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Autor: Fairbairn, H. / Culwick, A.T.

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The Transmission of the Polymorphic Trypanosomes.

By H. FAIRBAIRN and A. T. CULWICK.

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The factors governing the transmission of the polymorphic trypanosomes by the tsetse fly have been the subject of much experimental work, but unfortunately the results have been largely contradictory. The purpose of this paper is to review some of this past work, pointing out the anomalies, and then to show how the contradictions can be resolved, and the facts fitted into a consistent picture of trypanosome transmission.

Literature.

The isolation of the infected flies. LLOYD, JOHNSON, YOUNG and MORRISON (1924), using the method of dissection described by LLOYD and JOHNSON (1924), were doubtful whether they were discovering all their T. brucei and T. gambiense infections. In one case three flagellates were found in the exudate at the cut ends of the glands, and careful examination by three workers could discover no forms through the unbroken walls of the glands. The infection was confirmed by staining. They quoted other examples of very light infections of the salivary glands which were missed by inspection, but where trypanosomes were found when the glands were teased up and stained.

DUKE (1933 a) found three instances of unilateral gland infections; and he also remarked that in light infections of the salivary glands—and such infections were commoner than was generally realised—the trypanosomes might be completely emptied through a wound such as was made when the salivary glands were cut in dissection, and the scanty parasites easily lost to view. Taylor (1932) and Burt (1946 a) failed to find trypanosomes in the salivary glands on the dissection of known infected flies; and it was for this reason that Taylor included the examination of the proventriculus in his dissections. The dissection of flies, therefore, is not a completely reliable method by which to determine the percentage of infected flies.

The length of the trypanosome cycle. Comparing the two methods, for the determination of the length of the trypanosome cycle

in the fly, (i) dissection of flies or (ii) feeding them on clean susceptible animals, Duke (1933 f) considered that the dissection method was the most accurate; but the method had to be directed specially to that end, and its proper application demanded large numbers of tsetse. He repeatedly stated that the trypanosome cycle was usually complete in 25-30 days (DUKE, 1928, 1931, 1933 f), and he dissected his flies as a rule on the 30th day (DUKE, 1930 b, 1933 b). Murgatroyd and Yorke (1937) agreed that it was exceptional for the trypanosome cycle to take more than thirty days; and Broom (1939) dissected his flies after 35 days, which, he said, gave sufficient time for the complete cycle of development of most species of trypanosomes. TAYLOR (1932) dissected his flies on or about the twenty first day, although he remarked that it was well known that some strains of T. gambiense took much longer to develop than others. He referred to his strain Gadau XV where salivary gland infections only appeared on the thirty-eighth day, and to strains Ayu 7 and 8 where it was only in dissections carried out from the forty-fifth day onwards that "100 per cent of the infections found were established in the salivary glands". Duke (1933 f) quoted the results of KLEINE and FISCHER, and said that their experiments were the most reliable evidence available of a cycle taking more than 40 days for completion at laboratory temperatures; and MURGATROYD and YORKE (1937) quoted VAN HOOF and HENRARD as saying that in most of their experiments the cycle was completed within 17-32 days, but in a few it was not completed until much later, 48-80 days.

In view of the proved differences in the length of cycle of various strains, it appears to us anomalous for research workers to determine beforehand when the cycle will be completed in any experiment.

The proportion of tsetse flies infected. Duke (1933 a) reviewing the results obtained by different observers by the dissection of "game tsetse", remarked: "It is not necessary for our purpose to attempt to distinguish between flies with infected glands and flies which merely have flagellates in the intestine and are consequently not infective. The important figure is the percentage of flies that contain established and developing flagellates", and from this review he drew conclusions about the relative transmissibility of T. rhodesiense and T. gambiense. LLOYD (1930) classified the infections encountered in his experiments as (a) light in the gut and likely to die out (b) heavy in the gut and likely to mature and (c) mature with salivary glands infected. Presumably his class (b) included all proventricular infections. In the dissection of his flies TAYLOR (1932) included the examination of the proventriculus as

a routine, as "it provides a valuable additional means of detecting maturing infections and is never involved in transitory gut infections". Duke (1933 f), however, said that Taylor had yet to prove that every fly with an infected proventriculus would, if it lived long enough, eventually develop an infection of its glands; and he, Duke, believed that in all but exceptionally transmissible strains there would always be a certain number of infected flies (i.e. with flagellates in the gut) in which no matter how long they lived, the glands never would become infected.

Murgatroyd and Yorke (1937) also criticised Taylor's results. From a review of their own and others' work they concluded that in only about 20 per cent of cases in which the proventriculus was involved were the salivary glands also invaded, and that this could not be explained on the hypothesis that the flies which exhibited only a "gut + proventriculus" infection had not lived long enough for the trypanosomes to reach the salivary glands. In their experiments establishment of the trypanosomes in the extraperitrophic space implied that in all probability the infection would reach the proventriculus, but proventricular infection did not necessarily imply that the salivary glands would eventually become infected, as suggested by Taylor. Van Hoof, Henrard and Peel (1937 c) and Burtt (1946 c) also came to this conclusion.

We consider that the percentage of infected flies should be calculated only on those flies in which the salivary glands have been invaded.

The effect of temperature. Kinghorn and Yorke (1913) showed that at a temperature of 60° F (15.5° C) the development of *T. rhodesiense* in *G. morsitans* proceeded up to a point, that the trypanosomes could persist in this stage for at least sixty days without the salivary glands being invaded, and that it was only when the flies were placed in an incubator at 80° F (26.7° C) that the salivary glands were invaded and the cycle was completed. They also compared transmission experiments in the Luangwa valley at laboratory temperatures of 75° F-84° F (23.9° C-28.9° C) with those in the laboratory at Ngoa at temperatures of 59° F-65° F (15° C-18.3° C), and with experiments at Ngoa in which the flies were kept in an incubator at 80.6° F-82.6° F (27.0° C-28.1° C), and they concluded that comparatively high temperatures, 75° F-85° F (23.9° C-29.4° C) were necessary for the completion of the developmental cycle of *T. rhodesiense* in *G. morsitans*.

DUKE (1928) doubted whether the seasonal differences in any given area would be sufficiently pronounced to produce a similar effect. LLOYD (1930) incubated flies, *G. tachinoides*, at 29.4° C-36.1° C "usually for a few days only, at the time of their infecting feed",

and he obtained greatly increased transmission rates of two strains of T. brucei. He was certain that there was an optimum temperature favouring development, and a critical temperature both above and below it. But he was unable to repeat his results; and he came to the conclusion that the factors which influenced the rate of infection in the tsetse by trypanosomes were so many that it was impossible to estimate with any degree of accuracy the transmissibility of any particular strain. DUKE (1930 c) in discussing LLOYD's work stated that the temperature factor was not important at Entebbe, though he later reported (DUKE, 1933 e) that he also had obtained greatly increased transmission rates of T. brucei, T. gambiense and T. rhodesiense by incubating G. palpalis at 95° F-98° F (35° C-36.7° C). TAYLOR (1932) maintained G. tachinoides at 37° C (97.6° F) for varying lengths of time during the four days that they were feeding on animals infected with T. gambiense. He tested twenty-six strains, and in every case he obtained a greatly increased infection rate (though his highest results could not be repeated). He also stated that prolonged incubation at 37° C after the concluding infecting meal had the effect of accelerating development of T. gambiense in the tsetse but without raising the infection rate further. He suggested that the temperatures at which experimental flies were infected in the laboratory were excessively low. In certain of his strains the salivary glands had not been invaded by the twentieth to the twenty-third days, and he thought it significant that these strains were tested at the coldest time of the year, when normal development in the tsetse was considerably retarded. Van Hoof and Henrard (1934) said that the length of the cycle through Glossina in the Leopoldville experiments showed that it was shorter than in Entebbe and probably longer than in Nigeria; and MURGATROYD and YORKE (1937) referring to this paper remarked that it was interesting to note that the experiments in which the authors obtained the most prolonged developmental cycles were performed during the cold season of the year. (Despite these observations, workers have largely ignored the effect on the infection rate and the length of the trypanosome cycle of the temperature at which experimental flies are kept.) Finally, BURTT (1946 a) compared flies which had emerged from pupae incubated at 30° C, with flies which had emerged from pupae kept at normal laboratory temperatures, the flies themselves being maintained at laboratory temperatures. He found that in the flies which had emerged from incubated pupae (a) there was not a single failure to secure a cyclical transmission, (b) there was a significant increase in the transmission rate and (c) there was a significant shortening of the length of the trypanosome cycle in the fly.

The fly or the trypanosome. Muriel Robertson (1912) considered that, given reasonably favourable conditions of temperature and moisture, it was the strain of trypanosomes and not the fly that within a relatively wide range played the deciding rôle in limiting the number of infected glossina. Duke (1928) said that the tsetse itself was neutral, and that if the appropriate forms of the parasite were taken up by the fly they would develop; but later (Duke, 1930 b) he wrote "the weight of experimental evidence tends rather to show that in only a comparatively small percentage of tsetse can any development of trypanosomes take place, this percentage varying according to the adaptability possessed by the strain"; and he said that "the view that the trypanosome is the variable factor rather than the fly seems to me to be based on sound reasoning". Finally (Duke, 1933 a), he considered that certain individuals among the tsetse population were specially fitted to act as intermediate hosts of the pathogenic trypanosomes. VAN HOOF, HENRARD and PEEL (1937 a) compared the transmission of T. gambiense by a strain of G. palpalis which they had bred for many years in their laboratory, with flies which had hatched from pupae collected in the bush, and found no difference. TAYLOR (1932) considered that the effect of high temperature during the infecting meal could be regarded as partly due to a simple acceleration of the multiplication by fission of the trypanosomes taken up in the infecting meal, and partly to a reduction in the mortality which always followed the sudden change of conditions undergone by the blood trypanosomes. Dissection of incubated tsetse during the first few days following infection showed massive gut infections, very much heavier than those in the control series kept at room temperature. Duke (1933 e) criticized Taylor's view, and considered the action of temperature was mainly due to the effect produced on the vital processes of the tsetse, and, he went on, "we must assume that for the completion of the cycle in Glossina a temperature above a certain minimum is necessary, whatever the species of tsetse".

The sex of the fly. Duke (1930 a) examined his records of the transmission of T. gambiense and T. rhodesiense by G. palpalis and found that of 12,737 male flies, 581 contained "flagellates-developmental forms", and that 178 of the latter had salivary gland infections, the corresponding figures for the female flies being 11,727, 656 and 183 respectively. There was no significant difference between the two sexes. Later he had the data reexamined (Duke, 1933 d), basing his infection rate on all flies showing developmental forms in the gut (and this presumably included proven-

tricular forms), and found a significantly higher infection rate in the female flies. However, as Burtt (1946 b) pointed out, if the infection rate was calculated on the number of flies with infections of the salivary glands, there was not a statistically significant difference between the sexes. Corson (1935) reported, without comment, a transmission of T. rhodesiense by G. morsitans in which twenty-one out of forty male flies had salivary gland infections, but only five out of thirty-three females. Burtt (1946 b) found that there was a significantly greater infection rate with T. rhodesiense in male than in female G. morsitans, and that this sex ratio did not differ significantly in the two categories of pupal treatment (i.e. whether incubated or kept at laboratory temperature). VAN HOOF (1947) transmitting T. gambiense by G. palpalis, also found a greater frequency of salivary gland infections in male than in female flies—32.4 per cent males against 21.4 per cent females.

The infecting meal. Duke (1935) considered that a preliminary feed or two by newly hatched flies on clean blood before they fed on an infected animal made no apparent difference to the eventual number of infected flies found in dissection. Van Hoof, Henrard and Peel (1937 b), however, denied this and maintained that a preliminary feed on clean blood reduced the infection rate in the flies. (It may be mentioned that a statistical analysis of the figures quoted in the Tropical Diseases Bulletin shows that the results from the monkey were highly significant, but that the results from the guinea-pig were not.) Muriel Robertson (1912, 1913) considered that a feed on clean blood shortly after the infecting feed reduced the percentage of flies infected, by "the clearing out of the trypanosomes by the new feed"; but to obtain uniformity in experimental procedure with newly hatched flies, she recommended that the infecting feed should be the first one.

Experimental.

For some years experiments have been conducted in this laboratory to determine the effect of temperature and humidity on the transmission of *T. rhodesiense* by *G. morsitans*.

Pupae of *G. morsitans* are collected in the bush by natives and are carried some miles to our headman who buys them. (The collections were originally made in Kondoa-Irangi District, but since August, 1948, they have been done in Singida District.) The pupae are placed in a mosquito-net bag which is put into a perforated tin; and once a week the tin is carried to the nearest post-office 15-20 miles away and posted to us, arriving 3-4 days later.

During the period June, 1945, to December, 1948, we received 82,120 pupae from which 58,400 flies emerged. It is impossible to say how old the pupae are when they reach us ¹, but it will be appreciated that they are exposed to considerable variations in temperature after they have been collected.

On receipt, the pupae are placed in wide-mouthed bottles closed with mosquito-gauze. For the standard laboratory maintenance the bottles are inverted and stand on wire netting over water in a tray on the laboratory bench. When the pupae are being incubated, the bottles are inverted on the shelves of the incubator which contains a dish of water. Every morning, at the same hour, the flies which have emerged during the previous 24 hours are transferred to "Bruce" boxes, which are always kept in trays on wire netting over water, and exposed to the varying laboratory bench temperature.

KINGHORN and YORKE (1913) considered that humidity had no influence on the transmission rate, and our work confirms this view but shows that humidity has a marked influence on longevity.

After Burt (1946 b), working in this laboratory, found that there was a significant difference in the infection rate of the male and female flies, the sexes were kept seperate. Evidence soon accumulated to suggest that the length of the trypanosome cycle also differed in the two sexes. In many of Burtt's experiments, therefore, the flies may have been examined before the cycle was complete in one sex, which would, of course, have given a false picture of the relative infectibility of the two sexes.

From June, 1945, therefore, an experiment has consisted of two boxes of male flies (33 in a box) and two boxes of female flies (33 in a box). The flies emerged over the same period from the same batch of pupae, and were maintained under identical conditions. The newly emerged flies were always fed on an infected animal without a preliminary feed on clean blood. After the four boxes had been applied to the infected host for a period of 5-10 days, the male flies were fed daily on one "indicator" animal and the female flies on another. The indicator animals were sheep, rabbit or guinea-pigs for the Sheep line of trypanosome, Cercopithecus monkeys for the Monkey line, and monkeys, rabbits or guinea-pigs for the Antelope line; and the blood of these indicator

¹ When Professor R. Geigy, of Basle, visited this laboratory recently, he dissected a large number of pupae which had arrived that morning by post, and he found that about half of them were dead. He said that the remaining live pupae corresponded to the stage of development reached after 3 weeks by pupae of G. palpalis, which had been maintained at a constant temperature of 26° C in his laboratory.

	TA	BLI	Ε Ι.
Summary	of	the	Experiments.

	Tota	l	Successful transmission						
Pupal maintenance	Number of experiments	Number of flies used		Flies used	Flies isolated	Flies infected			
Male									
at laboratory temperatures	138	9091	99	6531	1828	116			
incubated at 280 C	41	2697	31	2037	686	56			
incubated at 30° C	57	2795	57	2795	1242	217			
Female									
at laboratory temperatures	136	8991	63	4214	636	17			
incubated at 280 C	40	2640	20	1320	249	7			
incubated at 30° C	_	Manhamon	38	1904	647	70			

animals was examined three times a week by stained thick films in order to determine when trypanosomes first appeared. The duration of the trypanosome cycle for each sex was thus the period from the first application to the host of a box of newly emerged flies to the date when trypanosomes were first found in the blood of the particular indicator animal less the average incubation period of the disease in that animal. From an examination of a large amount of data we calculated these average incubation periods as being 14 days in sheep, 13 days in guinea-pigs, 7 days in rabbits and 6 days in monkeys. When the indicator animal was found to be infected, the surviving flies were placed singly in bottles and were induced to probe on to glass slides whenever they were hungry, as described by Burtt (1946 c). By this technique even very lightly infected flies can easily be picked out—we have actually isolated flies which only extruded three or four metacyclic trypanosomes in a whole drop of saliva—while flies which had proventricular forms could be observed during their whole life, and the progress of the infection followed.

From March, 1943, until August, 1944, a proportion of the pupae had been incubated at 30° C, though the flies which emerged were maintained under standard laboratory conditions (Burtt, 1946 a). Although the sexes were mixed in the boxes, it has been possible to go through Burtt's data and to select experiments in which only male flies were infected, thus allowing us to calculate their infection rate and length of cycle. We selected other experiments in which both male and female flies were infected, and from these we were able to calculate the infection rate of the female flies.

However, owing to the very few experiments in which only female flies were infected sufficient data were not available to calculate the length of cycle in the female flies.

During the whole of 1948 the pupae were again incubated, but this time at 28° C, and the sexes were segregated.

We therefore have male and female flies which emerged from pupae exposed to varying temperatures on the laboratory bench, or from pupae incubated at 28° C or 30° C respectively, the flies in all cases being kept on the laboratory bench and exposed to varying temperatures. The numbers in each group are shown in Table I.

A preliminary statistical analysis showed that our host animals (whether sheep, monkey or antelope) had no significant effect on the infection rate or the length of cycle, and the data from the three lines have therefore been grouped.

The Infection Rate.

The infection rate can be expressed either as the percentage of the flies at the start of the experiment which become infected, or as the percentage infected of those surviving to be isolated. The former method suffers from the objection that the infection rate will depend on the length of the cycle, the longer the cycle the less the chance of isolating infected flies, since all the time flies are dying. Since, however, infected flies live as long as non-infected ones, the calculation of the infection rate as a percentage of the survivors obviates this difficulty and gives a figure independent of the cycle length.

In this paper the infection rate is calculated solely on salivary gland-infected flies as isolated by the probe technique, and is the percentage of such flies among those surviving to be isolated.

In a number of experiments no infected fly was isolated, although the infection of the indicator animal meant that at least one must have been present. The fly (or flies) had died during the incubation period of the indicator animal. This raises certain difficulties in dealing with the regression of infection on temperature. One is faced with three alternatives:

- a) Each experiment (i.e. two boxes of male or female flies) can be treated as an observation at a given temperature. In this case, the successful transmissions in which no fly was isolated give a value of zero for the infection rate, and are therefore not distinguished from the complete failures to transmit, which is biologically absurd.
- b) Secondly, as at least one infected fly must have been present originally, one can overcome this biological difficulty by treating

TABLE II a.

The number of male G. morsitans used, and the percentage infected, under various conditions of maintenance.

	Median laboratory temperature, ^o C, during the cycle													
Method of maintenance	Number of flies	21.9° and under	22.00-22.40	22.5° – 22.9° (and under)	23.0^{0} – 23.4^{0}	$23.5^{0} - 23.9^{0}$	24.00-24.40	24.50-24.90 (and over)	$25.0^{\circ} - 25.4^{\circ}$	25.50-25.90	26.00 – 26.40 (and over)	26.50-26.90	27.00 and over	Total
Pupae and flies at laboratory temperature	used isolated infected ⁰ / ₀ infected			330 120 6 5.0	746 217 8 3.7	660 146 1 0.7	654 164 10 6.1	858 261 18 6.9	1122 291 23 7.9	858 288 21 7.3	1303 341 29 8.5			6531 1828 116 6.35
Pupae incubated at 28º C Flies at laboratory temperature	used isolated infected ⁰ / ₀ infected	396 157 14 8.9	198 40 1 2.5	396 148 16 10.8	330 102 8 7.8	132 26 4 15.4	7	191 46 6 13.0						2037 686 56 8.2
Pupae incubated at 30° C Flies at laboratory temperature	used isolated infected ⁰ / ₀ infected			97 42 5 11.9	154 82 14 17.1	453 222 28 12.6	291 128 18 14.1	150 64 21 32.8	409 189 35 18.5	496 201 42 20.9	187 86 21 24.4	148 66 6 9.1	410 162 27 16.7	2795 1242 217 17.5

TABLE II b.

The number of female G. morsitans used, and the percentage infected, under various conditions of maintenance.

	Median laboratory temperature, ^o C, during the cycle													
Method Number of of maintenance flies		21.90 and under	$22.0^{0} - 22.4^{0}$	22.5° 22.9° (and under)	23.0^{0} 23.4^{0}	23.50 - 23.90	24.00 - 24.40	24.5° – 24.9° (and over)	$25.0^{0} - 25.4^{0}$	$25.5^{0} - 25.9^{0}$	26.0°-26.4° (and over)	$26.5^{\circ} - 26.9^{\circ}$	27.0º and over	Total
Pupae and flies	used			132	366	779	627	858	594	726	132			4214
at laboratory	isolated			16	63	123	74	149	48	142	21			636
temperature	infected			0	1	2	2	5	1	5	1			17
temperature	$^{0}/_{0}$ infected			0.0	1.6	1.6	1.7	3.4	2.1	3.5	4.8			2.7
Pupae incubated	used	330	132	330	198	66	66	198						1320
at 280 C	isolated	94	31	71	26	0	13	14						249
Flies at laboratory	infected	1	0	1	1	0	1	3						7
temperature	$^{0}/_{0}$ infected	1.1	0.0	1.4	3.8	0.0	7.7	21.4						2.8
Pupae incubated	used				134	96	263	146	217	383	213	101	351	1904
at 30° C	isolated				52	11	103	47	89	125	85	32	103	647
Flies at laboratory	infected				3	1	8	5	10	12	10	5	16	70
temperature	⁰ / ₀ infected				5.8	9.1	7.8	10.6	11.2	9.6	11.8	15.6	15.5	10.8

these experiments as though one fly had actually been isolated, adding one fly to those surviving, and calculating the infection rate in this way. There are obvious mathematical objections to this.

c) However, the complications introduced by these zero values can be overcome by treating all the experiments in a given temperature *group* as one observation of the infection rate. This is open to neither biological nor mathematical exception, and is therefore the method we have adopted here. The data, grouped in this way, are shown in Tables II a and II b.

In examining the regression of infection rate on temperature, we have used the mean median temperature to which the flies were exposed during the trypanosome cycle, grouped at intervals of 0.5° C. This is referred to throughout as the temperature of fly maintenance. The temperatures of pupal maintenance have already been described. The results of the analyses are shown in Fig. 1, where it will be seen that the infection rates of the sexes differ markedly.

Male Flies. With both the pupae and the male flies maintained at laboratory temperatures, there was a significant positive regression of infection rate on temperature (P < \cdot 001). The regression coefficient was \pm 1.85% \pm 0.49% per $^{\circ}$ C, the mean rate being 6.35 per cent at 24.6° C.

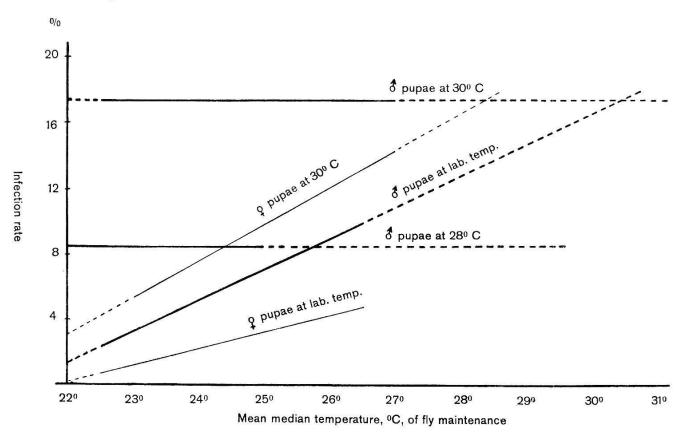


Fig. 1. The effect of temperature on infection rate.

When, however, the pupae had been incubated at 28° C, there was no significant regression, but the average infection rate was raised to 8.2 per cent (at 23.1° C). Similarly, when the pupae had been incubated at 30° C variation in the temperature of fly maintenance did not affect the infection rate, which was now 17.5 per cent (at 25.2° C), significantly greater than the value for flies from pupae incubated at 28° C (P < \cdot 01) 2 .

It should be noted that if the regression line for standard laboratory maintenance of pupae and flies is extrapolated, it reaches a value of 17.5 per cent infection rate at a temperature of 30.5° C.

These results show that it is the pupal temperature alone which influences infection rate; and that whatever the change may be which renders a male fly susceptible to infection, it is complete before the fly emerges.

Female Flies. With female flies, on the other hand, we found that the infection depended upon both the pupal temperature and the temperature of fly maintenance. Raising the pupal temperature raised the average infection rate, while raising the temperature of fly maintenance increased the infection rate for any given pupal temperature. The details are:

Pupal Maintenance	Linear Regression Coefficient	Mean at Temperature of
Laboratory temperatures	$+\ 1.05\%\ \pm\ 0.26\%$	2.7% 24.5° C
Incubated at 28° C $^{\circ}$ (?)	+ $8.15%$	2.8% 23.2° C
Incubated at 30° C	$+ 2.2 \% \pm 0.36\%$	10.8% 25.4° C

The change which renders a female fly susceptible to infection therefore takes place in two parts, one in the pupae and the other in the fly. There is no reason to think that this alteration is fundamentally different in the two sexes: only in the male the process is complete by the time the fly emerges from the pupae whereas in the female it is not.

The Length of the Trypanosome Cycle.

The length of the trypanosome cycle is influenced by both temperature and the sex of the fly.

² After this paper was written, pupae were incubated at 27° C. In 14 experiments 924 male flies were fed on infected sheep, 349 flies survived to be isolated and 21 were infected, an infection rate of 6.0 per cent.

³ This result for female flies from pupae incubated at 28°C is unreliable, being, calculated on only 7 flies, three of which came from one temperature group.

Male Flies. The effect of pupal temperature and the temperature of fly maintenance on the length of the cycle in male flies is shown in Fig. 2.

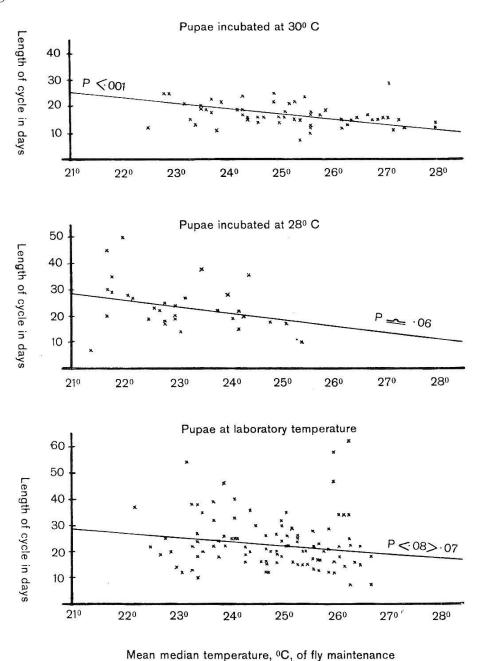


Fig. 2. The regression of cycle length on temperature of fly maintenance (male flies).

When the pupae are maintained at varying laboratory temperatures, the variance of the cycle length is large ($\sigma=\pm$ 19.8 days), and the regression of cycle length on temperature of fly maintenance is not significant (P < · 08 > · 07). By incubating the pupae at 28° C the variance in the cycle length is reduced ($\sigma=\pm$ 15.4 days), but the regression of cycle length on temperature still does not quite reach the 5% level of significance (P Ω · 09). When, how-

ever, the pupae are incubated at 30° C, the variance in the cycle length is small ($\sigma = \pm 8.5$ days), and the regression of cycle length on temperature of fly maintenance is highly significant (P < \cdot .0001).

The large variance of cycle length in flies from pupae maintained at laboratory temperatures is not surprising considering their varied history before arrival at Tinde. It appears, however, that when the pupae are incubated at a higher temperature, even though this is only during the very last stage of the pupal period, the heterogeneity in the emergent flies is much reduced.

The regression coefficients of cycle length on temperature of fly maintenance, referred to above, are as follows:

Pupal Maintenance	Linear Regression Coefficient
Laboratory temperatures	$-$ 1.58 \pm 0.89 days per $^{ m o}$ C
Incubated at 28° C	-2.37 ± 1.26 days per $^{ m o}$ C
Incubated at 30° C	-2.00 ± 0.36 days per _0 C

It would be erroneous to conclude that only in the last case did the temperature of fly maintenance influence the cycle. The three regression coefficients are of the same sign and of similar values; and the failure of the first two to reach significance may be due to the large variances involved.

Taking the values as a whole, the most probable interpretation is that, whatever the pupal temperature, an increase in the temperature of fly maintenance shortens the cycle in male flies.

The mean cycle lengths, reduced to a common temperature of fly maintenance of 24.6° C, and their corresponding standard errors, were calculated for all classes of pupal maintenance. They were:

Pupal Maintenance	Mean Cycle Length
Laboratory temperatures	$23.34\pm1.96~\mathrm{days}$
Incubated at 28° C	$19.92\pm2.52~\mathrm{days}$
Incubated at 30° C	$18.06\pm1.17~\mathrm{days}$

It will be seen that in flies from pupae incubated at 30° C the length of cycle was significantly shorter than in flies from pupae kept at laboratory temperatures. Flies from pupae incubated at 28° C had a cycle of an intermediate value.

Female Flies. As already stated, the data were insufficient to calculate the length of cycle in female flies from pupae incubated at 30° C. In flies from pupae kept at laboratory temperatures the regression coefficient of cycle length on temperature of fly maintenance was — 0.15 ± 0.59 days per °C; and in flies from pupae incubated at 28° C the regression coefficient was \pm 3.86 \pm 2.23

days per ^o C. These statistically non-significant values of *opposite* sign show that the temperature of fly maintenance had no influence on the length of the cycle in female flies.

The mean cycle lengths and their standard deviations were:

Pupal Maintenance	Mean Cycle Length	σ
Laboratory temperatures	$28.5\pm1.68~\mathrm{days}$	\pm 13.3 days
Incubated at 28° C	$22.8\pm2.29~\mathrm{days}$	\pm 10.2 days

By incubating the pupae at 28° C, the variance in the cycle lengths was also reduced as in the males; and there is a suggestion that the mean cycle length too was shortened (P < \cdot 07 > \cdot 06), although the difference does not reach the 5% level of significance.

Comparing male and female flies from pupae kept at laboratory temperatures, the cycle length is probably longer in the female flies $(P \Omega \cdot 05)$.

From these results we conclude that the length of the trypanosome cycle depends on conditions in the fly which are affected by temperature. In the female, the action of temperature, if any, is completed before the fly emerges from the pupa, but in the male it is not. Compared with the effect of temperature on the infection rate, the behaviour of the sexes has been reversed.

The Proventricular Forms.

When the indicator animal had been infected and the surviving flies were being isolated by the probe technique, a number of flies were found which only extruded proventricular forms. Thirty-three flies, all males, probed at least once again and lived long enough for the development of the infection to be followed.

Twenty-six flies came from pupae kept at laboratory temperatures, and in fourteen of them metacyclic trypanosomes eventually appeared in the salivary glands, after an average period of 11 \pm 2.0 days. The remaining twelve flies were still negative when they died from 6 to 22 days after probing for the first time.

The other seven flies emerged from pupae incubated at 28° C, and in six of them metacyclic trypanosomes appeared after an average period of 5 ± 1.3 days. The seventh fly was still negative when it died 26 days after its first probe.

The proportion of flies which developed a salivary gland infection was 53.8 per cent and 85.6 per cent respectively. The difference is not significant statistically. On the other hand, the period taken for metacyclic trypanosomes to appear in the salivary glands was significantly shorter $(P < \cdot 01)$ in flies which had emerged from

incubated pupae than in flies which had come from pupae kept at laboratory temperatures. The temperature of fly maintenance was examined and was found to have no effect on the time taken for metacyclic forms to appear in the salivary glands.

It is, therefore, the pupal temperature which determines the speed at which proventricular forms develop into metacyclic trypanosomes. There is a strong suggestion too that pupal temperature also determines the proportion of proventricular infections which will mature, though our evidence on this point is not conclusive.

The Incidence of T. rhodesiense in Western Tanganyika Territory.

Are these laboratory conclusions on the effect of pupal temperature borne out by our knowledge of the incidence of Rhodesian Sleeping Sickness in nature? Fairbairn (1948) has demonstrated a significant correlation between temperature and Sleeping Sickness cases one month later. (The cases were diagnosed in settlements which had all been made some years previously and were fly-free. Each settlement had a hospital with a competent microscopist, and it is considered that practically every case in the area was diagnosed). The number of new infections rose to a maximum at the end of the hot, dry season; and he concluded that this rise was probably due to the combined effects of occupation, leading men into the surrounding fly bush at that time of year, and of an increased proportion of infected flies encountered, owing to the high temperatures then prevailing.

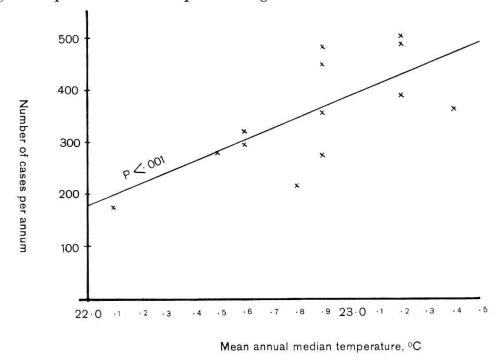


Fig. 3. The regression of the annual number of Sleeping Sickness cases in the Western morsitans Fly Belt on the mean annual median temperature at Tabora.

To eliminate the seasonal factor, the *annual* number of Sleeping Sickness cases in the Western *morsitans* Fly Belt was compared with the mean annual median temperature at Tabora, which is in the centre of the fly belt. The figures are given in Table III, and the result of the analysis is shown graphically in Fig. 3.

The regression of casese an temperature was found to be significant $< \cdot 001$). Reference to Fig. 1 will show that this result must be due primarily to the effect of temperature on the pupae, and only secondarily, and only in the case of the female, to the effect of temperature on the adult fly.

TABLE III.

The annual number of Sleeping Sickness cases diagnosed in the Western morsitans Fly Belt.

	1936	1937	1938	1939	1940	1941	1942	1943	1944	1945	1946	1947	1948
Number of Cases .	320	175	275	482	487	361	279	264	448	354	388	296	503
Mean Annual Median Temperature ⁰ C at													
Tabora	22.6	22.1	22.9	22.9	23,2	23.4	22.5	22,8	22.9	22.9	23.2	22.6	23.

Discussing his results Taylor (1932) wrote: "It is probable that in seeking the reason why sleeping sickness should be endemic in one area and epidemic in another, one should look for it rather in the nature of contact between the population and tsetse than in differences in the transmissibility of the *T. gambiense* strains in the two areas." Contact between man and fly is undoubtedly important in the epidemiology of Sleeping Sickness; but our results show that variations in temperature are also vital.

Discussion.

For the laboratory transmission of T, rhodesiense by G, morsitans we suggest that the pupae should be incubated at 30° C in an incubator containing a dish of water, and that the flies should be maintained at 28° C and approximately 80% relative humidity. Under these conditions about 17 per cent of both sexes should be infected (Fig. 1), with a trypanosome cycle in the male flies of about 12 days, and the longevity of the flies will be satisfactory.

It will be seen that under these conditions of maintenance there is no difference in the infection rate of the sexes. The difference reported by Burtt (1946 a) only becomes apparent when tempera-

tures of pupal and fly maintenance are considerably lower than those recommended.

If the regression line in Fig. 3 is extrapolated, it reaches a value of zero cases at a mean annual median temperature of 21.1° C. Similarly in Fig. 1, extrapolating the regression lines for "normal" male flies, "normal" female flies and for female flies from pupae incubated at 30° C gives zero infection rates at 21.2° C, 21.9° C and 20.5° C respectively. The agreement between these four temperatures is close.

Re-examining the experiments reported by Kinghorn and Yorke (1913), in which they compared the transmission rates in the Luangwa valley with those at Ngoa (a) at laboratory temperatures and (b) with incubated flies, it is of interest to note that the laboratory-bred flies, used in the latter experiment, had emerged from pupae which had also been incubated (page 206), the pupal temperature being 85° F (29.4° C). Taylor (1932) stated that his flies were obtained from "daily collections of wild puparia", and that the great majority of the experimental work was therefore confined to the dry season months of November to June. It is suggestive that the experiments yielding the high infection rate with strain Ayu V were undertaken during May to August, 1931, when some of the pupae at least had probably been exposed to high temperatures in the soil during April and May. LLOYD (1930) merely said that he used G. tachinoides "bred in the laboratory". A difference in the temperature to which the pupae had been exposed would explain his failure in Experiment liii to reproduce the high infection rate of Experiment xliv by only incubating his flies. NASH (1948), criticising Burtt's pupal work, remarked "it would seem probable that he was employing a temperature which would never occur in the pupal environment"; but NASH (1942, Table VI) has himself recorded mean maximum temperatures of $90.3^{\circ} \mathrm{\ F\ } (32.4^{\circ} \mathrm{\ C})$ at the edge, and $86.5^{\circ} \mathrm{\ F\ } (30.3^{\circ} \mathrm{\ C})$ in the centre of forest islands. Whether the exposure of pupae to an intermittent temperature of 32° C has the same effect as exposure to a constant temperature of 30° C remains to be worked out.

In view of Vanderplank's success (quoted by Burtt, 1946 a) in raising the infection rate of *T. congolense* in both *G. morsitans* and *G. swynnertoni* by increasing the pupal temperature, we suggest that the effect of raising the pupal temperature is probably a general one, and is not confined to a single species of fly or trypanosome.

TAYLOR (1932) stated that prolonged incubation of the flies after the concluding infection meal had the effect of shortening the trypanosome cycle. Averaging the temperature of maintenance, the five successful transmissions of LLOYD (1930) can be tabulated as follows:

Experiment Number	Temperature ⁰ C	Length of Cycle
xxxvi	26.1	20 days
xlv	28.2	20 days
liii	29.9	17 days
xliv	31.3	16 days
xxix	32.3	13 days

and statistical analysis shows that there is a very significant negative regression of cycle length on temperature. Our results, at least for male flies, are in agreement.

Duke (1930b) has recorded strains of T. gambiense in man which, he says, were not transmissible by G. palpalis. VAN HOOF and HENRARD (1934) commented on the differences in the length of the trypanosome cycle at Entebbe, Leopoldville and in Nigeria. DUKE (1930 c) considered that the temperature factor at Entebbe was not important; but he recorded (DUKE, 1933 e) that the mean monthly maximum dry bulb temperature in his laboratory varied from 74.5° F (23.6° C) in July to 83° F (28.3° C) in February, 1931. At Tinde Laboratory, on the other hand, the mean monthly maximum laboratory temperature varied from 26.9° C to 31.9° C, while the mean monthly median temperature was 21.7°C to 28.1°C (Burtt, 1947). The mean annual maximum screen temperature for 15 years recorded by the East African Meteorological Department was 25.7° C at Entebbe and 27.2° C at Kondoa-Irangi, the area from which our pupae came. It will be seen that our pupae and flies were exposed to higher temperatures than those of Duke. Both the pupal and fly maintenance temperatures Duke used were low, which we have shown to favour a long cycle. By his insistence on dissecting his flies at 25 to 30 days, Duke would have missed all transmissions with cycles of 35-80 days which TAYLOR (1932), VAN HOOF and HENRARD (1934) and ourselves have shown to be common. Fairbairn and Burtt (1946) found even at Tinde Laboratory that there were periods of two months' and three months' duration in the rains and in the cool, dry season, when it was difficult to secure cyclical transmissions by G. morsitans; and had their flies been dissected at 30 days (instead of being isolated after an indicator animal was found to be infected), they might have been tempted to class their strains as being non-transmissible.

The host animal, in some cases, appears to have an influence on the infection rate in the fly. Corson (1935, 1936, 1938) recorded

infection rates of 60 per cent, 33.3 per cent, 47 per cent and 52 per cent when G. morsitans were fed on the Southern reedbuck, Redunca arundinum infected with T. rhodesiense. VAN HOOF, HENRARD and PEEL (1937 a) and VAN HOOF (1947) state that the monkey, Cercocebus galeritus agilis, gives greatly increased infections of G. palpalis with T. gambiense and T. brucei. On the other hand, in our experiments reported here, there was no statistically significant difference in the infection rate of flies fed on sheep, Cercopithecus monkeys or Thomson's gazelle. Burtt (1946 a) says that the host animal has an influence, but it is difficult to decide whether his view is correct or not, since his experiments with different hosts were not always carried out at the same time of the year, and so the temperature conditions were not comparable. Fairbairn and Burtt (1946) reported that the Bohor reedbuck, Redunca redunca, gave high infections rates in G. morsitans, while DUKE (1933 g) stated that "change from one species of host animal to another may also be attended by great changes in the transmissibility of a strain". Both these statements, however, require to be confirmed, as they ignore the effect that the temperature of pupal and fly maintenance may have had.

The relative rôle of the trypanosome and of the fly in the transmission of the disease involves a number of variables, e.g. the temperature of pupal and fly maintenance, perhaps the nature of the infected host animal, the strain of the trypanosome and the species and sex of the fly. In the past, all these factors have not been taken fully into consideration at the same time, and unless they are it is impossible to compare the efficiency of, say, G. morsitans and G. palpalis as vectors of trypanosomiasis, as has been done by DUKE (1933 c, 1936); nor can one be sure that T. rhodesiense has a shorter cycle in the fly than T. gambiense (DUKE, 1933 f), or that T. rhodesiense is as a general rule more easily transmissible by G. palpalis than T. gambiense (Duke, 1933 g). Robertson (1912) believed that the strain of trypanosome was the deciding factor in infecting the tsetse, and she wrote (ROBERTSON, 1913) "Early infectivity (of the tsetse) is generally a character found in a strain which produces many positive flies." But both early infectivity (i.e. a short cycle) and a large number of positive flies can be produced merely by incubating the tsetse pupae at 30° C (Burtt, 1946 a). Taylor (1932) found massive gut infections in his incubated tsetse, very much heavier than those in the control series kept at room temperature; and he considered that the effect of temperature was to stimulate the trypanosome. But our experiments, conducted in parallel, show that the same trypanosome gave different infection rates, and different lengths of cycle, when ingested by male and female flies kept under identical conditions, and that in the males the temperature of fly maintenance had no effect on the infection rate, proving that it was the conditions in the fly which alone determined the infection rates and cycle lengths we observed.

Two conclusions can be drawn from our results.

1) Since raising the pupal temperature produces in the male fly an increase in infection rate *and* a decrease in the length of the cycle, it follows that a rise in pupal temperature allows male flies to become infected both more easily and more quickly.

NASH (1948) assumed that since in nature "the females live considerably longer than the males (they) must therefore be considered to be the more dangerous sex". Besides ignoring differences in the activity of the sexes, NASH's statement presumes that the two sexes do not differ markedly in their infection rates. We have, however, shown that over most of the temperature range the male is a far more efficient transmitter of disease than the female, and it is only at the highest temperatures that the latter becomes equally dangerous, temperatures at which NASH (1936) himself has shown that the longevity (presumably of both sexes) "is curtailed to a month or less".

2) Since infection rate and cycle length—which we have just seen are governed by conditions in the fly—are affected differently by temperature, it follows that at least two distinct processes in the fly are involved, one influencing infection rate, the other cycle length. The time taken for the proventricular forms to invade the salivary glands, which obviously influences cycle length, is controlled in male flies by pupal temperature alone. Length of cycle as a whole, however, in male flies is governed by both pupal and fly-maintenance temperatures. The process controlling cycle length must therefore itself be divisible into two; and so in the production of salivary gland infections there are at least three processes in the fly influenced by temperature.

Hoare (1931) has shown that the peritrophic membrane in the adult tsetse extends as an unbroken tube for some distance into the hind-gut; and in order that T. grayi should reach the extraperitrophic space of the midgut, the trypanosomes have to pass backwards inside the membrane to its termination in the hind-gut, then leave the lumen of the mebrane and migrate forwards in the extraperithrophic space to the midgut. Taylor (1932) and Yorke, Murgatroyd and Hawking (1933) agree that the polymorphic trypanosomes probably reach the extraperitrophic space in the same way. Robertson (1913) stated that a feed of clean blood reduced

the number of infected flies by the clearing out of the trypanosomes by the new feed; and YORKE, MURGATROYD and HAWKING (1933) state that after the first feed on a clean animal, trypanosomes are no longer found *inside* the peritrophic membrane, but that they may be found in great numbers multiplying in the space *outside* the membrane. For a tsetse to become infected the trypanosomes must reach the extraperitrophic space, and we have seen that the trypanosomes can be caused to reach this space in a large number of adult flies merely by incubating the pupae. The conclusion, therefore, is that the increase in pupal temperature has caused some change in the fly enabling the trypanosome to reach the extraperitrophic space more readily.

We do not know whether this change is a physiological one, influencing the intestinal secretions, or an anatomical one, affecting, for instance, the structure of the peritrophic membrane, or both. Hoare (1931) considered that the membrane was a continuous tube extending for some distance into the hind-gut. Wigglesworth (1929), on the other hand, described the peritrophic membrane as constantly present in the anterior segment of the midgut, but variable in the middle segment, where it might exist in the form of occasional shreds, or be absent altogether. In the light of our work, it is interesting to note that whereas Hoare was working with laboratory-bred flies in the comparative cool at Entebbe, Wigglesworth was at Sherifuri, Northern Nigeria, in April and May at the height of the hot dry-season, and in Sierra Leone in July.

Hoare (1931) states that Chatton and his collaborators found a peritrophic membrane in the larvae of *Drosophila* spp., and Dr. C. H. N. Jackson, of the Tsetse Research Department, Shinyanga, allows us to mention that he has found a fully-formed peritrophic membrane in the gut of the first instar larva of *G. swynnertoni*. Yorke, Murgatroyd and Hawking (1933) state that when the trypanosomes, developing in the extraperitrophic space, migrate forwards to the proventriculus, they return to the lumen of the proventriculus by passing through the fluid part of the membrane at the point where it is being secreted from the epithelial ridge. If this is the case, then pupal temperatures, by altering the secretory mechanism and therefore the consistency of this chitinous membrane at this point, might well influence the ability of the trypanosomes to reach the lumen of the proventriculus.

Finally, when the trypanosomes do reach the lumen of the proventriculus, they may remain there without proceeding to invade the salivary glands, as we have confirmed by using the probe technique. Their ability to develop further is, we have shown in

the case of the male fly, influenced by pupal temperature alone—and, again, this may be due to physiological or anatomical changes, or both.

Within the temperature range we have used, we can find no evidence of any direct effect of temperature on the trypanosome. The influence of the temperature of fly-maintenance on the cycle length in the male fly, and on the infection rate in the female, appears to be merely a continuation of a process begun in the pupa. Our results demonstrate conclusively that the fundamental factor governing the transmission of the polymorphic trypanosomes is the condition of the fly, which in turn is primarily determined by the temperature to which the pupa has been exposed.

Summary.

- 1. The literature on the transmission of the polymorphic trypanosomes is reviewed, and attention is drawn to the many contradictory statements therein.
- 2. An experimental procedure, and a statistical method of analysing the results, is described which eliminates many of the previous errors.
- 3. The Tinde strain of T. rhodesiense has been transmitted by G. morsitans, under known conditions of temperature, for three and a half years. The effect of temperature differs with the sex of the fly, and each sex must therefore be considered separately.
- 4. In male flies there is a very significant positive regression of infection rate on pupal temperature; but the infection rate is not influenced by the temperature to which the adult flies are exposed.
- 5. In female flies, the infection rate is governed both by the pupal temperature and the temperature of fly-maintenance.
- 6. As pupal temperature rises the variance of the length of the trypanosome cycle in male flies decreases. The mean length of the cycle is significantly shorter in male flies, which have emerged from pupae incubated at 30° C, than in flies which have come from pupae kept at laboratory temperatures. In the former flies also there is a very significant negative regression of cycle length on the temperature at which the adult flies have been maintained.
- 7. The data on the length of the trypanosome cycle in female flies were scanty, owing to the difficulties in securing transmissions by this sex, but there is evidence that the cycle is longer in the female than in the male fly at the temperatures we used. The length of cycle in the female fly is reduced by increasing the pupal temperature, but it is not influenced by the temperature to which the adult fly is exposed.

- 8. The temperature to which the pupae have been exposed also determines the time taken for the proventricular trypanosomes to invade the salivary glands of male flies.
 - 9. No data were available to study this question in female flies.
- 10. For easy laboratory transmission of T. rhodesiense by G. morsitans we recommend the incubation of the pupae at 30° C in an incubator containing a dish of water, and the maintenance of the adult flies at 28° C and approximately 80% relative humidity.
- 11. An examination of the number of Sleeping Sickness cases diagnosed in the Western *morsitans* Fly Belt of Tanganyika Territory shows that there is a significant positive regression of cases on the mean annual median temperature at Tabora. This result must be mainly due to the effect of temperature on the pupae.
- 12. The male fly is a far more efficient transmitter of the disease than the female, except at the highest temperatures.
- 13. It is pointed out that the many anomalous and contradictory statements in the literature can be explained and reconciled if the effects of varying pupal temperature are taken into consideration.
- 14. The fundamental factor governing transmission is the condition of the fly, which is primarily determined by pupal temperature.

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Zusammenfassung.

- 1. Es wird eine Uebersicht gegeben über die bisherigen Veröffentlichungen betreffend die Uebertragung polymorpher Trypanosomen und dabei auf mancherlei Widersprüche in den aufgestellten Behauptungen hingewiesen.
- 2. Es werden ein Weg für das experimentelle Vorgehen und eine statistische Methode zur Analyse der Resultate beschrieben, dank welchen manche der bisherigen Irrtümer vermieden werden können.
- 3. Der Tinde-Stamm von T. rhodesiense ist während 3½ Jahren unter bekannten Temperaturbedingungen mittels G. morsitans experimentell übertragen worden. Der Einfluß der Temperatur wirkt sich je nach dem Geschlecht der übertragenden Fliege verschieden aus, so daß jedes Geschlecht getrennt betrachtet werden muß.
- 4. Bei Verwendung männlicher Fliegen wird eine deutlich positive Regression der Infektionsrate in Relation zur Puppen-Temperatur festgestellt; dagegen wird die Infektionsrate durch die Temperatur, welcher die adulten Fliegen ausgesetzt werden, nicht beeinflußt.
- 5. Bei den weiblichen Fliegen wird die Infektionsrate beeinflußt von der Aufenthalts-Temperatur sowohl der Puppen als auch der Imagines.
- 6. Steigt die Puppen-Temperatur, so verkürzt sich bei den männlichen Fliegen die Variationsdauer des Trypanosomen-Zyklus. Die absolute Dauer des Zyklus ist deutlich kürzer bei männlichen Fliegen, welche aus Puppen geschlüpft sind, die in 30° C gehalten wurden, als bei solchen Fliegen, welche aus Puppen stammen, die sich in Laboratoriumstemperaturen entwickelten. Außerdem zeigt sich bei den Fliegen der ersten Kategorie auch eine negative Regression der Dauer des Zyklus entsprechend der Temperatur, in welcher die Fliegen nach dem Schlüpfen im Adultzustand gehalten wurden.
- 7. Bezüglich der Dauer des Trypanosomen-Zyklus bei weiblichen Fliegen konnten nur wenige Resultate erhalten werden, was auf die Schwierigkeit zurückzuführen ist, in diesem Geschlecht Speicheldrüseninfektionen zu erzielen. Jedoch geht aus den Ergebnissen mit Sicherheit hervor, daß bei allen verwendeten Temperaturen der Zyklus bei den Weibchen vergleichsweise länger dauert als bei den Männchen. Bei Erhöhung der Puppen-Temperaturen verkürzt sich in den weiblichen Fliegen die Dauer des Zyklus; er wird jedoch nicht beeinflußt durch die Aufenthalts-Temperatur der Imagines.
 - 8. Die Temperatur, in welcher männliche Puppen aufgezogen

worden sind, bestimmt die Zeit, welche von den Proventrikel-Formen der Trypanosomen benötigt wird, um in die Speicheldrüsen vorzudringen.

- 9. Für die weiblichen Fliegen konnten über den letzten Punkt keine Angaben erzielt werden.
- 10. Für sichere Laboratoriums-Infektion von G. morsitans mit T. rhodesiense empfehlen die Autoren, die Puppen bei 30° C in einem mit einer Wasserschale versehenen Brutschrank zu halten. Den Imagines sagt die Haltung in 28° C und in einer relativen Luftfeuchtigkeit von ungefähr 80% am besten zu.
- 11. Eine Ueberprüfung der Häufigkeit der Schlafkrankheitsfälle, welche im westlichen Morsitans-Fliegen-Gürtel von Tanganyika diagnostiziert worden sind, führt zur Feststellung eines deutlich positiven Rückganges der Fälle in Relation zur mittleren Jahrestemperatur in Tabora. Dieses Resultat muß wohl in erster Linie mit dem Temperatureffekt auf die Puppen in Zusammenhang gebracht werden.
- 12. Die männliche Glossine ist ein wirksamerer Ueberträger der Schlafkrankheit als das Weibchen, ausgenommen bei besonders hohen Temperaturen.
- 13. Die Autoren weisen darauf hin, daß wohl manche sich widersprechende Angaben in der Literatur aufgeklärt und darauf zurückgeführt werden können, daß mit wechselnden Puppen-Temperaturen gearbeitet worden ist.
- 14. Der Hauptfaktor, welcher die Uebertragung beherrscht, ist die Disposition der Fliege, die ihrerseits primär von der Puppen-Temperatur bestimmt wird.

Résumé.

- 1º Les auteurs passent en revue la bibliographie concernant la transmission des trypanosomes polymorphiques et attirent l'attention sur les nombreuses contradictions qui se trouvent dans ces conclusions.
- 2º Ils décrivent ensuite un procédé expérimental et une méthode statistique pour analyser les résultats qui permettront d'éviter bien des erreurs antérieures.
- 3º La souche Tinde de T. rhodesiense a été transmise expérimentalement pendant trois ans et demi par G. morsitans dans des conditions de températures connues. L'effet de la température diffère selon le sexe de la mouche transmetteuse, de sorte qu'il faut considérer chaque sexe séparément.
- 4º Chez les mouches mâles on note une très nette regression du taux d'infection en relation avec la température pupale; mais le taux d'infection n'est pas influencé par la température à laquelle sont exposées les mouches adultes.

- 5º Chez les mouches femelles le taux d'infection est influencé par la température de maintien appliquée aux pupes aussi bien qu'aux imagos.
- 6º Lorsque la température pupale monte, la durée du cycle trypanosomial se raccourcit chez les mouches mâles. La durée absolue du cycle est nettement plus courte chez des mouches mâles écloses de pupes maintenues à 30° C., que chez des mouches sortant de pupes élevées dans les températures du laboratoire. En outre, on constate chez les mouches de la première catégorie également une très nette regression négative de la durée du cycle en relation avec la température dans laquelle les mouches ont été maintenues à l'état adulte.
- 7º Les dates obtenues au sujet de la durée du cycle trypanosomial chez les mouches femelles sont plus rares, ce qui s'explique par la difficulté d'obtenir des transmissions dans ce sexe. Mais il en résulte toutefois clairement que dans les températures appliquées le cycle est plus long chez la mouche femelle que chez la mouche mâle. La durée du cycle se réduit chez la femelle lorsqu'on élève la température pupale, mais, par contre, la durée n'est pas influencée par la température à laquelle est exposée la mouche adulte.
- 8° La température à laquelle des pupes mâles ont été exposées, détermine aussi le temps que les formes proventriculaires des trypanosomes nécessitent pour envahir les glandes salivaires.
 - 9° Ce dernier point n'a pas été étudié chez les mouches femelles.
- 10° Pour la transmission aisée de T. rhodesiense par G. morsitans au laboratoire, les auteurs recommandent d'élever les pupes à 30° C. dans une étuve contenant un bassin d'eau et de maintenir les imagos à 28° C. et dans une humidité relative d'environ 80 %.
- 11º Lorsqu'on examine la fréquence des cas de maladie du sommeil diagnosé dans le morsitans fly belt oriental de Tanganyika, on constate qu'il y a une regression positive très nette des cas en relation avec la température annuelle moyenne de Tabora. Ce résultat doit être mis en rapport principalement avec l'effet de la température sur les pupes.
- 12º La Glossine mâle est un transmetteur plus efficace de la maladie du sommeil, sauf à des températures très élevées.
- 13. Les auteurs font remarquer que beaucoup de contradictions rencontrées dans la littérature peuvent sans doute être éclaircies et ramenées au fait que les auteurs ont travaillé avec des températures pupales variantes.
- 14º Le principal facteur qui prédomine la transmission, réside dans la disposition de la mouche, qui est influencée primairement par la température pupale.