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PRELIMINARY RESULTS OF AN INVESTIGATION INTO THE AETIOLOGY OF SANDFLY FEVER.

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INTRODUCTION.

During the past nine years in the Peshawar district, with an average strength of 19,758 troops, the average number of admissions to hospital for sandfly fever has been 1,456 per year. In any one year over this period the number of admissions for both British and Indian troops combined has been 2,218, for British troops alone 1,526, and for Indian troops 790; the smallest number of admissions, on the other hand, has been 767 for all troops, and for British troops alone 285, and for Indian troops 267. In seeking a broad explanation for this wide variation in the incidence it has been found that relatively high figures are always associated with the presence of unsalted troops in the district, and low figures when the troops are salted. In other words, the number of admissions to hospital in any season bears a very close relationship to the degree of immunity possessed by the troops serving in the district.

The disease accounts for 13·6 per cent of all admissions to hospital, and occupies the second place amongst the more prevalent diseases.
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Malaria accounts for the greatest number of admissions, but, whereas many cases of malaria are contracted outside the district, this is not so with sandfly fever.

The disease, which is a non-fatal one of short duration, is endemic in the district, and occurs in epidemic form during the summer months, reaching its maximum usually in June/July, then declining fairly rapidly, with perhaps a slight rise in September, and disappearing in October.

It is known that a very definite degree of immunity is developed amongst those troops which have experienced one season in an endemic area, and that troops fresh to India and unsalted troops in general suffer very heavily during their first year in such an area. If, from the point of view of immunity, troops are divided into three groups, (i) troops fresh to India, (ii) unsalted troops in general, and (iii) salted troops, it is found that the difference in the degree of susceptibility amongst the groups is striking.

(i) Troops fresh to India.—During the season 1933 the table given below was prepared and illustrates the degree of susceptibility of troops in their first year of service in this district.

<table>
<thead>
<tr>
<th>Service in India</th>
<th>Total Admissions during the Months:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Under 1 year</td>
<td></td>
</tr>
<tr>
<td>2 years</td>
<td>9</td>
</tr>
<tr>
<td>3 years</td>
<td>5</td>
</tr>
<tr>
<td>4 years</td>
<td>6</td>
</tr>
<tr>
<td>5 years</td>
<td>3</td>
</tr>
<tr>
<td>6 years</td>
<td>8</td>
</tr>
<tr>
<td>7 years</td>
<td>6</td>
</tr>
<tr>
<td>8 and over</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>27</td>
</tr>
</tbody>
</table>

This table shows how susceptible troops are with less than one year's service. In Landi Kotal, during the same season, forty-eight per cent of the total number of cases occurred amongst British troops with less than one year's service. The figures for Landi Kotal and Peshawar closely correspond.

(ii) Unsalted Troops.—A good illustration of the susceptibility of unsalted troops can be given by a study of the conditions in Landi Kotal where the incidence of sandfly fever is high. Normally a British battalion serves one year in this station, and Indian troops two years. The British battalion, which was posted there in 1933, and which came from another district in India, may be considered an unsalted battalion, since out of a strength of 834 only 17 men had had sandfly fever in districts other than Peshawar. The total admissions from this battalion for the year under
consideration were 389, which is approximately 50 per cent of their average strength of 779.

(iii) Salted Troops.—On the other hand, in 1933, the Indian troops with a strength of 1,677 were spending their second season in Landi Kotal, and may be considered as salted. Among these Indian troops, which during this season did not consist of Gurkhas, who are highly susceptible, there were only 151 admissions, which was nine per cent of their strength. This low figure amongst the Indian troops must, of course, be assessed, bearing in mind the fact that British troops are more susceptible to sandfly fever than either Gurkha or Indian troops, as shown by McCombie Young, Richmond and Brendish [9].

It is estimated that the total number of “sick days” from sandfly fever in this district approximates to at least 20,000 per year.

The figures which have been given show that sandfly fever is an extremely important disease, and that it accounts for a very considerable wastage of man power. Owing to the fact that the greatest number of cases occurs amongst troops that come from non-sandfly fever areas, the disease is of special importance in a district such as Peshawar, where it may be necessary to concentrate troops drawn from parts of the country outside endemic areas.

OBJECT OF THE PRESENT INVESTIGATION.

The present investigation has been undertaken to show: (i) That the disease known as sandfly fever in the Peshawar district agrees with the clinical description of the disease; (ii) that it is not associated with the presence in the blood of a leptospira, or any other visible micro-organism.

The transmission of the disease by sandflies, researches into the communicability of the disease by blood inoculations, and the question of a filter passing virus as the cause, form the subject of a separate communication now in the press.

CLINICAL OBSERVATIONS.

An analysis has been made of the medical case sheets of 2,000 cases that have occurred in the district during 1932 and 1933, and in practically every case the symptoms have conformed to the classical description of the disease. That is, the disease is characterized by sudden onset, rapid rise of temperature to somewhere in the region of 102° to 103° F., slow pulse, flushed face, injected conjunctivae, frontal headache, pains behind the eyeballs, backache, pains in the muscles, and a general feeling of being “very ill.” Temperature falls on the second or third day, after which the patient feels much better, but convalescence is not rapid, the individual being “off duty” for ten to fourteen days. Sometimes the onset is gradual, the man feeling “out of sorts” the day before reporting sick. This is more noticeable amongst the Indian than the British troops. The typical case, however, is one of fever, flushed face, injected conjunctivae, backache and pains all over the body.
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It was found that the duration of pyrexia in 74.1 per cent of the cases lasted from two to three days; that there was a secondary rise in 8 per cent of the cases, lasting about twenty-four to thirty-six hours, after the initial fever of two to three days duration; and that in only 1.5 per cent of the cases was there a continuous fever of six days and over.

The following table gives in greater detail the types of pyrexia together with the duration and the percentages of cases in which they occurred.

<table>
<thead>
<tr>
<th>Types of fever</th>
<th>Duration</th>
<th>Percentage of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple</td>
<td>1 day</td>
<td>4.8 per cent</td>
</tr>
<tr>
<td></td>
<td>2 days</td>
<td>36.7</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>38.5</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>9.5</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2.1</td>
</tr>
<tr>
<td>Continuous fever</td>
<td>over 6</td>
<td>1.5</td>
</tr>
<tr>
<td>Recurrence of fever, or</td>
<td></td>
<td>8.0</td>
</tr>
<tr>
<td>cases showing terminal</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Blood Picture.—Total and differential counts were carried out on the third day of the disease in 478 cases. These were done in the clinical side-rooms of three different hospitals and also in the District Laboratory. The third day of the disease was chosen as a likely time when a blood examination might assist in the diagnosis of the disease. In 46 per cent of the cases the total white counts were in the region of 5,000, and in 24.5 per cent in the region of 6,000. The following table gives in detail the total white counts and the percentage of cases which gave these results.

<table>
<thead>
<tr>
<th>Total white counts</th>
<th>Percentage of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,000 W.B.C.s per c.mm.</td>
<td>1.5</td>
</tr>
<tr>
<td>4,000</td>
<td>16.0</td>
</tr>
<tr>
<td>5,000</td>
<td>46.0</td>
</tr>
<tr>
<td>6,000</td>
<td>24.5</td>
</tr>
<tr>
<td>7,000</td>
<td>10.5</td>
</tr>
<tr>
<td>8,000 and over</td>
<td>1.5</td>
</tr>
</tbody>
</table>

The majority of the cases gave a polymorphonuclear leucocyte count in the region of 65 per cent. There were no changes considered as being of real diagnostic value amongst the other cells.

The findings during the investigation in 1932 (carried out by one of us, A.S.) were similar to the above.

Laboratory Work.

The laboratory work in this report is mainly concerned with investigation into the culturing of blood for possible micro-organisms, and animal experiments.

During the season 1932 an investigation was carried out (by one of us, A.S.), and in three cases an organism belonging to the spirochaetal group...
was discovered in culture. This organism was from 6 to 9\(\mu\) in length and 0.5\(\mu\) broad. It had six to eight regular fine coils and pointed extremities. The coils appeared to be fixed and rigid, and motility was never observed. It could be stained by Fontana's method. In the stained specimens it resembled \(T. pallidum\) somewhat, except that the coils were more open and drawn out. It was an extremely delicate organism, and difficult to subculture, but subcultures were successfully obtained and the organism was kept alive for several months. The organism could not, however, be recovered when injected intraperitoneally into guinea-pigs. A somewhat similar organism with well-marked spiral twists, which showed no motility, has been described by McCombie Young, Richmond and Brendish [9]. They obtained it from a sandfly fed six days previously on an infected case.

In consequence of this spirochetal organism having been isolated in 1932, the Medical Directorate at Simla decided to carry out a further investigation during the season 1933, to find out whether or not this spirochetal organism had any bearing on the aetiology of the short fevers that occur in the Peshawar district.

**Blood Cultures.**—During the season 1933, blood cultures were taken on the first day of the disease in 470 cases.

The technique adopted was that advocated by Taylor and Goyle (1931) [10], for the cultivation of leptospires. Fletcher's medium (1928) was the culture medium used for the majority of the cases. Most of it was prepared in the District Laboratory, Peshawar, but a certain amount was received from the Central Research Institute, Kasauli. The medium was issued to the hospitals in sealed glass capsules containing about four to five cubic centimetres. Sinton's method, as used for the cultivation of malaria parasites, was also employed for culturing the blood.

In the wards of the hospitals blood was withdrawn from the patient's vein by means of a syringe, and the glass capsule containing the medium inoculated with 0.5 cubic centimetre of whole blood. Immediately this was done, the glass capsule was sealed in a flame, and despatched to the laboratory where it was incubated at a temperature of 25° to 26° C. The object of using sealed glass capsules was to minimize the possibility of contaminations during the long period of incubation.

Many difficulties were experienced in preventing contaminations in spite of the precautions adopted. One of the chief difficulties was to prevent air-borne contaminations. Peshawar is an extremely dusty place, the whole of the Peshawar Vale being enveloped in a haze of dust for many months of the year. Consequently, the media room in the laboratory throughout the season, even although the work was carried out in a room with tightly fitting windows and door, was far from being free of dust. As a result, it was found necessary to carry out work, such as tubing the medium and subculturing, inside a specially constructed box with glass windows, the air inside being sterilized. When this box was taken
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into use the contaminations, especially from moulds, were fewer. In the wards of the hospitals, however, with fans going and general movement, a still atmosphere was more difficult to obtain.

The glass capsules used were purchased locally, and a certain amount of difficulty was experienced by the hospitals in opening and sealing them. The reason being that the glass was very brittle and generally of poor quality, the stems in particular being extremely fragile and inclined to shatter and not fracture in a regular manner. This was found to be a point of considerable importance as particles of shattered glass were liable to drop inside the capsules and contaminate the medium. A certain number of capsules of good quality glass were very kindly given us by the Director of the Central Research Institute, Kasauli, which solved many of the difficulties.

Each capsule was incubated for a period of fifteen days before being opened and examined. Specimens for examination were withdrawn from the capsules by means of fine capillary glass tubes, after which the capsules were sealed and replaced in the incubator for a further period of fifteen days. Approximately 50 per cent. of the capsules were examined a second time after thirty days incubation, and 25 per cent. a third time after forty-five days.

Each specimen was examined with dark-ground illumination, a period of roughly ten minutes being spent over each specimen.

**Findings.**—In not a single case was a leptospira found, and in 69 per cent. of the cases the cultures were sterile. Of those that were contaminated, the organisms were found to be extraneous contaminations. In only one case was a pathogenic organism found; that was from a case of staphyloccocal septicæmia. Many artefacts were seen, such as granular and globoid bodies, and also many bodies resembling spirochætes, but these were all very definitely the pseudo-spirochætes of the blood as described by Knowles and Das Gupta [8].

**Direct Examination of the Blood.**—Examinations of the blood, both in the stained and unstained state, failed to reveal the presence of an organism.

**Animal Experiments.**—The animals used were rabbits and guinea-pigs. Each animal was observed over a period of fourteen days after each experiment, during which time the temperature was taken night and morning. Inoculations were carried out with:

(i) Whole blood injected intravenously, intraperitoneally and subcutaneously.

(ii) Cultures from cases of clinical sandfly fever that showed bodies which might possibly have been some phase in the life cycle of a spirochæte, injected as in (i).

(iii) Emulsions of sandflies, both fed and unfed, injected as in (i) and also intradermally. In addition, from one rabbit that had received an intradermal injection, after allowing a period of five days to elapse for possible incubation in the animal, a volunteer human subject was inoculated by scarification of the skin with a drop of the rabbit's blood.
None of the animals developed any definite fever, and none of them showed any signs of disease. No leptospiroæ or other organisms were recovered from their blood or tissues. The volunteer human subject suffered no untoward effect, but it should be mentioned that the volunteer was residing in the endemic area and had had sandfly fever twelve years previously.

Examination of Sandflies.—Both fed and unfed sandflies were examined with the dark-ground illumination, and Dr. I. M. Puri, who was carrying out the entomological side of the investigation, kindly dissected out the gut and mouthpieces for the examinations. The results were in all cases negative for leptospiroæ and spirochætes. Pseudo-spirochætes of the blood were observed in certain of the fed sandflies.

The Spirochæte isolated in 1932.—A search was made to determine where this spirochæte came from, and the Peshawar tap-water was examined for leptospiroæ by Hindle's [5] coprozoic method of cultivation, with negative results. In addition, twenty-two rats were examined in 1932 and sixteen in 1933 with negative results.

SUMMARY AND CONCLUSIONS.

The short fevers in the Peshawar district:—

(i) Conform to the clinical description of sandfly fever.
(ii) The causal agent is not a leptospiroæ or other visible micro-organism.

Acknowledgments.—We desire to express our thanks to Colonel E. C. Hodgson, D.S.O., K.H.P., who initiated this investigation; to Colonel E. W. C. Bradfield, C.I.E., O.B.E., V.H.S., for his stimulating advice; to Lieutenant-Colonel I. M. Macrae, C.I.E., O.B.E., I.M.S., O.C., I.M.H., Peshawar, Lieutenant-Colonel T. H. Scott, D.S.O., M.C., R.A.M.C., O.C., B.M.H., Peshawar, and Lieutenant-Colonel A. C. Munro, I.M.S., O.C., I.M.H., Landi Kotal, for placing the clinical material at our disposal. We are also indebted to Dr. I. M. Puri, M.Sc.Punjab, Ph.D.Cantab., F.E.S., for assisting us with the examination of the sandflies.

REFERENCES.