

The Susceptibility of the Rodents, *Gerbillus gerbillus* and *Gerbillus pyramidum*, to Experimental Typhus Infection

J. C. SNYDER, C. J. D. ZARAFONETIS, AND WEI T'UNG LIU.*

From the Cairo Unit of the United States of America Typhus Commission.

It is the purpose of this report to describe experiments which indicate the susceptibility of 2 rodents, *Gerbillus gerbillus* Olivier and *Gerbillus pyramidum* Geoffroy, to experimental typhus infection.[†] The rodents were supplied to the Cairo Unit of the United States of America Typhus Commission¹ through the courtesy of the Giza Ophthalmological Laboratory. They were trapped on the desert near Cairo, Egypt, where they occur in large numbers. Dr. Kamal Wassef, of the Department of Zoology, College of Science, Cairo, identified the two species.² The common Egyptian name of the species *G. gerbillus* is "far baiyoudeh"; of *G. pyramidum*, "far dimsi."

Preliminary tests with suspensions of infected lice taken from patients suffering from typhus fever showed that large numbers of rickettsiæ produced a fatal infection in gerbilles of both species by the intranasal and the intraperitoneal routes. The animals died 3 to 8 days after inoculation. Typical intracellular rickettsiæ were found in smears at autopsy. Further studies were made with the Breinl strain of classical louse-borne typhus in suspensions of infected yolk sacs.³ It was found that the animals succumbed more regularly to smaller doses of rickettsiæ when the intravenous rather than the intraperitoneal route was used. The tail veins of both species are suitable for inoculation. When the gerbilles

died several days after intravenous inoculation, smears of heart's blood occasionally contained a few large mononuclear cells with their cytoplasm entirely filled with densely packed rickettsiæ.

The smaller of the two species, *G. gerbillus*, was found to be more susceptible to fatal infection than the larger species, *G. pyramidum*, even when animals in the same weight range were selected for comparison (Table I).

The greater susceptibility of *G. gerbillus*, indicated by the experiment shown in Table I, was confirmed in another test in which the infective material was incubated for an hour at 37°C with normal rabbit serum for one group of gerbilles and with typhus immune rabbit serum for another. All of the gerbilles of both species survived the inoculum containing rickettsiæ and immune serum. Only 2 of a group of 12 gerbilles of the species *G. gerbillus* survived the inoculum containing rickettsiæ and normal serum, whereas, 6 of 7 *G. pyramidum* survived the same inoculum.

The specific protective effect of typhus

TABLE I.
Comparison of the Susceptibility of *Gerbillus gerbillus* and *Gerbillus pyramidum* to Experimental Typhus Infection by the Intraperitoneal and Intravenous Routes (Breinl strain of louse-borne typhus).*

Route of Inoculation	Concentration of infected yolk sac, %	Ratio of No. of survivors to total No. inoculated in each group	
		<i>G. gerbillus</i>	<i>G. pyramidum</i>
I.P.	20	1/4	3/4
	5	2/4	4/4
I.V.	20	0/4	4/4
	5	1/4	3/4
	1 1/4	4/4	4/4

*All rats were in the 20-25 g weight range at the time the test was started. Each rat received 0.25 cc of the stated concentration of yolk sac suspension in saline, via tail vein. No anesthesia was used. The experiment was arbitrarily terminated at the end of 7 days.

* Technical Expert of the National Health Administration of China.

† Gear and Davis have described the susceptibility of gerbilles of the closely related genus *Tatera* to experimental epidemic and murine typhus infection. See: Gear, J., and Davis, D. H. S., *Trans. Roy. Soc. Trop. Med. and Hyg.*, 1942, **36**, 1.

¹ Bayne-Jones, S., *Army Med. Bull.*, 1943, **68**, 4.

² Anderson, J., *Zoology of Egypt: Mammalia*, London, 1902, pp. 252, 255.

³ Cox, H. R., *U. S. Pub. Health Rep.*, 1938, **53**, 2241.

immune serum was fully confirmed in several experiments with the species *G. gerbillus*. The sera used in the tests were taken from patients suffering from louse-borne typhus fever at various stages in their illness and in convalescence. Protective antibodies were demonstrated in the sera of patients toward the end of the febrile period and in one case as long as 3 years after the illness. Tests were not made at longer intervals. Protective antibodies were likewise demonstrated in some, but not all of the sera from persons who had received varying amounts of Breinl strain typhus vaccine (Cox type).

Both species were found to succumb to the Wilmington murine strain of typhus as well as to louse-borne typhus. Cells packed with rickettsiæ were found to be much more numerous in smears of peritoneal exudates made when the former strain was used.

The two infections produced a complete cross-immunity to one another. Gerbilles of both species, when tested 2 weeks to 2 months after a sub-lethal dose of either murine or louse-borne typhus, were immune to a dose of the other strain which killed all of the control non-immune animals in 3 to 7 days.

Relatively small amounts of killed rickettsial vaccine (louse-borne strain) administered subcutaneously in 2 doses one week apart, conferred immunity in gerbilles of the species *G. gerbillus* to at least one "certainly fatal dose" of a similar strain.

Specific complement fixing antibodies⁴ were demonstrated in the blood of both species after inoculation with sub-lethal doses of louse-borne strains. The same result was obtained with the Wilmington murine strain. In the few tests which have been run thus far, there was very slight cross fixation between sera from gerbilles which had received either murine or louse-borne typhus. In some instances the complement fixing antibodies were observed several months after the infecting dose of typhus. The longest interval tested in these studies was 5 months and a

positive result was obtained in that instance. Neither species was found to have developed positive Weil-Felix tests (proteus OX-19) as a result of infection with either louse-borne or murine typhus in the few tests completed at this time.

Since there has been a considerable amount of typhus fever in the region in which these rodents abound, the question has been entertained as to the possible incidence of typhus as a natural infection of the species. Inoculation of the brains of both species into guinea pigs and eggs in several trials has not disclosed evidence of typhus infection in either species, although an agent pathogenic for guinea pigs, possibly *Spirillum minus*, was encountered in the species *G. pyramidum*. The guinea pigs which had a febrile response after inoculation of gerbille brains did not develop complement-fixing antibodies to typhus, nor were they immune to challenge with known typhus strains. The sera of both species have been examined shortly after arrival in the laboratory and as yet no evidence has been obtained that the animals had previously suffered from typhus infection. When large doses of rickettsiæ were given, the control animals regularly died, and the gerbilles which had recovered from experimentally induced typhus had no evidence of sickness. This phenomenon, observed repeatedly, suggests that the two species in question either are not infected with typhus in nature, or if so, only rarely. The length of survival of rickettsiæ in the organs of gerbilles which have survived experimental typhus infection has not yet been determined.

The species *G. gerbillus*, in one or two instances, seemed to be slightly susceptible to the toxic effect of rickettsiæ.^{5,6} Usually, however, the yolk sac suspensions employed in these studies did not contain a sufficiently large number of rickettsiæ to have had a very definite toxic effect.

⁴ Plotz, H., Wertman, K., and Bennett, B. L., from the Division of Virus and Rickettsial Diseases, Army Medical School, Army Medical Center, Washington, D.C., 1943, to be published.

⁵ Gildemeister, E., and Haagen, E., *Deut. Med. Wchschr.*, **66**, 878.

⁶ Bengston, I. A., Topping, N. H., and Henderson, R. G., communication dated 11 July, 1942, Division of Infectious Diseases, National Institute of Health.

TABLE II.
Comparison of the Susceptibility of *Sigmodon hispidus hispidus** and *Gerbillus gerbillus*† to Experimental Typhus Infection.

Concentration of infected yolk sac (Breinl strain of louse-borne typhus) %	Ratio of No. of survivors to total No. inoculated in each group	
	<i>S. hispidus</i>	<i>G. gerbillus</i>
10	0/3	3/4
2.5	1/3	3/4
0.63	2/3	4/4
0.16	3/3	4/4

* The cotton rats were inoculated intracardially.

† The gerbilles were inoculated in the tail vein. Both species were inoculated with amounts which corresponded to their sizes: 0.1 cc of the stated yolk sac concentration for each 20 g of body weight.

In both species, a small rod-like form was frequently observed in the blood. It usually occurred in oval clusters approximately the size of a red blood cell. It stained red with Machiavello's stain. It was not found inside cells. Attempts to cultivate it in nutrient broth and in the yolk sac membrane of developing chick embryos were unsuccessful. However, its appearance was somewhat similar to that of typhus rickettsiæ, and the presence of the latter was only considered certain when smears showed the typical intracellular forms.

One experiment was conducted to compare the susceptibilities of *G. gerbillus* and the eastern cotton rat, *Sigmodon hispidus hispidus*.⁷ The results shown in Table II indicate that *G. gerbillus* was slightly less susceptible to fatal infection than *Sigmodon hispidus hispidus*.

The species *G. gerbillus* proved to be useful for various purposes, particularly the demon-

stration of cross immunity between standard laboratory strains and newly isolated strains, the survival or death end point being superior to the interpretation of fever curves in guinea pigs.

Summary. *G. gerbillus* and *G. pyramidum*, two rodents which abound in Egypt, succumb to large doses of either murine or louse-borne typhus rickettsiæ by the intranasal, intravenous, or intraperitoneal routes. Protective antibodies in the sera of persons who have recovered from typhus fever and in the sera of persons who have been vaccinated against typhus fever have been demonstrated in tests with the species *G. gerbillus*. Both species were useful in various types of experiments in which survival or death was the end point. Neither species has yet been found to be infected with typhus under natural conditions. Specific complement-fixing antibodies were demonstrated in the sera of both species after inoculation with murine or louse-borne typhus. *G. gerbillus* was found to be slightly less susceptible to fatal infection than the Eastern cotton rat, *Sigmodon hispidus hispidus*.

The authors wish to thank the Giza Ophthalmologic Laboratory for supplying the gerbilles, Dr. Kamal Wassef for identifying the two species, the Egyptian Ministry of Agriculture for making available the fertile hens' eggs used in preparation of the infective material in the study, the Director of the Cairo Fever Hospital, Dr. Demerdash Bey, for facilitating the establishment of the United States of America Typhus Commission laboratory in his institution, and Dr. Andrewes of the National Institute of Health, Hampstead, England, for sending the cotton rats.

Sergeant Dworkowitz and Corporals Stearman, Hogan, and Cassell took part in this study and their assistance is gratefully acknowledged.

⁷ Snyder, J. C., and Anderson, C. R., *Science*, 1942, **95**, 23.