(7) Experimental animals are immune to the disease.

We have no conclusive evidence to offer either for or against this statement but certain of our experiments would appear to suggest that at least in the case of monkeys it may be possible to reproduce the disease by inoculation.

The experiments conducted by us consisted in passaging human blood of sandfly fever cases into monkeys (Macacus rhesus), and monkey blood into humans and monkeys. For convenience these different experiments will be considered seriatim in the groups indicated above but, before doing so, it will be necessary to make a few remarks on certain fallacies which had to be guarded against and various factors which had to be taken into consideration in interpreting our results.

In a disease such as sandfly fever, where one is dependent for diagnosis on clinical signs and subjective symptoms, it is essential that these signs and symptoms should be typical and unequivocal before the assertion is made in any particular case that the condition induced as the result of a certain experiment is really sandfly fever. When one is dealing with animals the subjective symptoms are ruled out as unascertainable and diagnosis has to be made on clinical signs alone. This would be fairly satisfactory were they present in typical form and degree, but if we are dealing with an animal but mildly susceptible to the disease, it is likely that the signs as well as the symptoms will be modified in kind or degree or both. This is the less remarkable when we consider that in man himself, who is highly susceptible to sandfly fever, modified attacks may and do occur. It is on account of these considerations that we lay no claim actually to have transmitted the disease to monkeys but merely point out that some of our experiments suggest that such was the case, albeit the disease was mild in degree and the signs more evanescent than in the more highly susceptible human being.
Sandfly Fever on the Indian Frontier

The main factors on which we had to rely in making our diagnosis in the case of monkeys were the temperature and the incubation period and the latter had to be gauged by the former alone.

Two of us have had a very large experience of dealing with monkeys owing to the numbers used by us in experiments in connection with rabies, and we were therefore fully cognizant of the variations in temperature exhibited by monkeys and considered it essential to include an elaborate system of controls in our experiments. To do this we decided to take all the temperatures at the same hour each day, to take the temperatures of normal monkeys at the same time, and to employ control monkeys into which normal monkey and human bloods had been inoculated in the same amounts as the infected blood into the experimental monkeys. In addition, the temperatures of twenty normal monkeys were taken at the same time in order to establish a provisional mean normal temperature for the time of day and other conditions prevailing.

![Chart 3](https://example.com/chart3.png)

The continuous bold line in Chart 3 shows graphically the temperatures of twenty normal monkeys taken at the same time of day (4 p.m.) superimposed on the maximum temperatures of eighteen of the experimental monkeys used in this investigation. It will be seen that the variations of the normal temperature lie within one degree Fahrenheit. The average temperature of these twenty monkeys taken at the time stated was $102.3^\circ F$.

Experiments in which Human Blood of Sandfly Fever Cases was Inoculated into Monkeys.—The human blood specimens used in these experiments were in some cases sent from Landikotal or Peshawar and had therefore undergone a three-day exposure to the heat of the plains as well as a long railway journey. In other cases the specimens were freshly drawn blood from cases of sandfly fever induced in Kasauli by inoculation of the Peshawar bloods.

The only clinical indication available of successful infection of the monkeys was a rise of temperature which persisted for some time above the point considered the daily maximum under normal conditions. As a
control against the possible thermogenic effect of inoculation of blood alone certain controls were given inoculations of normal human blood. This was found actually to cause a slight rise of temperature and therefore cases in which supposedly infective bloods caused no greater rise than in these controls were not looked upon as positive results. This, however, does not rule out the possibility that their blood may have contained the virus, as certain of our results seemed to indicate this as a possibility. Thus, the blood of monkeys I and II in which the temperature rose only slightly above 103°F. (although this was higher than the normal human blood control monkeys) produced definite fever in monkeys IV, V, and VII and therefore presumably contained the virus.

Using, then, temperature as our criterion we found that out of 11 monkeys inoculated with the blood of sandfly fever cases 5 contracted definite fever, while 6 showed no fever or only a transient rise as compared with the control monkeys which received an inoculation of normal human blood.

In combined Chart 4 we have given the temperatures of these five monkeys which appeared to show a definite short fever of greater degree than would be caused by injection of normal human blood alone. The temperature chart of a monkey receiving an equal amount of normal human blood is also given for comparison, and, as a background to the charts, the provisional normal temperature for monkeys is given as a straight line at 102.3°F.

A glance at the individual charts will show that there is a distinct difference between the temperatures of monkeys VIII, IX, and X and that of the control monkey, but much less in the case of monkeys I and II.

The incubation period appears shorter than in the case of similar experiments with human beings but it has to be remembered that a much greater volume of blood relative to body weight was inoculated in the case of the monkeys.

Experiments in which Supposedly Infective Monkey's Blood was Inoculated into Monkeys.—Seven experiments were carried out in this series. In each case the monkey received into the loose tissues of the abdomen 5 cubic centimetres of freshly drawn blood of a monkey infected from a human case. Three out of the seven monkeys became infected judging by the criteria laid down by us for the animal experiments.

The charts of these monkeys are given in combined Chart 4 together with the chart of one of the control monkeys inoculated at the same time with the same amount of normal monkey blood.

It will be seen that there is an incubation period of three or four days and that the fever lasted for one to five days. The control monkey showed the relatively small rise of temperature which has been previously mentioned.

Experiments in which Supposedly Infective Monkey's Blood was Inoculated into Humans.—Three such experiments were carried out. In each
Chart 4.—The temperature charts of monkeys infected by inoculation of human and monkey infective blood.
case the monkey used had been infected by means of blood sent from Peshawar. In two of the cases 10 cubic centimetres of citrated-glycerinated blood, sent as such from Peshawar, was used for infecting the monkeys (monkeys IX and X). In the third case the infection of the monkey (monkey VIII) was brought about by 10 cubic centimetres of a filtrate through an Ll3 Chamberland candle of a mixture of citrated blood and citrated-glycerinated blood of the same individual. The filtration was performed in Kasauli after receipt of the bloods from Peshawar. All these monkeys became infected, as judged by the criteria for infection in

<table>
<thead>
<tr>
<th>NAME</th>
<th>DIAL-INFECTED FROM MONKEY NO VIII</th>
</tr>
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<tbody>
<tr>
<td>DAYS</td>
<td>1  2  3  4</td>
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<tr>
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<td>105 104 103 102 101 100 99 98</td>
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**SIGNS AND SYMPTOMS RECORDED**

- **HEADACHE**
- **VERTIGO**
- **MALAISE BEFORE FEVER**
- **GENERALISED PAINS IN BODY**
- **LOSS OF APPETITE**
- **BRADYKARDIA**
- **INJECTED CONJUNCTIVAE**

**NOTE**

The pulse rate of this volunteer, after convalescence, was 72 per minute.

**PULSE RATE** 76 68 64 68 68 68 68 68

**INCUBATION PERIOD:** Five days

**INFECTIVE MATERIAL.** 5 c.c. of fresh whole blood from infected monkey

Chart 5.—Sandfly fever induced in human volunteer by inoculation of infective monkey's blood.

animals we have already discussed. The temperature charts are shown on combined Chart 4. From each of these monkeys 5 cubic centimetres of freshly drawn whole blood was inoculated into a human volunteer in the loose tissues of the abdomen.

In two cases the results were negative as regards rise of temperature. One of them complained of headache and vertigo on the sixth day from the date of infection—temperature normal. The next day he complained of headache and pain at the site of inoculation. The symptoms cleared up on the third day. The other complained of headache and vertigo on the seventh day after infection—temperature 98·8° F., pulse 88—site of injection swollen and tender. Cleared up next day.
In the third case the volunteer contracted a definite, though modified, attack of sandfly fever as will be seen by a reference to Chart 5. Apart from the low temperature in this case the other signs and symptoms of sandfly fever were quite typically manifested. In recording our findings in the human cases we adopted, at the beginning of our experiments, a pro forma which included all the signs and symptoms of sandfly fever described by various observers or seen in our own experience. A part of this pro forma is given with Chart 5 completed for the patient in question as an indication of the completeness of the evidence that the case really was a mild case of sandfly fever.

In the pro forma the signs and symptoms which we consider most important in the diagnosis of sandfly fever are underlined and it will be seen that most of these were shown by the patient. The incubation period was five days and the duration of the fever three days. Malaise, headache and injected conjunctivae were marked symptoms. Extremely characteristic was the slow pulse which maintained a low level throughout the fever and for some days afterwards. The pulse rate of this individual has since returned to its normal rate of seventy-two per minute.

The fact that the attack was a comparatively mild one may have been due to the attenuation of the virus by passage through a monkey but, on the other hand, it may have been due to a relative immunity, for several of our volunteers who were inoculated with infective human blood complained of malaise and vague muscle aches yet did not actually develop fever and so have been put down by us as failures in infection.

To exclude the possibility that the inoculation into human subjects of normal monkey blood might produce the results recorded in these cases, two human controls were each inoculated in a manner similar to the volunteers with five cubic centimetres of freshly drawn normal monkey blood. The temperature, pulse and symptoms were recorded daily for one week. There was no rise of temperature and the only complaint was slight tenderness at the site of inoculation on the day following the operation.

(8) No visible causative agent has been demonstrated in the blood by direct microscopical or cultural examination.

In all the typical cases of sandfly fever induced by us in Kasauli the blood was very carefully examined microscopically. With the exception of one case which will be mentioned in the next section no visible microorganisms were encountered. The freshly drawn blood of six of the induced cases was cultured in Fletcher's medium. Six tubes of medium were inoculated from each case, about 0.75 cubic centimetre of blood per tube being used. In no case was any causative micro-organism demonstrated and the cultures remained sterile.

(9) A Leptospira has been isolated from cases of so-called sandfly fever.

As stated in the last section the bloods of all the cases of sandfly fever induced at Kasauli were carefully examined. In one stained blood slide
from volunteer No. 28 a single definite spirochetal organism was seen. Further slides taken from the same case failed to reveal any more organisms. Inoculation of blood into Fletcher's medium had been carried out at the same time at which the blood slide was made. These cultures remained sterile. It was thought that the most probable source of the single organism was the distilled water used in diluting the Leishman's stain used. A sample of the water in the bottle was centrifuged and the deposit stained and examined. Scrapings from the stopper of the bottle were similarly examined and the samples were also examined by dark-ground illumination. These examinations showed the presence of a variety of bacteria and, although no leptospira was noted, it is considered that the water was the probable source of the one specimen seen in the stained blood slide.

SUMMARY AND CONCLUSIONS.

(1) Blood specimens of sandfly fever cases from the Indian Frontier sent to Kasauli produced typical sandfly fever in human volunteers when inoculated subcutaneously.

(2) Fresh blood from a locally induced case in Kasauli produced typical sandfly fever in another volunteer when inoculated subcutaneously.

(3) Fresh whole blood, citrated whole blood, glycerinated whole blood, citrated glycerinated blood and filtrates of blood were all found capable of inducing the disease by subcutaneous inoculation.

(4) Inoculation of infective human blood appears to have induced sandfly fever in monkeys.

(5) Inoculation of infective monkey's blood appears to have induced a mild but definite attack of sandfly fever in a human volunteer.

(6) Inoculation of infective monkey's blood appears to have induced sandfly fever in monkeys.

(7) The bites of sandflies fed on cases of sandfly fever on the Indian Frontier appear to have induced attacks of sandfly fever when fed upon volunteers in Kasauli.

ACKNOWLEDGMENTS.

In conclusion, we desire to express our thanks to Colonel E. W. C. Bradfield, C.I.E., O.B.E., I.M.S., Assistant Director of Medical Services, Peshawar District, for the great interest taken and assistance given by him in this preliminary investigation. Our thanks are also due to Colonel I. M. Macrae, C.I.E., O.B.E., I.M.S., Officer Commanding, Indian Military Hospital, Peshawar, Lieutenant-Colonel A. Campbell Munro, M.D. (Glasg.), D.P.H., D.T.M. (Camb.), I.M.S., Officer Commanding, Indian Military Hospital, Landikotal, and Lieutenant-Colonel T. H. Scott, D.S.O., M.C., M.B., R.A.M.C., Officer Commanding, British Military Hospital, Peshawar, for putting at our disposal all the clinical material required in the investigation.
To Captain A. Sachs, M.B., R.A.M.C., we are greatly indebted for the laboratory facilities provided by him at the District Laboratory, Peshawar. It should be noted here, also, that it was due to investigations carried out by this officer in the previous year that the researches on sandfly fever were instituted by the Army medical authorities.

Dr. I. M. Puri, M.Sc.(Punjab), Ph.D.(Cantab.), F.E.S., who was working on the sandfly investigation under the Indian Research Fund Association, was of the greatest assistance to us and, by supplying us with laboratory-bred flies and arranging for the feeding of these on cases of sandfly fever, rendered possible the work on transmission by the bites of the flies.

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