THE MULTIPLICATION OF THE VIRUS OF MEXICAN TYPHUS FEVER IN FLEAS

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Plate 19

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In two preceding publications, (1, 2) the writers, with Zinsser, have reported the discovery of the virus of Mexican typhus fever in rats trapped in Mexico City, and have described the conditions under which the virus can be conveyed from rat to rat by the rat louse, *Polyplax spinulosus*. These investigations brought definite proof that there is an animal reservoir of typhus virus outside the human body from which epidemic spread can occur under suitable conditions. The appearance of epidemics after prolonged typhus-free intervals is far more readily explained by the existence of such an animal reservoir than by the older hypothesis of a continuous maintenance of the typhus virus in an apparently free community by the occurrence of inapparent human infections. It is hardly conceivable that, in the prolonged interepidemic periods, all or even the majority of human cases which bridge the interval between two epidemic outbreaks should remain clinically inapparent.

In order to discover the means of transmission of the disease from rat to man, it became necessary to study in detail the blood-sucking arthropods which infest the living rats in Mexico City. An investigation of several months in which insects were collected from trapped rats revealed the following ectoparasites.

Mites: *Laelaps echidninus*
Liponyssus bacoti[1]

[1] We are indebted to Dr. Ewing of the U. S. Department of Agriculture for the identification of this mite.

Lice: *Polyplax spinulosus*
Fleas: *Xenopsylla cheopis*
Leptopsylla musculi
Ceratophyllus fasciatus
Ctenocephalus canis (rare)
Ctenocephalus felis (rare)

Of the mites, *Laelaps echidninus* was shown to be unable to transmit the disease, as no virus could be found in this species in spite of prolonged contact with infected rats. This mite does not ingest blood during its larval and nymphal stages, and of the adults only the female has been reported to suck blood.

Liponyssus bacoti, on the contrary, takes blood during its larval, nymphal and adult instars. When put on rats, it becomes engorged with blood in less than 48 hours, as a rule, whereupon the nymphs and adult stages drop off and crawl to a hiding place, where they moult or lay eggs according to their respective developmental stages. The larvae may remain several days on the host before they crawl off. Within less than a week most of the mites are ready for another meal. In two experiments, in each of which we used numerous mites and examined them between 9 and 10 days after an infectious meal, we were not able to observe *Rickettsiae* or to demonstrate the virus of typhus in this species.

There seem to remain, therefore, only the fleas, which had already been shown by Dyer, Rumreich and Badger (3) to be able to harbor the virus of endemic typhus in the southern United States. Of the fleas found on our rats, all except *Leptopsylla musculi* have been reported to attack man more or less readily. We have, therefore, tested all of the above mentioned species systematically, in order to find out in which of them the virus of Mexican typhus is able to survive and to multiply. At the same time, we tried to determine which of these fleas would be most likely to be implicated as a vector from rat to rat and from rat to man. Our methods varied somewhat according to the species of flea which was to be investigated.

When rat fleas were tested (*Xenopsylla, Ceratophyllus, Leptopsylla*) they were, as a rule, put on the rats some time before infecting the latter with a heavy dose of guinea pig tunica emulsion. In the experiments with cat and dog fleas (*Ctenocephalus*) the starved insects were put on the rats immediately after the febrile

period had made its appearance, in view of the difficulty of keeping these two species on rats for any length of time. The rats were kept in high glass jars covered with fine mesh gauze. The average room temperature was around 20°C. The temperature in the jars oscillated between 26° and 28°C. during the daytime. In all these experiments, with the exception of those carried out by Dr. Castaneda with dog fleas, a typhus strain isolated from wild rats in Mexico City was used.

Experiments with Leptopsylla musculi.—

Large numbers of this species were found on two wild rats (*Mus rattus rattus*) caught in the vicinity of Mexico City. The rats were killed and put in a jar con-

Protocol 1

Guinea Pig 1

Date	Temperature	Remarks
	°C.	
July 14	39.2	Inoculated
" 15	39.3	
" 16	39.2	
" 17	40.2	
" 18	39.6	
" 19	40.5	Scrotum red and swollen
" 20	40.8	
" 21	40.6	
" 22	39.7	
" 23	39.7	
" 24	39.6	
" 25	39.0	
" 26	39.1	
" 27	39.0	

This animal was preserved for immunity test with Nicolle's strain of African typhus.

taining three young white rats. 24 hours later, on June 28th, the dead rats were removed and the three white rats, Nos. 1, 2 and 3, inoculated with tunica emulsion. On July 7th, one flea was removed, smeared and stained with Giemsa solution. Numerous small red staining organisms were observed in this smear. They were indistinguishable from *Rickettsia prowazeki* as found in typhus-infected fleas. On July 11th, large numbers of these organisms were observed in the smears of two other fleas. Three fleas were removed on that day and prepared for histological examination. On July 14th, two fleas were emulsified and inoculated into Guinea Pig 1. The reaction of this animal is shown in Protocol 1.

On July 15th Rats 1, 2 and 3 were killed and a fresh rat, No. 4, put in the jar. When the fleas had changed to the new rat, the others were removed. On July

20th, it was noticed that the number of fleas had decreased considerably on this rat. Five were recovered and smeared. Numerous typical *Rickettsiae* were found in all of them. On August 5th, Rat 4 was killed and its brain inoculated into a guinea pig. No symptoms resulted from this inoculation.

Experiments with Ceratophyllus fasciatus.—

Ten specimens of this species were procured from several wild rats (*Mus decumanus*) together with other fleas. The rats were killed and combed over a pan containing ice cold water. The fleas, anesthetized by the cold, were removed with a hair brush and classified. The collected specimens of *Ceratophyllus fasciatus* were then put on Rat 5, which several days later was inoculated with a tunica

Protocol 2

Guinea Pig 2

Date	Temperature	Remarks
	°C.	
Aug. 12		Inoculated with 3 fleas
" 13	38.7	
" 14	39.0	
" 15	40.0	Scrotum red and very swollen
" 16	39.5	
" 17	40.0	
" 18	40.2	Killed, autopsy typical. Tunica hemorrhagic, covered by a thick layer of fibrin. *Rickettsiae* present

Tunica emulsion inoculated into Guinea Pigs 3 and 4 produced typical typhus, with scrotum swelling within 4 days and numerous *Rickettsiae* in Guinea Pig 4. Guinea Pig 3 was preserved for immunity test with Nicolle's strain.

emulsion very rich in *Rickettsiae*. The results obtained with this species were in every respect identical with those of the preceding experiment. In smears of fleas examined around the 12th day after the inoculation of Rat 5 *Rickettsia prowazeki* was found in all of them, and the inoculation of an emulsion of three fleas into Guinea Pig 2 was followed by the typical picture of Mexican typhus. Two fleas were prepared for histological examination. The reaction of the guinea pig is shown in Protocol 2.

Experiments with Xenopsylla cheopis.—

This species was the most common flea found by us on rats in Mexico City during the spring and summer of 1931. It was therefore given special attention, its infectivity during a prolonged period after feeding on infected rats being thoroughly

investigated. Fleas collected in the harbor of Vera Cruz were used in one experiment, and fleas collected from rats in Mexico City in two additional experiments. 100 per cent of them were found to be infected with *Rickettsia prowazeki* when

Protocol 3

Guinea Pig 4

Date	Temperature	Remarks
	°C.	
July 9	39.0	
" 10	39.0	
" 11	40.4	Scrotum ++
" 12	40.3	" ++
" 13	40.2	" ++
" 14	40.3	" ++
" 15	40.2	" +
" 16	40.0	" +
" 17	40.0	" normal
" 18	39.7	" "
" 19	39.5	
" 20	39.4	
" 21	39.0	
" 22	39.0	
" 23	38.9	

Preserved for immunity test with Nicolle's strain.

Guinea Pig 5

Date	Temperature	Remarks
	°C.	
July 9	38.9	
" 10	38.8	
" 11	40.2	Scrotum ++
" 12	40.5	" +++. Killed. Numerous petechiae in the skin and subcutaneous tissue. Pronounced edema and hemorrhage around site of inoculation. *Rickettsiae*: numerous intra- and extracellular. Cultures negative

examined between the 9th and 10th days after the beginning of the febrile periods of the rats. The inoculation of emulsions of one and a half to two and a half fleas on the 13th,[2] 24th and 33rd days, respectively, into male guinea pigs was followed

[2] Counted from the day the rats had begun to show fever.

in each instance by the typical picture of Mexican typhus after a very short incubation period, indicating an extraordinary multiplication of the virus. Protocols 3 and 4 illustrate the reaction of two sets of guinea pigs inoculated with *Xenopsylla cheopis*.

Protocol 4

Guinea Pig 6

Date	Temperature	Remarks
	°C.	
July 28		Inoculated at 7 p.m.
" 29	39.3	
" 30	40.4	" " 10 a.m.
" 31	40.7	Scrotum +
Aug. 1	40.6	" ++. Killed. Autopsy typical. *Rickettsiae* in tunica ++++. Tunica into two guinea pigs which came down with typical picture of Mexican typhus

Guinea Pig 7

Date	Temperature	Remarks
	°C.	
July 28		Inoculated at 7 p.m.
" 29	39.3	
" 30	40.0	" " 10 a.m.
" 31	40.4	Scrotum ++
Aug. 1	40.7	" ++
" 2	40.6	" ++
" 3	40.3	" ++
" 4	40.0	" normal
" 5	39.3	
" 6	39.2	

Preserved for reinoculation with Nicolle's strain.

Experiment on Infection of Fleas on Inoculated Rats

June 23. Two white rats previously infested with *Xenopsylla cheopis* of Mexico City inoculated with tunica emulsion.

June 25. Both rats have fever.

June 29. Both rats dead. Replaced by healthy Rat 8.

July 6. One flea examined. *Rickettsia prowazeki* present.

July 8. Three fleas emulsified and inoculated into Guinea Pigs 4 and 5—see Protocol 3).

July 11. Weil-Felix of Rat 8 negative. One *Xenopsylla* prepared for histological examination.

Protocol 5

Guinea Pig 8

Date	Temperature	Remarks
	°C.	
Aug. 3		Inoculated
" 4	38.8	
" 5	39.0	
" 6	39.2	
" 7	40.2	
" 8	40.0	
" 9	40.4	Scrotum +
" 10	39.8	
" 11	40.1	
" 12	40.0	
" 13	39.6	
" 14	39.0	
" 15	39.0	
" 16	39.2	

Preserved for immunity test with Nicolle's strain.

Guinea Pig 9

Date	Temperature	Remarks
	°C.	
Aug. 3		Inoculated
" 4	39.1	
" 5	39.0	
" 6	39.4	
" 7	40.0	
" 8	40.6	Scrotum ++. Killed. Autopsy typical. Numerous endothelial cells heavily infected with *Rickettsiae*. Two successive passages in guinea pigs produced typical infections

July 13. Weil-Felix of Rat 8 negative.

July 14. Weil-Felix of Rat 8 positive 1–40.

July 15. Rat 8 found dead. Brain inoculated into a guinea pig which died from peritonitis on July 21st. Fresh rat, No. 9, put in cage.

July 25. Rat 9 dead. Replaced by fresh rat, No. 10.

July 28. Eleven remaining fleas removed. Five emulsified and inoculated into Guinea Pigs 6 and 7 (see Protocol 4). Three fleas smeared and numerous *Rickettsiae* found. Three fleas prepared for histological examination.

Experiments with Ctenocephalus felis.—

About 100 fleas collected from cats were put in a jar containing two white rats which 3 days previously had received a heavy dose of tunica emulsion. 3 days later only a few fleas could be observed on the rats, most of them having been killed. 8 days after the beginning of the experiment one flea was smeared and few, but typical, *Rickettsiae* found. On the 10th day only a few fleas were still found on the rats. One of them was prepared for histological examination. The two remaining ones were emulsified and inoculated into Guinea Pigs 8 and 9, as indicated in Protocol 5.

Experiments with Dog Fleas.—

The fleas found on dogs in Mexico City are *Ctenocephalus canis*, *Ctenocephalus felis* and *Pulex irritans*. In several experiments in which numerous dog fleas were put on infected rats, nearly all of them were destroyed by the hosts within a day or two. These fleas bothered the rats greatly, and the rats did not spare any effort to get rid of them as quickly as possible. Whereas rat fleas settle preferably on the heads of the rats, especially behind the ears and in the intermandibular groove, *Ctenocephalus* and *Pulex* settle on the abdomen, on the back and even on the legs, where they are reached easily by the teeth of the rats. In several experiments, the few fleas which still remain on the rats after 8 to 9 days turned out to be nearly always *Ctenocephalus felis*. *Rickettsiae* were found in them each time. In one instance, however, a female *Ctenocephalus canis* was found on a rat on the 11th day after the beginning of the experiment. It was emulsified and inoculated into Guinea Pig 10.

Experiment to Determine the Immunity to the Nicolle Strain of North African Typhus Fever of Guinea Pigs Inoculated with Infected Fleas

Guinea Pigs 1, 3, 4, 7, 8 and 10, all of them infected with flea material at various times, were used in this experiment. The preliminary history and the date of inoculation as well as the materials inoculated into these guinea pigs are described in preceding experiments, where the corresponding numbers of these animals can be found.

On August 23, 1931, these six animals were inoculated with an emulsion of one-tenth of the brain of a guinea pig representing the 302nd passage of Nicolle's strain of North African typhus fever. At the same time, four control guinea pigs were

inoculated with corresponding amounts of the same material. The amount of brain inoculated represented from 300 to 500 infectious doses for a guinea pig.

In order to economize space, we may summarize this experiment in the following way: None of the animals that had been flea-inoculated at a previous time and had developed typical Mexican typhus fever at that time showed a temperature reaction characteristic of the European or African typhus fever, though observed for 18 days. The highest temperature reached by any of them during this period was 39.3°C., or 102.74°F.

Protocol 6

Guinea Pig 10

Date	Temperature	Remarks
	°C.	
Aug. 1		Inoculated
" 2	39.4	
" 3	39.2	
" 4	39.4	
" 5	39.6	
" 6	40.4	
" 7	40.6	Scrotum +
" 8	39.8	" normal
" 9	40.2	" +
" 10	40.0	
" 11	39.8	
" 12	39.6	
" 13	39.2	
" 14	39.2	
" 15	39.0	

Preserved for immunity test with Nicolle's strain.

The four controls all had temperatures approaching or exceeding 40°C. or 104°F. on the 6th day, and between the 6th and 14th days two of them continued to run temperatures fluctuating between 40.3° and 40.8°C., or 104.5° and 105.5°F. The two others were used on the 10th day for transfers showing a fever of 40.6° and 40.8°C., respectively, when they were killed on the 5th day of continuous fever.

It is plain to be seen that the controls all passed through a typical typhus fever of the Nicolle type, whereas none of the flea animals developed a typhus reaction. It is obvious, therefore, that the animals which had been inoculated with a Mexican virus that had passed through fleas were immune to a subsequent inoculation with the Nicolle strain.

Transmission Experiments.—

These experiments were greatly hampered by an epizootic among our rats. In one rat a slightly positive Weil-Felix reaction was observed 15 days after infected fleas had started to feed on it. Unfortunately, the rat was found dead the next day, and the inoculation of its brain into a guinea pig produced peritonitis in the latter. The inoculation of Rats 4 and 10, respectively, was not followed by any symptoms.

Histological Examination of Fleas Fed on Typhus-Infected Rats

The following fleas were examined.

3 Xenopsyllae.—33 days after fever had been noticed in the inoculated rats, 29 days, respectively, after replacing the infected with a normal rat.

1 Xenopsylla.—13 days after the febrile period had started in the rats, 9 days, respectively, after the fleas had changed to a normal rat.

3 Leptopsyllae.—16 days after being put on infected rats.

2 Ceratophyllus fasciatus.—12 days after feeding on infected rats.

1 Ctenocephalus felis.—10 days after feeding on infected rats.

The fleas were killed by applying a drop of chloroform to the place where they were observed, and then carefully removed by a pair of soft forceps. The legs were clipped off as closely as possible to the body, then the head was removed with a razor blade, and finally the two last abdominal segments were cut off. The fleas were then fixed in Regaud's fluid for 12 hours, kept for 6 hours in sterile water, and then passed through the alcohol series, employing not more than 2 hours for this last step. After the absolute alcohol, they were transferred to oil of cedar wood, where they were kept for at least 24 hours before being transferred to paraffin of a melting point not less than 58°C. Serial sections 4 microns in thickness were cut through the whole length of the fleas. The sections were stained by the method of Wolbach, Todd and Palfrey, changing the Giemsa solution once and staining in the incubator for 12 to 16 hours. Alkalinization of the water was omitted. Perfect series of sections were obtained from several fleas. After fixation in Regaud's fluid, the *Rickettsiae* take an intensive purplish red color with Giemsa solution, and very little care has to be taken in differentiating the sections in 96 per cent alcohol, as the *Rickettsiae* retain the stain even better than most of the cellular structures of the insect's body.

The picture observed in the fleas was alike in all the species examined. The cells of the stomach and the epithelial lining of the Malpighian tubules are the sites of multiplication of *Rickettsia prowazeki* in the fleas. (By "stomach" we understand that part of the gut which is situated between the proventriculus and a place just in

front of the union of the four Malpighian tubules with the intestine.) In the epithelial lining of the stomach, *Rickettsiae* settle first in the basal part, where the cells adhere to the membrana propia. From there, they spread to the rest of the cells, filling them in dense masses. Such heavily infected cells protrude into the lumen of the stomach as large, distally clubbed structures. In the fleas killed 16 days after feeding on infected rats, only a few cells could be found which were entirely free from infection, most of them showing a massive invasion by *Rickettsiae*. The cells of the small intestine remain *free* of *Rickettsiae*, with the exception of those which surround the opening of the Malpighian tubules into the intestine. In comparing sections of heavily infected lice with our sections of fleas, one is at once struck by the large numbers of free *Rickettsiae* in the lice's guts and the enormous swelling of the stomach cells in these insects. These free organisms originate from the greatly swollen and disintegrating epithelial cells. In our fleas, no such discharge of *Rickettsiae* into the lumen of the gut could be observed. Indeed in perfect sections, with no indication of damage to the cells done during the process of sectioning, only few organisms were seen free in the lumen of the stomach. It was astonishing, also, that in the hind gut, which receives the discharge of a stomach in which practically every cell was found to be infected, no *Rickettsiae*, or at the most a few only, could be found. Only in a specimen of *Xenopsylla cheopis* sectioned more than a month after feeding on infected rats were fairly numerous *Rickettsiae* seen in the hind gut near the opening of the Malpighian tubules. In one flea a few *Rickettsiae* were observed in the lumen of the proventriculus and in the distal part of the esophagus. The scarcity of *Rickettsiae* in the contents of the stomach of the fleas can only be explained by the presence of a peritrophic membrane. This membrane covers the cylindrical epithelium of the stomach as a well developed hyalin structure. It is the continuation of the chitinous membrane which lines the esophagus and the proventriculus (it may well be called chitinogenous membrane). This membrane reaches distally to a place immediately proximal to the union of the Malpighian tubules with the gut. Proximally, it increases slightly but steadily in thickness up to the corner where the proventriculus joins the stomach. Here the membrane assumes a decidedly chitinous aspect. Even here the cells beneath

the membrane may be heavily infected with *Rickettsiae*. There cannot be any doubt that this membrane prevents the discharge of organisms from the heavily infected cells into the lumen of the stomach. No organisms could ever be observed within this structure, suggesting a passage from the cells directly into the cavity of the stomach. How, then, did *Rickettsiae* gain entrance to the body of these cells, which are covered by a membrane apparently impermeable for particulate matter? We have already mentioned that the first signs of multiplication of them is noticed in the basal part of the cylindrical cells of the stomach, and not in the surface, which is covered by the peritrophic membrane. The same behavior of *Rickettsiae* is observed also near the union of the stomach with the esophagus, where this membrane already looks like chitin. *Rickettsiae* could not have entered the cells by penetrating the membrane here. As mentioned above, the peritrophic membrane reaches through the whole stomach to a place immediately in front of the union of the Malpighian tubules with the small gut. At this point, the heaviest infection of the cells of these tubules is noticed with regularity. From here, the infection spreads proximally into the stomach, each cell apparently infecting the next by contact beneath the peritrophic membrane. Distally, from the union of the gut with the Malpighian tubules, the epithelial cells of the gut remain free from *Rickettsiae*. Not all of the Malpighian tubules were found to be infected in each flea. When they were infected, however, the number of *Rickettsiae* within their cells decreased progressively from the place of their union with the gut, where the cells are greatly swollen and crowded with *Rickettsiae* to the extent of nearly blocking the lumen of the tubules up to their ends, where isolated groups only of organisms were observed in the protoplasm of the Malpighian cells. It is evident, therefore, that the number of *Rickettsiae* eventually excreted by the fleas' feces must depend more on the number of infected Malpighian tubules and on the intensity of their infection than on the degree and number of infected stomach cells, because the Malpighian cells are not covered by a membrane. Salivary glands, salivary ducts and the reproductive organs remain free from *Rickettsiae*.

A comparison of the sections of fleas killed between 12 and 16 days after feeding on infected rats with sections of fleas killed more than a

month after first feeding on infectious blood gave results which may prove of significance. Whereas in the 12 and 16 day fleas the infection of the cells with *Rickettsiae* was without exception extensive and evenly distributed over the whole stomach, in the latter the most distal part only showed heavy infection, and from this point the number of *Rickettsia*-infected cells decreased progressively up to the entrance into the esophagus. In addition to this, we observed a change in the configuration of the mucosa of the stomachs of the infected fleas. In many places the cylindrical epithelial lining was found to be replaced by masses of more or less concentrically arranged cells. Within these masses, mitotic cell division was repeatedly observed, but no signs of infection with *Rickettsiae* were seen. Undoubtedly these cell masses represent centres of rapid cell regeneration of the mucosa which had been partly destroyed by the infection. When one compares sections of normal fleas with sections of infected fleas, especially when the infection has been one of long standing, a considerable difference in the number of stomach cells is at once apparent in the two sets of fleas. Whereas in the normal stomach the cylindrical cells are densely packed, one pressing closely to the other, in the infected fleas the cells are more isolated, so that many have room enough to spread out more or less flatly on the membrana propria. Because of this, the lumen of the stomach in infected fleas is considerably wider.

Examination of Normal Fleas.—

From each lot of fleas which was to be used for our experiments, a number were reserved for microscopical examination, in order that we might become acquainted with the normal microbic flora.

Rickettsia prowazeki is easily recognized by the experienced by its morphology, and—more reliably—by its peculiar affinity for Giemsa's stain and for Castaneda's stain. When stained with Giemsa solution, the organisms stain red with a faint hue of purple, and with Castaneda's method they take a clear blue coloration. Ordinary bacteria do not stain blue by Castaneda's method, and with Giemsa solution they stain from blue to dark purple. This holds true when Giemsa stain is used for smears as well as for sections, provided that fixation is practiced in Regaud's fluid.

No organisms that took the coloration peculiar to *Rickettsia prowazeki* were observed in any of the normal fleas. *Rickettsia*-like microbes were observed in several fleas, especially in *Ctenocephalus*, but they were decidedly larger than

Rickettsia prowazeki and invariably took a dark purplish blue coloration with Giemsa stain; and when Castaneda's method was applied, they took the counterstain. Moreover, the examination of sections demonstrated that these organisms, nearly always rather scarce in number, were without exception extracellular in the lumen of the gut. A confusion with *Rickettsia prowazeki* was, therefore, easily excluded.

DISCUSSION

An astonishing fact is disclosed by the preceding experiments, namely that the Mexican typhus virus develops and multiplies with extraordinary regularity in all varieties of fleas observed on rats in Mexico City when these fleas are fed on infected white rats. The susceptibility of all observed varieties of fleas was such that not a single insect failed to contract an infection with *Rickettsia prowazeki*. Fleas are in this respect even more susceptible than *Pediculus humanus* and *Polyplax spinulosus*, since with lice a certain percentage always fails to contract the infection in feeding experiments. The type of the *Rickettsia* infection in fleas is, in principle, the same as in lice. In both cases there is a considerable multiplication of *Rickettsiae* in the epithelial cells of the intestinal canal. In fleas there is, in addition, a progressive invasion of the Malpighian tubules, organs which remain uninfected in lice. In the salivary glands and in the genital organs of fleas no *Rickettsiae* were seen.

In one important respect there is a fundamental difference between the course of *Rickettsia* infection as it occurs in lice and in fleas, respectively. While the organisms continue to multiply both in *Pediculus* and *Polyplax*, leading to death of the insect within a short time, in fleas the intestinal infection is apparently arrested, and the insects are capable of regenerating the lining epithelium. This explains the fact that, in experiments with *Xenopsylla cheopis*, the fleas remained alive for over a month after infection. Whether all fleas are capable of overcoming the intestinal infection we cannot say at the present time, since this cannot be determined until extensive investigations have been carried out upon laboratory strains of fleas of known ages. In one flea of the *Leptopsylla musculi* variety, we observed an extensive secondary invasion of all organs with a large bacillus. A constant occurrence in all varieties of fleas was the extensive multiplication of *Rickettsiae* in the Malpighian tubules, particularly at the proximal

ends. Such multiplication was proportionate to the length of time after infection that the flea was killed, an observation which reminds one of the behavior of *Dermacentroxenus rickettsi* in *Dermacentor andersoni*. It is not impossible that this indicates that fleas are more closely adapted to the typhus virus than are lice, which regularly succumb to the infection within a relatively short period. Because of such louse mortality, one of the writers (Mooser (4)) suggested as early as 1929 that the louse was probably not the original vector of typhus. It may have appeared on the scene relatively late in the evolution of the disease, and not yet have had sufficient time, in the biological sense, to convert *Rickettsiae* by adaptation into harmless saprophytes. Our results in this paper, as well as in the preceding ones cited at the beginning, invite the conclusion that typhus, which until recently was regarded as a specific disease of man, was originally and exclusively a disease of rodents, from which—in the course of time—it has been transmitted to man by fleas, or possibly by some variety of tick. A similar evolutionary cycle has, according to Nicolle and Anderson (5), occurred with the relapsing fevers, which, originally passing from rodent to rodent by vectors of the *Ornithodorus* species, later adapted themselves to the cycle of man to louse and louse to man. The longevity of fleas marks them as potentially more dangerous factors than lice. From a practical point of view, however, they are usually much less dangerous than the latter. In regions where endemic typhus occurs, it is rare to find two cases of the disease in the same household. Maxcy has called attention to this repeatedly, and the same observation has been made again and again in Mexico during the warm months.

In view of the extraordinary ease with which fleas can be infected from rats, it is astonishing that these insects are relatively so harmless. A partial explanation may be found in the following considerations.

From the observations recorded above it appears to be clear that in fleas, as well as in lice, the virus leaves the body of the insect only with the feces. The salivary glands remain uninfected, and in the esophagus we have seen the virus only in the posterior region. We have also observed that the lumen of the flea's intestine contains a small number of *Rickettsiae* only, as compared with the contents of the louse intestine. Apparently the peritrophic membrane of the flea

prevents the discharge of any considerable amounts of *Rickettsiae* into the lumen, in consequence of which, the flea feces are likely to be far less infectious than those of the lice. Again, we may assume that isolated flea bites are much less irritating to most human beings than are those of body lice, and lead to far less scratching and consequent inoculation. Again, the body of most fleas is much more strongly chitinized than that of lice, and in consequence the danger of crushing a flea against the skin is much less than is the case with lice.

The likelihood of transmission of the virus from rat to man in endemic regions by fleas is further diminished by the disinclination of the rat fleas *Xenopsylla* and *Ceratophyllus* for the human host. Although flea-infested rats have been handled by us almost every day in the course of this work, only once has a rat flea been found on an investigator, and on this occasion he had opened a glass container within which a rat had died during the night. Since typhus—in contradistinction to plague—does not kill rats under ordinary conditions, there is little occasion for the fleas to leave the normal host.

The variety of flea which is significant in the transmission of the virus from rat to man probably depends upon climatic conditions. In tropical and subtropical regions, the *Xenopsylla* varieties are probably the important ones, while in more northerly climates the significant fleas are probably *Ceratophyllus fasciatus*. *Ctenocephalus canis* and *Ctenocephalus felis* are probably of little importance, owing to their scarcity in rats. *Leptopsylla musculi* does not appear to infest man at all. In view of the ease with which we have been able to infect with the typhus virus all varieties of fleas with which we have worked, it is more than likely that *Pulex irritans* is no exception.[3] It is obvious, however, that this variety of flea cannot be of epidemiological importance; otherwise, it would be hard to understand why secondary infections are so rarely noticed in well managed hospitals and private houses, which cannot even with the greatest care be kept entirely free of fleas. Although, therefore, we can no longer maintain the rule cited by Otto and Munter, "without lice, no typhus," it is nevertheless still true that without lice there is no epidemic typhus.

[3] Since this paper was written *Pulex irritans* has actually been infected by allowing it to feed upon human typhus cases and upon infected guinea pigs.

SUMMARY AND CONCLUSIONS

The virus of Mexican typhus has been shown to multiply abundantly in the following species of fleas: *Xenopsylla cheopis, Ceratophyllus fasciatus, Leptopsylla musculi, Ctenocephalus canis, Ctenocephalus felis.*

In all fleas, *Rickettsia prowazeki* was demonstrated within the epithelial cells of the stomach and within the cells of the Malpighian tubules. Whereas in infected lice enormous numbers of these organisms are discharged from the disintegrating cells into the intestinal content, only few *Rickettsiae* are found in the lumen of the fleas' intestines. They are held back by the peritrophic membrane, which covers the mucosa of the entire stomach. *Rickettsiae* seem to enter the lumen of the gut almost exclusively by the route of the Malpighian tubules. Observations were made which seem to indicate that the fleas recover from the infection and that they are able to regenerate the partly destroyed intestinal mucosa. An explanation is given for the relative harmlessness of fleas as vectors of typhus.

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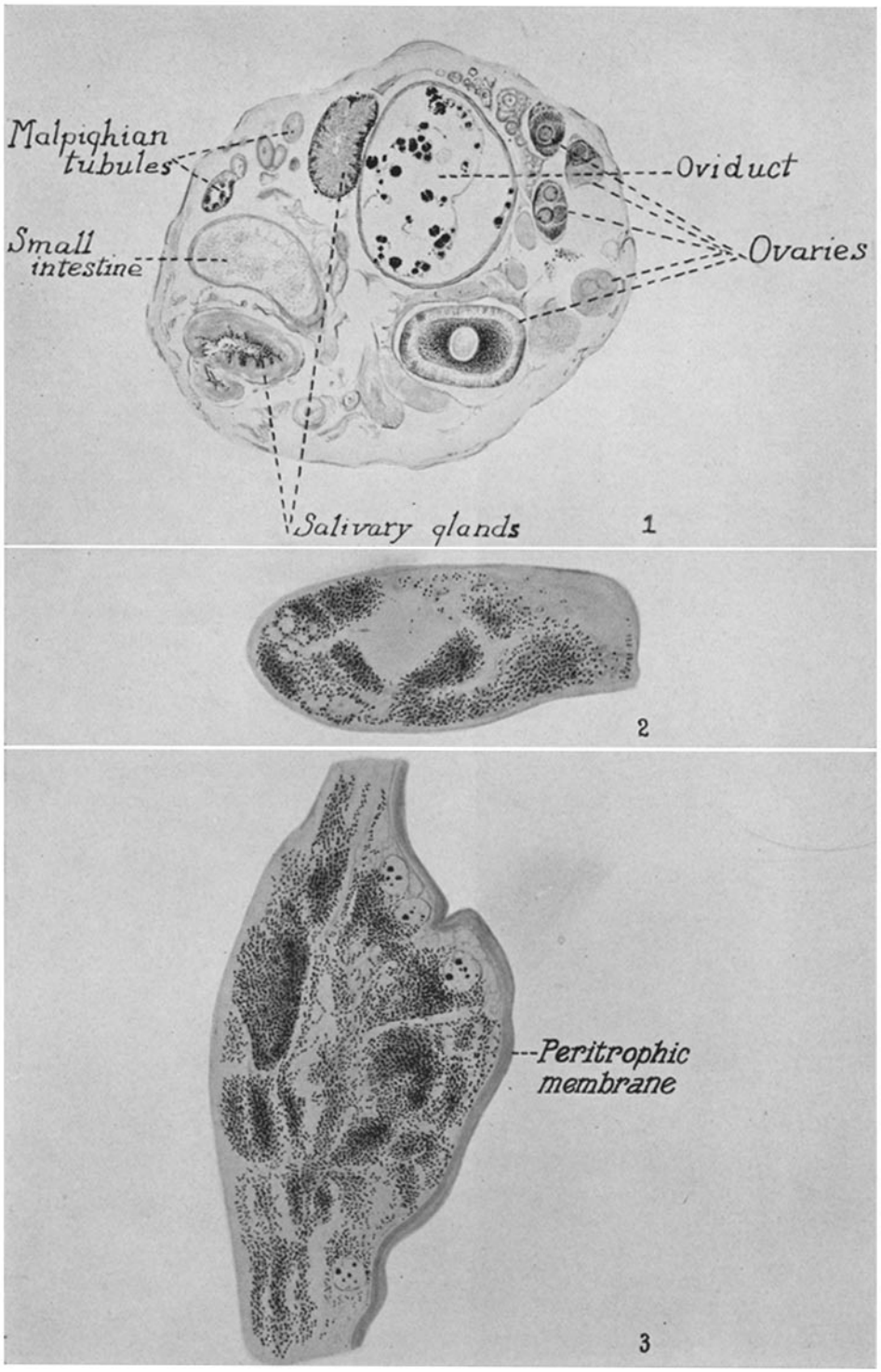
EXPLANATION OF PLATE 19

The fleas were fixed in Regaud's fluid and stained with Giemsa's solution.

FIG. 1. Cross-section through posterior abdominal end of *Xenopsylla cheopis*. One Malpighian tubule is infected with *Rickettsia prowazeki*. ×80.

FIG. 2. Heavily infected Malpighian tubule of the same flea at high power magnification. ×900.

FIG. 3. Same flea. Heavily infected cells of the stomach. Note the absence of organisms from the peritrophic membrane. ×900.



(Mooser and Castaneda: Virus of Mexican typhus fever in fleas)