On September 9th he showed me some peculiar "bodies" which he had found by this method in syphilitic chancre scrapings. In consequence, I have followed this method, and here describe the bodies I have seen while working with the jellies myself.

I make the jellies as follows:

Agar, 1 gram; sodium chloride, 0.5 gram; polychrome methylene blue, 4 c.cm.; distilled water, 100 c.cm.; boil, filter, and store in test tubes. When required for use, the jelly is melted and a small quantity is poured on to a slide to cover an area of about a square inch and allowed to cool and set.

The syphilitic material to be examined is obtained from a hard chancre; a drop of the scraping is mixed with an equal quantity of a 3 per cent. solution of sodium citrate in water, and placed on a cover-slip, which is inverted on to the surface of the set jelly. Or a hard, "shotty" syphilitic gland is punctured with a Bayly needle and the gland squeezed; after a minute the needle is withdrawn, and the material contained in it is expelled into the citrate solution as before.

On the jelly the cells, leucocytes, epithelial cells, lymphocytes, stain a general blue; the "bodies" stain

red, and this serves to distinguish them readily. I have examined 25 chancres and 22 glands by this method, and have found the bodies in every syphilitic case. They may be described as (1) extracellular, (2) intracellular. The former consist of two forms: (a) Oval bodies 2 to 10 microns in diameter, containing a granular nucleus and one or two chromatin dots (Fig. 1); (b) bodies varying in size from 6 to 15 microns, in which I can find no definite nucleus, but they are completely filled with granules sometimes in violent movement (Fig. 2). (2) The intracellular bodies may be divided into two classes also: (c) A red-staining mass, embedded within the cytoplasm of large cells, containing several definite round or pear-shaped chromatin structures (Fig. 3). Sometimes this mass appears to have burst when the chromatin structures are found free inside the cell cytoplasm (Fig. 4); (d) a large mononuclear cell containing similar red-staining masses (Fig. 5), which show within their interior either chromatin rods (Fig. 6), or a skein or tangle (Fig. 7) of chromatin threads. Apparently these intracellular bodies burst out of the cells, for the threads can sometimes be seen near, but outside, the cells attached to a common chromatin ring (Fig. 8).

In addition to the above I have seen certain other forms about 10 microns in diameter, which stain very deeply and

contain five to six chromatin granules. This form, which is free, frequently seems to divide into six daughters, each containing a chromatin granule. Occasionally in the glands, but frequently in the chancres, refractile bodies about a micron in size are found; each contains two chromatin dots. It is possible that this is but a phase, incompletely stained, of the free forms already described.

I am of the opinion that these "bodies" are parasites because of their peculiar staining reaction and because they develop while included within the cytoplasm of the cells of the chancres and glands. Up to the present I have only seen them in syphilitic subjects.

I beg to express my thanks to Mr. E. H. Ross for showing me the technique of the jelly method, and to the staff of the London Lock Hospital for permitting me to use the material obtained from their patients.

ON THE INFLUENCE OF METEOROLOGICAL CONDITIONS ON THE DEVELOPMENT OF TRYPANOSOMA RHODESIENSE IN GLOSSINA MORSITANS.

BY

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(Sixth Interim Report of the Luangwa Sleeping Sickness Commission, British South Africa Company.)

In a previous communication¹ various observations were recorded which appeared to indicate decisively that the developmental cycle of *T. rhodesiense* in *Glossina morsitans* is influenced to a marked degree by the meteorological conditions to which the flies are subjected. It is unnecessary to repeat in detail the experiments upon which this conclusion was based, but the evidence may be briefly summarized.

1. At Nawalia, in the Luangwa Valley, where a relatively high mean temperature was experienced during the greater part of the year (75–84° F.), *T. rhodesiense* was readily transmitted at laboratory temperatures by both "bred" and "wild" *Glossina morsitans*.

2. At Ngoa, on the Congo-Zambesi watershed, where, during the cold season of the year, the mean temperature was much lower (59–63° F.), we were unable to transmit the parasite by means of *Glossina morsitans*.

3. The trypanosome was readily transmitted at Ngoa during the cold season by flies which were kept in an incubator at a mean temperature of about 80° F.

4. Corroborative evidence in support of this contention was afforded by an analysis of the valley transmission experiments, which showed that the largest proportion of infective flies was obtained in the hottest season of the year. Further, in the Luangwa Valley a larger percentage of "wild" *Glossina morsitans* were found to be infective during the hot season; and lastly, the percentage of flies naturally infected was very much greater in the valley than on the plateau.

During the past months further experiments were completed which indicated in the most decisive manner that the development of *T. rhodesiense* in *Glossina morsitans* is directly dependent on the temperature to which the flies are subjected.

A further attempt to transmit the parasite by *Glossina morsitans* at laboratory temperature was commenced on July 24th; 160 "wild" flies, previously shown to be non-infective by feeding on a healthy monkey, were utilized. They were fed for three days on a heavily infected guinea-pig, and afterwards for thirty-seven days on healthy monkeys, none of which became infected.

In all, therefore, a total of 680 flies were used in six attempts to transmit the human trypanosome by *Glossina morsitans* on the Congo-Zambesi watershed at mean temperatures ranging from 59 to 65° F., without obtaining a single infective fly, whereas at Nawalia, in the Luangwa Valley, of 330 flies used in similar experiments, 6, and probably 10, became infective, at mean temperatures ranging from 75 to 84° F. Details of these experiments are given Tables I and II.

The incubator experiments described in the previous paper were repeated, but laboratory-bred flies were used instead of "wild" ones. Attention was drawn to the fact

TABLE I.—Synopsis of Transmission Experiments with *T. rhodesiense* and *Glossina morsitans*, carried out at Nawalia, Luangwa Valley, Northern Rhodesia.

No.	Date on which Started.	Season.	Number of Flies Used.	Variety of Flies Used.	Result.	Duration of Developmental Cycle: Days.	Mean Temperature during Developmental Cycle.	Absolute Maximum during Developmental Cycle.	Absolute Minimum during Developmental Cycle.	Relative Humidity per Cent.
1	Aug. 20, 1911	Dry (Commence- ment of rains ..)	26	Bred	Infection	13	° F. 75.1	° F. 90.0	° F. 60.5	35.0
2	Nov. 14, 1911		16	"	"	15	83.5	93.5	74.5	44.8
3	Nov. 14, 1911		57	Wild	"	11	83.8	93.5	74.5	44.8
4	Dec. 29, 1911	Rainy	20	Bred	Negative	—	78.2	87.1	72.8	74.5
5	Jan. 12, 1912	"	42	Wild	"	—	78.0	85.7	72.8	74.2
6	Jan. 12, 1912	"	42	"	"	—	78.0	85.7	72.8	74.2
7	Jan. 12, 1912	"	23	Bred	Infection	19	77.9	85.7	72.8	75.4
8	Feb. 13, 1912	"	104	Wild	"	25	77.3	86.0	71.0	66.5

In the cases of the unsuccessful experiments, the mean temperature and the relative humidity have been calculated for the first thirty days only.

TABLE II.—Synopsis of Transmission Experiments with *T. rhodesiense* and *Glossina morsitans*, carried out at Ngoa, Congo-Zambesi Watershed.

No.	Date on which Started.	Season.	Number of Flies Used.	Variety of Flies Used.	Result.	Duration of Developmental Cycle: Days.	Mean Temperature during Developmental Cycle.	Absolute Maximum during Developmental Cycle.	Absolute Minimum during Developmental Cycle.	Relative Humidity per Cent.
1	May 18, 1912	Dry	116	Wild	Negative	—	° F. 62.7	° F. 74.5	° F. 50.5	52.0
2	June 13, 1912	"	90	"	"	—	59.1	72.4	42.0	52.0
3	June 14, 1912	"	119	"	"	—	59.1	72.4	42.0	52.0
4	June 26, 1912	"	19	Bred	"	—	60.2	71.8	42.0	52.0
5	July 11, 1912	"	176	Wild	"	—	62.0	72.0	49.1	50.0
6	July 24, 1912	"	160	"	"	—	64.7	76.1	51.0	47.0

that the low temperatures obtaining at Ngoa in the cold season were very unfavourable to the pupation of *Glossina morsitans*; in fact, so slow was the process that, in spite of the large number of pupae at our disposal, we were unable to procure sufficient flies for experimental purposes. The difficulty was all the greater as many of those which did emerge were malformed, and quickly died. In order to obtain a sufficient number of "bred" flies for this experiment we resorted to the expedient of placing the pupae, some of which had been deposited over two months previously, in the incubator (85° F.). Within three or four days a large number of flies were procured.

The experiment was commenced on August 8th with 30 "bred" flies, to which were added 12 on the 9th, 11 on the 10th, and 3 on the 11th. These groups were fed for four, three, two, and one days respectively on a heavily infected guinea-pig, and afterwards on healthy monkeys. Parasites were found in the blood of the animal on which the flies of the fourth group were fed on the eighteenth day after the insects fed on the guinea-pig; so that, allowing five days for the incubation of the disease in the monkey, the fly was infective on the thirteenth day. The monkey on which the flies in Group 1 were fed became infected on the twenty-sixth day of the experiment, so that the duration of the developmental cycle of the parasite in the fly would be twenty-one days. As we cannot be certain that the infective fly fed on the infected guinea-pigs on each of the four days, the latent period in the insect may be anything from seventeen to twenty-one days. The animals on which Groups 2 and 3 were fed did not become infected.

It will be seen from Table III that the total number of flies used in the incubator experiments was 189, namely, 133 "wild" and 56 "bred." Of the "wild" flies 3 became infective, and of the "bred" 2, that is, 2.6 per cent. of the total number used. This figure is, however, hardly a fair estimate, as of the 133 "wild" flies only 78 were alive at the end of the first seven days of the experiment. This heavy mortality was probably due to the sudden change from the cold external air to the warm, dry atmosphere of the incubator. As a general rule, in our transmission experiments it was found that about 10 per cent. of the flies died during the first week. This was approximately the case in the incubator experiment in which "bred" flies were used, as, owing to the fact that the insects were hatched out in the incubator, they were not subjected to any sudden change of atmospheric conditions. We consider, therefore, that had the mortality of the flies in these two experiments been the customary 10 per cent. instead of over 40 per cent., the proportion of infective flies would be 3 of 90, instead of 3 of 133, or 3.3 per cent. instead of 2.2 per cent. This figure, 3.3, approximates closely to that obtained in the incubator experiment in which "bred" flies were used—namely, 3.5, and also to that obtained in the valley experiments—3.5.

The results of these series of experiments—namely, those carried out at Nawalia at laboratory temperatures 75–84° F., those made in the laboratory at Ngoa at from 59–65° F., and the incubator experiments at Ngoa 80.6–82.6° F.—show in a most conclusive manner that comparatively high temperatures—75–85° F.—are necessary

TABLE III.—Synopsis of Experiments to Transmit *T. rhodesiense* by Means of *Glossina morsitans* kept in Incubator.

No.	Date on which Started.	Season.	Number of Flies Used.	Variety of Flies Used.	Result.	Duration of Developmental Cycle: Days.	Mean Temperature during Developmental Cycle.	Absolute Maximum during Developmental Cycle.	Absolute Minimum during Developmental Cycle.	Relative Humidity per Cent.
1	June 30, 1912	Dry	61	Wild	Infection	14	° F. 80.6	° F. 87.8	° F. 74.5	36.0
2	July 1, 1912	"	72	"	"	13	80.6	87.8	74.55	36.0
3	Aug. 8, 1912	"	56	Bred	"	13 and 21	82.6	89.2	77.0	72.0

TABLE IV.—Synopsis of Experiments showing the Effect of Raising the Temperature on the Development of *T. rhodesiense* in *Glossina morsitans*.

No.	Date Started.	Season.	No. of Flies Used.	Variety of Flies.	Laboratory Conditions.				Date on which Flies were put in the Incubator.	No. of Flies Alive at the time they were first put in the Incubator.	Incubator Conditions.				Remarks.
					Mean Temperature.	Absolute Maximum.	Absolute Minimum.	Relative Humidity per Cent.			Mean Temperature.	Absolute Maximum.	Absolute Minimum.	Relative Humidity per Cent.	
1	June 13th, 1912	Dry	90	Wild	°F. 59.1	°F. 72.4	°F. 42.0	52.0	July 24th, 1912 (41st day)	42	°F. 85.0	°F. 90.5	°F. 77.0	36.0	Three flies infective on 48th day.
2	June 14th, 1912	"	119	"	59.1	72.4	42.0	52.0	August 14th, 1912 (61st day)	38	83.0	91.0	77.0	72.0	One fly became infective about the 72nd day.

for the completion of the developmental cycle of *Trypanosoma rhodesiense* in *Glossina morsitans*.

In our previous paper we discussed the question whether any climatic factor other than temperature might possibly exert some influence on the development of the trypanosome in the fly. We referred especially to the relative humidity of the atmosphere, as the most favourable season of the year in the Luangwa Valley for successful transmission experiments appeared to be that in which the relative humidity was extremely low. Owing to the fact that the data at our disposal were insufficient, we were unable to come to any definite conclusion.

In order to decide the point, the relative humidity of the atmosphere in the incubator in the "bred" fly experiment described above, was kept at from 70–72.5 per cent. As the incubation period of the parasite in the flies (thirteen and seventeen to twenty-one days respectively), and also the percentage of infective flies obtained (3.5 per cent.) were approximately the same as those in other experiments in which the relative humidity was very low, we can only conclude that this factor does not exert any appreciable influence on the developmental cycle of *T. rhodesiense* in *Glossina morsitans*.

The following experiments were devised with a view to ascertaining more definitely the influence of temperature on the development of the parasite.

EXPERIMENT 1.

Two batches of "wild" *Glossina morsitans* (Batch A consisting of 95, and Batch B of 119) in which the possibility of the presence of an infective fly had previously been excluded by feeding the insects on clean monkeys, were fed for three consecutive days on a guinea-pig infected with *T. rhodesiense*. After being starved for a day, each batch was fed on a healthy monkey until the fortieth day after the first feed on the infected animal. Neither of the monkeys became infected. Batch A, in which there were 42 flies still alive, was placed in the incubator, whilst Batch B, in which there were now 58 flies, was kept at laboratory temperature. The sudden change from the laboratory to the warm, dry air of the incubator proved very fatal to the flies in Batch A, and on the forty-third day only 6 were alive. From the forty-first to the forty-seventh day the flies in this batch were fed on a monkey, and from the forty-eighth day on a rat. The rat became infected on the fifty-third day, so that, allowing five days for the incubation of the disease in the animal, Batch A contained an infected fly on the forty-eighth day after the first feed on the infected guinea-pig, and eight days after being placed in the incubator. As the monkey died on the forty-seventh day, we are unable to state whether the fly became infective before the forty-eighth day. The four flies still alive on the fifty-third day were fed on four clean rats, and three of these became infected. The monkey on which Batch B was fed was still negative at the end of sixty days, when there were 38 flies alive.

EXPERIMENT 2.

This is really a continuation of the former experiment. The 38 flies in Batch B were placed in the incubator on the sixty-first day after the first feed on the infected guinea-pig, and were fed from the sixty-first to the seventy-fifth day on a healthy monkey (No. 443). Unfortunately the animal died on the seventy-sixth day, so that we were unable to determine with certainty whether any of the flies became infective. All the flies were dissected as they died, and one was found to harbour trypanosomes in the gut and salivary glands. Animals inoculated with the contents of these structures became infected with *T. rhodesiense*. As in our experience all flies in which salivary infection was observed were capable of infecting animals with the human trypanosome, we may assume that had the monkey (No. 443) lived a few days longer it would have been found to be infected.

In Experiment 1 the relative humidity of the air in the incubator was very low (36 per cent.), while in Experiment 2 the relative humidity was comparatively high

(72 per cent.). In addition to confirming the view that a relatively high temperature is essential to the completion of the developmental cycle of *T. rhodesiense* in *Glossina morsitans*, and that the relative humidity of the atmosphere is not an important factor, these experiments afford more definite information. It is apparent that the earlier stages of the development of the parasite in the fly can occur at comparatively low temperatures (60° F.), and that trypanosomes can persist in this stage for at least sixty days. It is obvious that the developmental cycle of the parasite is not complete, since the flies are non-infective, and inoculation of the gut contents into susceptible animals is followed by negative results. For the completion of the cycle it is necessary for the temperature to which the flies are subjected to be raised to a considerable extent (75–85° F.).

It is interesting to note that the flies in Batch A (Experiment 1) became infective eight days, possibly less, after being placed in the incubator. This is three days less than the shortest incubation period observed in any of our successful transmission experiments, a fact which supports the view that the developmental cycle of the parasite in the fly had proceeded to a certain point at laboratory temperature (60° F.) before the insects were subjected to the higher temperature (80° F.) of the incubator.

The facts brought to light in this investigation afford a satisfactory explanation of some observations which have hitherto appeared to be contradictory. Kleine² was unable to transmit *T. gambiense* by *Glossina morsitans* on the Victoria Nyanza (altitude 3,700 ft.), whereas Tauts³ was successful on Lake Tanganyika at a lower altitude (2,680 ft.). Although we were unable to transmit *T. rhodesiense* experimentally on the Congo-Zambesi watershed in the cold season, nevertheless we found that a certain percentage of wild flies were infective. This apparent discrepancy may be explained on the assumption that the flies became infective during the warm season, and that a certain number survived into the colder season of the year.

CONCLUSIONS.

1. The developmental cycle of *T. rhodesiense* in *Glossina morsitans* is to a marked degree influenced by the temperature to which the flies are subjected. High temperatures (75–85° F.) favour the development of the parasite, whilst low temperatures (60–70° F.) are unfavourable.

2. The first portion of the developmental cycle can proceed at the lower temperatures, but for its completion the higher temperatures are essential.

3. The parasites may persist in the fly at an incomplete stage of their development for at least sixty days under unfavourable climatic conditions.

4. These observations afford an adequate explanation of the extremely long latent periods of trypanosomes in *Glossina* which have occasionally been observed by various workers.

5. The relative humidity of the atmosphere has apparently no influence on the development of the trypanosome in *Glossina morsitans*.

Ngao, N. Rhodesia,
September 10th, 1912.

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