Original Communications.

SANDFLY FEVER ON THE INDIAN FRONTIER.¹
A PRELIMINARY NOTE ON SOME LABORATORY INVESTIGATIONS.

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MATERIALS AND METHODS.

SANDFLY FEVER, under its multiplicity of names, appears to have a world-wide distribution. The similarity of the clinical manifestations throughout the range of the disease is prima facie evidence that the disease, wherever met with, is one definite entity, but this evidence would be strengthened were it supported by a like similarity in laboratory findings.

Laboratory findings have already been recorded by various workers, and it has been our endeavour to repeat these observations, as far as possible, in order to demonstrate the identity, or at least essential similarity, of the Indian form of the disease with that occurring in other parts of the world.

It was obvious to us that much of the work involved in repeating these observations in India could not conveniently be carried out within an area

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where sandfly fever was endemic. It was therefore decided that materials for investigation—blood from sandfly fever cases and sandflies fed on such cases—should be collected in Peshawar and immediately despatched by post or messenger to Kasauli. Here the actual experimental work could then be carried out in a known non-endemic area where there would be no necessity for protecting the volunteers used from bites of sandflies other than those used in the experiments.

Even these precautions did not remove all the complications as we would have wished to do in order to make the experiments perfect. Thus, we had to utilize as volunteers such Indians on the staff of the Pasteur Institute, Kasauli, as were willing to offer themselves. Some of these, who were hill-men, had presumably never had sandfly fever and were therefore considered good subjects; others, however, were plainsmen and had at various times been in areas where they might at some time or other have had sandfly fever and so gained a certain immunity. These were difficulties which there was no way of surmounting with the facilities available to us.

As it was not known in what form blood was likely to conserve most efficiently the infective principle it was decided to send it in the three following forms:

(a) Citrated whole blood, i.e. 5 cubic centimetres of blood in 15 cubic centimetres of 2 per cent sodium citrate solution.

(b) Glycerinated whole blood, i.e. 5 cubic centimetres of blood in 5 cubic centimetres of 50 per cent glycerine.

(c) Citrated-glycerinated blood, i.e. 5 cubic centimetres of (a) mixture in 5 cubic centimetres of 50 per cent glycerine.

As the work was carried on during the middle of the hot weather, and the journey from Peshawar to Kasauli occupied approximately twenty-four hours, and as the blood was taken on the day previous to its despatch and sent either by messenger or post without any special precautions for cooling it, it will be evident that there was every likelihood of any but a fairly resistant virus dying out between the taking of the blood at Peshawar and its use in Kasauli. The average interval elapsing between the two operations was sixty hours.

Previously Recorded Laboratory Findings.

The most important laboratory findings as recorded by previous workers on sandfly fever are enumerated below and will later be considered in more detail in relation to our own observations.

(1) Blood taken from a patient on the first day of the fever when inoculated into man produces a typical attack of the fever after the usual incubation period (Doerr, Franz and Taussig, 1909; Birt, 1910).

(2) Blood passed through a fine filter can still convey the typical fever when inoculated into a susceptible person (Doerr, Franz and Taussig, 1909; Birt, 1910).

(3) Phlebotomus flies fed upon a patient suffering from the fever, when
taken to a non-endemic area and fed on susceptible persons, convey the disease to the latter (Doerr, Franz and Taussig, 1909; Birt, 1910).

(4) The flies do not become infective until seven to ten days after their meal on a fever case (Doerr, Franz and Taussig, 1909; Birt, 1910).

(5) The incubation period varies between three and a half days and seven days (Doerr and Russ, 1909; Birt, 1910).

(6) The virus retains its infectivity for a week in vitro (Birt, 1910).

(7) Experimental animals are immune to the disease (Birt, 1910).

(8) No visible causative agent has been demonstrated in the blood by direct microscopic or cultural examination (Doerr, Franz and Taussig, 1909; Birt, 1910).

(9) A Leptospira has been isolated from cases of so-called sandfly fever (Whittingham, 1921; Nervoort, 1922).

In order to give the clearest account of our own findings and to indicate where they confirm or differ from those recorded by the other workers referred to, it has seemed to us most convenient to deal with them seriatim under the headings we have used above in summarizing the conclusions of these workers.

(1) Blood taken from a patient in the first day of the fever when inoculated into man produces a typical attack of the fever after the usual incubation period.

The impression one gathers from the literature quoted is that the virus has disappeared from the peripheral circulation by the second day of the disease. It was on this account that the majority of the blood samples sent to Kasauli from Peshawar for test were taken on the first day of the disease. In Table I given below are recorded the results of inoculating the bloods into volunteers in Kasauli from the point of view of the infectivity of the blood on the first and second days of the fever:

<table>
<thead>
<tr>
<th></th>
<th>Number of Infections obtained by Inoculating into Volunteers the Blood of Sandfly Fever Cases taken on the First and Second Days of the Attacks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of bloods used</td>
</tr>
<tr>
<td>First day bloods</td>
<td>10</td>
</tr>
<tr>
<td>Second day bloods</td>
<td>4</td>
</tr>
<tr>
<td>Totals</td>
<td>14</td>
</tr>
</tbody>
</table>

* Modified Cases.—No case was considered as a "positive" unless there was actual fever. Those cases in which the fever was only of moderate intensity and short duration were called "modified cases." The discomfort, in the form of various aches, pains and general malaise of some of the cases was considerable, and often lasted for some days beyond the actual duration of the fever.

It will be seen from the table that, contrary to the impression conveyed by previous work, there is probably a considerable percentage of cases in which blood is still infective on the second day of the fever. No investigation on this point was made beyond the second day of fever.

The dose of the inoculum varied with the concentration of the blood,
an attempt being made in each case to give the equivalent of 0.7 cubic centimetre of whole undiluted blood.

As regards the best method of sending blood for the experiments the numbers used were too small for us to come to any decision but, in practice, successful transmissions of fever to man or monkey were produced by all the methods mentioned as well as by direct inoculation of fresh whole blood from fever cases to volunteers. The latter process was carried out by inoculating the blood of fever cases produced in Kasauli from the Peshawar bloods into a second series of volunteers. The actual figures for these experiments are given below in Table II:

<table>
<thead>
<tr>
<th>Condition of Blood</th>
<th>Number of Bloods Inoculated</th>
<th>Number of Fever Cases Produced</th>
<th>Typical</th>
<th>Modified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrated</td>
<td>14</td>
<td>7</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Glycerinated whole blood*</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citrated-glycerinated blood</td>
<td>10</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Fresh whole blood</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td>25</td>
<td>9</td>
<td>7</td>
<td>2</td>
</tr>
</tbody>
</table>

* Glycerinated whole blood was used for injection in monkeys only.

(2) Blood passed through a fine filter can still convey the typical fever when inoculated into a susceptible person.

The quantities of blood received by us were comparatively small and, being mostly used in the direct inoculation experiments, not much was available for filtration. Such experiments as were done, however, showed that the infective virus was capable of passing a comparatively fine filter and retaining its virulence. The filters used by us were L3 and L5 Chamberland candles.

The results of the experiments are given in Table III. The dose of filtrate used for inoculation varied from 3.8 cubic centimetres to 10 cubic centimetres:

<table>
<thead>
<tr>
<th>Condition of Blood before Filtration</th>
<th>Number of Filtrates Inoculated</th>
<th>Number of Fever Cases Produced</th>
<th>Typical</th>
<th>Modified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrated whole blood</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Citrated-glycerinated blood</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td>6</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

The temperature charts of all the cases of sandfly fever induced in Kasauli by the inoculation of blood, whatever its form, or filtrates of blood, are shown in Chart 1.

(3) Phlebotomus flies fed upon a patient suffering from the fever when conveyed to a non-endemic area and fed on susceptible persons convey the disease to the latter.

Eight batches of laboratory-bred P. papatasii which had been fed on cases of sandfly fever at Peshawar or Landikotal were received by post or
<table>
<thead>
<tr>
<th>NAME</th>
<th>NAJOO</th>
<th>GROHARI</th>
<th>KACHWARA</th>
<th>GOYAK</th>
<th>KISHAN BAI</th>
<th>PADA</th>
<th>SATLAL</th>
<th>MOULOO</th>
<th>SUNMOO</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAYS</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>TEMPERATURE</td>
<td>105</td>
<td>103</td>
<td>101</td>
<td>99</td>
<td>97</td>
<td>95</td>
<td>93</td>
<td>91</td>
<td>90</td>
</tr>
</tbody>
</table>

**PULSE RATE**

- Extirpated Glycerinated Blood
- Extirpated Blood
- Filtered Extirpated Blood
- Filtered Blood
- Filtered Glycerinated Blood
- Extirpated Blood
- Filtered Blood

**CONDITION OF INFECTING DOSE OF BLOOD**

- 3.4 cc.
- 2.8 cc.
- 2.8 cc.
- 3.8 cc.
- 3.8 cc.
- 2.8 cc.
- 1.0 cc.
- 2.8 cc.

**INJECTION PERIOD**

- 3 days
- 4 days
- 5 days
- 6 days
- 7 days
- 8 days
- 9 days
- 10 days

**SYMPTOMS**

- Headache 9 cases
- Bradycardia 5
- Malaria-like fever 7
- Injected conjunctivitis 7
- Loss of appetite 7
- Generalised pains in body 6
- Pain behind eyes 5
- Vertigo 1

**TOTAL SYMPTOMS**

- Crisis 1
- Pseudo-crisis 6

**CHART 1.**—The temperature charts of, and other clinical observations made on cases of sandfly fever induced in volunteers by inoculation of infective blood.
passenger train in Kasauli. These flies were sent in the mosquito despatch boxes devised by Barraud (1929). The total number of flies despatched was 110. Of these 69 arrived alive, 32 were dead and 9 were not accounted for. Of the 69 flies which arrived only 19 fed on volunteers. Eight volunteers were used. As the flies when received in Kasauli had fed only three or four days previously it was considered that the third and subsequent feeds were

<table>
<thead>
<tr>
<th>NAME</th>
<th>KINATA-A CASE OF FEVER INDUCED BY SANDFLY BITES</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAYS</td>
<td>1</td>
</tr>
<tr>
<td>TEMPERATURE</td>
<td>105</td>
</tr>
<tr>
<td>SIGNS AND SYMPTOMS</td>
<td>MELAISE BEFORE FEVER</td>
</tr>
<tr>
<td>PULSE RATE</td>
<td>88</td>
</tr>
<tr>
<td>INCUBATION PERIOD</td>
<td>10 DAYS</td>
</tr>
</tbody>
</table>

CHART 2.—Sandfly fever induced by bites of sandflies.

most likely to be the infective ones. The second feed (or first in Kasauli) was given to keep the flies alive and the third and subsequent feeds were on the most suitable volunteers. Of the 8 volunteers, all were fed upon once, 5 were fed upon twice, 1 was fed upon three times, 1 was fed upon four times, and 1 was fed upon five times.

Two volunteers developed fever, their histories being as follows:

Volunteer Ram Das, aged 9, male.—On August 22 three flies which had originally fed on sandfly fever cases in Landikotal between August 13 and 16 were fed upon the volunteer. One of the flies took only a partial feed. This fly was again fed on the volunteer on the succeeding day.

On August 25 the volunteer developed fever which lasted for three days. Unfortunately, the boy lived in a village and did not report sick until the third day so that no record of his temperature was made. When seen on August 27 the fever was subsiding and the temperature was normal on the morning of August 28. On the 27th the patient's face was flushed, the eyes were suffused and he complained of pains all over the body and headache. The pulse rate was slow, eighty per minute. No coryza, cough or
any other symptoms which could account for the fever were present at any
time. We have no doubt in our minds that this was a genuine case of
sandfly fever transmitted by the bites of sandflies fed at Landikotal six to
nine days previously on sandfly fever cases. It is unfortunate that owing
to the non-reporting of the illness the temperature was not recorded.

Volunteer Kimla, aged 11, male.—On September 21 one fly which had
originally fed on a sandfly fever case in Landikotal on September 16 was
fed upon the volunteer. The feed was partial. On September 22, 23, and
24 three, two and two flies respectively of the same batch were fed; all
these feeds were partial.

On October 5, ten days after the last feed, the volunteer complained of
fever. The fever lasted for three days. There was complaint of pain
behind the eyes, which were strongly injected, pains all over the body and
headache. The pulse rate was markedly slow considering the degree of
fever and the age of the patient, being 80 per minute. Chart 2 shows the
progress of the fever in this case.

If this case was a genuine one of sandfly fever the incubation period
was longer than usual and for this reason we make the diagnosis with
reserve.

(4) The flies do not become infective until seven to ten days after their
meal on a fever case.

In the two cases of fever recorded by us above the flies may be said to
have become infective between the minimum of six days and the maximum
of eight days.

(5) The incubation period varies between three and a half and seven days.

In the case of fevers following feeds by infected sandflies the incubation
period varied in the two cases recorded by us between minima and maxima
of three days and ten days. In our series of inoculation experiments the
minimum and maximum incubation periods for typical cases of the fever,
as measured by the interval elapsing between the time of inoculation and
the first onset of fever, were three and six days, the average time for the
seven positive typical cases being 4·4 days. In one of the two modified
attacks the incubation period was three days and in the other five days.

(6) The virus retains its infectivity for a week in vitro.

In all our experiments we endeavoured to use the bloods sent from
Peshawar as soon as possible after receipt as we were not, at the time,
especially concerned to see how long the virus survived in vitro, but rather
to utilize the blood while the virus was still alive.

In one case, however, 10 cubic centimetres of the filtrate from a L13
Chamberland candle from a mixture of specimens of citrated whole blood
and citrated-glycerinated blood of a sandfly fever case, kept in the ice-chest
for fourteen days, was inoculated into a monkey and appeared to be virulent.
This experiment is dealt with later in the section dealing with animal
experiments.

(To be continued.)