

*Experiments to Ascertain if Trypanosoma gambiense during its Development within Glossina palpalis is Infective.*

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It will be remembered that the injection of chopped-up tsetse flies (*Glossina morsitans*) a short time after feeding on an infected animal, did not give rise to nagana. As this was thought to be an interesting fact, and difficult of explanation, experiments were carried out on the same lines with *Trypanosoma gambiense* and *Glossina palpalis*.

If tsetse flies (*Glossina palpalis*) are fed upon an animal whose blood contains *Trypanosoma gambiense*, the trypanosomes can be found living within the intestines of some of the flies for several days after they were ingested. In a small percentage (0·5 to 2·0 per cent.) of flies so infected, active trypanosomes may be found swarming in their intestines on any day between the seventh and the fiftieth day, or even longer, after they have been fed upon an infected animal.

A series of experiments were undertaken, to ascertain if *Trypanosoma gambiense* retained its power of causing Sleeping Sickness when inoculated, subcutaneously, into monkeys, throughout its period of multiplication within the fly, especially during the interval of some 20 days in which the bites of infected flies are harmless. In some of these experiments Lake-shore flies were used; in others, laboratory-bred flies.

The flies were fed upon a monkey whose blood contained many trypanosomes. After a pre-determined time had elapsed, the wings and legs of the infected flies were cut off, and the bodies were either chopped up and brayed in a mortar with saline solution (0·8 per cent.), or the alimentary canal alone was removed. In many instances the gut was proved by microscopical examination to be heavily infected with trypanosomes before it was inoculated into a monkey. In flies thus found to be infected, the salivary glands also were carefully removed and washed thoroughly in several changes of normal saline solution. They were then broken up and injected, by means of a sterile syringe, into the subcutaneous tissue of the groin of a monkey.

The following table shows the result of the individual experiments as carried out with Lake-shore flies:—

Table I.—*Glossina palpalis* and *Trypanosoma gambiense*.

Date.	Expt. No.	No. of hours after infected feed.	No. of flies injected.	Result.	Incubation period in monkey.
1909.					
Mar. 10 .....	521	$\frac{1}{2}$ hour	2	+	8 days.
Jan. 6 .....	420	$\frac{3}{4}$ "	1	—	
" 6 .....	421	1 "	2	—	
Feb. 18 .....	518	1 "	2	—	
Mar. 19 .....	604	1 "	2	+	6 "
" 19 .....	605	$1\frac{1}{2}$ hours	2	+	6 "
Feb. 18 .....	519	2 "	2	—	
June 22 .....	1038	3 "	1	+	6 "
" 22 .....	1043	3 "	1	+	6 "
Mar. 29 .....	622	4 "	8	+	7 "
June 11 .....	984	4 "	5	+	8 "
" 16 .....	1009	4 "	5	+	6 "
" 22 .....	1039	6 "	1	+	6 "
" 22 .....	1044	6 "	1	+	6 "
" 22 .....	1045	7 "	1	+	9 "
" 22 .....	1041	8 "	1	+	9 "
" 22 .....	1046	8 "	1	+	10 "
" 22 .....	1042	9 "	1	+	9 "
" 22 .....	1047	9 "	1	+	9 "
" 26 .....	1154	16 "	1	—	
" 26 .....	1155	16 "	1	—	
" 26 .....	1156	17 "	1	+	13 "
" 26 .....	1157	17 "	1	—	
" 26 .....	1158	18 "	1	+	9 "
" 26 .....	1159	18 "	1	+	13 "
Mar. 20 .....	600	19 "	5	—	
June 26 .....	1161	19 "	1	—	
Mar. 4 .....	524	23 "	6	—	
" 5 .....	585	44 "	6	—	

*Remarks.*—Twenty-nine experiments were carried out with Lake-shore flies and *Trypanosoma gambiense*. Eighteen of these experiments yielded a positive result, and were almost uniformly successful up to 18 hours after the infected feed. It seemed to be immaterial whether the fly was introduced whole or whether the alimentary canal alone was injected. In several instances where the gut of the fly was examined in the fresh state, swarms of flagellates natural to the fly, i.e. *Trypanosoma grayi*, were found, but their presence did not appear to modify the virulence of *Trypanosoma gambiense*.

The following table shows the result of the experiments as carried out with laboratory-bred flies:—

Table II.—*Glossina palpalis* and *Trypanosoma gambiense*.

Date.	Expt. No.	No. of hours or days after infected feed.	No. of flies injected.	Result.	Incubation period in monkey.
1909.					
Aug. 24 .....	1545	1 hour	1	+	7 days
" 24 .....	1546	2 hours	1	+	7 "
Feb. 18 .....	520	4 "	2	—	
Aug. 24 .....	1547	4 "	1	+	7 "
Sept. 14 .....	1681	1 day	3	—	
" 23 .....	1756	2 days	3	+	12 "
" 26 .....	1723	3 "	15	—	
Oct. 2 .....	1713	4 "	10	—	
" 3 .....	1783	5 "	11	—	
" 4 .....	1752	6 "	10	—	
*Sept. 28 .....	1721	7 "	1	—	
*Nov. 16 .....	1906	8 "	2	—	
Oct. 7 .....	1788	9 "	10	—	
*Nov. 17 .....	1909	9 "	1	—	
Oct. 8 .....	1795	10 "	8	—	
" 9 .....	1799	10 "	8	—	
*Nov. 18 .....	1911	10 "	1	—	
* " 19 .....	1913	11 "	2	—	
* " 29 .....	1923	11 "	1	—	
Oct. 11 .....	1807	13 "	5	—	
Nov. 26 .....	1864	14 "	30	—	
*Nov. 15 .....	1905	14 "	2	—	
*Oct. 3 .....	1781	15 "	1	—	
" 27 .....	1865	15 "	12	—	
" 19 .....	1838	16 "	34	—	
" 29 .....	1872	17 "	12	—	
*Dec. 13 .....	1939	17 "	1	—	
Oct. 30 .....	1873	18 "	12	—	
*Dec. 14 .....	1940	18 "	2	—	
Nov. 1 .....	1877	20 "	12	—	
* " 16 .....	1951	20 "	1	—	
Nov. 2 .....	1879	21 "	20	—	
" 4 .....	1886	23 "	15	—	
Dec. 5 .....	1887	24 "	10	—	
* " 20 .....	1958	24 "	1	+	8 "
" 6 .....	1889	25 "	22	—	
*Sept. 28 .....	1749	28 "	1	—	
* " 29 .....	1759	28 "	1	+	11 "
*Nov. 9 .....	1866	30 "	1	+	12 "
" 4 .....	1884	34 "	1	—	
* " 11 .....	1897	36 "	2	+	5 "
* " 16 .....	1907	37 "	1	—	
* " 4 .....	1885	42 "	1	—	
*Oct. 8 .....	1791	46 "	2	+	9 "
*Nov. 3 .....	1881	49 "	1	—	
* " 11 .....	1898	51 "	1	—	
* " 4 .....	1856	53 "	1	—	

*Remarks.*—In these experiments the intestines only of the infected flies were inoculated. In the experiments marked with an asterisk the gut was first proved, by microscopical examination, to contain swarms of living *Trypanosoma gambiense*, and then immediately injected into a monkey. It will be seen there are 47 experiments in which a total of 296 laboratory-bred flies were inoculated, subcutaneously, into monkeys at various intervals after the flies had fed upon blood infected with *Trypanosoma gambiense*. The injection into a monkey of a single fly, one, two, and four hours after an infected feed, caused Sleeping Sickness. Three flies inoculated two days after their infected feed produced a like result. Between the 2nd and the 24th days

after the flies had fed on infected blood, 249 of them were inoculated; the result was negative in every case, although 15 of these flies proved, by microscopical examination, to be swarming with living *Trypanosoma gambiense* at the time of inoculation. In 13 experiments, 14 flies proved, microscopically, to be infected, were inoculated, between the 24th and 53rd days after they had fed upon an infected animal, and in five of these a positive result was obtained.

The following table shows the result of subcutaneous injections of washed salivary glands dissected from laboratory-bred flies whose intestines contained swarms of *Trypanosoma gambiense* :—

Table III.—*Glossina palpalis* (Salivary Glands) and *Trypanosoma gambiense*.

Date.	Expt. No.	No. of days after infected feed.	No. of flies from which glands injected.	Result.	Incubation period in monkey.
1910.					
Apr. 14 .....	2376	2 days	3	—	
" 15 .....	2376	3 "	1	—	
" 17 .....	2376	6 "	2	—	
" 18 .....	2376	7 "	3	—	
" 22 .....	2394	8 "	2	—	
" 25 .....	2394	11 "	2	—	
Jan. 26 .....	2099	14 "	2	—	
" 31 .....	2114	21 "	1	—	
Feb. 2 .....	2114	23 "	1	—	
" 7 .....	2148	28 "	1	—	
" 9 .....	2148	30 "	1	—	
" 13 .....	2177	36 "	4	+	8 days.

*Remarks.*—The results of the microscopical examination of the salivary glands show that trypanosomes do not appear in the glands until about the 30th day. Out of 12 experiments 11 were negative, and one was positive. The glands from 19 infected flies were injected subcutaneously, between the 2nd and 30th days after the flies had fed upon an infected animal; the results were all negative. In the positive experiment the salivary glands from 4 infected flies were injected into a healthy monkey 36 days after the flies had fed on a monkey suffering from Sleeping Sickness, with the result that the former animal contracted the disease.

#### *Conclusions.*

1. *Trypanosoma gambiense* may retain their virulence, as ascertained by direct inoculation into susceptible animals, for a period of two days after they are ingested by *Glossina palpalis*.
2. After the trypanosomes have been within the gut of the fly for two days the power of infecting animals with Sleeping Sickness, when inoculated subcutaneously, is lost for a period of 22 days.
3. *Trypanosoma gambiense* regains the power of infecting by direct inoculation after it has been about 24 days within the intestine of the fly.
4. The number of days, during which the virulence of the trypanosomes contained in the fly is lost, roughly coincides with the time that the infected fly is incapable of transmitting Sleeping Sickness by biting susceptible animals.
5. There is some evidence that the salivary glands of the fly are invaded by virulent forms of the parasite 36 days after the fly has fed upon infected blood.