3. *Larva.*—With six legs, light or deep orange in colour, very active, feeds on vertebrate host. In the breeding tube if the superficial layer of sand is not too moist, the larvæ hide in the sand for a variable length of time. The addition of a few drops of water to such a tube makes them emerge from hiding and climb up the sides of the tube. On the vertebrate host the larvæ remain attached for 3 to 5 days and then drop off. In another day or two, they assume the next stage.

4. *Nymphochrysalis.*—A quiescent stage in which the nymph develops within the larval integument. It lasts for about 7 days.

5. *Nymph.*—With 4 pairs of legs, orange in colour like the adult, but smaller in size and with the genital organs not fully developed. Very active, lives on the eggs of insects and after 7 to 14 days assumes the next stage.

6. *Imagochrysalis.*—This stage is the second resting stage in the cycle of development. The adult develops within the nymphal integument. This stage lasts for 7 days and then the adult emerges.

7. *Adult.*—Larger in size than the nymph, has a heavy coat of greyish hair and folds on the integument on the dorsum of the abdomen. Genital organs fully developed with 3 pairs of suckers. Sexes are separate and easily distinguishable. Has four pairs of legs of which the anterior pair is larger than the others. Very active and feeds like the nymph on insect eggs.

In plate V microphotographs of the different stages in the life cycle of *T. deliensis* are given.

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TRANSMISSION OF *RICKETTSIA ORIENTALIS* BY THE BITE OF THE LARVÆ OF *TROMBICULA DELIENSIS*

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ALTHOUGH *T. deliensis* has, for some years past, been suspected to be the vector of typhus of the XK serological type in India and Malaya, experimental proof to substantiate the theory has not been obtained so far. The chief difficulty in the way of doing that was the lack of a satisfactory method for breeding mites in the laboratory and obtaining larvæ in sufficient numbers for use in transmission experiments. The fact that it is only during the larval stage that these mites feed on a vertebrate host, necessitates the rearing of at least two generations of larvæ in the laboratory—the first generation for infecting with a known strain of *R. orientalis* and the second generation (bred out of the infected first generation) for transmitting infection by their bites to clean animals. In a previous paper we reported success in the breeding of *T. deliensis* under artificial conditions using mosquito eggs as food for the nymphs and adults. By this technique it is possible to obtain several generations of larvæ without difficulty. Using the technique we conducted transmission experiments to find out if *T. deliensis* is the vector of *R. orientalis* or not in the Barrackpore area. In 5 out of 8 experiments performed positive transmission was achieved through the bite of *T. deliensis* larvæ. Details of the experiments performed and the results obtained are given below :

Technique of infecting mites.—Larval mites originally obtained from rats in the Barrackpore area and bred out in the laboratory were used. First generation mites which were assumed to be clean were fed on mice infected with *R. orientalis* and the engorged mites dropping from the mice collected and bred out again. The larvæ coming out of these infected parents were used in transmission experiments.

The strains of *R. orientalis* used in these experiments were from two sources—human and rodent. The human strains had originally been isolated from human cases of XK typhus and subsequently maintained in the laboratory in white mice. The rodent strain was isolated from *Rattus rattus* caught in the area and identified as *R. orientalis* on the basis of serological and pathogenicity tests.

The mice used in these experiments belonged to the Haffkine Institute breed of white mice. They are susceptible to *R. orientalis* infection and, when injected intraperitoneally with an inoculum containing *R. orientalis*, live usually for about ten days and then succumb to the infection. Depending upon the dose of *R. orientalis* and the resistance of the mouse, this period varies between 6 and 15 days with an average of about 10 days.

In order to be certain that the infected mice used for feeding clean larvæ would live for at least 3 days after the larvæ had attached themselves to the mouse, infected mice which had been inoculated 4 to 6 days previously were used for the purpose. This worked well as the majority of infected mice lived long enough to enable the mites attached to them to get well engorged and drop off.

The infected engorged larvæ were collected and reared up to the adult stage and their progeny (larvæ) obtained for transmitting infection to clean mice. The progeny are infected as there is transovarian transmission of infection from

parent to offspring. Since the transmission experiments were started late in the breeding season, *i.e.* October, it is unfortunate that the number of larvæ available was not as plentiful as we would have liked. At this time the number of larvæ found on rats also is minimal. At the time of report (January) the number of larvæ emerging from the breeding tubes are small, but it is expected that with the advent of the warm weather an increased production of larvæ will result and a larger number of mice would be used in transmission experiments.

Before conducting the final transmission experiment and in order to make sure that trans-ovarian transmission of infection does occur from parent to offspring one batch of 15 larvæ collected from tubes containing adults which as larvæ had fed on infected mice were emulsified and injected intraperitoneally into a mouse. The mouse succumbed on the 15th day of inoculation and showed the characteristic pathological lesions of XK typhus, *i.e.* ascites and enlarged spleen. Rickettsia were demonstrated in the endothelial cells of the peritoneal scrapings.

Technique of feeding infected larvæ on clean mice.—The animal was enclosed in a comfortably fitting wire mesh tube. The infected unfed larvæ were collected from the breeding tubes and placed on the hairs of the mouse. The wire tube with the mouse was then kept in a cage which was suspended over a dish of water. The mouse was so confined for a few hours each day in order to enable as many larvæ as possible to get attached unhindered by movements or scratching of the mouse. The cage was suspended over a dish of water in order that engorged mites which dropped off the mouse may be recovered. Each mouse was used for feeding mites from 10 to 14 days and as many larvæ as were obtained during that period were used. Thus each experimental mouse was given a large number of infective bites over a period of time. Usually after 8 to 15 days of the commencement of feeding, the mouse was labelled and kept apart and observed daily for any sign of illness.

All experimental mice showing signs of illness were sacrificed and examined for *R. orientalis* in peritoneal scrapings and if negative an emulsion of brain was inoculated intraperitoneally into two fresh mice. If no sign of illness was noted in 30 days after the commencement of experiment, the mouse was sacrificed and the brain emulsion inoculated into two mice as above. These mice were watched daily for any sign of illness and if they got ill they were sacrificed and the above procedure repeated. This had to be done as ordinarily in nature the disease in rodents is an inapparent infection. Furthermore, due to the uncertainty of the dose of infection that is actually given through the bites of larvæ, and the route of infection being subcutaneous, the experimental mouse cannot be expected to develop severe illness and die.

Result.—Up to date 8 mice have been used in transmission experiments. These have been bitten by several infected larvæ. The results of the first six mice which have been sacrificed are given in the table below :—

TABLE

Number of mouse	Period of feeding	Number of fed larvæ collected	Date of sacrifice and first passage	Date of sacrifice and second passage	Date of sacrifice and third passage	Date of sacrifice and fourth passage	Result	
1	20-10-48 to 3-11-48.	11	24-11-48	7-12-48	23-12-48	6-1-49	Negative so far	
2	4-11-48 to 19-11-48.	30	7-12-48	23-12-48	..	..	Negative so far	
3	20-11-48 to 3-12-48.	60	Sick on 21-12-48, passaged.	Both ill sacrificed, 30-12-48.	..	..	Rickettsia +++	Ascites and enlarged spleen.
				31-12-48	..	..	Rickettsia +++	
4	30-11-48 to 10-12-48.	29	23-12-48	One died on 9-1-49.	..	..	No rickettsia	
				Other well	..	..	Negative so far	
5	10-12-48 to 19-12-48.	38	Sick on 28-12-48, passaged.	One died on 5-1-49.	..	..	Rickettsia ++	
				Second sacrificed.	..	..	Ascites ++	
							Spleen ++	
6	19-12-48 to 27-12-48.	78	Sick on 4-1-49, passaged.	One sick on 11-1-49.	..	..	Rickettsia ++	Spleen enlarged.
				Other sick on 12-1-49.	..	..	Rickettsia ++	Ascites present.

Numbers 7 and 8 have also proved to be positive.

From the tabular statement of results it will be seen that 5 out of the 8 mice which were bitten by infected larvæ contracted the infection. Nos. 2, 5, 6 and 7 were positive while nos. 1, 2 and 4 were negative. It will be noticed that the positive mice received more infective bites than the negative ones. Although in no instance were rickettsia found microscopically in the mouse actually bitten by the larvæ they were shown to be present in the next passage mouse. The positive mice (nos. 2, 5, 6, 7 and 8) looked sick at the time of sacrifice though in no instance did it appear that the mouse would have succumbed to infection if not sacrificed. The infection in rodents in nature appears to be an inapparent one in which the organisms persist in the brain of the animal. Strains of rickettsia identical with human XK strains have been isolated by us from the brains of apparently healthy wild rats. It may be so in mice also when infection is transmitted through the bites of infected larvæ and inapparent infection results. So far, we have not been able to find any natural rickettsial infection in the breed of mice that we are using in transmission experiments. Under these circumstances the positive transmission obtained should be accepted pending the demonstration of transmission of infection to man through the bites of infected larvæ.

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TYPHUS IN CALCUTTA

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IN a previous communication 10 cases of typhus fever among civilians in Calcutta were reported from this Department (Lowe, 1946). Since then we have encountered 25 more cases the clinical observations on which are presented in this paper.

The patients.—The present series included 17 Bengalees and 8 non-Bengalees of whom two were Anglo-Indians. The youngest patient was 11 years and the oldest 61 years old, 18 (72 per cent) being between 11 and 30 years. This shows the maximum prevalence during adolescence and in young adults. There were 15 males and 10 females indicating that both sexes were almost equally affected; female accommodation is more limited in our hospital. The occupation of 14 patients was as follows : Three medical men, 4 clerks, 1 darwan and 6 college students; the rest were women and children, or gave no information regarding their occupation.

Twenty patients belonged to the city proper while the remaining five came from outside, of whom one patient, a woman, was on her way to Eastern Pakistan and contracted the disease during her short stay in Calcutta, but members of the family accompanying her were unaffected. Only 2 patients—nos. 4 and 9 in table I—gave a history of similar type of fever in their families. A reference to the dates of admission shows a definite seasonal incidence, 18 cases occurring from July to September with the maximum of 8 in the month of August.

Clinical features

These were recorded in a *pro forma* specially prepared for the study of enteric cases admitted to this hospital. The information was obtained by questioning and examining patients. The important symptoms and signs and their relative frequency as observed in this series are shown below :—

Principal symptoms and signs	(*per cent*)
Fever	100
Headache with pain in the body ..	96
Cough	72
Deafness	64
Sore throat	48
Injected eyes	44
Rash	36
Lymphadenitis	28
Palpable spleen	24
Mental dullness and apathy ..	24
Marked toxæmia	32
Bradycardia	12
Eschar	12

The *onset* was sudden or insidious, the former being commoner. An abrupt attack of fever with headache and pain in the body was the common mode of onset (60 per cent). Occasionally, there was chill and rigor (16 per cent) specially when the temperature rose rapidly simulating malaria and rarely associated with nausea and vomiting. In a proportion of cases the disease came on slowly with such symptoms as headache, lassitude and weakness, possibly accompanied by mild fever for 3 or 4 days. Cough was a common complaint, the throat being congested and sore in nearly half the cases. Flushing of the face and sometimes of the body was common. Eschar often with regional adenitis may be seen.

Fever.—The temperature during the height of illness was of continuous type in 11 cases, of remittent type in 12 and irregular and intermittent in 2. The maximum temperature varied from 101° to 105.5°F., and in 16 it was above 103°F. The duration of fever varied from 14 to 35 days. Severely ill patients usually had a