normal animal.
They were found containing some blood and dug into the skin of the guinea pig
on Mar. 9th and induced to let loose by snipping the skin just in front of the imbedded sucking apparatus. This was done because we had been told by Dr. Parker that infected ticks become more potent in virus after feeding. From Mar. 9th up to Mar. 12th they were kept at 30° in an incubator.

On Mar. 12th, 14 days after intrarectal injection, two ticks treated as above were washed in alcohol, eviscerated and the viscera injected into the guinea pig labelled

<table>
<thead>
<tr>
<th>Exp. 3 T1</th>
<th>Dermacentor andersoni</th>
<th>Mar. 12, 1931</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day of disease</td>
<td>1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18</td>
<td></td>
</tr>
<tr>
<td>Temp.</td>
<td>106° 105° 104° 103° 102° 101° 100° 99°</td>
<td></td>
</tr>
</tbody>
</table>

**Chart 3.** Results in guinea pig injected with *Dermacentor andersoni* viscera, as described in Experiment 3.

<table>
<thead>
<tr>
<th>G.P Control 2</th>
<th>Dermacentor andersoni (adults)</th>
<th>Mar. 9, 1931</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day of disease</td>
<td>1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16</td>
<td></td>
</tr>
<tr>
<td>Temp.</td>
<td>106° 105° 104° 103° 102°</td>
<td></td>
</tr>
</tbody>
</table>

**Control Chart 3.** Control guinea pig injected with normal ticks of same lot as those injected into guinea pig on Chart 3.

Experiment 3, T 1. A control of the same lot of ticks, in which the viscera of four uninfected insects were used, was done on Mar. 9th.

The guinea pig inoculated with the two infected ticks treated as described above showed a temperature of 104° on the 7th day, with typical swelling, and was castrated. Plentiful *Rickettsiae* were found in the tunic.
The temperature of this animal dropped to 102° on the 15th day, when it was
reinoculated with Mexican typhus virus and found immune, controls of course
being done with the same virus.

The subsequent passages of this tick virus is indicated in the follow-
ing protocol.

**Tick Experiment 3 (Protocol)**

*Dermacentor andersoni* adults infected rectally with tunica material on Feb. 26th.
2 of these ticks fed for 2½ days on normal guinea pig, Mar. 6th to Mar. 9th.
Kept at 30° C. until Mar. 12th. Viscera injected into

*Guinea Pig T 1*
(See Chart 3, Experiment 3, T 1. Temperature and swelling on 7th day.
One testicle removed on 9th day. *Rickettsiae* +. Reinoculated on
15th day and found immune. Tunica of 9th day into

*Guinea Pig T 2*
Swelling and temperature 105° on 6th and 7th days

<table>
<thead>
<tr>
<th>Exp. 4 T1</th>
<th>Dermacentor nitens (nymphs)</th>
<th>Mar. 9, 1931</th>
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</thead>
<tbody>
<tr>
<td>Day of disease</td>
<td>1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21</td>
<td></td>
</tr>
<tr>
<td>Temp</td>
<td>99° 100° 101° 102° 103° 104° 105° 106°</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Swelling</td>
<td>No swelling</td>
</tr>
<tr>
<td></td>
<td>Calibrated</td>
<td>Rickettsiae</td>
</tr>
</tbody>
</table>

*Chart 4. Reaction in guinea pig inoculated with viscera of Dermacentor nitens
nymphs, as described in Experiment 4.*

*Experiment 4.*—We had received through the kindness of Dr. Iglesias a number
of ticks of two varieties from the neighborhood of Vera Cruz. Some of these were
*Amblyomma*, others were nymphs of *Dermacentor nitens*. Unfortunately, many of
these ticks were adult females which were in the process of laying eggs and died in
the course of unsuccessful experimentation, most of them dying within a few days
after intrarectal injection. The only insects of this lot that were successfully
handled were two *Dermacentor nitens* nymphs which were injected intrarectally.
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with Mexican typhus virus on Feb. 27th, and whose viscera were inoculated intraperitoneally on Mar. 9th into a guinea pig charted as Experiment 4, T 1.

This guinea pig developed on the 6th day typical swelling and a temperature of 104.2°. One testicle was removed on that day and Rickettsiae typical in appearance and position were found. The temperature continued for 16 days, the swelling subsiding. On Mar. 27th this animal was reinoculated with Mexican typhus virus and found immune. See Chart 4.

The subsequent course of this experiment is described in the following protocol.

Tick Experiment 4 (Protocol)

Two Dermacentor nitens nymphs were rectally injected with typhus virus on Feb. 27th, 1931. Kept at room temperature. On Mar. 9th, eviscerated and viscera injected into

Guinea Pig T 1
Swelling and temperature 104.2° on 6th day. One testicle removed. Plentiful Rickettsiae. Reinoculated, and found immune on Mar. 24th. Material of removed testicle inoculated into

Guinea Pig T 2
Typical disease. Rickettsiae ++. Immune on reinoculation

Guinea Pig T 3
Typical disease. Rickettsiae ++ Immune on reinoculation

Four control guinea pigs into which the viscera of presumably normal ticks had been injected without arousing reactions were subsequently inoculated with typhus virus and found susceptible.

Not all attempts to pass the virus through ticks were successful. In addition to the positive experiments above recorded, failures should be reported as follows:

1. Two Dermacentor adults were intrarectally injected with virus on February 28th, 1931. Their viscera inoculated into a guinea pig on March 9th produced neither infection nor immunity.

2. Two Dermacentor adults treated exactly as were the two described in Experiment 2, except that feeding on a normal animal was not carried out in the interval between rectal infection and visceral inoculation, likewise were negative. This may have some importance in that Dr. Parker tells us that infected Rocky Mountain spotted fever ticks may increase in virulence after feeding.

3. Dermacentor andersoni nymphs allowed to feed on typhus infected and benzolized rats did not produce typhus fever on guinea pig injection 6 and 14 days respectively after the last date of feeding.
All but one of the guinea pigs unsuccessfully inoculated in this manner were subsequently infected with typhus virus and reacted typically, thus adding further controls to show that the normal ticks used by us contained no virus that could either simulate typhus fever in the guinea pig or produce immunity.

DISCUSSION

Our experiments have shown that the virus of Mexican typhus fever rectally injected into ticks of three varieties (Dermacentor nitens, an Amblyomma not further identified and Dermacentor andersoni) will remain alive and potent in these insects for as long as 14 days, the limit of our experiments.

Guinea pigs injected with the viscera of such ticks develop the temperatures, scrotal swellings and tunica lesions characteristic of Mexican typhus infections. Typical and plentiful Rickettsiae were always found, brain lesions—though few and small—were seen in one of two animals examined, and six guinea pigs so infected were found immune on subsequent inoculation with the virus itself in amounts which caused severe and characteristic reactions in control animals.

These experiments, added to Nicolle's fundamental discovery regarding lice, Dyer's observations on rat fleas and our own work with bedbugs, make a fourth group of blood-sucking insects which can harbor the virus of typhus fever. Our work, it is true, was carried out by experimental injection and not by the natural methods of infection and transmission by feeding. It does not, therefore, justify the deduction that ticks can become typhus vectors in the course of their natural existences. The experiments, however, render this a distinct possibility. That the louse transmits typhus fever from man to man under epidemic conditions and, perhaps in most regions in endemic cases, is, of course, unquestionable in the light of the classical investigations of Nicolle and his collaborators (4), of Ricketts and Wilder (5) and innumerable subsequent students of the disease. That the louse is not likely to account for the sustained endemic prevalence of the disease in regions like the southern United States has been pointed out by Maxcy.

Maxcy's suggestion that there is an animal reservoir has been upheld by the rat flea observations of Dyer. The fact, however, that the
virus can survive in a number of widely differing insects gives rise to the thought that the flea may not be the only possible vector.

Ticks can take blood from a variety of animals associated with man; they are widely distributed, at least in American and Mexican typhus regions; they have suggested themselves as possible transmitting agents to those who have made epidemiological studies of the American disease (Maxcy (1), Halliday, personal letter), and they convey the closely related disease, Rocky Mountain spotted fever. Our experiments add another fact in favor of the possible incrimination of ticks, together with bedbugs and fleas, in the epidemiological cycle which keeps typhus fever prevalent in places and at periods in which man to man transmission is unlikely.

The viability of the virus in ticks naturally leads to the thought that dogs may represent the animal reservoir. Two experiments with dogs failed to show that these animals could either contract the disease or harbor the virus in an “inapparent” form. Rats and mice, therefore, in view of Dyer’s work, are the most likely animal sources.

CONCLUSIONS

Mexican typhus virus can be passed through ticks by the method of rectal injection.

The virus will remain alive in the ticks for at least 12 days.

These studies, together with one of our preceding publications and the work of Dyer, demonstrate that there are at least three insects—bedbugs, fleas and ticks—which must be considered as possibilities in conveying typhus fever from an animal reservoir to man. Our work will be continued by a study of rats and mice caught in typhus regions such as Mexico City and its immediate vicinity, with a search for the virus in these rodents as well as an analysis of the insects found upon them or in the localities in which they are concentrated.

REFERENCES