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The Susceptibility of the Rodents, *Gerbillus pyramidum* and *Gerbillus gerbillus*, to Experimental Tsutsugamushi Infection (Scrub Typhus)*

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The purpose of this report is to present experimental evidence which indicates the susceptibility of 2 rodents, *Gerbillus pyramidum* and *Gerbillus gerbillus*, to tsutsugamushi infection. The susceptibility of these animals to epidemic and murine typhus infection has been reported recently from this laboratory,[1] and the description, identification, source, and supply of the rodents used in these tests are the same.

Origin of tsutsugamushi strains used. Four strains of *R. orientalis*† were available for trial in these rodents. They are designated Imphal (= Imphal 8), Imphal 13, Calcutta, and Ceylon with the names indicating the locality in which they were isolated. Their derivations are as follows:

Imphal (= Imphal 8). Lt. Col. M. H. P. Sayers, R.A.M.C., injected 3 guinea pigs with a suspension of pooled blood clots which had been obtained from 5 patients with tsutsugamushi-like disease (on their 8th, 11th, 12th, 13th, and 20th days of disease, respectively). Rabbits were inoculated intra-ocularly with peritoneal washings from 2 of the 3 guinea pigs. The rabbits reacted typically and the strain was maintained in other rabbits for 7 generations, and then transferred to white mice. On January 7, 1944, 3 of the 3rd generation rabbits inoculated with this strain were received in Cairo, and on Feb. 19, 1944, infected mice of the 5th mouse passage were received. The latter line of this strain is named "Imphal 8."

Imphal 13. Capt. Parry, R.A.M.C., isolated this strain from blood taken from patients on the 4th and 7th days of disease by inoculating guinea pigs and then transferring to white mice and rabbits. Fourth generation mice of this strain were also received in February, 1944.

Calcutta. Major M. T. Parker, R.A.M.C., obtained this strain from the blood of a patient in his 12th day of disease. Washed blood clot material was inoculated intra-ocularly into a rabbit which showed iritis on the 11th day. One of the third passage rabbits was received in Cairo on January 7, 1944.

Ceylon. Major R. B. Lucas, R.A.M.C., isolated this strain and sent it to Dr. S. R. Savoor for purposes of identification. Dr. Savoor identified it as a strain of *R. orientalis* and sent infected mice of the 3rd generation to Cairo in June, 1944.

Passages of these strains in the Cairo laboratory. All 4 of these strains have been tested in gerbilles, and the passages carried out in the Cairo laboratory to the time of this writing are summarized as follows:

Imphal (= Imphal 8). The rabbit line of this strain was carried through 7 additional generations, making a total of 10 rabbit passages for it. Intra-ocular passages were made, and of the 26 animals used, half gave the characteristic response to the infection.[4] Passages to gerbilles were attempted from each rabbit generation except the 10th, by intraperitoneal inoculation of 0.1 cc of anterior chamber fluid from an infected rabbit's eye showing the characteristic involvement. The gerbilles infected in this manner usually died

* The terms "tsutsugamushi" and "scrub typhus" are considered synonyms for the same disease after the conclusions reached by Lewthwaite and Savoor.[2] The former term is used here because of its priority in the nomenclature of the disease.

[1] Snyder, J. C., Zarafonetis, C. J. D., and Liu, W. T., to be published.

† The choice of the name, *R. orientalis*, is based upon the recent re-evaluation of rickettsial terminology by Philip.[3]

[2] Lewthwaite, R., and Savoor, S. R., *Brit. J. Exp. Path.*, 1936, **17**, 448.

[3] Philip, C. B., *Am. J. Hyg.*, 1943, **37**, 301.

[4] Lewthwaite, R., and Savoor, S. R., *Brit. J. Exp. Path.*, 1936, **17**, 1.

in 14 to 19 days, though in one instance death occurred on the 5th day. Of 12 such attempts, 11 were successful in establishing the infection. The one failure occurred in the case of a rabbit from which material taken 2 days previously had successfully infected a gerbille. Following its establishment from infected rabbits into gerbilles, the strain was passed through 15 generations of infection in gerbilles of the species *G. pyramidum*.

The *Imphal 8* branch of this strain was carried through another mouse passage, 3 rabbit passages, and 2 series of 3 gerbille passages (*G. pyramidum*) before storing it frozen at −70°C. One of the 2 series of gerbille passages was maintained by using peritoneal washings for inocula, and the other by using 10% brain suspension.

The *Imphal 13* strain was carried through another mouse passage, as well as 6 passages in *G. gerbillus* and 2 in *G. pyramidum* before being stored frozen at −70°C.

The *Calcutta* strain was maintained for 4 additional passages in rabbits and then established in the species *G. pyramidum* in which it has been carried through 14 successive passages.

The *Ceylon* strain was established directly in *G. pyramidum* from the original infected mouse received in Cairo. Peritoneal washings from the mouse were used to start one series, and a pooled suspension (10%) of its brain and spleen to begin a second series. Three generations of infection were carried out by transferring brain suspensions, and 8 passages have been made with peritoneal washings.

Inocula used and routes of infectivity. As indicated above, 10% suspensions of infectious brain and/or spleen material have been used for passage with success, but, for convenience, peritoneal washings were usually used. About 3-5 cc of peritoneal washings were made from a single gerbille, sacrificed when moribund or freshly dead from the infection. One cubic centimeter of peritoneal washings was inoculated intraperitoneally into each of the next passage gerbilles. This usually killed all the inoculated animals in 9 to 12 days, though deaths occurred as early as 5 days and as late as 36 days after inoculation in isolated instances. Following intraperitoneal inoculation with brain or spleen suspension (10%), the average time of death was delayed 2 to 3 days longer than when peritoneal washings were used.

Most inoculations were made intraperitoneally, though subcutaneous and intravenous routes were tried in a few instances and found to be successful.

Cultures of the various inocula on plain and blood agar plates and in broth media failed to give growth.

The disease in gerbilles. The disease manifestations in these two rodents are essentially the same. Following inoculation, the course is usually not remarkable during the first 8 to 10 days. Towards the latter part of this interval, the infected animal eats less well and there may be ruffing of its fur. Then there appear signs of progressively increasing dyspnea, weakness, and ataxia. Its activity is markedly diminished and it eats poorly or not at all. If the animal is forced to run about in its cage, marked dyspnea and tachypnea result. The difficulty in respiration is perhaps the most prominent feature of the disease. The signs of illness may become gradually accentuated over 2 or 3 days, terminating in the death of the animal, but oftentimes the fatal course is completed in 24 hours or less after the first evidence of illness is noted.

Almost 300 gerbilles have been used in these studies, and all which showed manifestations of illness died. This 100% fatality is due to the virulence of the disease for these rodents as well as to the overwhelming infective dose contained in the inocula used. Titrations of the infectious material have been done twice with the Ceylon strain. In one instance, peritoneal washings in dilutions through 10-3 resulted in the death of all the test animals within 22 days after intraperitoneal inoculation. A second titration using pooled brain and spleen material made up in peritoneal washings was fatal through 10-2, and killed 3 of 4 animals inoculated with 10-3. Thus, the inocula used for the routine passages carried many times the minimal lethal dose.

Autopsy findings in gerbilles. Autopsy almost invariably revealed a sticky peritoneal exudate often up to 1 cc in amount. The liver and especially the spleen were routinely found

to be enlarged and covered with a white fibrinous exudate. The other abdominal organs were grossly normal. Collapsed lungs due to a relatively large amount of pleural effusion were generally encountered. There were no other striking findings.

Findings in stained smears of the exudates. Smears of the peritoneal and pleural exudates were usually stained with a simple 1% aqueous methylene blue solution. This was adopted after many attempts with Macchiavello's method had uniformly given the blue stain to the rickettsiæ instead of the basic fuchsin as had been expected from experience with other rickettsial organisms. Giemsa stain was satisfactory, but had the disadvantage of being slower.

Using these staining methods, the peritoneal exudate revealed numerous cellular elements including serosal cells, monocytes, and some lymphocytes and polymorphonuclear leukocytes. The serosal cells frequently exhibited a highly vacuolated cytoplasm. The vacuoles were usually small and numerous, and sometimes indented the margin of the nucleus to give it a punched-out appearance. Within the cytoplasm of these cells could be seen varying numbers of minute pleomorphic rickettsiæ. These appeared at times as minute rods, and at other times as cocci or coccobacilli, occurring singly or in pairs. Less frequently, the organisms were found in the cytoplasm of the monocytes, but the lymphocytes and polymorphonuclear leukocytes were rarely observed to contain them.

The pleural exudate contained similar cell types, but fewer of them. Rickettsiæ were present in some of these cells as well.

It should be noted that the manifestations of all these strains were essentially alike in the experimental animals used. There were no differences in the incubation period, course, average time of death, autopsy findings, or in the appearance of the rickettsiæ in the stained smears of exudates.

Discussion. One of the major difficulties of experimental work with tsutsugamushi disease is the establishment of the identity of the rickettsiæ. The circumstantial evidence pertaining to the 4 strains used in these studies strongly indicates they are *R. orientalis.* All the strains were isolated by experienced workers from patients suffering from an illness clinically diagnosed as tsutsugamushi disease. Their behavior in experimental animals and the microscopic appearance and staining properties of these intracytoplasmic organisms are compatible with the descriptions given for other strains.[4] In addition they fail to grow in ordinary culture media. Cross-immunity tests in gerbilles have not been possible due to the lack of survivors from infection with these strains. The complement-fixation test for tsutsugamushi disease recently described by Bengston[5] should prove to be an important ancillary aid in establishing the relationship of these and other strains, but as yet the antigen is not readily available. For the present, however, it appears to be fairly safe from the evidence cited to conclude that the strains tested in these studies are *R. orientalis.*

Certain potential disadvantages in the use of trapped gerbilles for experimental rickettsial work have been mentioned in a previous communication.[1] These include the occasional occurrence in the blood of both species of extracellular clusters of small rod-like forms which stain red with Macchiavello's method. With this staining method, the latter organisms offer no problem in differentiation from *R. orientalis* which fail to stain with basic fuchsin, but instead hold the methylene-blue counterstain.

Spirillum minus infection, which is occasionally encountered in these rodents, may give rise to confusion, particularly in *B. proteus* OX-K agglutination tests. Savoor and Lewthwaite[5] have described significantly high OX-K agglutination titers in sera from humans and rabbits infected with *Sp. minus.* These workers also cite Felix in stating that wild rodents may often have high OX-19 and OX-K agglutination titers. However, Weil-Felix tests on sera from normal *G. gerbillus* and *G. pyramidum* have failed to give significant OX-19 or OX-K titers in the animals so far tested. The lack of survivors to infection with these strains of *R. orientalis,* however,

[5] Bengston, I. A., Pub. H. Rep., U. S. Pub. Health Service, assigned publication date Feb. 11, 1944.

has prevented the testing of sera from infected gerbilles.

The possibility of naturally occurring infections of these species with *R. orientalis* has been discounted since this disease has never been known to occur in Egypt.

As already indicated, these rodents have been shown to be susceptible to epidemic and murine typhus infection. The finding described herein, namely, that they are also susceptible to tsutsugamushi infection enhances their value as experimental animals. Since infection results in death of these rodents, they are particularly suitable for titrations of tsutsugamushi inocula, thus making possible their use in vaccine assay, in neutralization tests, and in the trial of therapeutic drugs. They are satisfactory animals for strain maintenance and yield a rich source of infectious material. Their value in strain isolation cannot be estimated as no attempts have yet been made with them. They can, of course, receive a greater amount of inoculum than may be placed in the anterior chamber of a rabbit's eye. Furthermore, it was found that a much higher percentage of successful transfers resulted from rabbit-to-gerbille than from rabbit-to-rabbit passages. This made the gerbilles much more useful, both as for certainty of infection and as a richer source of infectious material for further passage.

It is not intended to imply, however, that these animals possess any great superiority over laboratory-bred white mice for the study of tsutsugamushi disease. Rather, *G. pyramidum* and *G. gerbillus* were discovered to be suitable substitute animals in the absence of a supply of white mice.

Summary. Experimental evidence indicates that the two rodents, *Gerbillus gerbillus* and *Gerbillus pyramidum* are readily susceptible to large doses of scrub typhus rickettsiæ. One hundred percent of the animals succumbed to the disease when inoculated by the intraperitoneal, subcutaneous, or intravenous route. Smears of the peritoneal and pleural exudates showed varying numbers of minute phomorphic rickettsiæ within the cytoplasm of the cells. It was found that a much higher percentage of successful transfers resulted from rabbit-to-gerbille than from rabbit-to-rabbit passages. For this reason gerbilles were found to be much more useful both as for certainty of infection and as a richer source of infectious material for further passage than rabbits. They are suitable substitute animals in the absence of a supply of white mice.

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Evidence that Menstrual "Toxin" and Canine "Necrosin" are Identical

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The similarities between the toxic material in menstrual discharge[1] and the more recently described injury factor, termed "necrosin," of pleural exudates[2] suggested to us that these substances might be identical.[3] It is merely for the sake of brevity that either is referred to as a single substance, since neither has been

[1] Smith, O. W., and Smith, G. V., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 100.

[2] Menkin, V., *Science*, 1943, **97**, 165.

[3] Smith, O. W., and Smith, G. V., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 285.