numbers only after the 4th or 5th day. After the 9th day they became mostly extracellular and began to disappear.[15]

*Summary.* A typhus virus was recovered from the blood of a patient with typhus fever and was found to conform to the murine type in its behavior in the guinea pig, rat and mouse.

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## II. Typhus Virus Isolated from Rats and Rat-fleas in Typhus Houses.*

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In our previous communication,[1] a virus of the murine type isolated locally from the blood of a patient with typhus fever was described. Recently Wu and Zia[2] recovered a typhus fever virus from the pooled brain emulsion of 3 out of 139 rats trapped from different households in this city, thus verifying the assumption that the rat serves as a reservoir host for the disease in this locality. It appeared to us, that to complete the evidence of a rat-flea-man cycle of transmission, it would be necessary to recover typhus fever virus from rats and rat-fleas caught in the houses of typhus fever patients who were suspected, on clinical and epidemiological grounds, to be suffering from the murine variety of the disease. This communication reports the observations on 2 rat-strains and 3 flea-strains of typhus fever virus obtained in this manner.

In October, 1938, four typhus fever patients from widely scattered parts of the city were seen in this hospital. They were cleanly in their habits and gave no history of contact with the body louse. Their disease ran a very mild clinical course. In the homes of 2 of the patients whose Weil-Felix reaction titer exceeded 1:2560, there was evidence of heavy infestation with rats. These were trapped for study. One rat† was caught on October 19th in the house of the

* The authors are indebted to Dr. K. H. Pang for growing one of the rat-strains on Maitland's and Zinsser's tissue media.

1 Liu, W. T., and Chung, H. L., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 350.

2 Wu, C. J., and Zia, S. H., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 163.

† Six other rats were caught in the same house but were not brought to us for study.

first patient‡ who was admitted on October 6th on the 9th day of the disease. It was killed with chloroform, and 3 fleas were collected from the body. The fleas (after being washed in 70% alcohol and rinsed in distilled water) and the brain of the rat, which was aseptically removed, were ground in sterile physiological saline and separately inoculated into the peritoneal cavity of 2 guinea pigs. After an incubation period of 11 days the guinea pig inoculated with the emulsion of rat brain developed fever (above 40°C) and scrotal swelling. It was killed on the second day of fever, and typical Rickettsia bodies were seen in smears prepared from scrapings of the tunica. From the tunica vaginalis of a guinea pig of the second generation Rickettsiae were also grown on Maitland's and Zinsser's tissue media.[3] The virus has now been passed through 5 generations of male guinea pigs. The infection is invariably manifested by fever after an incubation period of from 4 to 6 days and by marked scrotal swelling. A large number of Rickettsia bodies were found in the tunica vaginalis of all the animals examined. Four albino rats were inoculated with the virus. All developed a febrile reaction (38.2°-39.6°C), lasting from 3 to 6 days, after an incubation period of 3 or 4 days. The sera of 2 of these rats killed 10 days after infection, *i. e.*, 2 days after the subsidence of fever, agglutinated Proteus OX19 at a dilution of 1:320. The guinea pig inoculated with the 3 fleas also developed fever after an incubation period of 7 days. Scrotal swelling in this guinea pig was slight, but in animals of subsequent generations it became very pronounced. This flea virus has now been passed through 6 generations of guinea pigs.

In the house of the second patient§ who was admitted on October 18th on the fourth day of the disease, one rat was caught on November 5th. This rat harbored 5 fleas. The brain emulsion of the rat was inoculated intraperitoneally into a guinea pig which developed fever after an incubation period of 6 days. Although there was no scrotal swelling, the tunica vaginalis was congested and hemorrhagic. Rickettsia bodies were demonstrated in smears from this tissue. Four of the 5 fleas, after being washed in 70% alcohol

‡ When we heard of this patient, the temperature was already returning to normal. Therefore, no attempts were made to isolate the virus from the blood.

[3] Zinsser, H., Wei, H., and Fitzpatrick, F., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 285.

§ Five cc of blood were taken from this patient on the 7th day of the disease and inoculated intraperitoneally into a guinea pig which died 4 days later. Another guinea pig inoculated in the same manner with 5 cc of blood taken on the 12th day of illness did not develop the disease.

and rinsed in distilled water, were suspended in sterile physiological saline and inoculated into another guinea pig. After an incubation period of 9 days, it developed fever and scrotal swelling. Rickettsia bodies were demonstrated in the tunica vaginalis. The 5th flea was identified as *Xenopsylla cheopsis*. We have now carried both the rat-strain and the flea-strain through 6 generations of guinea pigs which have thus far retained a characteristic Neill-Mooser reaction. On November 12th another 2 rats, which harbored 2 and 3 fleas respectively, were caught in the same house. A guinea pig inoculated with the 5 fleas developed fever but without scrotal swelling after an incubation period of 5 days. On subsequent passages, however, slight scrotal swelling appeared in the inoculated animals, and typical Rickettsia bodies were found in the tunica vaginalis. This virus has been successfully passed through 6 generations of guinea pigs. Each of 2 guinea pigs was inoculated with the whole brain of one of the 2 rats, and observed for 3 weeks. They failed to show any fever. However, each of these 2 guinea pigs, when later inoculated intraperitoneally with one-third of a mixed emulsion of the brain and the tunica vaginalis of a guinea pig infected with the flea virus obtained from the rat trapped in the house of the first patient, developed fever for one day but showed no scrotal swelling. It seems obvious that they were partially immune as a result of the previous inapparent infection. The fact that the brains of these 2 rats were less heavily infected with typhus virus than the fleas recovered from them might mean that the latter had only recently migrated to these rats from a typhus-infected rat, presumably following its death, so that there was not enough time for the new hosts to develop a heavy infection. Another possibility is that, as a medium for growth and multiplication of the Rickettsiae in question, the rat-flea was actually better than the rat brain. A fourth rat caught on November 27th died shortly after it was brought to the laboratory. Four fleas were collected from this animal. Two of the fleas were crushed, smeared on 2 glass slides and stained with Zinsser's basic fuchsin solution. An enormous number of morphologically typical Rickettsia bodies, chiefly extracellular, were found in one of the smears. The other 2 fleas are being fixed for section.

*Summary.* Five strains of typhus virus were isolated from rats and their fleas caught in the houses of 2 typhus fever patients who were suspected, on clinical and epidemiological grounds, to be suffering from the murine variety of the disease.