That for the diagnosis of X2 cases the Weil-Felix reaction is not always dependable and animal inoculation is not also of much value. It is possible that the complement-fixation test will be found more helpful, but it has not been tried with local strains.

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THE BREEDING AND MAINTENANCE OF *TROMBICULA DELIENSIS* IN THE LABORATORY FOR EXPERIMENTAL PURPOSES

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(From the Bengal Typhus Enquiry at the All-India Institute of Hygiene and Public Health, Calcutta, partly financed by the I. R. F. A.)

THE interest in the life history of the mite *Trombicula deliensis* lies in the fact that it is suspected to be one of the vectors of typhus of the XK serological group in India and Malaya. Before the last great war, fevers of the typhus group were known to exist in certain limited areas in India and the Far East, but the distribution of troops over wide areas in the East during the second world war revealed the widespread nature of the endemic foci of typhus of this type. Bengal which was supposed to be free of such a disease was soon shown to possess many endemic foci, some immediately around Calcutta. Investigations in these foci revealed that rats were infected with *Rickettsia orientalis* the causal agent of XK typhus and that they were also heavily parasitized by larvæ of *T. deliensis* the suspected vector. This gave us an opportunity to find out if *T. deliensis* was the carrier of *R. orientalis* in this area. In that connection we had to breed *T. deliensis* in the laboratory. Before one can obtain certain evidence of the rôle of this mite transmission, it is necessary to breed the mite in captivity so that laboratory-bred larval mites will be available in sufficient numbers for use in transmission experiments. This had not been done till now.

Nagayo *et al.* (1917) were the first to attempt the breeding of Trombiculids. They claimed to have obtained a few adults by feeding nymphs on melon, potato, etc. Our efforts to breed *T. deliensis* using such material as food proved fruitless. We found that the nymphs which were easily obtained were able to find some nutriment from silt. Enriching the silt with bacteria and protozoa did not secure the desired result. At this stage our attention was directed to an article by Wharton and Carver (1946) in which they described the development of a species of Neoschongastia by feeding the nymphs on the eggs of insects. Mosquito and sandfly eggs were used by us to feed the last few nymphs remaining at the close of the 1947 season, and it was soon apparent that this food was suitable for nymphs and adults of *T. deliensis* as well.

T. deliensis is most prevalent around Calcutta, just after the first rains in June. It can be found in adequate numbers on the ears of rats up to November after which the numbers diminish very rapidly. As soon as sufficient numbers of *T. deliensis* larvæ were obtained, the breeding of these mites was begun, *i.e.* about the middle of June 1948, and the following method of breeding was practised with satisfactory results :—

Wild rats collected from rural areas were etherized and those found to be harbouring larval mites in their ears were placed in small wirenetting cages suspended over a dish containing water. Larval mites as they engorged dropped off and fell into the water. On the surface of water the mites could easily be detected with a lens and picked up with a fine brush. The engorged mites were transferred to tubes 3 inches by 1 inch containing about an inch or more of fine moist sterilized sand and closed with well fitting straight sided corks covered with a piece of white cotton cloth. Provided the sand was sufficiently moist, the majority passed into the first resting or nymphochrysalis stage in from a few hours to two days. The nymphochrysalis stage lasted for 7 or 8 days after which the nymphs hatched out. As soon as nymphs were found in a tube, some mosquito eggs were added; the easiest obtainable being culex egg rafts, and these were broken up before delivery into the tube. Nymphs require a fair degree of moisture for satisfactory development. When newly emerged they are cream coloured and very active. Nymphs as well as adults avoid light and always seek the shelter of any irregularity on the surface of the sand. The making of a few trenches or holes in the sand of the tubes is beneficial, as the mites hide in these places and do not endeavour to escape from the tube to such an extent as when there are no such shelters for them to hide in. Despite this, practically from every tube a certain number are lost due to being crushed between the sides of the tube and the cork.

The nymphal stage lasts from 7 to 14 or more days. Though food and moisture are equally available some seem to develop very much slower than others. It is not unusual to see the 3 stages of nymph, imagochrysalis and adult in the same tube which was charged with a batch of engorged larval mites collected on the same day. As the nymphs feed and develop they assume a distinct

brick red or dull pinkish colour in contrast to the pale yellow or cream colour of the newly hatched individual. After feeding for 7 or more days the nymph assumes a second resting stage, the imagochrysalis, which lasts for a further period of 7 days after which the adult emerges. The adults may be distinguished from the nymphs by their larger size, heavy coat of greyish hair and the folds in the integument on the dorsum of the abdomen—features distinguishable with a hand lens. Microscopically the genital organs are seen to be fully developed.

The females deposit their eggs indiscriminately—a few at a time and continue to oviposit for about 5 months. Adults grown in June are still ovipositing in December. The number of eggs or the exact time after maturation when ova are laid has not been determined yet. From a tube containing one pair of mites 158 larvæ have been collected in 28 days so that at least 5 to 6 eggs would appear to be laid each day. Larvæ may be expected in the tubes any time 20 days after adults are seen. One of the greatest difficulties experienced so far in breeding is the control of fungi, certain kinds of which smother and kill the mites; in others the powdery spores get entangled in the hairs and embarrass the adult mites leading to sluggishness and later death. Clearing of the fungus results in a considerable loss of eggs and deutova; eggs and deutova are easily obtained by suspending the fungal debris removed from the surface of the tubes in a saturated solution of sugar. Against a dark background, the eggs which look pearly white are globular with a shell which is pitted much like a miniature golf ball. The deutova are brownish in colour and irregular in shape and when examined under the microscope are seen to consist of the orange coloured larva enclosed in a membrane with the remains of the shell still attached.

The time from egg to deutovum is 7 days and from deutovum to larva 7 days in *T. deliensis*.

In the case of larvæ that are infected with rickettsia, the following method was used to control the escape of larvæ from breeding tubes during handling and manipulation.

Tubes containing adults are kept in a receptacle which in turn is placed in a large enamel iron dish containing a solution of dettol. The rim of the dish is carefully ringed with an adequate supply of soft paraffin. When tubes have to be examined for collection of larvæ and feeding, a second large dish is used with a vaseline ring just below the rim of the vessel. In the dish are three petri dishes of water—one is used for placing the tube for examination, the second contains a smaller dry petri dish on which the corks are placed for collecting such larvæ as have come up on the cork and the third contains a deep solid watch-glass with water for receiving the larval mites which are collected from the corks and sides of the tube. Although larvæ can move easily over the surface of the water, especially if there is the slightest current of air, they move quicker on anything solid. When collected and placed in the watch-glass with water, they usually tend to bunch up together in the middle of the watch-glass from where they can be easily picked up in batches and placed on the hair of the victim to be fed on.

The technique of feeding larval mites on mice is as follows: If sufficient mites are found in a single breeding tube, a mouse enclosed in a wire gauze tube is placed in contact with the rim of the open tube for a few hours. This attracts the larvæ which attach themselves to the mouse. When large numbers of tubes and larvæ are available it is more convenient to collect the larvæ in a watch-glass as described above and place them directly on the hair of a mouse enclosed in a small wire gauze cage 4 inches by 4 inches which is placed in a dish of fine sand. The dish of fine sand and the cage are placed in a larger container with water up to a certain level. Both dishes are protected by vaseline rings.

When the single tube method is used or when small numbers of larvæ are collected and placed directly on the hairs of a mouse enclosed in a small wire mesh tube, the mouse on release (*i.e.* in 2 or 3 hours) from the tube, is transferred to a cage which is suspended over a dish of water. This is done in order that any engorged mite or unattached ones that drop off the mouse may be received in the water and collected from there. When the second method is used, *i.e.* where the mouse is left in the 4 inches by 4 inches cage on sand, the mouse is changed every 48 hours or so, because larval mites take anything from 48 hours to 4 or 5 days to engorge; and if they drop into the sand they cannot be easily recovered from there. The largest numbers drop off on the 3rd day. Mice removed from the feeding cage are kept in the same manner as infested rats for the collection of the engorged larvæ.

In white mice the larvæ seem to attach themselves to any part of the head and body and not mainly to the ears and legs as in rats and shrews. Infected mice dying within 3 days of feeding have been found with numerous larvæ attached to the outer surfaces of the ears, round the mouth and on the legs.

The colony of mites which was started in June 1948 is now in the third generation and a few mites reared in June are still alive and breeding in December.

A brief description of the different stages in the life cycle of *T. deliensis* is given below :—

1. *Egg.*—Round, pearly white in colour when seen against a dark background. The shell is pitted like a miniature golf ball. The egg stage lasts 7 days.

2. *Deutovum.*—Brown in colour with the egg-shell broken and the larvæ remaining quiescent enclosed in a membrane. This stage lasts for a further period of 7 days and then the larva emerges.

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

PLATE V

THE BREEDING AND MAINTENANCE OF *T. DELIENSIS* IN THE LABORATORY FOR EXPERIMENTAL PURPOSES : K. V. KRISHNAN, R. O. A. SMITH, P. N. BOSE, K. N. NEOGY, B. K. GHOSH ROY & M. GHOSH. (O. A.) PAGE 39