FEVERS OF THE TYPHUS GROUP IN THE BHIM TAL AREA, KUMAUN HILLS, U.P., INDIA.

BEING A REPORT OF AN INVESTIGATION CARRIED OUT INTO THE ALLEGED INCIDENCE AND NATURE OF TYPHUS GROUP FEVERS IN THE BHIM TAL AREA, KUMAUN HILLS, JULY, 1936.

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(Continued from p. 167).

THE FIELD WORK.

Material was collected by a system of extended drives with a 400-yard frontage through the district, using coolies and a large number of dogs. Each drive was on a definite bearing which extended from one end of the area to the other, and was so arranged that an overlap occurred on the return drive. In this way the entire area was covered and should have resulted in no infected zone being missed.

The routine adopted for this portion of the investigation was as follows: From early morning until sunset was occupied in the collection of material; at the conclusion of the day’s drives the entire bag was counted and sorted into convenient lots, and the animal experiments commenced.

The methods of collecting material in the drives were as follows:
(1) The deticking of all animals and cattle which fell within the drive;
(2) dragging the area with coarse sheets of white cloth and flags;
(3) deticking the dogs used at the conclusion of each drive.

These methods are simple and require little comment. As regards “dragging,” this merely offers a large area of coarse material to which insects or ticks harboured in the local scrub or undergrowth may attach themselves, and is particularly designed for the collection of “seed” ticks, e.g. larvæ, etc. The dogs used were of especial value in negotiating difficult country, as they were deticked at the end of each drive and provided a large amount of material. Each coolie had one or more dogs in addition to a flag or a long sheet of material which he dragged with him through the undergrowth.

By these means some thousands of ticks, larval and otherwise, were collected from the area, but only 1,000 were retained for experimental purposes and were made up of a selected number from each zone.

The details of animal experiments and number of ticks used will be found in Table II.
Table II.

<table>
<thead>
<tr>
<th>Animal series</th>
<th>No. of ticks unfed and inococ. per animal, intraperitoneally</th>
<th>No. of ticks fed on each animal</th>
<th>No. of ticks fed and inococ. per animal, intraperitoneally</th>
<th>Total ticks used per animal</th>
<th>Total ticks used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea pigs (10)</td>
<td>45</td>
<td>5</td>
<td>50</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>Rabbits (4)</td>
<td>45</td>
<td>5</td>
<td>50</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>White rats (6)</td>
<td>10</td>
<td>3</td>
<td>13</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>White mice (6)</td>
<td>5</td>
<td>1</td>
<td>6</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>(26)</td>
<td></td>
<td></td>
<td></td>
<td>814</td>
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</table>

From the above table it will be seen that in all 26 animals were employed and 814 ticks. As regards the animals, almost every type of laboratory animal was used which had been found by previous workers on the typhus problem throughout the world to respond in some appreciable manner to the known types of the typhus virus. Further, the ticks employed were a pooled collection representative of every zone in the Bhim Tal area, at least 60 per cent of which were either *R. sanguineous* or *Hyalomma aegyptium*. These two species had been incriminated by Megaw as the possible vectors, and his contention has been given a certain support by so distinguished an entomologist and student of tropical disease as Strickland in a brilliant study of the potential vectors (Strickland, 1927).

Further, *R. sanguineous* is itself the proved vector of the tick-borne typhus fever of the Mediterranean littoral, e.g. the Fievre Boutonneuse (Durand and Conseil). And finally it belongs to the same tribe as *Dermacentor andersoni* Stiles, one of the proved vectors of the tick-borne typhus fever of America.

All animals used were subjected to control, temperature and weight records, in addition to control Weil-Felix tests for some time prior to being employed in the experiments.

These experiments consisted of three stages: (1) The preparation of the emulsions of unfed ticks and their injection intraperitoneally into the experimental animal; (2) The feeding of ticks on the experimental animal for a number of days; (3) the preparation of emulsions of the fed ticks and their injection into their host.

**Details of the Tick Emulsion Injection.**—All ticks used were sterilized externally and then thoroughly ground in a sterile mortar and made into a suitable emulsion in normal saline and injected under strict asepsis intraperitoneally into the experimental animal. In certain cases the experimental animal received a blood injection intraperitoneally prior to receiving the emulsion; this was done in view of recent work which suggests that this procedure increases the chance of establishing a strain of the virus (Zinnser 1934).

**Details of the Feeding Experiments.**—For the feeding experiments fine wire gauze was used in the shape of a straw hat after the manner employed in these studies in America (Woolbach, 1919). The purpose of this portion of the experiments was two-fold—to investigate the potentialities of tick
transmission by feeding, and to obtain a re-activated tick virus should such exist, as suggested in recent studies of the tick virus in America (Parker, 1933). After three to four days feeding the ticks were removed from their host, emulsified in saline and injected into their host intraperitoneally when presumably a re-activated virus was introduced, if such were to be found in the ticks.

This completed the field work and all the animals, together with the equipment, were returned to the Brigade Laboratory, Bareilly, for the further stage of the investigation.

LABORATORY WORK.

The details connected with this portion of the investigation may be said to consist of a study of the reaction of the various animals to the feeding and inoculation experiments carried out at Bhim Tal. The details for the individual animal have been tabulated. The problem was investigated not only from a purely clinical aspect as revealed by daily temperature records and weekly weight records but also from the serological and histopathological side. All animals had a weekly Weil-Felix test carried out, and on the death of the animals smears and sections from suitable tissues were made and examined for the presence of rickettsia after staining with Giemsa as recommended by Woolbach, 1921, and Covell, 1936. A limited number of attempts at passage were undertaken. For each series of animals a control animal was maintained so that it was possible to check any apparent "reactions" in animals under experiment. The value of these control animals is particularly noticeable as regards ranges of normality in temperature. It is noticeable as far as guinea-pigs are concerned and to a less extent rabbits that the accepted standards as recorded in the "System of Bacteriology" (Privy Council, Vol. 19), are definitely not applicable to the plains of India during the hot weather. Covell considers a temperature of 102°F. in a guinea-pig as definitely abnormal. This is apparently true for the hill station in India; but in the plains the writer has found that this is not the case and it has not been possible to consider a temperature of 103°F. or under as evidence of a febrile response in guinea-pigs. Further it should be noted that wide fluctuations of two to four days' duration are quite common in normal guinea-pigs and rabbits and cannot be regarded as a specific febrile response unless the other lines of investigation confirm this. It may seem that unnecessary stress is being laid on a divergence from normal of only one degree; nevertheless the writer has been struck by the frequency by which the specific febrile responses of previous observers have involved no greater divergence; this one degree is of very considerable importance.

The two temperature charts are from guinea-pigs, one inoculated with blood from a human case and one with brain emulsion from a wild rat. The charts were kindly given to the writer by Covell as examples of the
specific febrile response in guinea-pigs infected with the typhus virus. From these it will be immediately appreciated how important this one degree divergence happens to be. These charts, when compared with our results, show that “even in Covell’s” cases the febrile responses are in no way different to the normal fluctuation found in many guinea-pigs.

In Covell’s series rickettsiae have been found and the Weil-Felix, though never impressive, has nevertheless been such as to suggest the possibility that these febrile responses were in fact abnormal and associated with a typhus infection.

It remains to consider in general terms the reactions of the various animals employed.
THE REACTION IN THE EXPERIMENTAL ANIMALS.

It is clear that beyond slight fever in some of the guinea-pigs and rabbits on or about the end of the second week in no case was there evidence to warrant the assumption that it was secondary to a typhus virus infection—there is little to note about these reactions. They certainly are in no way similar to the reactions as noted by Anigstein (1933) and Woolbeck (1919). The few attempts at passage that were made were uniformly negative, in this connection it might have been better had brain emulsion been used more frequently instead of blood. This would have meant killing the animals before a thorough serological analysis could be carried out, and this was not done as frequently as the writer would have liked. There is little, however, to note about the reactions in any of the animals and certainly nothing to suggest they had acquired the typhus virus. In no case was any organism remotely resembling rickettsiae seen in smears and sections from brain, testicle, tunica vaginalis, or spleen.

(To be continued.)