EXPERIMENTS ON THE CONVERSION OF TYPHUS STRAINS

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It is a well established fact that the strains of Mexican typhus which have been established in laboratory animals during recent years differ in several points from all the well studied strains of Old World typhus, in spite of complete cross-immunity between the two kinds of strains. Regular scrotal swelling in guinea pigs with numerous rickettsiae in the tunica vaginalis, and a febrile, often fatal disease in rats with numerous rickettsiae in the tunica, distinguish Mexican strains clearly from Old World strains of typhus. Old World strains produce scrotal lesions only rarely, the lesion as a rule is mild, and rickettsiae can be found in the animals only with great difficulty and very irregularly. In rats they are said to cause inapparent infections only. Since our Mexican strains isolated in previous years from man show all the characteristics of strains obtained from wild rats in nature, we call them murine strains, whether they have been isolated from rats or from man. Recently we were able to isolate from a long standing epidemic of typhus several strains which correspond closely to strains of Old World typhus. We call them epidemic strains. We avoid the word endemic for those strains which cause scrotal lesions in guinea pigs and fever in rats for two reasons: first, we were able to isolate from short lived, rapidly checked epidemics in Mexico City only strains which are in every respect identical with strains obtained from isolated endemic cases and from rats in nature; second, from isolated cases in Europe only typical strains of Old World or epidemic typhus have been reported so far. The finding of typical epidemic strains in Mexico where previously only murine strains have been isolated by us, seems to be in agreement with the hypothesis of Nicolle that there must exist in Mexico two varieties of typhus, the Mexican variety of murine origin and the historic (1) louse-borne
typhus imported from abroad. We, however, are of the opinion
that the murine strains represent the original form of the virus,
whereas the epidemic strains are the product of a secondary adapta-
tion by a long standing transmission in the cycle man-louse-man.
The transformation of murine strains into epidemic strains is not
amenable to an experimental demonstration. The reverse, however,
namely the transformation of epidemic non-orchitic strains into strains
which show the murine characteristics, can be tried in the laboratory.
The present paper deals with experiments which show that the prop-
erties which characterize the murine strains are contained in latent
form in the epidemic strains, and that by proper handling the original
murine traits become clearly manifest in the epidemic strains.

Material and Methods

The murine strains do not require any description on this occasion.
They have been described in earlier papers (2) and are entirely
identical with strains of endemic typhus in the southern United States,
with strains isolated from rats and rat fleas by Dyer and his associa-
tes (3), from rats and rat fleas in France, Greece, and Syria (4),
and with strains of so called Machurian typhus (5). Our epidemic
strains, however, deserve a detailed description because they are the
first non-orchitic strains of typhus which we were able to establish
in Mexico.

In 1932 an epidemic of typhus started among an Indian tribe in the State of
Oaxaca. This epidemic spread slowly northwards among the native population
along their paths of trade. In February, 1933, when the epidemic had assumed
alarming proportions among the Mixtec Indians in the Sierra of Oaxaca and in
several large Aztec Indian settlements in the adjoining parts of the State of Puebla,
we decided to visit these regions with the aim of isolating virus strains from a long
standing, serious epidemic. We were especially interested in finding out whether
strains from such a long standing epidemic in Mexico corresponded to the murine
type of the virus as did those isolated from short lived, rapidly checked epidemics
in the capital, or whether they corresponded experimentally to strains of historic
(1) Old World typhus. The conditions we met there were typical of epidemic
typhus. The Indians were covered with head and body lice and in many huts
we found two to four patients ill with severe typhus. In others we found one or
several acutely ill, with other members of the family lying around as convalescents.
Many of them were mourning one or several members.

Five strains of virus were established: one from the blood of a patient in Chila,
three from blood of various patients in Zinacatepec, and one from lice collected
from a patient on the 10th day of the disease also in Zinacatepec, in the State of Puebla. White rats, as well as guinea pigs, were used for isolating the strains. Each material was inoculated into two or three rats, and into two or three guinea pigs. The superiority of the rat was revealed by the fact that of the four blood strains obtained, all four were established from rats and only one in addition from guinea pigs. The louse strain was obtained both from guinea pigs and rats. In all, blood samples from six patients in the 1st week of illness were inoculated into the animals, giving a positive take in rats of 66.66 per cent of the cases, and a positive take in guinea pigs of 16.6 per cent. 15 days after inoculation, the rats which had not shown any sign of illness during that time were killed, and the strains established in guinea pigs by inoculation of brain emulsions. The louse strain showed scrotal swelling in guinea pigs in the first three transfers, with rickettsiae in the tunica. Then the phenomenon disappeared for several months entirely, but recently has been appearing again in an occasional transfer guinea pig. On no occasion was the lesion so pronounced as we are accustomed to find in our previously isolated murine strains. Blood Strain 2 showed a transient scrotal reaction in the first passage in guinea pigs, and since February, 1933, this symptom has appeared twice again. The other three blood strains never produced the slightest scrotal involvement. In guinea pigs all five strains caused high continuous fever of from 8 to 9 days' duration, after an incubation period lasting from 7 to 8 days.

Histological examination of the brains revealed nodular lesions with great regularity. In rats the two strains which caused occasional scrotal involvements in guinea pigs produced frequently a mild short fever without any other clinical symptoms; whereas the three other strains ran in rats a purely inapparent course, and correspond therefore exactly to strains of historic Old World typhus in respect to their experimental behavior. No rickettsiae could be found in rats inoculated with these strains. All five strains, however, as could be expected, showed complete cross-immunity to our murine strains, and rat to rat transmission was easily accomplished with *Xenopsylla cheopis*.

The experiments were carried out with these five epidemic strains. The method employed for their transformation is based on the following observations.

In examining sections of lice and fleas infected with the virus of typhus, it is observed that *Rickettsia prowazeki*, the causative agent, multiplies only within the epithelial cells of the mid-gut; *i.e.*, in cells which come in frequent contact with fresh blood. Never has there been observed in insects the slightest invasion of the muscular apparatuses, the genital organs, the fatty tissue, nor of the salivary glands. Only in fleas the lower parts of the Malphigian tubules near their union with the gut are frequently found to be invaded by *Rickettsia prowazeki*. When guinea pigs and rats are inoculated subcutaneously, *Rickettsia prowazeki* develops in the endothelial cells of the blood vessels only. Absolutely never does it invade cells which lie beyond the endothelial lining of a blood vessel. When animals are
inoculated intraperitoneally with a murine strain, *Rickettsia prowazeki* multiplies abundantly within the endothelial cells of the tunica vaginalis. Examination of guinea pigs killed during the incubation period revealed that infected endothelial cells of the tunica vaginalis are found only where the serosa covers superficially situated blood vessels, especially along those vessels which are situated in the groove separating the fatty body from the testicle proper. Such places seem to constitute the primary site of multiplication of *Rickettsia prowazeki* in the tunica vaginalis. When a guinea pig with a fully developed scrotal reaction is killed, the infected serosa cells are found to be distributed over the entire tunica vaginalis. At that time the scrotal sac is found to be filled with coagulated blood or plasma.

It seems evident from these observations that in the mammal, as well as in the insect, *Rickettsia prowazeki* is in some way dependent for its intracellular development on the presence of fresh blood which has to come in close contact with the susceptible cells. Why the rickettsiae of Old World typhus and of our recently established epidemic Mexican strains show so little tendency to grow in the processus vaginalis of guinea pigs and especially of rats where the rickettsiae of the murine variety multiply so abundantly, is a problem yet to be solved. A working hypothesis was, however, conceived which seems to have brought us a step forward in the understanding of the conditions involved. Since in murine strains scrotal lesions in guinea pigs occur regularly, whereas they are the exception in epidemic strains, we thought that the rickettsiae of the murine strains were less hemophilic than the rickettsiae of the epidemic strains, being able to multiply in the processus vaginalis when a transudation of a small quantity of blood or plasma occurs through the superficially situated blood vessels on account of intraperitoneal inoculation of foreign material. The ensuing specific inflammation on the sites of the primary multiplication of *Rickettsia prowazeki* along the superficially situated blood vessels causes a more or less abundant exudation of plasma which collects in the scrotal sac and creates favorable conditions for the spreading of the organism over the entire endothelial lining of the processus vaginalis. For the rickettsiae of the epidemic strains, which apparently are much more hemophilic than those of the endemic strains, the conditions for growth in the peritoneal cavity are not favorable. To be able to multiply within the endothelial cells they seem to need a steady and abundant supply of fresh blood, a condition present only in blood vessels. With this idea in mind, we decided to inject daily fresh blood into the peritoneal cavity of
guinea pigs and rats previously inoculated by the same route with the virus of the non-orchitic epidemic strains. Fresh guinea pig blood, whole or defibrinated, was used in nearly all of the experiments. Since in the endemic strains multiplication of rickettsiae takes place almost exclusively in the tunica of guinea pigs and rats, we included an endemic strain in our experiments in order to find out whether this method produced in rats a general infection of the endothelial lining of the peritoneal cavity. Full grown white rats were used in all experiments.

Experiments with a Murine Strain

Two rats were inoculated intraperitoneally with tunica and testicular washings from a guinea pig killed when scrotal swelling was at its height. Immediately after and from then on every morning the rats received 3 cc. of whole guinea pig blood intraperitoneally. A control rat received the same amount of blood every day. When the inoculated rats looked very ill they were killed and smears made from the visceral and parietal peritoneum. Both rats showed an extensive invasion of the peritoneal cells with *Rickettsia prowazeki*. The majority of the cells were found to be crowded full with organisms and very numerous extracellular rickettsiae were spilled from disintegrated cells. Protocol 1 illustrates the course of fever in these animals.

**Experiment I. Protocol 1**

<table>
<thead>
<tr>
<th></th>
<th>Rat 1</th>
<th>Rat 2</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>July 15</td>
<td>°C.</td>
<td>°C.</td>
<td>°C.</td>
</tr>
<tr>
<td>inoculated</td>
<td>B 37.2</td>
<td>B 37.3</td>
<td>B 37.1</td>
</tr>
<tr>
<td>July 16</td>
<td>B 37.1</td>
<td>B 37.3</td>
<td>B 37.4</td>
</tr>
<tr>
<td>July 17</td>
<td>B 37.4</td>
<td>B 37.5</td>
<td>B 37.3</td>
</tr>
<tr>
<td>July 18</td>
<td>B 38.2</td>
<td>B 38.1</td>
<td>B 37.5</td>
</tr>
<tr>
<td>July 19</td>
<td>B 38.2</td>
<td>38.3</td>
<td>B 37.4</td>
</tr>
<tr>
<td>July 20</td>
<td>B 38.1</td>
<td>Looks ill, killed.</td>
<td>B 37.6</td>
</tr>
<tr>
<td></td>
<td>il, killed.</td>
<td>Rickettsiae</td>
<td></td>
</tr>
<tr>
<td></td>
<td>++ + +.</td>
<td>Spleen very</td>
<td></td>
</tr>
<tr>
<td></td>
<td>large.</td>
<td>Much brownish</td>
<td></td>
</tr>
<tr>
<td></td>
<td>exudate in peritoneum.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Few coagula</td>
<td>Few coagula</td>
<td></td>
</tr>
</tbody>
</table>

± = Very few infected cells.
+ = Few infected cells.
++ = About 10 per cent cells infected.
+++ = Between 20 to 50 per cent cells infected.
++++ = The majority of cells infected.
B = Blood injection.
This experiment was remarkable inasmuch as it was conducted with females in which rickettsiae can as a rule be found only with difficulty. In no instance did we see such large numbers of rickettsiae in the peritoneal cavity of rats which had been inoculated directly from a guinea pig. Only x-ray-treated rats may show such a heavy infection of the peritoneal cavity (6). From the peritoneal exudate of Rat 1 three new rats were inoculated. Two of them received a daily blood injection; the third served as an infected control without blood.

**Experiment II. Protocol 2**

<table>
<thead>
<tr>
<th></th>
<th>Rat 4 ♀</th>
<th>Rat 5 ♂</th>
<th>Rat 6 ♂</th>
</tr>
</thead>
<tbody>
<tr>
<td>July 20</td>
<td></td>
<td></td>
<td>37.3</td>
</tr>
<tr>
<td>inoculated</td>
<td>B 37.2</td>
<td>B 37.2</td>
<td></td>
</tr>
<tr>
<td>July 21</td>
<td></td>
<td>B 37.4</td>
<td>37.2</td>
</tr>
<tr>
<td>July 22</td>
<td>B 37.4</td>
<td>B 37.5</td>
<td>37.3</td>
</tr>
<tr>
<td>July 23</td>
<td>B 38.4</td>
<td>B 38.5</td>
<td>38.4</td>
</tr>
<tr>
<td>July 24</td>
<td>B 38.3</td>
<td>B 38.6</td>
<td>38</td>
</tr>
<tr>
<td>July 25</td>
<td>B 38.2</td>
<td>B 38.5</td>
<td>38.1</td>
</tr>
<tr>
<td>July 26</td>
<td>B 38</td>
<td>Died in the evening.</td>
<td>Dying. Rickettsiae, tunica +. Rickettsiae, peritoneum ++. Spleen very large</td>
</tr>
<tr>
<td>July 27</td>
<td>B 37</td>
<td>Rickettsiae, peritoneum +++. Spleen very large. A good deal of exudate</td>
<td>Spleen very large</td>
</tr>
</tbody>
</table>

Two more transfers were made from rat to rat with and without additional blood injections. In both instances the rats died between the end of the 3rd and the end of the 4th day with heavy rickettsiae infection of the peritoneal cavity. Although in the animals injected with blood considerably more rickettsiae were found, in the animals not injected with blood the infection had also spread over the entire peritoneal cavity in the last transfer.

**Experiments with the Epidemic Strains of Mexican Typhus**

*A. Epidemic Blood Strain 1.*—Experiments were carried out first with a strain which, since its isolation, had never shown the slightest
scrotal involvement in guinea pigs, nor had frequent examinations of
the tunics of transfer animals ever shown any rickettsiae. This strain
constantly showed numerous typical brain lesions in guinea pigs. In
rats it caused an inapparent infection only when brain of transfer
guinea pigs was inoculated, and on no occasion did we find rickettsiae
in such rats.

A transfer guinea pig received a daily blood inoculation intraperitoneally. On
the 7th day this animal showed fever and a typical scrotal swelling was observed,
for the first time in this strain, with very numerous rickettsiae in the tunica.
Tunica and testicular washings from this animal were inoculated into two white
rats followed by a daily injection of 3 cc. of guinea pig blood. Two male guinea
pigs were also inoculated with the same material without consecutive blood in-
jection. Both guinea pigs ran a typical non-orchitic course characteristic of this
strain. Protocol 3 shows the course of infection in two rats.

**Experiment III. Protocol 3**

<table>
<thead>
<tr>
<th></th>
<th>Rat 7</th>
<th>Rat 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>July 18 inoculated</td>
<td>°C.</td>
<td>°C.</td>
</tr>
<tr>
<td>July 19</td>
<td>37.3</td>
<td>37.2</td>
</tr>
<tr>
<td>July 20</td>
<td>37.7</td>
<td>37.4</td>
</tr>
<tr>
<td>July 21</td>
<td>37.3</td>
<td>37.8</td>
</tr>
<tr>
<td>July 22</td>
<td>38.6</td>
<td>37.6</td>
</tr>
<tr>
<td>July 23</td>
<td>38.8</td>
<td>39.0</td>
</tr>
</tbody>
</table>

Sick, killed. Rickettsiae, tunica
+++ +. Rickettsiae, peritoneum
+++ +. Spleen very large

Looked sick in the evening, killed.
Rickettsiae, peritoneum
+++

From Rat 7 three male guinea pigs were inoculated intraperitoneally with
peritoneal exudate, and two male rats with the same material. The latter re-
ceived a daily guinea pig blood injection, whereas the guinea pigs did not receive
any blood. All three guinea pigs reacted with fever after an incubation period
of 3 days only, and in all of them a typical scrotal lesion appeared between the 4th
and 5th day. Protocol 4 illustrates the course of the disease in these two rats.
From Rat 9 three male guinea pigs and two female rats were inoculated and the rats treated with daily blood injections. The three guinea pigs developed fever after an incubation period of 3 days only, and typical scrotal involvement appeared in all of them. The rats showed fever on the 3rd day and were moribund on the 4th with heavy rickettsiae infection of tunica and peritoneum.

After a few more transfers through rats, the strain, which previously had caused only inapparent infection in rats, killed them now regularly between the 4th and 5th day, with or without the application of blood. In this strain also repeated transfers from rat to rat caused a diffuse invasion of the peritoneal cavity with rickettsiae even when no blood was injected, but a heavy infection could as a rule be found in those animals only in the tunica; whereas in the blood-treated animals a heavy rickettsiae infection was found over the whole peritoneal cavity.

The experiments with this typical non-orchitic epidemic strain demonstrated clearly that by the blood method the strain assumed characteristics found hitherto only in endemic murine strains; i.e., scrotal lesions in guinea pigs and a fatal course of disease in rats, with numerous rickettsiae in the peritoneal cavity.

**B. Epidemic Louse Strain.**—The same procedure was followed with this strain as that which was applied to the epidemic blood Strain 1; namely, inoculating a transfer guinea pig with a daily dose of blood. No scrotal lesion appeared, but the tunica was found to
be edematous and hemorrhagic on the 1st day of fever although no rickettsiae could be found. Tunica emulsion and testicular washings from this animal were inoculated into rats, followed by a daily dose of 5 cc. of blood. One rat died on the 6th day from peritonitis. Two others had fever on the 8th and 9th days; blood injection was discontinued on the 9th. The next day when the rats were very ill they were killed, and numerous rickettsiae were found in the tunica and in the peritoneum. From one of these animals transfers were made to other rats and the strain then became highly virulent following the third transfer, with enormous numbers of rickettsiae in the blood-treated animals. In guinea pigs inoculated with peritoneal exudate of such rats, scrotal lesions appeared with great regularity; but when transfers were made then from guinea pig to guinea pig, the strain rapidly reverted to its non-orchitic original course.

C. Blood Strains 2, 3, and 4.—Strains 2 and 3 could also be adapted to rats with the blood method. A highly fatal disease resulted in these animals, with the same enormous invasion of the peritoneal cavity by rickettsiae. The inoculation from such rats into guinea pigs regularly caused a scrotal involvement, but on further transfers from guinea pig to guinea pig the strains rapidly reverted to their original non-orchitic course. Both these strains were adapted to rats by inoculation of guinea pig brain followed by blood injections, instead of using tunica as was done in the previous experiments. The epidemic blood Strain 4 behaved differently from the other epidemic strains inasmuch as we have not yet been able to convert it. On no occasion did it cause fever in rats and only exceptionally could a few rickettsiae-infected cells be found in the peritoneal cavity of animals infected with this strain.

Experiments with Nicolle's African Strain of Historic Typhus

This well known strain which we owe to the courtesy of Professor Charles Nicolle, of Tunis, has been kept in our laboratory since July, 1931. In transfer guinea pigs this strain causes as a rule only a rare and transitional scrotal reaction. Occasionally, however, a lesion may be observed which looks exactly like that observed in our murine strains. When transfers into rats are performed and brains of such rats inoculated back into guinea pigs, scrotal lesions may
appear in nearly 50 per cent of these animals; but on further transfers from guinea pig to guinea pig the lesion becomes rare again. When brain of guinea pigs infected with this strain is inoculated into rats, no fever is observed and no rickettsiae can be found in the peritoneal cavity. The disease remains entirely inapparent. When, however, tunica exudate from a guinea pig with scrotal involvement is inoculated into rats, a short fever may be observed occasionally, and rickettsiae, although as a rule not numerous, are found in the tunica of such rats. It is this strain which Professor Nicolle has compared with one of our Mexican rat strains, coming to the conclusion that the two types of strains behave entirely differently in rats (1). He admits for his strain only a purely inapparent infection in rats as he does for all Old World strains. That this is not entirely the case we have already demonstrated (7). On no occasion, however, had we observed in this strain previous to the present experiments a serious course of the disease in rats even when they had been inoculated with a large amount of tunica from an occasional orchitic guinea pig. Another difference between the murine strain and the Tunisian strain to which Nicolle gives much importance is his observation that while the Mexican murine strain can be carried indefinitely in transfers from rat to rat, the Tunisian strain breaks off sooner or later in such rat transfers. Nine successive transfers in rats were the longest series that he was able to obtain (8). Since Nicolle’s strain is one of the best studied Old World viruses, we thought that it would be of particular significance if we succeeded in transforming this strain into a strain highly pathogenic for rats with the characteristics of our Mexican murine strain.

Two separate strains of Nicolle’s virus which assumed murine characteristics with our blood method were established. One strain was started from the tunica of a guinea pig with scrotal involvement, and the other from the brain of a guinea pig without scrotal involvement. The guinea pig from which the first strain was started had been inoculated with brain of a rat and was then injected daily with 5 cc. of guinea pig blood. On the 7th day, when the guinea pig showed a suggestive scrotal lesion, it was killed and the tunica in which few rickettsiae were found was inoculated into rats. These rats received a daily dose of 3 cc. defibrinated guinea pig blood. The rats did not show any signs of illness, but when killed on the 5th day typical infected endothelial cells were found in the tunics. By further transfers from rat to rat using tunics and 3 cc. of guinea pig blood,
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typical fever curves appeared in the rats, and in the animals of the third transfer enormous numbers of rickettsiae-infected cells were observed in the tunica smears. In the general peritoneal cavity, however, they were scarce or absent. We increased, therefore, the daily blood dose to 5 cc. of whole blood every day in the succeeding transfers. Most of the rats so treated now died around the 5th day, with a general heavy rickettsia infection of the whole peritoneal cavity.

Infections were as severe as those found in our Mexican strains, and frequently almost 100 per cent of endothelial cells were found to be crowded full of rickettsiae. Inoculation from such heavily infected rats into guinea pigs caused pronounced scrotal swelling in the great majority of them, and in rats it caused a serious fatal disease, often without fever. After several transfers with the blood method, the strain became so virulent that an additional injection of blood was no longer necessary. Some rats showed paralysis of the hind legs shortly before death, and extensive specific lesions were found in the medulla oblongata of these rats. The second strain which behaved entirely analogously was obtained by inoculating brain and blood of a guinea pig into several rats and then making transfers with the blood method from rat to rat.

Protocols 5 and 6 give the course of rats of the sixth and seventh transfers of Nicolle's strain.

Protocol 5

<table>
<thead>
<tr>
<th>Rat 132 ♀</th>
<th>Rat 133 ♂</th>
<th>Rat 134 ♂</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aug. 21</strong></td>
<td>Inoculated</td>
<td><strong>Aug. 22</strong></td>
</tr>
<tr>
<td>37.3</td>
<td>B 37.4</td>
<td>B 37.4</td>
</tr>
<tr>
<td>37.3</td>
<td>B 37.5</td>
<td>36.4</td>
</tr>
<tr>
<td>36.6. Ill</td>
<td>36.3</td>
<td>Looked ill during a.m.</td>
</tr>
<tr>
<td>Aug. 26</td>
<td>Spleen enlarged. Rickettsiae, tunica +++. Rickettsiae, peritoneum ±</td>
<td></td>
</tr>
</tbody>
</table>
CONVERSION OF TYPHUS STRAINS

Protocol 6

<table>
<thead>
<tr>
<th></th>
<th>Rat 137♂</th>
<th>Rat 138♂</th>
<th>Rat 139♂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aug. 23</td>
<td>°C.</td>
<td>°C.</td>
<td>°C.</td>
</tr>
<tr>
<td>Inoculated</td>
<td></td>
<td>Inoculated</td>
<td>Non-infected blood control</td>
</tr>
<tr>
<td>Aug. 24</td>
<td>B 37.3</td>
<td>B 37.2</td>
<td>B 37.3</td>
</tr>
<tr>
<td>Aug. 25</td>
<td>B 37.2</td>
<td>B 37.1</td>
<td>B 37.1</td>
</tr>
<tr>
<td>Aug. 26</td>
<td>B 39.2</td>
<td>B 38.5</td>
<td>B 37.3</td>
</tr>
<tr>
<td>Aug. 27</td>
<td>B 38.2</td>
<td>B 38.3</td>
<td>B 37.4</td>
</tr>
<tr>
<td>Aug. 28</td>
<td>35.3. Very ill</td>
<td>35.5. Moribund</td>
<td>B 37.2</td>
</tr>
<tr>
<td>Rickettsiae, tunica, mostly naked nuclei of endothelial cells. Very numerous extracellular rickettsiae</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In guinea pigs the inoculation of peritoneal exudate of such rats regularly produced scrotal lesions as already mentioned. With the addition of blood the scrotal lesion appeared in nine successive guinea pig transfers. In guinea pigs not inoculated with blood, the strain reverted rapidly to its original non-orchitic course. The strain was kept in rats through fifteen successive transfers when the experiment was discontinued on account of lack of rats. No diminution of virulence was observed in the fifteenth transfer, as three of the four rats of the last transfer died at the end of the 5th day. The fourth rat recovered in spite of a severe paralysis of the hind legs.

The Result of Prolonged Transfers in Rats of the Adapted Epidemic Strains

Whereas the adapted epidemic strains reverted rapidly to their original non-orchitic course when put back into guinea pigs after only a few transfers in rats, they assumed definite murine characteristics after prolonged transfers in rats. The inoculation of these strains back into guinea pigs from rats now regularly caused a scrotal lesion which did not disappear on further transfers in guinea pigs, and also in rats they now behaved constantly like our murine strains even
when brain of guinea pigs was inoculated. The same observation in
respect to rats was made with the strain of Nicolle, although this
strain ran a non-orchitic course in guinea pigs in most of the animals.

The Staining of Rickettsiae in Smears from Rats and Guinea Pigs

The best results are invariably obtained with Giemsa’s stain. This method,
however, has the disadvantage that on prolonged staining which is frequently
necessary, very numerous reddish staining cellular debris are stained which makes
the observation of extracellular rickettsiae difficult and sometimes impossible.
Somewhat less of these debris are seen when the smears after fixation with methyl
alcohol are treated during 5 minutes with ether, and then are washed again with
methyl alcohol. Careful differentiation with alcohol-xylol as recommended by
Nigg and Landsteiner (9) frequently gives good results. Castaneda’s method (10),
as well as Lépine’s modification of it (11), demonstrate the intracellular rickettsiae
very well as a rule, when a good brand of methylene blue or azure II is used. The
extracellular rickettsiae, however, frequently stain very poorly with this method.
Lépine’s original method (12) does not stain rickettsiae at all. In rats which
were examined many hours after death, rickettsiae as a rule stained very poorly
and most of the endothelial cells were found to be autolyzed. This poor staining
was as a rule accompanied by clumping of the intracellular rickettsiae into red gran-
ular masses simulating inclusion bodies in virus diseases. Also the extracellular rick-
ettsiae examined many hours after the death of the rats were recognized only with
difficulty because their form was ill defined and they stained much redder than
usual.

DISCUSSION

The finding of epidemic strains of typhus in Mexico which corre-
spond experimentally to strains of historic Old World typhus seems
to support the ideas of those who hold that there exist two varieties
of typhus in Mexico: one, the New World type derived from the rat
reservoir in nature, and the other, the historic Old World, purely
louse-borne type, imported from abroad. This hypothesis has indeed
been advanced recently by Nicolle (1). His main reason for not
accepting the hypothesis that Old World typhus may also be originally
derived from rats is the following: (1) typhus existed in Europe be-
fore the continent had been invaded by rats; (2) the murine type of
virus can be differentiated clearly from historic typhus by the different
reactions which it induces in laboratory animals, especially in rats.
Now it has been shown beyond doubt that the murine type of virus
can be transmitted by the human louse (13) and that the virus of
epidemic Old World typhus can be transmitted experimentally from rat to rat by the rat flea (7). Moreover, from short rapidly checked epidemics of typhus in Mexico City we were never able to obtain strains which did not correspond entirely to the murine type of virus. The same type of virus was obtained constantly from isolated cases during interepidemic periods and from wild rats in nature (14). There cannot exist, therefore, the slightest doubt that the murine virus actually does cause epidemics, and we were able to show that the virus isolated from the brains of wild rats multiplies abundantly in the human louse (15). We have, then, this situation in Mexico: from rats, from endemic cases of typhus, and from cases during short epidemics, murine strains only could be isolated; whereas from a long standing serious epidemic, strains were obtained which correspond to strains of historic Old World typhus. But not all of our epidemic strains agree completely with Nicolle’s strain of Old World typhus. Whereas two of them cause a mild but typical fever in rats, three of them induce only inapparent infections in that species. Immunologically however all of them are identical. Non-orchitic strains of typhus have, without doubt, been isolated previously, all of them in times of long standing serious epidemic, by Gavino and Girard (16), Anderson and Goldberger (17), and Olitsky, Denzer, and Husk (18). It was in Mexico, indeed, that the work of Nicolle and his collaborators on the infection of the guinea pig with typhus was first confirmed, and it is not conceivable that the conspicuous scrotal reaction characteristic of all murine strains should have escaped the observation of so many investigators.

The finding, during a serious epidemic of typhus, of epidemic strains which correspond exactly to strains of historic Old World typhus and of strains which experimentally lie between typical epidemic strains and orchitic murine strains is in our opinion of great significance. After long series of transfers in rats with our blood method, all these epidemic strains, including that of Nicolle, with one exception assumed the typical murine characteristics. In later transfers the addition of blood was not necessary to cause a serious disease in rats with plentiful rickettsiae in the peritoneal cavity. This abundant multiplication of rickettsiae in rats is absolutely characteristic of all strains of the murine type, and the transformation of the non-orchitic
epidemic strains into strains of high virulence for rats shows clearly that there does not exist any fundamental difference between the two types of strains. When the epidemic strains were put back in guinea pigs, after only a few passages in rats, they invariably reverted to the non-orchitic form. When, however, these strains were kept in long series of rat transfers, they were found to have become definitely murine. Only Nicolle's strain could not be kept definitely orchitic in guinea pigs after long rat transfers, although it showed a definite and apparently permanent reversion to the murine type inasmuch as it is now highly virulent for rats, a property which Nicolle (1) considers to be the only valuable characteristic in differentiating historic Old World typhus from the murine New World typhus. But even before the epidemic non-orchitic strains had been adapted fully to rats, their murine traits could be occasionally elicited. When brain of a guinea pig of such a strain is inoculated into rats, a purely inapparent infection follows. When, however, tunica of an animal with an occasional scrotal swelling is inoculated into rats, a fever of short duration appears frequently. Since scrotal swelling in Old World strains had not been observed before one of us had published his results with Mexican typhus (2, 3) the conclusions of European investigators were based on inoculations with brain emulsions. Now it is a well established fact that a scrotal involvement is accompanied by an enormous accumulation of the virus of typhus in the tunica vaginalis. It is evident, therefore, that fever or no fever in rats depends entirely on the doses of virus inoculated and so cannot be a specific sign for differentiating strains of the murine type from strains of historic typhus.

Our hypothesis that the rat is the natural original carrier of the virus of typhus, murine and epidemic, seems to be well supported by the result of our experiments. We consider the epidemic variety to be the product of a long standing propagation of the murine type in the cycle man-louse-man; and it is thus not astonishing, and in complete agreement with our hypothesis, that the rat is the proper experimental animal for reverting epidemic strains into murine strains. The mechanism which causes the reversion of epidemic strains to the murine type of strains we consider to be the result of progressive selection during the transfers from rat to rat. All epidemic strains contain the
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murine properties in a latent form, because scrotal lesions in guinea pigs show up occasionally in them, and the injection of tunica emulsion from such orchitic animals may cause fever in rats and rickettsiae in the tunica. This we take to mean that epidemic strains contain in small number individual rickettsiae having the properties of murine strains. These are individuals endowed with recessive properties. In transfers from rat to rat with the blood method, these recessive individuals of rickettsiae become more and more numerous by progressive selection until the strain has reverted to murine. In guinea pig transfers, the individuals with murine properties may become suppressed again, and the strain then reverts to its epidemic form. That the murine strains contain an epidemic component of rickettsiae is evident from the observation which we have frequently made in past years that such strains may suddenly become non-orchitic for many generations. In one of our rat strains the orchitic component was lost nearly completely by prolonged transfers in guinea pigs. Inoculation of brain emulsions from such non-orchitic guinea pigs only rarely caused fever in rats, but transfers from rat to rat restored to the strain its original orchitic properties. Here again we see the ability of the rat to restore the murine qualities of the strain which had been lost in long transfers through guinea pigs, an animal which naturally is not a host of the typhus virus. A phenomenon is therefore observed in guinea pigs similar to that which can be observed during times of epidemic in Mexico. In long transfers through an unnatural host, in this case man, the original murine components are suppressed. One of our epidemic strains, in which the murine qualities could not be restored, seems to have lost its murine components altogether.

On various occasions one of the writers has pronounced the opinion that the human louse cannot be the natural vector of typhus, and man therefore is not the natural host. The louse is in the biological sense so little adapted to _Rickettsia prowazekii_ that it succumbs invariably to the infection within a relatively short time. In hot weather an infected louse survives but a few days, whereas the rat flea as shown by Dyer and his associates (19) and by Mooser and Castaneda (20) remains definitely infected without apparent harm. This we take as definite evidence that the human louse has appeared on the scene of typhus relatively late and that it has not yet had
time to become a highly adapted biological vector. That Old World
typhus can be transmitted indefinitely at least in cold countries by
the human louse we are not in a position to question. What we do
question seriously, however, is the opinion of European workers that
this is the only means by which historic typhus is preserved. On
several occasions we tried to infect lice on very mild cases of Mexican
typhus without the slightest success. No lice became infected. The
number of infected lice is in our experience in direct relation to the
severity of a case of typhus. There is little chance for lice to become
infected on cases which run a mild short fever, which can be diagnosed
as typhus only with the help of the Weil-Felix reaction, and there can
be no doubt that inapparent human infections are even less liable to
infect lice. On account of these experimental results we question
the great importance which Old World investigators give to inap-
parent infections as a reservoir of the virus during long interepidemic
periods. There is in addition no reason to believe that during long
interepidemic periods all cases of typhus should be inapparent.
It seems to us more than likely that what is happening in Mexico
must happen the world over; i.e., the epidemic adaptation of the
original murine virus to the secondary unnatural cycle man-louse-
man. The circumstance that the murine virus has also recently
been found in Greece and in Syria where epidemics of typhus have
occurred frequently, and the existence of endemic murine typhus in
rats and man side by side with historic typhus in such a typhus-
ridden country as Manchuria, is of great significance in this re-
spect. Nicolle (1) made the statement that the murine virus kills
lice in such a short time that epidemics caused by this type of virus are
doomed to die out quickly. He states furthermore that the virus of
historic typhus survives much longer in lice. We have no experience
with Old World virus in lice, but we know from the literature that it
kills them also within from 10 to 14 days. From experiments with
typical murine strains (21) we know that the longevity of lice depends
entirely on the number of rickettsiae ingested. When concentrated
tunica exudate is introduced by the method of Weigl, the lice do
indeed die within a few days. The more the infective material is diluted,
however, the longer they survive. Lice fed on monkeys infected with
a murine strain (21) and lice fed on human volunteers inoculated
with the same strain lived 10 to 14 days (22). There does not exist, therefore, the slightest reason for rejecting the human louse as a vector in epidemic form of the murine virus.

That the rat constitutes a natural reservoir of endemic typhus in the southern part of the United States, a possibility clearly foreseen by Maxcy (23), has been demonstrated beyond the shadow of a doubt by Dyer and his associates (3). This important discovery will go far to explain the origin of human typhus everywhere. Indeed there is not a single circumstance which speaks against the hypothesis that the historic typhus of the Old World is also derived from the rat reservoir in nature. That this is the case for epidemic typhus in Mexico we consider to be definitely demonstrated. From short lived rapidly checked epidemics in Mexico, the murine virus was isolated in each instance; from a long standing epidemic, strains were isolated which correspond to the typical historic Old World type of virus and in addition other strains which lie intermediate between the two extremes. Moreover the epidemic strains could be induced to assume all the characteristics of typical murine strains by long standing transfers through rats with our blood method.

The observation that *Rickettsia prowazeki* is able to develop in the mammal as well as in the insect only within those cells which come into constant contact with fresh blood explains why typhus cannot be transmitted by ticks and by mites which take blood only at long intervals. It is therefore not astonishing that we were not able to confirm the results of Shelmire and Dove (24) with *Liponyssus baoi*. The virus may occasionally survive in these mites as it does in ticks for several days, but never could we demonstrate any multiplication of it and on no occasion was transmission accomplished with mites or ticks by the act of biting. *Rickettsia prowazeki* exhibits the most specialized type of parasitism which has yet been observed in a bacterial pathogenic organism. Not only is it dependent on blood while in the mammal host, but it continues to be dependent on mammal blood during its life in the insect vector. This dependency on mammal blood may explain to a certain extent why the virus of typhus exhibits so little specificity toward animals and toward blood sucking insects. Practically all rodents are susceptible to typhus as well as man, the apes, and the monkeys. Of the blood-sucking insects
Pediculus, Pedicinus, Polyplax, and all species of fleas so far tested are susceptible. All these insects take blood at frequent intervals, 2 to 3 times a day. A persistence of the virus and a probable multiplication of it has been observed in Cimex lectularius (25). Although fresh blood is necessary for the development of Rickettsia prowazeki within cells, the cells do not play a merely passive rôle in this respect. We were not able to cause a general infection of the peritoneal cavity of guinea pigs by the blood method. In tissue cultures and in the Maitland medium Rickettsia prowazeki multiplies only in endothelial cells and never, for instance, in fibroblasts. That plasma or fresh serum is also necessary in these cultures for the development of Rickettsia prowazeki has been shown by Nigg and Landsteiner (9). In fleas as well as in lice no multiplication occurs in the cells of the hind-gut which also come into contact with blood. It is interesting that in the intestines of insects the susceptible cells are those of the intestinal tract which are covered by the chitinous peritrophic membrane. In fleas this membrane is well developed, whereas in lice the structure is thin and delicate. One is inclined to suspect that the development of Rickettsia prowazeki in these cells is related in some way to the metabolism of chitosamine.

In a series of experiments not recorded in this paper, we tried to determine what blood constituent is responsible for the enormous increase of Rickettsia prowazeki in the peritoneal cavity of rats injected with blood. Tests were made with serum, with washed red cells, and with defibrinated blood of various animals. Only with defibrinated blood of guinea pigs did we get results comparable to those obtained with whole blood of guinea pigs. As a rule, however, whole blood gave better results. Horse blood, sheep blood, and beef blood were found to be far inferior to guinea pig blood. To exclude a non-specific action of guinea pig blood on rats, we should have tried experiments using rat blood. The limited number of full grown rats at our disposal prevented us from carrying out these experiments. The observation that Rickettsia prowazeki can survive and multiply only in cells which come in contact with fresh blood furnished the basis for our blood method. Our uniform good results with guinea pig blood, however, offer no definite proof that whole blood or a blood constituent is really the specific factor which enables the rickettsiae to grow
in the peritoneal cavity. Zinsser and his pupils have succeeded in causing an enormous multiplication of rickettsiae in rats by various methods. Their x-ray method especially gives excellent results as a rule. They were, however, not able by their methods to adapt the rickettsiae of an epidemic non-orchitic strain to the peritoneal cavity of rats. It is possible that the positive results obtained by Zinsser and Castaneda with murine strains are based on the same principles as our blood method. Intensive x-ray treatment increases permeability of the walls of blood vessels, with subsequent effusion of blood constituents. For murine strains, the increased permeability caused by x-ray treatment seems to furnish enough of these constituents to allow the rickettsiae to grow in the general peritoneal cavity. For the epidemic strains which are much more hemophilic, this method is inadequate. In our experiments with Nicolle's epidemic strain, it was necessary to increase the daily blood dose from 3 cc. to 5 cc. in order to bring about a generalized infection of the peritoneal cavity.

**SUMMARY AND CONCLUSION**

Two types of strains of typhus virus are observed in Mexico: first the murine type which is obtained from wild rats in nature, from isolated endemic cases, and from cases during short epidemic outbreaks, and second, the epidemic type of strains which is obtained from long standing serious epidemics. Some of these epidemic strains correspond entirely to strains of historic Old World typhus. Other strains which in their experimental behavior are intermediate between these two types of strains were isolated from the same epidemic. A method is described by which these epidemic non-orchitic strains can be converted into murine strains regularly causing scrotal lesion in guinea pigs and a highly fatal disease in rats. The same results were obtained with an Old World strain of epidemic typhus. The method, which consists of daily blood injections into intraperitoneally inoculated rats, is based on the observation that *Rickettsia prowazeki* multiplies only within cells which come in constant or frequent contact with fresh blood. It is concluded from our experiments that there does not exist any real difference between the virus of historic Old World typhus and the murine New World typhus. Both are considered to be of murine origin. The murine strains represent the
original form of the virus of typhus, whereas the epidemic strains are the result of a prolonged propagation in the cycle man-louse-man.

BIBLIOGRAPHY

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