IV—Climate and Tsetse Flies: Laboratory Studies upon *Glossina submorsitans* and *tachinoides*

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I—INTRODUCTION AND METHODS

Introduction—It is probably true that wild populations of *Glossina* have been more studied than populations of any other insect. Nearly all this work has been based on fly-counts, and it is beyond dispute that this method has given results of great value. But the fly-count is open to objection, for the number of flies caught is determined partly by the population (which we wish to measure), but also to an unknown extent by the activity of the insects at the time when the count is made. The effect of these two factors cannot at present be distinguished, and it is not likely that any improvement in the technique of the fly-count will enable the distinction to be made. All the conclusions that are based on fly-counts (and they are many and far reaching) are therefore to some extent speculative. It appears therefore that some entirely new way of studying the *Glossina* population is required.

In this paper it is suggested that the effect of climate upon *Glossina* is capable of study and analysis in the laboratory. Let it be admitted that the conditions are artificial, also that the authors have limited themselves to temperature and humidity, whereas in nature there are many climatic factors which influence the fly. In answer it is claimed that the methods employed justify themselves, and that if they were more widely used our knowledge of these insects would soon rest on a more solid base.

Several of the earlier workers on *Glossina* realized the possibility that the insect's climatic limits might be capable of study in the laboratory (see, for instance, ROUBAUD, 1909, *a*, *b*, *c*, 1910 ; MACFIE, 1912 ; STUHLMANN, 1907). But they had little technical equipment and an insufficiency of flies for their purpose. Since those days the experimental method seems to have fallen into disuse.

Entomologists who work upon tsetse flies are principally engaged in endeavouring to understand the seasonal and geographical fluctuations in their numbers, in devising methods of control, and in attempting to evaluate the effect of these methods. Such work relates to populations of wild flies. But there is a secondary group of problems, which may become more and more important ; they centre round the maintenance of a supply of clean flies in the laboratory at all times of year, for purposes of transmission, etc. It is thought that the facts here recorded will be of value to those who wish to carry out work of this nature. Our work was much hampered because so little is recorded about the biology of these insects in the laboratory.

The work here described was performed at the laboratory of the Tsetse Investigation at Gadau in the north of Nigeria (12° N., 10° E.). Gadau is only about 15 miles from Sherifuri, where LLOYD, JOHNSON, and others worked for several years, and there is no essential difference in vegetation, altitude, or climate between the two places. It is a matter for congratulation that the information from the field should be so complete, and the laboratory accommodation so good. Descriptions of the surrounding country may be found in the series of papers published by LLOYD, JOHNSON, and their collaborators. Climatological data have been published in several of these papers, particularly by LLOYD, JOHNSON, and RAWSON (1927) ; also TAYLOR (1932, *a*). But no considerable study of the climate has yet been undertaken, and it seems essential that this deficiency should be made good. Some of the published figures are from Six's thermometers, and other inaccurate instruments. The best account of the flora and of the communities into which it may be divided is given by LLOYD, LESTER, TAYLOR, and THORNEWILL (1933).

At Gadau, *Glossina submorsitans** and *tachinoides* are available in numbers, except during the rains (say, from May till September), and admirable arrangements exist for the regular supply of adults and puparia. The laboratory itself is solidly constructed of stone, and provided with running water, petrol air gas, and electricity. Indeed, Gadau is one of the few places in Africa where adequate laboratory facilities and large numbers of fly may both be obtained. Here we worked from early February till the end of June, 1933, one of us remaining till August in order to complete certain meteorological investigations. But nearly all the laboratory work was carried out before the middle of May when the rains began, and the numbers of available fly dropped suddenly, leaving several experiments still unfinished.

The work here described owes much to the help of the medical department of the Nigerian Government. In particular, it is a pleasure to acknowledge the kindness received from the Director of Medical Services, Dr. W. B. JOHNSON, C.M.G.; and from Dr. H. M. O. LESTER and his colleagues of the Tsetse Investigation at Gadau.

Methods—In the experiments in which puparia or flies were exposed to controlled conditions of climate, the desired temperature was obtained with incubators. Experiments at 30° C (86·0° F) were not easy, for the mean temperature of the laboratory was above that figure during our stay; fortunately our incubator was water-jacketed, so that we were able to keep it cool by adding chilled water two or three times a day. Occasionally the temperature in the incubator ranged 2° C above or below 30° C, but generally the range was less than 1° C; a continuous record of temperature was kept with a thermohygrograph. Work at 24° C (75·2° F) was performed in an incubator cooled with ice; the temperature rarely fluctuated more than 1° C from this figure.

Inside the incubator the insects were kept in wide-mouthed glass jars; a type is required in which the mouth is exceptionally wide, and the stopper robust and air-tight. Humidity inside the jar was controlled by potash solutions. In the potash we put a small folding stand made of strip nickel; this metal is not attacked by aqueous solutions of potash. Above this was a sheet of copper gauze, on which the container for the insects stood. When we were dealing with adult flies they were put in a cylindrical cage, made up from sheet celluloid, fastened with amyl acetate, the top and bottom of the cage being covered with mosquito netting with a sleeve at the top. It was necessary to use very fine netting to prevent the larvæ, which might be produced during the experiment, forcing their way through it into the potash solution. When puparia were the subject of experiment, they were kept in short lengths of glass tubing, one inch in diameter, closed below with mosquito net,

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* It appears best to refer to this insect as *submorsitans* rather than *morsitans*. Into the morphological question we cannot enter, but it seems that the biological differences are considerable. We therefore prefer to make the distinction, until the systematists have finally dealt with their side of the question.

held in place with celluloid cement, and plugged at the top with wool. These small containers were stored in fruit jars when constant humidity was desired. Individual puparia were marked with spots of oil paint, applied behind the line at which the cuticle ruptures when the adult emerges.

A description has been published elsewhere (BUXTON and MELLANBY, 1934) of a method of making the necessary solutions of potash without having resource to titration. In working at Gadau we made use of solutions of potash to give us the degrees of humidity shown in Table I. It will be remembered that the relative humidity over a solution of potash is almost constant over a wide range of temperature ; from this it follows that the saturation deficiency is different at every temperature.

TABLE I—Showing the relation of the specific gravity of an aqueous solution of potash (KOH) to the relative humidity and saturation deficiency of the atmosphere over it ; the figures for saturation deficiency are mm of mercury.

Specific gravity	1050	1130	1260	1360	1470	1500	1560
R. h. (per cent)*	98	88	65	44	24	19	11
37° C (98·6° F) Sat. def.†	1·0	5·6	16·5	26·3	35·7	38·0	41·7
30° C (86·0° F) Sat. def.†	0·6	3·8	11·0	17·7	24·0	25·6	28·1
24° C (75·2° F) Sat. def.†	0·4	2·6	7·8	12·5	17·0	18·1	19·8

* R. h. = Relative humidity. † Sat. def. = Saturation deficiency.

In determining the thermal death point of flies or puparia, use was made of apparatus similar in principle to that figured by MELLANBY (1932, *a*). It consists of a copper tank containing water which is maintained at the desired temperature and stirred continuously. In it stands a series of glass vessels, within which the humidity is controlled by solutions of potash. Insects can be put in a small cage of wire gauze and introduced into these vessels without removing them from the tank. In one of the vessels, which contains no insects, a thermometer is put with the bulb in the position in which the insects would be. The temperature is read and booked every five minutes throughout the experiment. By careful attention to stirring and to the gas burner, it is not difficult to keep the fluctuation of temperature below 0·2° C during an exposure of several hours.

All our flies, both those kept for breeding and those used in experiments, were fed every day on a sheep.‡ It is not likely that an infection with Trypanosomes makes any difference to the physiology of *Glossina*, but we eliminated this variable

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‡ It is possible that the precise food which is available makes a greater difference to *Glossina* than is yet realized, though it is well known that several of the species feed in nature from a considerable number of hosts, both cold- and warm-blooded. One of us left Gadau with laboratory bred puparia of *tachinoides* and *submorsitans* ; these hatched on the way down to Lagos, and by the time he reached England a considerable number of puparia of the next generation had been produced. So far all flies had been fed on man, and very few deaths had occurred in spite of the difficulties of the journey. But when the flies reached London they were fed on guinea-pig and many of them died in the next few days. It is not clear whether death was due to starvation or to taking unfamiliar food. But the possibility that individual flies develop particular preferences in regard to food merits further study.

by feeding bred flies on one sheep and wild flies on another. It is the careful daily feeding of flies, more than anything else, which limits the amount of work that can be done. The present investigation owes much to two Hausas, MALLAM LAWAL and MALLAM DA'U, for careful and conscientious work. The puparia used in the experiments were all bred in captivity, so that the age was known within 24 hours. The adults which produced them were kept in the damp and cool cupboard (p. 187). This is an important point, for it is shown below (p. 216) that if adults are kept at high temperatures they produce puparia which have a high mortality.

Previous experience has shown that it is rarely sound to work on batches of insects, except perhaps in preliminary experiments. It is difficult to obtain a group of uniform insects, so that it is best to record individual changes of weight, etc., in order that a statistical evaluation of the consistency of the data may be possible. This necessitates the accurate weighing of small quantities, an unfed *Glossina tachinoides* weighing about 10 mg and containing about 3 mg of dry matter ; but if many single flies are to be weighed the method must be rapid. (The present paper is based on about 8,900 separate weighings.) The only apparatus which is suitable is a torsion balance, but as each instrument has a limited range it is important to choose one which is appropriate to the work. We provided ourselves with one weighing up to 80 mg, which is suitable for *Glossina tachinoides* ; we weighed a number of *tachinoides* immediately after feeding, and none of them exceeded 54 mg. But if similar work is contemplated with *morsitans* or *palpalis*, it would be well to have a machine weighing to 100 mg. With such a machine one could only weigh to 0·2 mg which is not sufficiently accurate for dry weights, etc. ; it would be necessary, therefore, to have a second machine with a maximum of 20 or 25 mg. The dry weights, etc., quoted in the present paper are not accurate, for we could not weigh beyond 0·1 mg which is about 3% of the dry weight of a fly or puparium.

In the statistical study of the facts we have received much valued help from our colleague Dr. J. O. IRWIN. The reader unfamiliar with elementary statistics may misunderstand the phrase that the " difference between the means is not statistically significant." Statistical methods pay heed to the inconsistency or variation in the original data from which the means are derived. If a difference is found to be " not statistically significant," it means that it might occur, by chance, if the two groups of individuals had been taken from the *same* population without being subjected to different experimental treatment. In other words, one cannot be certain that the difference is due to the experimental conditions, though it may be ; the conclusion is not proved (though it may be very probable). But if a difference is " statistically significant," this means that even when one has allowed for the inconsistency in the data, the difference cannot be accounted for ; so that one feels justified in attributing the difference to the experimental conditions.

II—FACTORS WHICH LIMIT ADULT LIFE

It is our general purpose to define the conditions of climate which limit the life, reproduction, and feeding of *Glossina tachinoides* and *submorsitans*. It is probably best to set out the facts first, and to consider their physiological explanation later (p. 196). The simplest type of experiment is that which delimits the conditions of temperature and humidity which are rapidly fatal. From that we pass to the duration of life of flies which may or may not have been fed, prior to the experiment, and which are starved to death under controlled conditions. This leads finally to the most elaborate, and perhaps most valuable, type of experiment, in which flies are put under controlled conditions of temperature and humidity in a container, from which they are removed daily in order that they may be offered food.

Extreme climatic limits (*high and low temperature*)

Thermal death point—A series of experiments was performed with the purpose of delimiting the combinations of elevated temperature and humidity which are rapidly fatal to adult *Glossina.* The apparatus has already been described (p. 178). In the most complete series of experiments, groups of adult *submorsitans* which were less than a week old, and had been fed daily including the day of the experiment, were exposed for one hour. At the end of that period it was frequently difficult to say whether the flies were dead or alive ; results were therefore booked after 24 hours, and during that interval the flies were kept in the damp cupboard. In controls treated in the same way an occasional death occurred, so that if one of the experimental flies died it was disregarded. The result of a number of such experiments are shown in fig. 1. Each point on the graph is established by exposing five or more flies ; when all died a cross is shown ; when all lived, a circle ; this is partly blackened when some died. It is clear that 44·0° C (111·2° F) is fatal, irrespective of humidity ; at 43·0° C, and still more at 42·0° C the flies survive in the drier air, but not in the moister ; at 41·0° C (105·8° F) no deaths were recorded.

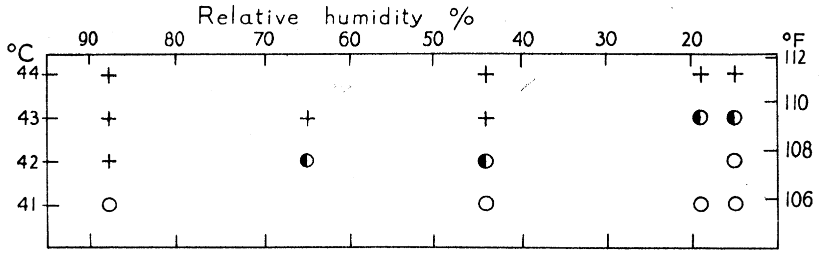

FIG. 1—Thermal death point of *Glossina submorsitans* exposed for one hour to controlled conditions of temperature and humidity. The cross indicates that all flies died ; the white circle that none died ; the black and white circle that some died.

A similar series of experiments was carried out, using *tachinoides* which were under a week old and had been fed daily. The results are essentially similar to those shown for *submorsitans*, fig. 1, and they need not be given in detail. Between 41° and 42° C a few flies die at all humidities ; at 42·4° to 43·6° C none survived except at the lowest relative humidity ; at 44·0° C all flies died. A few experiments were also performed with *tachinoides* unfed and under 24 hours old. So many of the controls died that it was difficult to distinguish deaths due to the experiment; but these young flies do not differ greatly from older flies which have been fed, though it seemed that the fatal temperature is about 1° C lower. It is clear from the above experiments that both species of *Glossina* possess the power of surviving a higher temperature in dry air than in moist, for an exposure of one hour. But if the period of the experiment is prolonged to three hours, the humidity of the air makes no difference to the lethal temperature. In experiments with fed *tachinoides*, it was found that only two or three out of 15 flies died at 39·2° C (102·6° F) at each of four humidities ranging from 11 to 88%; but at 40·4° C (104·7° F) all the flies died. It is probable that it is owing to evaporation of water that flies can survive dry hot air for limited periods; the matter is further investigated below (p. 202).

The lower temperature limit has been less precisely defined. It appeared unnecessary to control humidity, as evaporation, even in dry air, is so little at or about freezing point; it may be assumed that all the experiments were made in air which was saturated or nearly so. The work was carried out by confining *tachinoides* (several days old and well fed) to the lower ends of test tubes, which were set in a large thermos flask containing cold water or freezing mixture. The temperature was read on a thermometer inside a similar tube. It was found that exposures of up to two hours, to 8° or 4° C (46·4° or 39·2° F) produced no permanent ill-effect. Immediately after exposure the flies were numb, but they stood on their feet, and they subsequently moved and fed normally. After exposure to 0° C the flies appeared completely dead, but some rapidly recovered, the wings buzzing* violently at first. In many cases after an exposure of seven to ten minutes recovery was imperfect, the fly being ataxic and dying within a day or two; but a few flies made a perfect recovery after an exposure of four to seven hours. A temperature of −12° C (10·4° F) was invariably fatal after seven minutes.

MACFIE's studies (1912) on *Glossina palpalis* show that these insects are not killed by an hour at 7° to 10° C, but after a few minutes at 2·5° C, complete recovery does not occur. It is clear that *Glossina morsitans* can survive considerably lower temperatures; POTTS (1933) has shown that some individuals can recover after exposure to 0° C for 12 hours.

Duration of Life

(*a*) *Unfed flies*

(*b*) *Flies fed before experiment, but not subsequently*—Useful information might be obtained by starving flies under controlled conditions of temperature and humidity, and noting the duration of life. The method has given valuable results with other insects, though it seems difficult to obtain consistent results by it. (See, for instance, data on rat fleas, LEESON, 1932.)

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* MACFIE (1912) has suggested that the muscular effort of buzzing assists recovery by warming the fly. This may be so, but flies buzz when recovering from a general anæsthetic, and we regard the act as an expression of inco-ordination.

Working with *tachinoides* (and *submorsitans* when it was available), two series of experiments were performed. In the first we employed flies which had never fed, and were known to be under 24 hours old at the start of the experiment ; these are referred to as " unfed flies." The flies used in the second series were under a week old, and had been offered food every day, including the morning of the experiment ; these insects are called " fed flies." In both series the flies were put, in tens, into cages which were placed in fruit jars inside the incubator. Dead flies were removed and counted, and the sex booked, once in 24 hours.

Table II shows the type of result which is obtained by this method ; it gives the daily deaths of fed *tachinoides* exposed at 30° C (86·0° F) to relative humidities of 88 and 65% ; deaths are recorded daily under separate cages (A, B, etc.), and the total daily deaths are also expressed as a percentage of the total deaths. At first sight it appears that definite conclusions may be drawn from this table, for it seems evident that life is much longer at 65% r.h. than at 88%. But there are great inconsistencies between the separate cages which were exposed to the same experimental conditions. Clearly, therefore, the difference between the means of length of life requires to be tested for significance. It appears that it is appropriate to take the mean number of deaths on the first day, and see whether the figures differ significantly, making use of the *t* test (Fisher, 1932, p. 114). The value of *t* is 2·072 ; the probability that this value might occur by chance is just over 1:20. We conclude that the figures in Table II do not prove that humidity had any effect on the number of deaths during the first day of the experiment, though it is very probable that this was so.

Table II—Showing the number of deaths daily in cages (A, B, C, etc.), containing *Glossina tachinoides*, exposed to 30° C (86·0° F) and saturation deficiencies of 3·8 and 11·0 mm of mercury. The flies were offered food on the day when the experiment started, and for several days before.

	R. h., 88% (Sat. def., 3·8 mm)											R. h., 65% (Sat. def., 11·0 mm)							
Day	A	B	C	D	E	F	G	H	I	J	%	A	B	C	D	E	F	G	%
1 . . .	9	6	1	0	2	4	8	2	2	0	*34*	0	0	0	0	2	0	3	*7*
2 . . .	1	4	6	2	7	6	2	7	0	7	*42*	2	0	0	0	5	6	6	*27*
3 . . .			3	5	0			1	6	3	*18*	4	0	2	1	3	4	1	*21*
4 . . .				2	1				2		*5*	4	6	4	5				*27*
5 . . .				0							*0*		1	2	4				*10*
6 . . .				1							*1*		3	2					*7*

Total flies, 100.	Total flies, 70.
Mean life, 1·98 days.	Mean life, 3·27 days.
Value of χ^2, 27·8.	Value of χ^2, 13·5.

It is clear from Table II that considerable differences exist between cages which were kept at the same humidity. It is well to measure the degree of inconsistency, to discover whether it is so great as to imply that the population was heterogeneous.

The degree of inconsistency between cages at a single humidity may be appropriately measured by χ^2; we propose to test the hypothesis that the flies in all the cages at one humidity were drawn from a homogeneous population. The χ^2 formula gives a measure of the divergence between the " observed " facts and what would be " expected " on this hypothesis. An example will make this clearer. Taking the figures for 88% humidity, the number of deaths expected in each cage on the first day is 3·4. But in successive cages the deaths observed were 9, 6, 1, etc. Let successive values of x be $9 - 3{\cdot}4$, $6 - 3{\cdot}4$, etc., and let the expected number of deaths (3·4) be m. Successive values of x^2/m are then calculated for the ten cages; the sum of these values is χ^2. It is clear that if the separate cages give results which are consistent with one another, and therefore close to the mean, the value of χ^2 will be low. In the present example the value of χ^2 is 27·8, the number of " degrees of freedom " being nine. Reference to a table (FISHER, 1932) shows that so high a value of χ^2 would occur less than once in a hundred, if the flies had been drawn from a homogeneous population. The value of χ^2 for the experiments at 65% humidity is 13·5, and this also indicates heterogeneity.

Table II shows the effect of two different humidities on fed *tachinoides* at 30° C; similar experiments were performed at several other humidities, and the results are shown in fig. 2. Work of a similar nature was carried out at other temperatures, with unfed and fed *tachinoides*, and with unfed *submorsitans*. The χ^2 test shows that in most cases the figures (under any one set of conditions) are not homogeneous, and it seems unnecessary to set out the results at length. But certain results are obtained consistently, with flies of both species, fed or unfed, and these results may

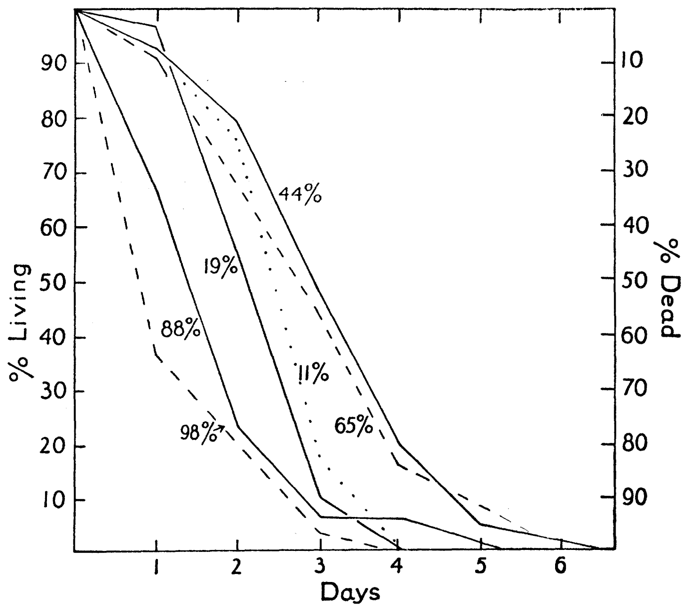

FIG. 2.—*Glossina tachinoides* (♂ and ♀) are fed daily for a week and then starved to death under controlled conditions of humidity, at 30° C (86·0° F). The figure shows the percentage living and dead, day by day, at each of six humidities.

be briefly summarized even if they are not statistically convincing. Table III gives the mean and extreme length of life under various experimental conditions. If the " extreme " is given as three days, it implies that the last fly was found dead on the third day of the experiment ; but flies were 0 — 24 hours old at the start, so that the age of such a fly is between 72 and 96 hours. The number of flies exposed under each set of conditions was between 40 and 100. The following conclusions appear to be justified :—

(1) Life is longer at intermediate humidities than in very dry or moist air. This is true of both species of fly, either fed or unfed, and at each temperature. At any particular temperature, the mean duration of life at the most favourable humidity is less than twice the duration at the least favourable, so that the effect of humidity is not very great. Tests by *t* show that many of the differences among fed flies are significant ; among unfed flies they are not.

(2) In all the experiments, which dealt with 1,510 *tachinoides* and 160 *submorsitans*, only two flies lived beyond the sixth day. It would be of interest to have figures of duration of life at 16° or 18° C (60·8° or 64·4° F), but we may safely conclude that *Glossina*, unlike many blood-sucking insects, cannot survive prolonged starvation. This confirms the work of several earlier writers, which was carried out under undefined conditions of humidity.

TABLE III—Giving mean and extreme length of life, in days, of flies (unfed and fed) of both species, under different combinations of temperature and relative humidity.

Species	State	Temp.			Relative humidity %					
		°C	°F		98	88	65	44	19	11
tachinoides	unfed	37	98·6	mean		1·00		1·05		1·00
				extreme		1		2		1
	,,	30	86·0	mean	1·45	1·29	1·80	1·44	1·71	1·61
				extreme	2	3	4	3	3	3
	,,	24	75·2	mean		3·16		3·95		3·02
				extreme		8		6		5
	fed	37	98·6	mean		1·04	1·02	1·29	1·27	1·31
				extreme		2	2	2	2	2
	,,	30	86·0	mean	1·63	1·98	3·27	3·47	2·64	2·96
				extreme	4	6	6	7	4	4
submorsitans	unfed	30	86·0	mean		1·58		2·86		2·62
				extreme		4		5		4

(3) The survival of *tachinoides* at 37° C (98·6° F) is of particular interest. Unfed flies nearly always die under 24 hours, and invariably (fed or unfed) under 48 hours. TAYLOR (1932*a*) has recently discovered that a very much higher proportion of *Glossina tachinoides* can be infected with *Trypanosoma gambiense* if the flies are kept at 37° C for a period, than at room temperature. In his experiments the flies were given infecting meals on four consecutive days, so that his figures are not strictly

comparable with ours ; in some experiments they were exposed to 37° C for 16 hours, during each of those days, and in others they were kept at the same temperature for the whole of the four days and nights " without any serious increase of mortality among the tsetse." There must be a considerable difference in technique between TAYLOR and ourselves, and the matter requires further study, particularly in view of the value of TAYLOR's discovery to protozoologists. Data on the length of life of flies at 34° to 35° C would be valuable.

(4) The effect of feeding on length of life is less than would be expected. Little difference between fed and unfed flies is observable at 37° C, though a difference might become apparent if the deaths were booked with greater frequency. At 30° C it seems that the fed *tachinoides* live about twice as long as the unfed. Both at 37° and 30° C the difference in length of life between fed and unfed flies is greater at lower humidities ; the fed flies can tolerate evaporation better than the unfed. Unfed *submorsitans* seem to live about twice as long as unfed *tachinoides* kept under the same conditions.

If work of this type is done again, the conditions should be more carefully standardized. It is not desirable to use unfed flies of any age up to 24 hours, for that is a considerable part of their total life. Flies known to be under six hours old (or less) should be used, and results booked every 12 hours. Working with fed flies, one should choose those which actually fed on the morning of the experiment (and not those which were given a chance of feeding, such as we used).

(*c*) *Flies fed daily throughout the experiment*—In this series the method was essentially that already described on p. 182 above. In each experiment three males and 15 females were confined in a celluloid cage which was placed in a fruit jar in the incubator. Every day the cage was taken out, and held against the sheep to give the flies an opportunity of feeding. This occupied considerably less than an hour ; we have reason for thinking that the control of humidity is re-established inside the fruit jar within one hour of the cage being returned to it, so that the flies were exposed to controlled conditions of temperature and humidity for at least 11/12ths of their lives. Every day, dead flies were removed and booked. Dead males were replaced by fresh ones, so as to keep the proportion of males up to about 1/5th of females ; dead females were not replaced. The experiments were discontinued at the end of 40 days.

A series of such experiments was made with *tachinoides*, two, three, or four cages being kept at each of five humidities at 30° C. The results for females only are shown in condensed form in fig. 3 ; the number of males used was not sufficient to give useful information. It appears that high humidities (88 and 65%) are most unfavourable to survival ; it may be added that during several experiments, which have not been included in the tabulation, the temperature in the incubator rose to 32° C (89·6° F) and this invariably caused the death of flies at 88% humidity. The figure also shows that middle humidities (44 and 19%) are most favourable to life. At the lowest humidity (11%) survival is intermediate between that at the highest and the middle humidities, and this is consistently so through a long period.

It is also apparent from the figure that there is little or no difference between 88 and 65% humidity or between 44 and 19%. The general resemblance between fig. 3 and fig. 2 will be observed.

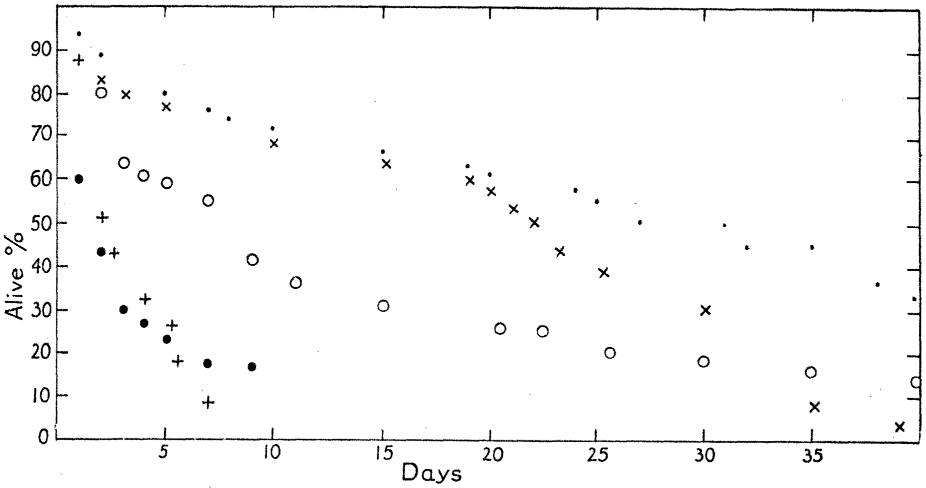

FIG. 3—*Glossina tachinoides* are kept at 30° C (86·0° F) and controlled humidity, but taken out and fed daily. The symbols show the survival of females at five different humidities. ● 88% humidity 30 ♀♀ ; + 65% humidity 60 ♀♀ ; · 44% humidity 66 ♀♀ ; × 19% humidity 30 ♀♀ ; ○ 11% humidity 44 ♀♀.

The symbols in fig. 3 show only the mean percentage survivals, each based on two to four cages (15 females in each). If the original data for separate cages at any one humidity are scrutinized, considerable variations are found. One must therefore test whether these discrepancies are great enough to invalidate the tentative conclusions which have been drawn. As a preliminary test, values of χ^2 for each humidity separately, on day 10 and day 20, were worked out. The values showed that each group of cages (at a single humidity) was heterogeneous, and this simple test was abandoned. The original data were then placed in the hands of our colleague, Dr. J. O. IRWIN, who applied the technique known as the analysis of variance. The result of his labours is to show that the variance between different humidities is greater than the average variance at the same humidity, on every day from the 3rd to the 10th. On days 8, 9, 10, and 15, the difference in variance is significant, as it is when the results for days 2—10 are added up and considered collectively. It seems unnecessary to examine the data beyond day 15. Going further into the matter, Dr. IRWIN has shown that for day 6 onwards, humidities of 19 and 44% give significantly larger numbers of survivors than humidities of 65 and 88% ; also, from day 8 onwards, the number of survivors at 11% is significantly larger than those at 65 and 88%. The difference between the number of survivors at 11% and at 19 and 44% is not significant on any day tested. One may say, therefore, that the conclusions already drawn from the study of fig. 3 are, in the main, supported by the statistician. But it is not proved that the difference between 11% and 19% (or 44%) is due to humidity.

A few experiments were performed with *tachinoides* at 24° C (75·2° F) and relative humidities of 88, 44, and 11%. It was not found possible to duplicate them and they need not be reported at length. It appears that at this temperature a humidity of 88% is less unfavourable than a lower humidity, though it is not so rapidly fatal as it is at 30° C. This is consistent with observations which have been made on many other insects, which are unfavourably affected by the combination of high temperature and relative humidity.

Similar experiments were performed with *submorsitans* at 30° C. Here again it was found that a high degree of humidity was unfavourable, for at 88% humidity, at which only a single cage was exposed, the original 15 females had become seven on the fifth day and four on the tenth, after which the experiment was discontinued. At lower humidities work was done at 44 and 11%, at each of which three cages (45 females in all) were exposed. Mortality in the two groups of cages was very similar, and the differences are not certainly significant, so that it seems sufficient to present the following data, showing the percentage survival of females exposed to the two humidities :—

Day	2	5	10	15	20	25	30	35	40
44% r.h.	95	80	73	71	60	56	49	40	29
11% r.h.	98	87	69	58	49	38	38	36	24

III—Factors which limit Reproduction

Preliminary Work

In our preliminary experiments the conditions of temperature and humidity were not precisely controlled, but the flies were kept in the small cages, already described, in a cupboard the walls of which were covered with sacking, wetted in the dry season. The climatic conditions in the cupboard were measured with a thermohygrograph ; the maximum temperature very rarely rose above 30° C (86·0° F), and the minimum was always above 20° C (68·0° F) during the months from February to June, during which this work was done ; the mean temperature rose from 24° C (75·2° F) in February to 28° C (82·4° F) in April, and 27° C (80·6° F) in May. The relative humidity fluctuated greatly, but was generally between 40 and 70%.

In all the experiments described here, groups of ten female and two male *Glossina* were kept in cages. In each cage the flies were all under 24 hours old at the start of the experiment. A record was kept of the deaths of females and of the production of puparia ; no record was kept of deaths of males, but when they died fresh males were introduced. Starting with eight cages (80 females) of *Glossina tachinoides* and with 76 female *Glossina submorsitans*, the percentage of survivors was as shown in Table IV. The figures may be compared with those published by Kleine and Fischer (1912) ; working on *Glossina palpalis*, they had considerably higher percentage survivals.

After the 90th day the few remaining female *tachinoides* died rapidly, the last survivor on the 105th day. The experiments with *submorsitans* were discontinued at the 40th day. It is felt that the figures show clearly that the experimental conditions were not unsuitable for either species.

Facts relating to reproduction are also shown in Table IV. The birth-rates have been standardized and are presented as puparia per 100 female-days. The figure in the table gives the birth-rate for a five-day period (for a ten-day period after 50th day). With *tachinoides*, the first puparium was generally found between the 16th and 19th day ; for *submorsitans* the period is a little longer, no puparia being found before the 19th day, and none till the 20th or 21st in some cages. After this point the normal production of puparia for *tachinoides* or *submorsitans* is between two and six per 100 female-days, but there are great differences between different cages. Our records only extend to 90 days (*tachinoides*) and 40 days (*submorsitans*), but up to that point there is no evidence of falling birth-rate. Indeed in *tachinoides*, the rate (puparia per 100 female-days) was consistently higher after the 45th day than before, but the figures are not reliable as the total number of females was then considerably reduced, and the temperature in the cupboard was rising.

TABLE IV—Showing the percentage survival at five-day intervals of 80 female *tachinoides* and 76 female *submorsitans* ; also birth-rates, expressed as puparia per 100 ♀ days

Day	5	10	15	20	25	30	35	40	45	50	60	70	80	90
♀ ♀ %—														
tachinoides . . .	92·5	91	87	82	80	74	69	69	67	67	54	43	27	10
submorsitans . .	97·5	89	88	85	84	73	68	64	?	?	?	?	?	?
Birth-rate—														
tachinoides . . .	0	0	0	1·9	2·5	3·6	3·9	6·4	4·5	6·1	6·1	8·8	7·8	13·6
submorsitans . .	0	0	0	4·2	2·8	3·3	3·0	4·8	?	?	?	?	?	?

Copulation, which appears to be mechanically perfect, is frequently observed between flies which are only a few hours old. But conception does not result even if the sexes are fed and kept together for the first 24 hours of life ; this was done with seven cages (*tachinoides*) and the females kept without males till the 45th day without producing puparia ; had males been present about 20 puparia would by then have been produced. On the 45th day males were introduced, and a normal production of puparia followed in each cage. But when males and females were kept together and fed daily for four days, and then separated, the production of puparia began about the 18th day and continued normally. It continued till about the 70th day, and in one instance till the 83rd day, showing that sperm can survive at least 70 days. Fertilization can occur by the fourth day of adult life, but not on the first day. As the first puparium is produced between the 16th and 19th day, it seems that the period of gestation of *Glossina tachinoides* at a temperature of about 24°—26° C is about a fortnight. This is borne out by the result of a series of experiments in which females were kept virgin till they were a month old ; males were then admitted and the first puparia were produced on the 13th or 14th day.

It appears probable that a considerable proportion of female *tachinoides* are barren, under the experimental conditions described. We have a record of one cage of ten females and two males which had produced no puparia by the 45th day, when the experiment was concluded ; the males had been changed once during that period. Three similar cages, started at the same time and kept under identical conditions, produced eleven, six, and one puparia respectively. The period of gestation is about a fortnight, which corresponds to about seven puparia per 100 female-days, if one pregnancy immediately succeeds another. But frequently the birth-rate is as low as two per 100 female-days. It seems easier to believe that some of the females are sterile, and that others are producing larvæ at fortnightly intervals, than that they are all intermittently reproductive. There are other grounds for thinking that sterility is common. The first puparium in a cage of *tachinoides* is generally produced between the 16th and 19th day. But one must wait till the 30th or 40th day before the total number of puparia is equal to the number of females in the cage. It is more probable that some of the females have produced two puparia by that time, than that the reproductive life of some of them begins very late. The results obtained with *Glossina submorsitans* are essentially the same. From these facts one may conclude that a proportion of the females of both species are probably sterile. The matter is of considerable importance to the laboratory worker, and requires fuller study.

The conditions under which stocks of *Glossina* can be maintained in the laboratory are now beginning to be clear ; the limiting conditions of temperature and humidity are discussed in the present paper. Much remains to be discovered with regard to sexual physiology. We do not know what proportion of males is optimal (see Potts, 1933, for *Glossina morsitans* in Tanganyika), and how many females may be kept in a cage of a given size without interference with nutrition and without causing abortion. We have shown that conception does not occur during the first day of adult life, but it is not yet known whether this is due to sexual immaturity of one sex or of both. Mackerras (1933) shows that the males of Calliphorines are potent on the first day of life, and that the females become so after a few days.

Reproduction under controlled conditions

Experiments have been described in which communities of flies were kept under controlled conditions of temperature and humidity and offered food daily ; the death-rate in such a community is much influenced by humidity, fig. 3. While those experiments were being performed, notes were kept of births. It was found that at 30° C the first birth (*submorsitans*) generally occurred on the 15th or 16th day. In experiments with *tachinoides*, the first puparium was found between the 13th and 29th day ; but even at a relative humidity of 44%, which is favourable for the reproduction of this species, the first puparia were noted on the 13th, 18th, and 24th days, in different cages. The cause of this irregularity is unknown (see,

TABLE V—Showing the production of puparia by *Glossina tachinoides* kept at 30° C (86·0° F) under controlled conditions of humidity, and fed daily. Each cage originally contained 15 females. ♀ signifies the mean number of females alive during the five-day period ; $p =$ actual number of puparia produced ; $b =$ puparia per 100 female-days.

	R. h., %																									
	88		65	44										19							11					
Days				A			B			C			Mean	A			B			Mean	A		B			Mean
	♀	*p*	♀	♀	*p*	*b*	♀	*p*	*b*	♀	*p*	*b*	*b*	♀	*p*	*b*	♀	*p*	*b*	*b*	♀	*p*	♀	*p*	*b*	*b*
11–15	5	0	0	11·0	2	3·6	13·0	0	0	10·8	0	0	*1·15*	5·0	0	0	14·0	0	0	*0*	5·4	0	9·4	0	0	*0*
16–20	4·4	0	0	10·6	5	9·4	12·4	2	3·2	10·0	0	0	*4·24*	4·4	1	4·5	14·0	0	0	*1·09*	4·2	0	9·0	0	0	*0*
21–25	2·6	0*	0	9·4	4	8·5	10·6	2	3·8	10·0	1	2·0	*4·67*	1·5	1	16·7	12·4	0	0	*1·47*	2·0	0	8·4	1	2·4	*2·13*
26–30	0		0	Accidentally destroyed			10·0	0	0	8·4	2	4·8	*2·17*	0			10·4	1	1·9	*1·92*	—		8·0	0	0	*0*
31–35	0		0				10·0	0	0	6·4	0	0	*0*	0			3·6	0	0	*0*	—		7·0	0	0	*0*
36–40	0		0				9·6	2	4·2	5·0	1	4·0	*4·11*	0			1·2	0	0	*0*	—		6·2	0	0	*0*
11–40		0	0	10·3	11	7·10	10·96	6	1·83	8·43	4	1·58	*2·85*	3·74	2	3·78	9·27	1	0·36	*0·90*	4·0	0	8·0	1	0·42	*0·34*

* One abortion.

however, p. 189 above). Very few abortions were observed. During the same experiments it was also noticed that *submorsitans* produces more puparia at a relative humidity of 11% than at 44%. For purposes of comparison it is convenient to standardize the birth-rate and reduce it to puparia per 100 female-days; the facts for the two species are shown in Tables V and VI. In these tables the production of puparia is given in five-day periods, ♀ signifying the mean number of females alive during that period, *p* the number of larvæ or puparia produced, and *b* the number of births per 100 female-days. Table V deals with *tachinoides*. It will be seen that at 88% humidity, no reproduction occurred in the single experiment. Four experiments were started at 65% humidity, but no females lived long enough to reach reproductive age. At 44% humidity reproduction was active in each of the three cages, and the mean birth-rate (*b*) is three times that for 19% humidity

TABLE VI—Showing the production of puparia by *Glossina submorsitans* kept at 30° C (86·0° F) under controlled conditions of humidity, and fed daily. Each cage originally contained 15 females. ♀ signifies the mean number of females alive during the five-day period; *p* = actual number of puparia produced; *b* = puparia per 100 female-days.

	R. h., %																			
	88	44									11									
		A			B		C			Mean	E			F			G			Mean
Days	♀	♀	*p*	*b*	♀	*p*	♀	*p*	*b*	*b*	♀	*p*	*b*	♀	*p*	*b*	♀	*p*	*b*	*b*
11–15	0	9·0	1	2·2	13·0	0	10·8	0	0	*0·61*	9·8	0	0	10·6	1	1·9	7·6	1	2·6	*1·42*
16–20	0	7·8	0	0	11·2	0	10·0	0	0	*0·0*	7·4	4	10·8	9·0	0	0	7·0	1	2·9	*4·28*
21–25	0	6·8	0	0	9·6	0	10·0	1	2·0	*0·76*	6·8	3	8·8	5·8	1	3·4	6·0	2	6·7	*6·45*
26–30	0	6·0	0	0	8·6	0	8·4	2	4·8	*1·74*	6·0	2	6·7	5·0	1	4·0	6·0	2	6·7	*5·88*
31–35	0	6·0	1	3·3	7·0	0	6·4	0	0	*1·03*	6·0	3	10·0	4·2	1	4·8	6·0	2	6·7	*7·40*
36–40	0	5·4	1	3·7	5·0	0	5·0	1	4·0	*2·60*	5·8	1	3·4	4·0	0	0	3·6	2	11·1	*4·00*
11–40	0	6·83	3	1·46	9·07	0	8·43	4	1·58	*0·96*	6·97	13	6·21	6·43	4	2·07	6·03	10	5·53	*4·62*

and nine times that for 11%. Furthermore, it must be remembered that the values of *b* are standardized birth-rates. But humidity affects not only this but also the actual number of surviving females, so that its effect on the number of births is greater than is at first sight apparent. For instance, taking the data in Table V for days 21—25, and comparing 44 and 11% humidity, the mean values of *b* are 4·67 and 2·13, roughly 2 to 1, but the mean numbers of females per cage are about 10 and about 5. The actual births are therefore about four times greater at the higher humidity. Table VI gives results obtained with *submorsitans*; it seems clear that the most favourable humidity for this species is 11%. This appears to throw light upon the effect of humidity upon wild populations of *Glossina*, and it is so important that the figures must be critically scrutinized before they are accepted. We propose to examine the following birth-rates *b* from Table VI (*Glossina submorsitans*) :—

	Humidity, 44%			Humidity, 11%		
Cage :	A	B	C	E	F	G
Days						
11–15	2·2	0	0	0	1·9	2·6
16–20	0	0	0	10·8	0	2·9
21–25	0	0	2·0	8·8	3·4	6·7
26–30	0	0	4·8	6·7	4·0	6·7
31–35	3·3	0	0	10·0	4·8	6·7
36–40	3·7	0	4·0	3·4	0	11·1

A glance is enough to show that the figures are higher at 11% humidity than at 44%, though at each humidity there are great inconsistencies between cages. The question to be solved is this : could the difference between the two humidities possibly be due to chance (*i.e.*, to the inconsistency of the material), or is it so great that we must attribute it to the experimental conditions, even when allowance has been made for the inconsistency? We then have two pairs of categories, two humidities and two values of *b* (positive and negative). The question is whether these categories are " independent " (Fisher, 1932, p. 87), or not.

The matter may be tested by χ^2, and it can be shown that the differences between 44 and 11% could not reasonably be attributed to chance, either for *submorsitans* (figures above) or for *tachinoides* (Table V) ; the difference between *tachinoides* at 44 and 19% are not significant (*i.e.*, they might or might not be due to chance). But when all the facts are considered, the effect of humidity on the birth-rate may be regarded as proved.

The information given in Tables V and VI suggests several questions of interest. It will be observed that even at the most favourable humidity the value of *b* is between two and six, the mean for *tachinoides* being 2·85 and for *submorsitans* 4·62. But in the preliminary experiments the birth-rate was also between two and six, though the temperature was materially lower (p. 188). The explanation may be that 30° C is a little above the optimum for reproduction ; or that exposure to unfluctuating conditions of temperature and humidity is not favourable. The matter can only be settled by further experiment. A third possibility exists, that the regulation of humidity by solutions of potash has some effect on the metabolism of the flies. Though the concentration of carbon dioxide is very low in the normal atmosphere, it is possible that in its absence, under experimental conditions, respiration is reduced. This question could easily be explored, if sulphuric acid or super-saturated salts were employed to regulate humidity.

A few experiments on *tachinoides* were carried out at 24° C (75·2° F) and relative humidities of 92, 44, and 11%. The results need not be given in detail. Puparia were produced under each of these conditions ; the first puparium appeared about the 26th to 30th day ; the rate of production of puparia *b* was below unity in each experiment.

IV—Factors which Affect Feeding

It has already been shown that *Glossina* lives longer and produces more offspring at middle humidities than in moist or dry air. It is of interest to know whether the flies also feed best at middle humidities, or whether they take most blood if they are kept in dry air, under conditions of greatest evaporation. The experiments were carried out with *Glossina tachinoides* of both sexes, known to be under 24 hours old. The work was done in May, when flies were scarce. It is desirable that it should be repeated on other species, and if this is done it would be well to treat the sexes separately. The wings were removed with the scissor-forceps supplied by Messrs. Flatters and Garnett, and the fly weighed ; it was then given an opportunity to suck human blood, and weighed immediately the meal was concluded. In most instances the second weighing was completed within one minute of the fly withdrawing its proboscis from the boy's arm, but even in this short space of time the insect frequently deposits a minute spot of fæces. The first time the flies were fed, before they had been exposed to controlled conditions of humidity, 11% of them refused to feed. The others fed, the mean weight of the feed being 9·52 mg and the mode in the 5—10 mg group. It seems, therefore, that the first feed is not much smaller than the subsequent ones, Table VII. After this first feed the flies were put at controlled humidity and 30° C, and weighed and offered food daily.

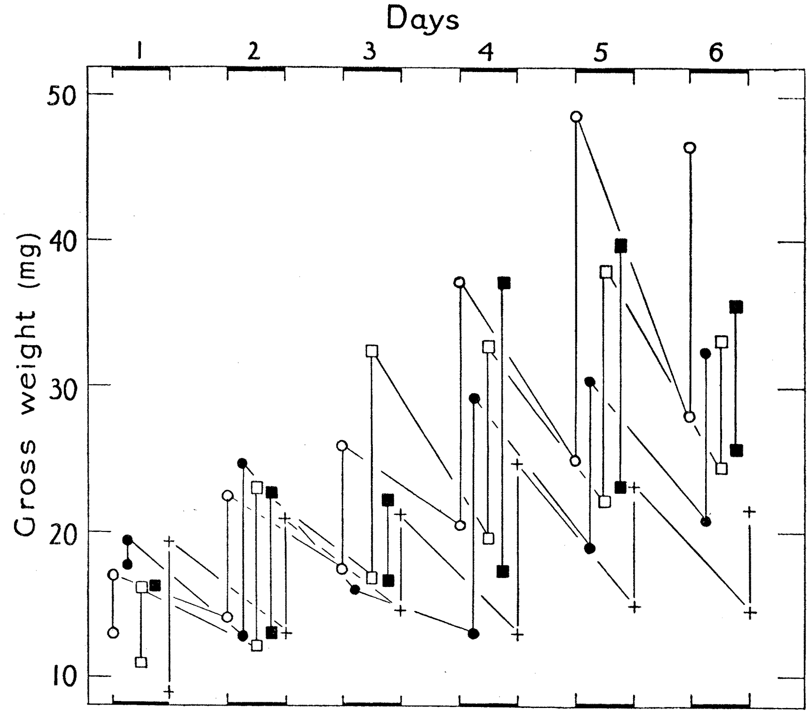

Fig. 4—Curves showing changes in gross weight of five individual *Glossina tachinoides* of both sexes. The flies were under 24 hours' old on day 1, and after the first feed were kept at 30° C (86·0° F) and 44% humidity, food being offered every day. Individual flies are distinguished by symbols.

In fig. 4 the weight curves of individuals kept at 44% relative humidity are given. It will be seen that each fly takes blood nearly every day. Fig. 5 gives similar curves for flies kept at 88% relative humidity and the same temperature.

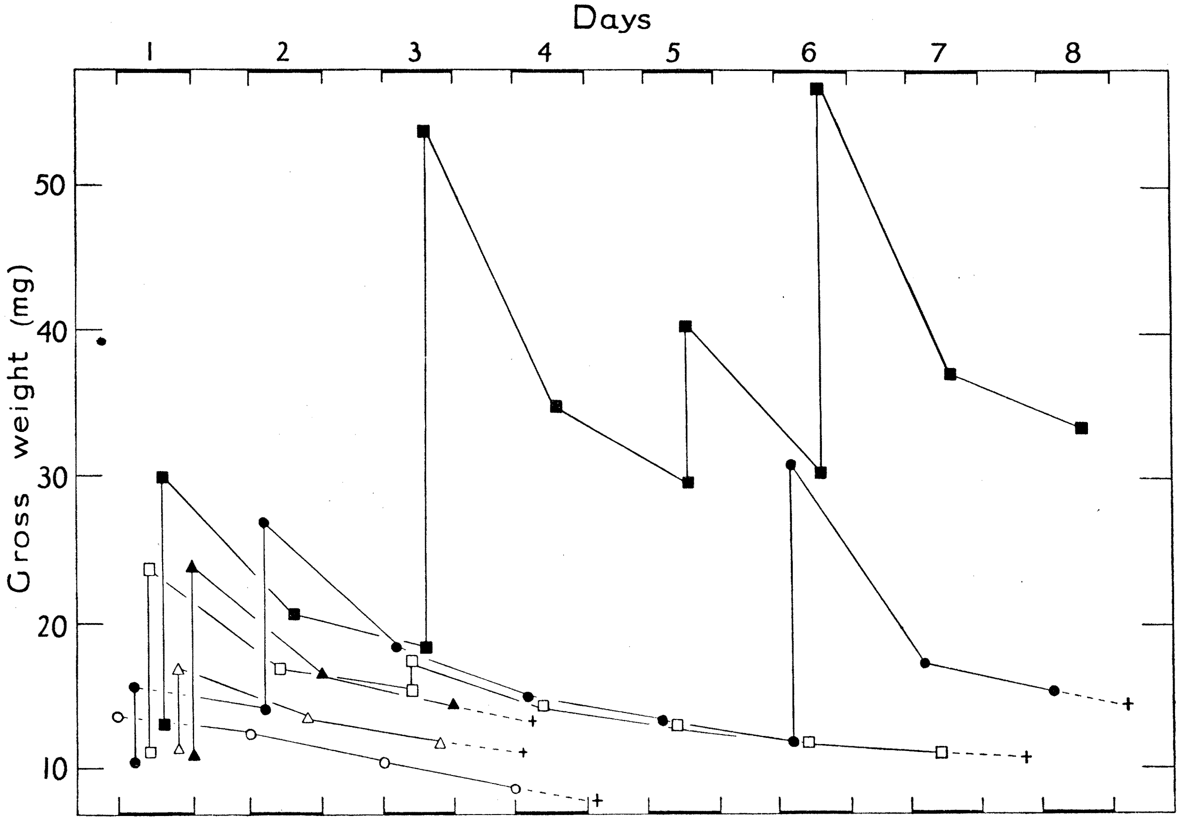

FIG. 5—Curves showing changes in gross weight of six individual *Glossina tachinoides* of both sexes. The flies were under 24 hours' old on day 1, and after the first feed were kept at 30° C (86·0° F) and 88% humidity, food being offered every day. Crosses indicate death.

Work of this type was carried out, at five different humidities at 30° C, and the results are summarized in Table VII. It must be understood that figs. 4 and 5 show the gross weight of fly and blood. But Table VII gives net weights of blood; the first feeds taken by the flies, before they had been subjected to controlled humidity, are excluded from the table. During the work it was observed that the mortality was higher at 88% humidity than at lower humidities; the proportion of nil feeds—that is to say, of occasions when the flies refused an opportunity to take blood—is also very high, 82%; indeed many of the flies entirely refused to feed after they had been exposed to this high humidity, and steadily lost weight, becoming ataxic and comatose before death (see also fig. 5). But on rare occasions, when the flies at high humidity fed, they took a normal weight of blood (indeed the mean is higher than at other humidities, but the number of feeds is low). Among the flies kept at the lowest humidity, 11%, the proportion of nil feeds is high, 39·5%. At the three intermediate humidities it is below 20%. As to positive feeds, they differ little at intermediate humidities, though there is a tendency for the flies to take rather less blood at 24% and 11% humidity, than at 44 or 65%.

The matter may be summarized in this way. Flies were kept at 30° C and under controlled conditions of humidity. The weight of food taken on 277 occasions is recorded. Those at 88% relative humidity generally refused to feed, and those at 11% frequently refused. At intermediate humidities they fed nearly every day. Humidity had therefore a considerable effect in determining whether they fed or not. But if they fed the weight of blood was but little affected by humidity, though there was a tendency for meals to be larger at 44 to 65% humidity than at 11 to 24%.

Knowledge of the amount of blood taken at a feed is clearly important, both to the insect physiologist, and also in relation to the infection of flies by trypanosomes.* It is therefore curious to find how little is on record ; this is no doubt because it is tedious to weigh flies on a chemical balance. The only available information appears to be as follows. Stuhlmann (1907) weighed four individual *Glossina fusca* ; before feeding they weighed from 51 to 96 mg, and they took from 79 to 258 mg of blood at a meal. According to Macfie (1912), male *Glossina palpalis* take about 20 mg and females about 28 mg, but one female took as much as 48·5 mg. Lester and Lloyd (1928) weighed five males and five females of *Glossina morsitans*, before and immediately after feeding ; a mean meal of a male was 34 mg, of a female 37 mg. Similar means for *tachinoides* were 30 mg (males) and 28 mg (females).

Table VII—Showing number of *tachinoides* used at 30° C (86·0° F) and different humidities, and number of feeds offered ; number and percentage frequency of nil feeds ; frequency distribution of weight of positive feeds ; mean weights of feed, and of positive feed.

	R. h., %				
	11	24	44	65	88
Sat. def., mm	28·1	24·0	17·7	11·0	3·8
No. of flies used	10	9	17	8	19
Total feeds	66	38	67	50	56
Blood taken, nil	26 (39·5)	7 (18·5)	6 (8·9)	8 (16)	46 (82%)
„ under 5 mg	4	3	1	2	2
„ 5–10 mg	20	16	20	10	2
„ 10–15 „	9	8	28	22	2
„ 15–20 „	6	3	10	6	2
„ 20–25 „	1	1	2	2	0
„ 25 mg & up.	0	0	0	0	2
Mean meal, mg	6·13	7·30	10·64	10·25	2·50
„ positive meal, mg	10·11	8·94	11·69	12·20	14·00

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* If it is desired to convert weights of blood, as given in this paper, into volumes, they should be divided by 1·06, the specific gravity of human blood.

V—Physiological Studies on Adults

Normal unfed flies

The preceding pages have been devoted to setting out a considerable body of fact. The reader now possesses some knowledge of the limits of temperature and humidity within which life is possible ; of the duration of life, of fed and unfed flies, under defined conditions of climate ; and of the effect of climate on reproduction and on feeding. We propose to study the physiological causes which may be supposed to underlie these effects. But before further experiments are discussed, it is necessary to establish the normal composition of flies which have not been subjected to experimental treatment. The mean wet and dry weight will be required, and also some knowledge of the variation about these means.

An attempt to establish a normal was made by taking flies known to be under 24 hours old and unfed, weighing them individually immediately after death, placing them in numbered tubes at 100° C, and recording their dry weight when it ceased to fall. The loss of weight is regarded as loss of water, though doubtless certain other volatile substances are lost. This work was done at intervals, for it soon became evident that there were considerable differences from month to month. There were not enough flies in June or July to carry the work through those months. A considerable number of *tachinoides* and a few *submorsitans* were weighed. The wet weights of *tachinoides* are shown as frequency distributions in Table VIII, which also gives the extreme weights observed month by month. Table IX gives the means and other constants of the wet and dry weights of the same species.

Table VIII—Giving frequency distribution of wet weight of 157 male and 120 female *Glossina tachinoides*, known to be under 24 hours old, and unfed ; also highest and lowest individual weights.

Weight	Males					Females					Total both sexes
mg	Feb.	Mar.	Apr.	May	Total	Feb.	Mar.	Apr.	May	Total	
15					*0*	1				*1*	*1*
14	1		1		*2*	8		1		*9*	*11*
13	4	1	1		*6*	5	3	4	1	*13*	*19*
12	9	4	2		*15*	3	11	11	2	*27*	*42*
11	11	4	10	1	*26*		12	15	8	*35*	*61*
10	6	19	10	3	*38*		7	7	4	*18*	*56*
9	1	14	22	7	*44*		8	3	2	*13*	*57*
8		6	8	1	*15*		1	2		*3*	*18*
7		6	5		*11*		1			*1*	*12*
Total	32	54	59	12	*157*	17	43	43	17	*120*	*277*
Highest mg	14·8	13·1	14·1	11·3	*14·8*	15·0	13·7	14·0	13·3	*15·0*	
Lowest mg	9·6	7·1	7·1	8·7	*7·1*	12·2	7·9	8·3	9·6	*7·9*	

TABLE IX—Giving mean wet and dry weights of normal *Glossina tachinoides* unfed, known to be under 24 hours old ; also certain constants.

Month	No.	Wet weights mg: Mean and standard error	Wet weights mg: Coeff. var.	Dry weights mg: Mean and standard error	Dry weights mg: Coeff. var.	Mean dry as % mean wet wt.
		Males				
Feb.	32	11·881 ± 0·202	9·47	3·631 ± 0·070	10·74	30·56
Mar.	54	9·913 ± 0·183	13·44	3·244 ± 0·051	11·41	32·72
Apr.	59	9·908 ± 0·184	14·15	3·088 ± 0·054	13·32	31·17
May	12	9·658 ± 0·225	7·74	2·900 ± 0·111	12·75	30·03
		Females.				
Feb.	17	13·859 ± 0·224	6·46	4·200 ± 0·083	7·88	30·31
Mar.	43	11·186 ± 0·204	11·84	3·565 ± 0·066	12·07	31·87
Apr.	43	11·047 ± 0·208	12·18	3·391 ± 0·049	9·34	30·70
May	17	11·182 ± 0·231	8·25	3·312 ± 0·204	24·68	29·62

Several facts of importance emerge from Tables VIII and IX.

(1) There is great variation in weight, even if individuals of the same sex and month are considered. Statistical tests must therefore be applied to experimental results in order to be certain that they are significant. As the distribution approximates to a normal curve, dispersion may be appropriately measured by the standard deviation.

(2) It is clear that the females are heavier than the males, and the difference, as tested by standard errors, is significant for each of the four months. Taking the mean male weight as 100, that of the female lies between 111·5 and 116·6 in different months. It seems that the proportion of dry matter is slightly less in the female than in the male.

(3) The wet weight of *tachinoides* killed in February is greater than in the succeeding months, and the difference between February and March is significant both for males and females, the standard error being used as a test ; the differences between March, April, and May are not significant. There are corresponding significant differences in the dry weights ; for both sexes the February value differs significantly from that for March or for May ; the differences between March, April, and May are not generally significant. It is a reasonable assumption that this difference is in some way due to climate. Reference to Table XXIII shows that from February to April the temperature in the open rose, and that the humidity was low. In May a little rain fell and the mean temperature was lower, with higher humidity. The puparia from which these adults were bred had been collected in nature, and subsequently kept in the laboratory in a cupboard of which the walls were lined with wet sacking so that the temperature and humidity were more equable than

outside. The seasonal difference in weight of flies is therefore probably due to something which affected the puparia, or even their parents, before they were brought into the laboratory. In experiments reported below (p. 216), we show that the humidity at which parents are kept seems to have no effect on the weight of the puparia they produce, but that temperature has an effect (though the results are not quite conclusive), high temperature producing light puparia. In any case, the observation that there is a seasonal difference in the weight of newly emerged flies is disturbing, for it suggests that flies at different times of the year may differ from one another in other ways, as yet unknown. One cannot assume that they are uniform, in working on their physiology or in using them in transmission experiments.

(4) The frequency distribution given in Table VIII shows that the range of weight is very great, even among individuals of the same sex and month. If one takes the extremes and compares the ratio of the heaviest to the lightest weight, it is generally about 1 : 1·5 or 1 : 2. But it is better to consider the standard deviation ; this gives a measure of the " scatter " of the group as a whole, the difference between every individual weight and the mean being taken into account in the formula. The standard deviations are high in proportion to the means Table IX ; for instance, the mean wet weight (males) for February is 11·881 mg, and its standard deviation 1·1246. The standard deviation expressed as a percentage of the mean is 9·47% ; this is known as the coefficient of variation. This coefficient enables us to make comparisons between the variability of different groups of figures. If, for instance, we wish to know whether males in February are more variable in respect of wet weight than of dry weight, we cannot get the information from inspecting the means or their standard deviations. But the coefficients of variation are 9·47 and 10·74, showing that wet weights and dry weights are about equally variable.

Table IX shows that for each month and sex, and for wet and dry weights, the value of the coefficient of variation is generally not far from 10. This is a high figure, and shows that there is a considerable degree of inconsistency between individuals in each group. This inconsistency may be inherent, or it may be partly due to keeping the flies for periods up to 24 hours before they are killed and estimated. (It will be remembered that 24 hours is a considerable part of the life of an unfed fly, see Table III. Unfed flies lose weight rapidly during their first 24 hours, see p. 200.) It was therefore decided to collect similar data from flies known to be less than two hours old ; it was found that emergence could often be precipitated if the pupæ were removed from the room (at about 30°—35° C) and put at 23° C for a few minutes. The work was done in May and the results will be found in Table X. As would be expected, these flies are heavier than flies of the same sex, the age of which is anything up to 24 hours, Table IX. The values of the coefficients of variation are a little less for flies under two hours old, but they are still high, ranging from seven to nine. This appears to show that most of the variation is inherent, and not due to the interval before the flies are killed.

TABLE X—Giving mean wet and dry weights of male and female *Glossina tachinoides*, killed (May) when they were under two hours old ; also certain constants derived from the same flies.

		Wet weight mg		Dry weight mg		Mean dry as % mean wet wt.
Sex	No.	Mean and standard error	Coeff. var.	Mean and standard error	Coeff. var.	
Male	29	10·859 ± 0·176	8·57	3·445 ± 0·054	8·30	31·72
Female	13	12·2 ± 0·256	7·27	3·546 ± 0·099	9·68	29·06

It was not possible to make such full studies on *Glossina submorsitans*, the supply of which was smaller and less regular. Sixty-two males and thirteen females, known to be under 24 hours old, were killed during March and April, and the frequency distribution of the wet weights in milligrams was :—

	12–	13–	14–	15–	16–	17–	18–	19–	20–	21–	22–	23–	24–	Total
Males . . .	1	1	2	3	6	4	13	11	8	6	4	2	1	62
Females . . .	0	0	0	0	0	0	4	5	3	0	1	0	0	13

The means and other constants of the wet and dry weights of the same individuals will be found in Table XI.

TABLE XI—Giving means and other constants of wet and dry weights of *Glossina submorsitans* killed (March and April) when unfed and less than 24 hours old

		Wet weight				Dry weight				Dry mean as % wet mean
Sex	No.	Mean and standard error	Coeff. var.	Max. mg	Min. mg	Mean and standard error	Coeff. var.	Max. mg	Min. mg	
Male	62	19·002 ± 0·2216	12·98	24·4	12·5	5·923 ± 0·0807	15·18	8·0	3·7	31·17
Female	13	19·623 ± 0·1933	5·02	22·0	18·6	5·769 ± 0·0596	5·25	6·5	5·3	29·40

Unfed flies in relation to humidity

It has already been observed, Table III, that the life of flies is generally longer at intermediate humidities than in moister or drier air. From this observation let us proceed to investigate the physiological effects of exposure to atmospheres of different humidity.

In the experiments which are now to be considered, unfed flies under two hours old were taken ; the work was done in May. The wings were clipped and the flies were weighed and placed in numbered tubes, under controlled conditions of humidity at 30° C ; 24 hours after the start of the experiment they were re-weighed. A few which died were rejected. Fig. 6 shows the loss of weight of the individual flies, and

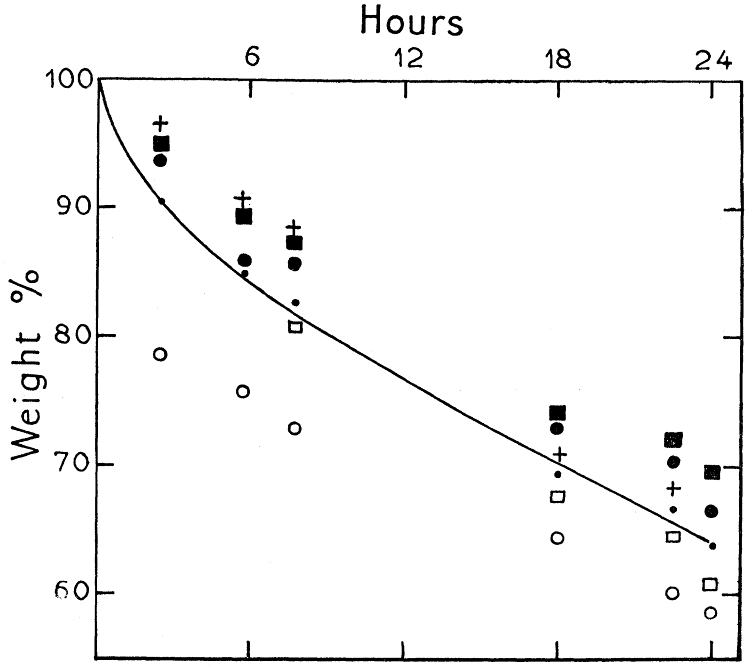

FIG. 6.—Loss of weight of a group of *Glossina tachinoides*, under two hours old at start of experiment, and kept at 30° C (86·0° F) and 44% relative humidity. The five individuals are distinguished by symbols. The small dots indicate mean values, through which the line is drawn.

the mean loss, during 24 hours' starvation at a saturation deficiency of 17·8 mm (relative humidity 44%). Similar work was done at three other humidities, Table XII. In spite of the fact that flies under two hours old were used, there is considerable lack of uniformity in the mean weights at the commencement of the experiments. The data given in Table XII under " original weight " show this. The mean original weight of the group at 11% relative humidity is significantly different from all the other means ; the differences between the others are considerable but not significant. We shall therefore proceed with the knowledge that the values obtained at 11% relative humidity are open to suspicion. It is clear that the flies kept in drier air have lost weight* more than those in moister ; this is well shown by the last column, which gives the final weight as a percentage of the original weight (the group at 11% humidity behaving irregularly). It appears that, as the number of flies in each group is small, the *t* test is appropriate for significance. If the differences between the final weights (either in mg or as a percentage of original weight) are tested, it is found that some differences between consecutive members of the series are not significant. But other differences (between 88 and 44%, 88 and 11%, and between 44 and 11%) are significant. The conclusion is justified

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* If unfed flies are observed during starvation, it will be noticed that most of them pass a small quantity of liquid excrement ; it seems that the quantity passed by different flies varies very considerably. One must not, therefore, presume that the loss of weight during the period of the experiment is due solely to evaporation.

that the differences between mean final weights are due to the experimental conditions and cannot be attributed to the variability of the material.

TABLE XII—Giving mean weights of groups of *Glossina tachinoides* before and after starving for 24 hours at 30° C (86° F) under controlled conditions of humidity ; flies were under two hours old, and unfed, at start of experiment.

R.h. %	Sat. def. mm	No.	Original weight mg Mean and standard error	Final weight mg Mean and standard error	Final as % original weight
88	3·8	9	$11\cdot4\dot{7} \pm 0\cdot254$	$9\cdot2\dot{4} \pm 0\cdot294$	80·54
65	11·0	8	12·2125 ± 0·275	9·2875 ± 0·372	76·60
44	17·8	5	12·7600 ± 0·590	8·1600 ± 0·467	63·94
11	28·1	11	10·6182 ± 0·269	$7\cdot5\dot{6}\dot{3} \pm 0\cdot244$	71·23

After the 24 hours' exposure the flies were killed and dried to constant weight. The differences between the wet and dry weights is taken to be water, though other volatile substances are lost. Subsequent extraction with ether divides the dry weight into two parts ; one part is soluble in ether and is recorded as fat, though no doubt the ether removes substances other than fat. We can now compare the groups of flies which were exposed to different humidities and see what effect the experiment has had upon their final composition. The flies which were killed when under two hours old, Table X, supply a control ; but as sex has been disregarded in the present experiments, we take the average of the two sexes as control. The figures for the experimental flies and for this control are given in Table XIII.

It would be unwise to build an elaborate interpretation on these figures, because the numbers are not great and because weighing was possible only to 0·1 mg. Moreover, the original weights of different groups differed greatly, Table XII, and one cannot estimate the loss which is due to excretion. But it seems that Table XIII goes some way to explain what occurs when insects are starved under different conditions of humidity. It has already been shown that there is considerable loss of weight in each group, Table XII, and that it is greater at the lower humidities. An unknown part of it is due to excretion, but it is easy to show that most of the lost weight is water. Consider, for instance, the data for control and for flies starved at 44%. From controls killed under two hours old, we know that the 12·76 mg of fly must have contained 8·90 mg water when the experiment started. But after exposure to 44% humidity these flies weighed 8·16 mg and lost 5·14 mg on drying, so that at least 3·6 mg of water had been evaporated during the experiment, the actual figure having been higher, because water of metabolism was being produced during the period. This figure, 3·76 mg, is equivalent to 29·5% of the insects' original weight. Similar calculations show that the greater part of loss of weight during all the experiments, even at the highest humidity, was water, see Table XIII, last column.

TABLE XIII—Taking final wet weight from previous table, and showing what parts are dry matter, ether-soluble "fat," and "water." Means (in mg) are followed by standard errors. Control is mean of two sexes, under two hours old (Table X).

			Means and standard errors (in mg)				Water	
R.h. %	Sat. def. mm	No.	Wet weight	Dry matter	Fat	Water mg	as % of wet weight	lost as % original weight
88	3·8	9	9·24 ± 0·294	3·40 ± 0·201	1·160 ± 0·178	5·84	63·20	18·9
65	11·0	8	9·2875 ± 0·372	3·05 ± 0·097	0·675 ± 0·070	6·24	67·2	18·7
44	17·8	5	8·1600 ± 0·467	3·02 ± 0·101	0·540 ± 0·040	5·14	62·99	29·5
11	28·1	11	7·5636 ± 0·244	2·60 ± 0·237	0·518 ± 0·038	4·96	65·61	23·0
Control	(13♂, 13♀)		11·5153 ± 0·229	3·58 ± 0·055	1·092 ± 0·053	8·04	69·73	—

But though so much water was lost, the percentage remaining in the flies at the conclusion of the experiments is not greatly different at the different humidities. In other words, these insects possess the power of compensating for loss of water from the body, so that though one group (88% relative humidity) lost about 20% of its original weight during starvation, while another group (44% relative humidity) lost about 36%, the proportion of water in their bodies at the end of the experiment was hardly different.* Inspection of Table XIII further shows that this regulation is brought about by using fats. In the group of flies exposed to 88% humidity, the amount of fat at the end of the experiment was actually more than in the controls ; this is probably accidental, the alternative hypothesis—that unfed insects synthesize fat during starvation—being improbable. At each lower humidity the amount of fat remaining at the end of the experiments is less and less ; nearly all the differences between groups of flies kept at different humidities, and between each group and the control, are significant by *t*. This shows that *Glossina* is one of those insects which compensate for evaporation by metabolising fat and so producing additional water.

It has been shown that even at 30° C there is considerable loss of water during 24 hours' starvation, and that it is greater in dry than in moist air. It is extremely probable that at temperatures over 40° C (104·0° F) the loss is greater, and the difference between loss in dry and in moist air accentuated. If this is correct it explains the observation that there is a difference of 2° C between the temperature which is fatal in moist air and in dry, fig. 1. An attempt was made to *prove* greater loss of water in drier air. Unfed male *tachinoides* were taken, when less than 24 hours old, and exposed to 41° C (105·8° F) for one hour at three different humidities.

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* The percentage of water is much higher in the controls than in any experimental group. It may be that evaporative loss is considerable even at so high a relative humidity as 88% (and *cf.* Mellanby, 1932, *b*). Alternatively, the fæces passed during the experiment may contain a high proportion of water, and this is included in the control, which was killed when two hours old.

The few which died were rejected, and the rest killed at once, weighed and dried. The control was furnished by flies taken unfed from the cages, on the same days. The results were inconclusive and need not be tabulated. The mean wet weights were lower at the two lower humidities than at the higher, and this may well have been due to greater evaporation, but only one of the differences between wet weights (that between the control and flies exposed to 11% humidity) is statistically significant. We should expect a disturbance in the percentage of dry matter, and, in fact, it rose from 32·9% after exposure, at a relative humidity of 88%, to 33·0 at 44% humidity, and 34·0 at 11% humidity. It seems, therefore, that there is greater evaporation in drier air, and that the increased metabolism of fat is not sufficiently rapid to compensate for it. But this conclusion cannot be regarded as proved, for the percentages of dry matter after exposure to different degrees of humidity are not significantly different from one another, nor from the control.

The preceding pages give a partial explanation of what happens when unfed *Glossina* are exposed to atmospheres of different humidity. During a 24 hours' exposure to 30° C, loss of weight is greater the drier the air ; but the proportion of water in the insect's body is maintained nearly constant by using more fat in dry than in damp air. It is also known that *Glossina* can survive a higher temperature in dry than in moist air, for an hour. It must be presumed that the insect does this by evaporating water, and lowering its body temperature, though this was not proved. It is difficult to think of any other mechanism by which the same result might be achieved ; indeed, there is definite evidence that cockroaches regulate their temperature in this manner (Necheles, 1924). But the experiments have failed to tell us why *Glossina* dies so soon in atmospheres of high humidity, even at such comparatively low temperatures as 24° and 30° C (see Table III and fig. 3). We expected to find that after exposure to 88% humidity the insects would contain a high proportion of water, which they failed to evaporate, and death might perhaps have been due to water-logging. It is already known from the work of Lester and Lloyd (1928) that if flies are caused to drink water, or fluids of low osmotic tension, the excess water acts as a tissue poison. But the data in Table XIII show that this does not occur in our experiments, for the insect's power of regulating the proportion of water in its body extends to this high humidity.

We can state definitely that death at high humidity is not due to the growth of moulds : we feel sure that its explanation is physiological.

Fed flies in relation to climatic factors

Considerable technical difficulties have already been encountered in studying the effect of different degrees of humidity upon flies which had never been fed, because individual flies are so unlike one another in weight and in the proportion of water in their bodies, Tables VIII, IX, and X. But if the flies are fed they become still more different from one another, for some take more than their own weight of blood within a few minutes, and others may not feed at all ; moreover, those which have fed lose weight very rapidly so that 24 hours later many of them are prepared to take another enormous meal ; the weight curve therefore fluctuates very rapidly and widely, fig. 4. The facts given in Table XIV show some of the inherent difficulties of the problem ; it gives wet and dry weights of *Glossina tachinoides* killed four hours after feeding, flies which refused to feed having been excluded. If the maxima and minima are compared, for either sex, and for wet or dry weights, the ratio is almost two to one. The same thing is more appropriately measured by the coefficients of variation which are about twice as great as in unfed flies, Tables IX, X, and XI. It is therefore certain that it will be difficult to study the effect of climatic factors upon flies which have fed. In the present paper no more can be done than to present a series of notes.

TABLE XIV—Showing mean wet and dry weights of male and female *Glossina tachinoides* killed about four hours after a meal. Flies were four to five days old ; they had been fed previously every day, and those which did not feed rejected.

		Wet weight				Dry weight				mean Dry as % mean wet
Sex	No.	Mean and standard error	Coeff. var.	Max. mg	Min. mg	Mean and standard error	Coeff. var.	Max. mg	Min. mg	
Male	14	18·743 ± 0·667	13·79	24·3	13·7	5·664 ± 0·263	18·01	7·6	3·8	30·22
Female	11	21·754 ± 0·677	10·79	26·9	18·4	6·754 ± 0·383	19·64	9·0	5·0	31·05

Feeding—Table XIV shows that four hours after feeding the proportion of dry matter is the same as in the unfed fly—that is to say, just over 30%. The proportion of solid matter in blood is 25%. It follows that during the first few hours after a feed a considerable concentration of the blood which has been swallowed must take place. It will be remembered that WIGGLESWORTH (1929) has already demonstrated a concentration of blood in the anterior segment of the midgut of *Glossina*, and that LESTER and LLOYD (1928) have shown that the rapid loss of weight which occurs soon after feeding is due in the main to the activity of the Malpighian tubes. The subject of excretion is probably of great importance, and is perhaps much affected by climatic conditions. It seems probable that a more intensive study of it, perhaps along the lines of recent work on *Rhodnius* (WIGGLESWORTH, 1931), might help to explain local and seasonal effects upon wild populations of *Glossina*. The volume of excreta is so great that it is quite impossible to study metabolism of the fed fly without including excretion.

Storage—In considering the effect of climate upon the fly which has fed, one must bear in mind the peculiar life-history of these insects. The larva completes the whole of its growth *in utero*. It does not feed as an independent organism, but pupates soon after birth. The puparium, in due course, liberates a fly, which is complete and full grown so far as external characters are concerned. It follows that the larva, at the time it leaves its mother's body, is equivalent to another complete fly, plus the material which is wasted in the larval and puparial skins, plus whatever is used during metabolism. *Prima facie*, therefore, one would suppose that the newly-hatched fly, in spite of appearing to be full grown, must be very different internally from a more mature insect.

It would be of interest to approach this problem from the chemical side, and to discover whether the result of feeding is that the fly accumulates stores of fat, carbohydrate or some other substance. Table XV shows the amount of fat (more precisely, of ether-soluble material) which has been found in *Glossina tachinoides* of various ages. It is to be understood that all the " fed " flies had been offered blood daily, including the day on which they were killed, so that their nutritional state was good. The period which intervened between the last meal and death was between three and six hours. It is already known, Table XIII, that the fly under two hours old contains approximately 1·0 mg of fat and that a considerable proportion of this is used up if the fly fasts for 24 hours. Table XV shows that if the fly is fed the amount of fat is increased, but only to a slight extent. This increase is observable by the third day (males). In view of the small number of individuals examined, one is not justified in concluding that there is any increase after this time. The figures for females lead to the same conclusion, but they must be accepted with caution, for they may be complicated by early pregnancy.

TABLE XV—Showing the amount of ether-soluble " fat " in *Glossina tachinoides*, unfed and after feeding daily for various periods. The figures give milligrams per fly.

Age	Sex	No.	Mean wet weight mg	Mean dry weight mg	Fat mg		
					Mean	Max.	Min.
2 hours unfed . .	♂	29	10·86	3·44	1·131	1·6	0·5
„ . .	♀	13	12·20	3·55	0·90	1·4	0·6
24 hours unfed . .	♂	8*	10·54	3·21	0·975	1·3	0·5
3 days fed	♂	9*	17·42	6·17	1·21	1·9	0·6
7 „	♂	5*	18·26	6·64	1·46	2·2	0·9
10 „	♂	10*	18·66	6·68	1·31	2·1	0·8
7 „	♀	7*	21·29	6·84	0·96	1·4	0·6
10 „	♀	7*	27·89	9·56	1·70	2·8	1·0

* All from one batch, in early April.

Very few facts relating to *Glossina submorsitans* are available. The following figures relate to wild *submorsitans* killed and dried immediately after capture :—

	Mean wet weight mg	Mean dry weight mg	Mean fat mg
Males	26·0	9·53	2·18
Females	34·5	13·8	4·31

As control the following may be used : a batch of 21 males under 24 hours old and unfed gave mean wet weight 17·70 mg, dry weight 5·52 mg, and fat 1·36 mg.

It seems, therefore, that in both species feeding causes an increase in fat. It would be of interest to know whether there is also an increase of glycogen or other carbohydrate.

Starvation—In a preliminary experiment a group of *tachinoides* was fed daily for a week. Those which fed on the last day of the week were selected ; some were killed at once and estimated for fat, the rest were kept fasting at 30° C and 44% relative humidity, and killed at intervals (44% is the optimum humidity—see Table III). In each group there were between four and ten individuals of each sex. The amount of fat per fly was found to be as follows :—

	At once	24 hours	48 hours	72 hours
Males	0·74	?	1·24	1·07
Females	1·13	1·26	1·34	0·87

No attempt was made to continue the experiment further than 72 hours, and only a small proportion of flies would have lived longer.

It is already known that in unfed flies, Table XIII, the rate at which fat is used is dependent on humidity. A corresponding preliminary experiment was carried out, with male *tachinoides*, in a good state of nutrition and a week old. The controls were killed immediately after feeding, and the rest after starving 24 hours at 30° C and three different humidities. The following mean figures were obtained (mg of fat per male) :—

Control	88%	44%	11%
0·67	0·62	0·80	0·92

This result is so contrary to expectation that the experiment was repeated, fat being estimated in individual flies ; it is unfortunate that on the second occasion all the flies at 88% humidity died during the period of 24 hours' starvation. The following figures show the number of flies in each group, and the mean weight of fat.

	Control	44%	11%
Number	10	8	7
Mean and S.E.	1·10±0·1716	0·34±0·0725	0·77±0·1603

The *t* test shows that the figures for fat are all significantly different from one another.

From these experiments, it appears that, after feeding, the amount of ether-soluble material in the fly increases, reaching a maximum at about 48 hours ; also, that if flies are kept at different humidities and examined after 24 hours, more fat is accumulated at the low than at the high humidity. At first sight it appears that the fed fly differs in this respect from the unfed (which has been shown to use its fat more rapidly in dry air than in moist), but perhaps the discrepancy will disappear in the light of further knowledge. In particular we require data as to the amount of fat present in flies after they have been kept for 48 and for 72 hours at different humidities. We also need precise information about the substances which are here crudely described as " fat."

It is already known that humidity has considerable effect both on the birth- and death-rates of communities of *Glossina*. If we are to understand the relation of the wild fly to the climate in which it lives, it is clearly of the first importance that the effect of humidity upon the fed fly should be the object of physiological study. It appears that work of this sort may elucidate a number of observations which have already been made in the field. JACKSON (1930, 1931, 1933) has shown that there are probably areas in which the fly feeds and others in which it breeds and rests after feeding. He distinguishes these areas in several ways, among them by noting the state of nutrition of the flies, and he shows by marking individuals that hungry flies pass from the breeding and resting place to the feeding ground. The state of nutrition of these flies is judged by rather elaborate criteria, and a considerable personal element must enter into the work. It is probable that physiological studies might give precision to these observations, and might provide simpler quantitative tests of the insects' nutritional state. We hold the view that the subject is hardly appropriate for study in the field until more attention has been devoted to it in the laboratory.

VI—PUPARIA

Introductory

The puparia were studied with reference to climatic conditions. The insect at this stage is exposed to a variety of climatic conditions which differ at different seasons and depend on the locality and the exact site of the breeding place. The effect of these conditions on the insect is seen, for example, in the variable rate of mortality among *Glossina* puparia (p. 212) collected in the field. In our work, temperature and humidity were controlled as in experiments on the adult fly (p. 177). The torsion balance was very useful in work on the puparia, much information being accumulated from records of weights on the day of pupation and at subsequent dates. Weighing was particularly valuable in ascertaining the time at which individuals died; puparia were weighed on the first day and at subsequent intervals so that the date of death, indicated by suddenly increased loss of weight, was observed. Also, puparia which failed to emerge were opened and examined and the stage of development at the time of death recorded.

Glossina tachinoides, being the species available in greater numbers, was the principal subject of experiment.

Duration of the puparial stage

The period from pupation to the emergence of the adult fly is considered in relation to the temperature and humidity in which puparia were kept and to the sex of the emerging adults. Puparia were examined and emergences recorded at regular intervals of 24 hours. In reckoning the duration of life, a puparium, found, for example, on 1st May, was 0 — 24 hours old, say 12 hours; so that, on 2nd May, the age of the puparium was $1\frac{1}{2}$ days and on 20th May $19\frac{1}{2}$, but a half-day is subtracted as being the age of the adult, leaving 19 days as the duration of the puparial stage.

Temperature—Table XVI shows the mean duration of the puparial stage in both species at 24° and 30° C (75·2° and 86·0° F). The duration of puparial life is very uniform under identical conditions and there is little difference between the means for the two species.

Table XVI—The duration of the puparial stage in *Glossina tachinoides* and *submorsitans* at 24° and 30° C (75·2° and 86·0° F); also that of four puparia of *submorsitans* at 27° C (80·6° F).

	Temp. °C	Sex	No. of puparia	Mean duration, days	Max., days	Min., days	Standard error	Coeff. of var.
Tachinoides	30	♂	49	21·8776	24	18	± 0·157	5·0
		♀	52	20·6346	22	19	± 0·093	3·3
	24	♂	33	33·9394	36	32	± 0·196	3·3
		♀	47	32·1277	35	29	± 0·203	4·3
Submorsitans	30	♂	20	23·4000	26	21	± 0·239	4·6
		♀	19	21·6316	24	20	± 0·193	4·0
	27	♀	4	24·5000	26	23		
	24	♂	38	33·9211	37	32	± 0·187	3·4
		♀	37	31·7568	35	30	± 0·211	4·0

Sex—As shown in Table XVI, the mean duration of the puparial stage of the males was consistently greater than that of the females in both species at each temperature. The differences, which lay between 1·25 and 2·16 days, were statistically significant in each case, as tested by the standard error.

Humidity—It has been stated by Pomeroy and Morris (1932, p. 515) that in *Glossina tachinoides* in the Gold Coast "the length of the pupal period is markedly affected by atmospheric humidity so that pupae deposited early in the dry season and those deposited late all appear to hatch out at about the same time, when rainfall causes an increase in atmospheric humidity." No experimental evidence in support of this statement is given, and it appears that they have not made a sharp distinction between proof and hypothesis. If their statement is correct, the matter is extremely important, and throws new light on the seasonal periodicity of *Glossina*.

There is no evidence in our experiments to show that the duration of the puparial stage in either species bears any relation to atmospheric humidity. Furthermore, out of a large number of puparia of both species kept at low humidities both at 24° and 30° C, none remained alive for more than a few days after the usual date of emergence. It is seen from Table XVI that no adult emerged more than three days after the mean date of emergence, although the groups of puparia were kept over a very wide range of humidity (98 to 11%). The following figures for *Glossina tachinoides* were obtained from puparia kept at 30° C. Clearly humidity has no effect, though the range of saturation deficiency is 0·6 to 28·1mm.

Relative humidity %	No. of emergences	Mean duration in days
98	10	22·0
88	66	21·2
65	55	21·3
44	5	22·4
19	5	21·8
11	2	22·5

Puparia of *Glossina submorsitans* at extreme humidities show similar means; for example, at 30° C 10 puparia at 19% relative humidity had a mean duration of 23·0 days and 11 at 88% relative humidity a mean of 22·6 days.

The fact that low humidity cannot prolong the life of puparia can be shown in another way. Fig. 7 shows the daily weights of some *tachinoides* puparia kept at 24° C and a relative humidity of 65%. Five emerged about the 34th day, and on the 35th day the remaining three, which died, had already begun to lose weight rapidly. Similarly, in other experiments there was no indication of prolongation of life beyond the usual period.

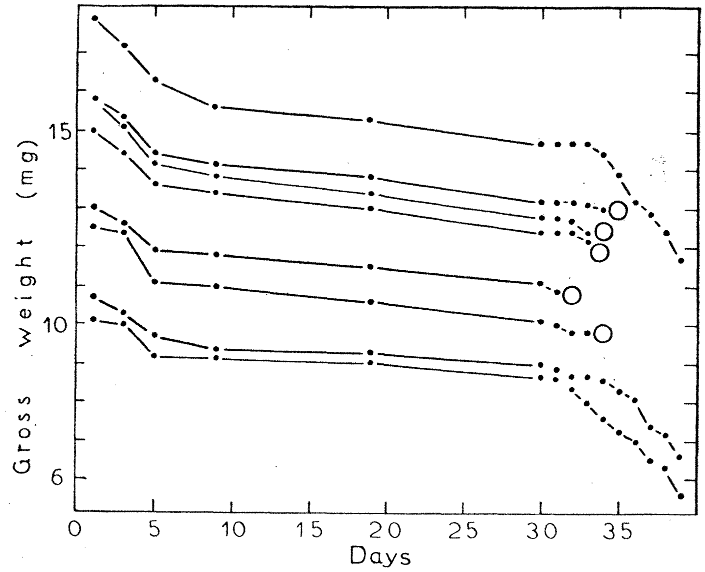

FIG. 7—Curves showing the changes in weight of eight puparia of *Glossina tachinoides* kept at 24° C (75·2° F) at a relative humidity of 65%. The circles indicate emergence; the remaining puparia died.

Weights of puparia on the first day

Glossina tachinoides—Fig. 8 shows the distribution of weights of 1,155 puparia bred in the laboratory and weighed within 24 hours of pupation. The mean was 15·1100 mg, and the extremes ranged from 6 mg to 23 mg. (Standard error ± 0·086, and coefficient of variation 19·2.) The distribution approximates to a normal curve. These puparia were obtained during a period of five months ; but the high coefficient of variation is not due to seasonal differences (see p. 222). None of the individuals included in this figure could have been described as abortions, for all had a regular shape and were rich brown. Abortions were also observed, but they can be easily distinguished from small puparia because they become wrinkled as they dry and they do not develop the colour which is characteristic of the puparium.

The range of weight is surprising, and we wished to know whether wild puparia differ so greatly from one another. This cannot be settled by weighing them, for the age is unknown and puparia lose weight as they mature. Judged by size, wild puparia of both species of *Glossina* are much more uniform than those produced in captivity ; moreover, it seems that there is little difference in mean size, for captive puparia may be either larger or smaller than wild ones.

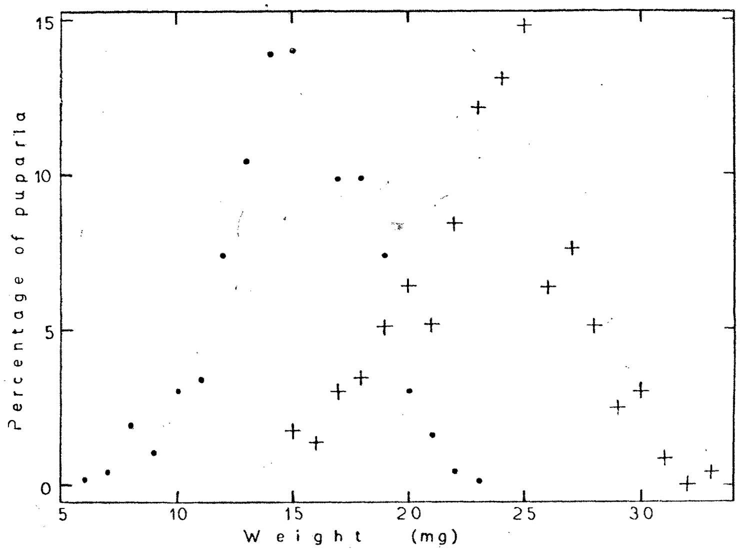

FIG. 8—The percentage distribution of weights of 1,155 puparia of *Glossina tachinoides* (dots) and 237 of *G. submorsitans* (crosses). All were weighed on the first day.

*Glossina submorsitans**—237 puparia had a mean weight of 23·4937 mg and a range of 15 to 33 mg. (Standard error ± 0·226 mg, coefficient of variation 14·8.) In fig. 8, the distribution of weights shows a considerable overlap between the two species so that out of 1,000 puparia of each species, 1,051 would be indistinguishable by weight as *tachinoides* or *submorsitans*. In nature this does not occur and wild puparia can be separated almost infallibly.

Sex of Adults emerging from the puparia.—It might be expected, from work on the adult fly (Tables VIII—XI), that puparia producing females would be heavier on the first day than those producing males. The data for puparia of both species bred during several months were examined, and it was found that the differences

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* It is curious that the only authors who have published weights of puparia of *G. morsitans* have misplaced a decimal point, and given figures which are ten times too high (POTTS, 1933 ; CARMICHAEL, 1933).

in weight between the two sexes were inconsistent and not statistically significant. There may be a significant difference in wild puparia, but it is probably masked in bred ones by their great variation in weight.

Weight of puparia and the climatic conditions in which the parents were kept.—Puparia of both species were obtained from adults kept at constant temperature and humidity, and from adults kept in cages under fluctuating conditions, the temperature being recorded.

Temperature—Of 35 puparia of *Glossina tachinoides* bred from parents kept at two temperatures in incubators, the mean weights were as follows :—

Temperature °C	No. of puparia	Mean weight mg	Standard error
30	23	12·1218	± 0·601
24	12	14·0083	± 0·671

The difference between these means was found by the *t* test not to be statistically significant, the probability of this difference occurring by chance being between 1 : 10 and 1 : 20. The figures are suggestive, but not conclusive.

Although the difference is not significant, we can examine data collected for puparia of both species kept in cages under fluctuating conditions of temperature. It cannot be regarded as conclusively proved that adults at a high temperature produce lighter puparia, but the following facts are available. Puparia of *Glossina submorsitans* were bred from adults in an incubator at 30° C, and in cages with monthly mean temperatures fluctuating between 24° and 28° C. At 30° C, 35 puparia had a mean weight of 21·2 mg, and at the lower temperature 237 puparia had a mean of 23·5 mg. The following monthly mean weights of *tachinoides* puparia bred in cages are compared with the approximate mean temperature $\left(\frac{\text{mean max.} + \text{mean min.}}{2}\right)$ of the cages in the previous month.

Month	Temp. in previous month, °C	No. of puparia	Mean weight mg	Standard error	Coeff. of var.
Feb.	About 24	22	16·6364	± 0·448	12·63
March	„ 24·7	488	15·1332	± 0·138	20·14
April	„ 24·9	264	15·5682	± 0·179	18·67
May	„ 28·1	178	14·6292	± 0·185	16·91
June	„ 26·9	88	14·4205	± 0·307	19·96

Humidity—Reproduction does not occur in captivity except over rather a narrow range of humidity (p. 189), so that some of the following mean weights of puparia from adults kept at 30° C are derived from small numbers.

R. h. %	*G. tachinoides* No. of puparia	*G. tachinoides* Mean weight mg	*G. submorsitans* No. of puparia	*G. submorsitans* Mean weight mg
44	15	11·45	3	19·57
19	2	14·75	6	22·28
11	6	12·82	26	21·32

There appears to be no relation between humidity and mean weight and the difference between the means of *tachinoides* at relative humidities of 11 and 44% is not statistically significant according to the *t* test.

Mortality of puparia

It is already known that the mortality in puparia collected in nature is variable and frequently high. It is sometimes over 50% and there is some evidence that the changes in mortality are seasonal (Chorley, 1929; Taylor, 1932*b*; Potts, 1933). The following laboratory experiments were carried out in order to discover what factors might cause mortality under natural conditions.

(*a*) *Mortality in relation to weight*—In early experiments it was found that puparia of *Glossina tachinoides* weighing less than 14 mg on the first day had a higher mortality than heavier puparia. Taking figures for puparia kept under favourable conditions (30° C and a relative humidity of 70% or over), the death-rate was as follows :—

Weight on first day, mg	No. of puparia	No. died	% died
20–22	7	2	29
18–20	31	4	13
16–18	47	14	30
14–16	37	9	24
12–14	39	18	46
10–12	13	8	62
8–10	15	14	93
6– 8	4	4	100

Subsequently all *tachinoides* puparia weighing less than 10 mg on the first day were discarded.

(*b*) *Temperature and humidity*—The effects of temperature on mortality can be divided into those of extreme high and low limits of temperature and those of moderate conditions of temperature and humidity with varying mortality.

Extreme limits : the thermal death points were ascertained when puparia were exposed for one hour in an atmosphere of 50% relative humidity in a water bath at a constant temperature. By weighing at intervals it was possible to ascertain whether death occurred soon after exposure to unfavourable conditions (and probably as a result of them) or at a subsequent date (p. 217). In the latter case, puparia were recorded as having survived exposure. After exposure, all puparia were kept at 24°—28° C in a relative humidity of 50%.

Table XVII shows the results of these experiments on both species. In nearly every instance the upper temperature limit is sharply defined. Two significant facts are observed : (*a*) that in both species the thermal death point of puparia exposed on the 15th day is higher by 2° C (3·6° F) than that of those on the 1st day ; and (*b*) that on both the 1st and 15th days the thermal death point of *Glossina* *submorsitans* is 1° C higher than that of *tachinoides*. Results of exposures on the 1st and 15th days are not comparable with those on the 22nd—27th, since in the latter emergences are recorded, and those which failed to emerge may have survived the experiment but died subsequently. The thermal death point of *submorsitans* puparia exposed on the 15th day is the same as that found by Nash for puparia exposed for a day on the surface of the soil (Nash, 1933*a*, p. 181). All puparia were killed when the maximum temperature of the soil rose to 45° C (113·0° F) during some part of the day.

At 37° C and a high relative humidity (80%), no puparia lived more than a few days ; 15 *tachinoides* and six *submorsitans* were used.

Puparia of *Glossina tachinoides* were exposed to a temperature of 0° C ; four out of six survived an exposure of one hour and four out of 20 an exposure of 24 hours.

Table XVII—The effect of exposing puparia of *Glossina tachinoides* and *submorsitans* to constant temperatures for one hour, at a relative humidity of 50%. Experiments were carried out with puparia under 24 hours old, about the middle of puparial life, and towards the end of puparial life.

	Temperature		Age of puparia, days					
			0		15		21–27	
	°C	°F	No. of puparia	No. survived	No. of puparia	No. survived	No. of puparia	No. emerged
Tachinoides	44	111·2	7	0	7	0	5	0
	43	109·4	7	0	6	6	8	0
	42	107·6	7	0	8	8	4	2
	41	105·8	15	10	8	8		
	40	104·0			8	8		
Submorsitans	46	114·8			4	0		
	45	113·0			6	0		
	44	111·2			4	4		
	43	109·4	5	0			4	0
	42	107·6	4	2			6	6
	41	105·8	4	4				

Mortality among puparia kept at moderate temperatures and different humidities : Table XVIII contains the data concerning mortality under less extreme conditions. Puparia of *Glossina tachinoides* and *submorsitans* were kept at 24° and 30° C at different humidities.

Temperature—Since humidity affects mortality (see below), we can only compare data at roughly the same saturation deficiency. If this is done, we see that for *tachinoides* puparia at relative humidities of 44, 65, and 88% (at which figures are available for two temperatures), the survival in each case was distinctly higher at 24° than at 30° C, a large number being available for comparison at 65%. At

7·8 mm of saturation deficiency at 24° C, the survival rate is greater than that at 3·8 mm at 30° C.

TABLE XVIII—Mortality of puparia of *Glossina tachinoides* and *submorsitans* kept under different conditions of temperature and humidity.

	Temp. °C	R. h. %	Sat. def. mm	No. of puparia	No. emerged	% emerged	Died early	Died late
tachinoides	30	98	0·6	12	11	92	0	1
		88	3·8	79	54	68	—	—
		65	11·0	84	55	65	16	13
		44	17·7	14	5	36	6	3
		19	25·6	13	4	31	5	4
		11	28·1	15	2	13	5	8
	24	88	2·6	16	16	100	0	0
		65	7·8	95	75	79	10	10
		44	12·5	7	6	86	1	0
submorsitans	30	88	3·8	14	11	79	0	3
		65	11·0	16	12	75	3	1
		44	17·7	13	10	77	1	2
		19	25·6	13	10	77	3	0
		11	28·1	7	3	43	1	3
	24	88	2·6	46	43	93	2	1
		65	7·8	12	12	100	0	0
		44	12·5	11	9	82	1	1
		19	18·1	20	14	70	5	1

Humidity—In *Glossina tachinoides* puparia at 30° C, the effect of humidity is clearly seen, the survival rate steadily falling with decreasing humidity. But even at the very low figure of 11% relative humidity, a few puparia can survive. The figures for *submorsitans* show that the emergence rate at 24° C is slightly higher than that at 30° C ; there appears to be no relation between survival and humidity except at the very low relative humidity of 11% at 30° C. Comparing the two species, it is seen that in six out of eight experiments, the emergence rate of *submorsitans* is higher than that of *tachinoides* under similar conditions, and that both temperature and humidity cause greater differences in mortality in *tachinoides* than in *submorsitans*.

Death occurred either during the first few days, or when the adult insect was developed. No relation can be seen between the time of death and either temperature or humidity, and this is true of both species. It may be concluded, therefore, that for puparia affected by unfavourable conditions, the beginning and end of puparial life are the most critical stages. In experiments on loss of weight (p. 217), it was found that those puparia which eventually died had a normal steady loss of weight until the last few days of puparial life, when the rate of loss suddenly increased. Owing to the frequency with which adults in experiments died without being able to emerge from the puparial cuticle, it was thought necessary to ascertain whether this occurred also under natural conditions ; 323 puparia of both species were examined within a few hours of being collected in nature. Of 176 puparia of *Glossina tachinoides*, 28 had died early or about the middle of puparial life and six had died as adults in the puparium, and of 147 *submorsitans*, 22 had died early and six late. The collection was made in the early rains during a period of increased breeding, as indicated by a preponderance of young puparia among the living ones. This breeding period was too recent to have allowed the accumulation of many dead old puparia, whereas the fresh condition of most of those which had died young indicated the recent rapid accumulation of this class. Therefore, at other seasons there would probably be a greater proportion of puparia which had died in a late stage under natural conditions.

(*c*) *Submergence*—Water-logging of the soil is sometimes a critical factor in the survival of puparia. In the laboratory, puparia of both species were submerged in completely water-logged soil to a depth of one inch for different periods at 30° C. The puparia were placed in glass cylinders, one inch long and covered at one end with mosquito netting. The cylinders were filled with soil, and submerged in water resting on a layer of the same soil. The soil used consisted of humus collected from a breeding place of both species among dense vegetation. Assuming that any mortality during submergence might possibly be caused by lack of oxygen and therefore affected by soil organisms, some puparia were submerged in soil sterilized for half an hour at 110° C, and others in aerated distilled water, the latter being kept at a depth of one inch by means of cotton wool. After the experiment the puparia were dried on filter paper and kept at 30° C and 50% relative humidity. All puparia were opened after the date of possible emergence, and those which had died very shortly before emergence were recorded as having survived submergence, but these only numbered 8 among 138 survivors.

Table XIX shows the result of this experiment, from which the following conclusions may be drawn :—

(1) Under the conditions of the experiment, submergence in water-logged soil for 24 hours or more is fatal for all *tachinoides* puparia 0 — 5 days old, whereas nearly all can survive six hours or less. There is no critical period of exposure, about half the puparia surviving a 12 hours' submergence.

(2) There is apparently no important difference between *tachinoides* and *submorsitans*.

(3) The age of *tachinoides* puparia has an important effect on survival, 18 out of 19 puparia about 15 days old surviving under conditions which killed all of seven puparia 0 — 5 days old. In experiments by Nash (1933*a*, p. 114) on submergence of *Glossina morsitans* puparia in water, it was found that " a submergence of up to four days is not fatal to tsetse puparia ; above four days it is fatal in every instance." Probably the mortality of our puparia with shorter exposures is partly due to the fact that they were only 0 — 5 days old at the time of exposure.

(4) Exposures in sterilized soil for 24 hours and 12 hours, and in distilled water for 24 hours, indicate a slightly higher survival under nearly sterile conditions, but

TABLE XIX—Survival rates of puparia of *Glossina tachinoides* and *submorsitans* submerged in water or water-logged soil and subsequently kept at 30° C, and a relative humidity of 50%.

Species	Conditions of experiment	Age of puparia days	Period submerged hours	No. of puparia	No. survived	% survived
tachinoides	Soil	0–5	48–96	83	0	0
			24	7	0	0
			12	20	12	60
			6	8	8	100
			3	23	23	100
			1	20	19	95
		About 15	24	19	18	95
	Autoclaved soil	0–5	24	10	2	20
			12	10	9	90
			3	11	11	100
	Distilled water	0–5	24	18	1	6
submorsitans	Soil	0–5	48–96	64	0	0
			24	14	3	21
			12	12	6	50
			6	30	26	87

more data would be desirable. It is recorded by POTTS (1933, p. 298), working with *Glossina morsitans*, that "immersion in water was not harmful," but it is not stated whether the puparia were held with the spiracles covered.

It is evident from what has been said that the puparium, at any rate of *tachinoides*, requires a high degree of humidity ; but it is also shown that submergence causes death. It is clear, therefore, that it would be profitable to study the relations which exist between the water content of the soil, and the humidity of the soil atmosphere. We carried out a few experiments by the method described by KEEN and RACZKOWSKI (1921), with recent improvements suggested by Dr. B. A. KEEN. The results do not appear to be strictly relevant to our purpose and need not be reported.

(*d*) *Mortality of puparia in relation to the temperature at which their parents were kept*—Up to this point, puparia have been exposed to certain conditions and the effect of these on mortality has been discussed. But it seems that the temperature at which the parent lives has also an effect upon the mortality of the offspring. *Glossina tachinoides* : Table XX shows the death-rates of puparia kept under favourable conditions (24° C, 65% relative humidity ; 30° C, 88% relative humidity) during the same period, but produced by adults kept at different temperatures. It is seen that about 80% of puparia from adults at 30° C died, whereas about 30% of those from 24° — 28° C died, and among those from adults at 24° C there was a still lower death-rate. The differences in mortality between puparia at 30° C and 24° — 28° C, and between those at 30° and 24° C are significant by χ^2. *Glossina submorsitans* : it is evident that there is no significant difference between the death-rates of the two groups of puparia, and both have a considerably higher survival rate than has *tachinoides* under similar conditions.

TABLE XX—Survival of puparia of *Glossina tachinoides* and *submorsitans* kept under favourable conditions, and produced by adults kept at different temperatures.

	Temp. in which parents were kept °C	No. of puparia	No. emerged	No. died
tachinoides	30	24	5	19
	About 24–28	76	54	22
	24	12	11	1
submorsitans	30	35	29	6
	About 24–28	26	23	3

We have dealt with a number of climatic factors which have been shown to influence mortality in the laboratory and probably do so under natural conditions. The only conditions encountered under which all puparia die are a rise of temperature to the thermal death point and subjection to prolonged submergence. The other factors are responsible for the death of different proportions of the puparia, but in all experiments some deaths were observed, although conditions were never fatal to every individual.

In nature adverse conditions must frequently reduce the number of puparia in any one habitat without killing them all, the extent of mortality depending on the degree of severity, the age of the puparia and their previous history. Possibly this might produce the kind of variation in mortality which is found in puparia collected from natural breeding places.

Metabolism of the puparium

A study of the mortality of puparia has shown that those of *Glossina tachinoides* and *submorsitans* which fail to emerge generally die either at the beginning or the end of puparial life (p. 214). By weighing the puparia, those which died early could soon be distinguished from those which eventually emerged. In fig. 9 are seen the percentage weights, on different days, of eight puparia, four of which died early. After the fifth day these could easily be distinguished from the survivors by their difference in weight. The puparia which died late generally began to lose weight rapidly after the usual date of emergence, but in a few this loss had already begun two or three days earlier. Therefore, in considering loss of weight, we will only take those puparia from which adults emerged.

Fig. 10 shows the mean weights on successive days of puparia of *Glossina submorsitans* kept at 24° C at four different humidities. All puparia were weighed within 24 hours of emergence and subsequent weights are expressed as percentages of the original. It is at once evident that more weight is lost at low than at high humidities. It is also seen that at each humidity there is a rapid loss of weight during the first four or five days followed by a steady but reduced loss till the end of puparial life. Very similar curves showing loss of weight at different humidities were

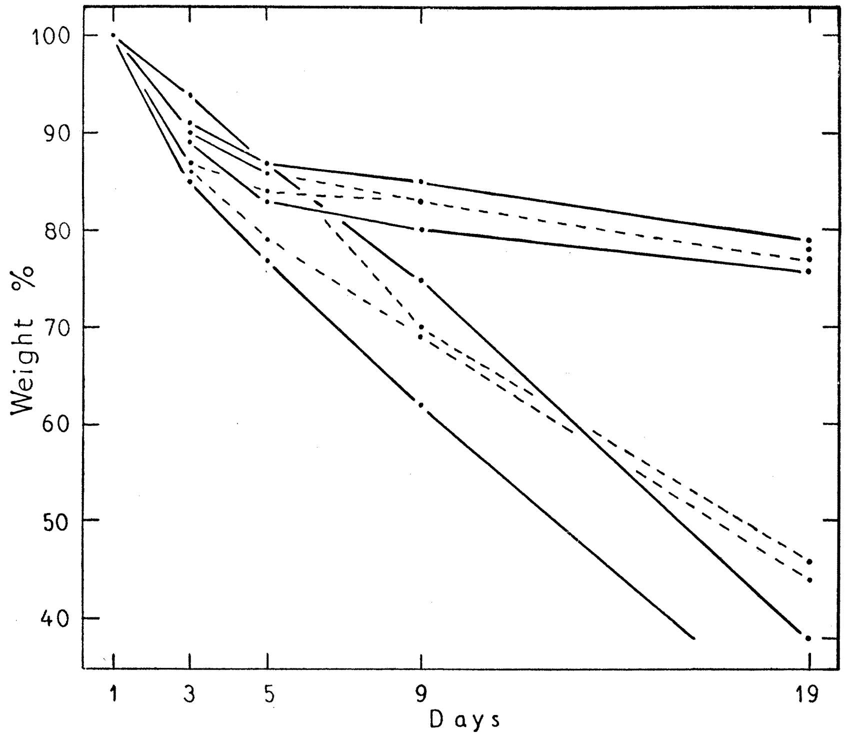

Fig. 9—Curves showing the changes in percentage weight of eight puparia of *Glossina tachinoides* kept at 30° C (86·0° F) and a relative humidity of 19%. Four died early.

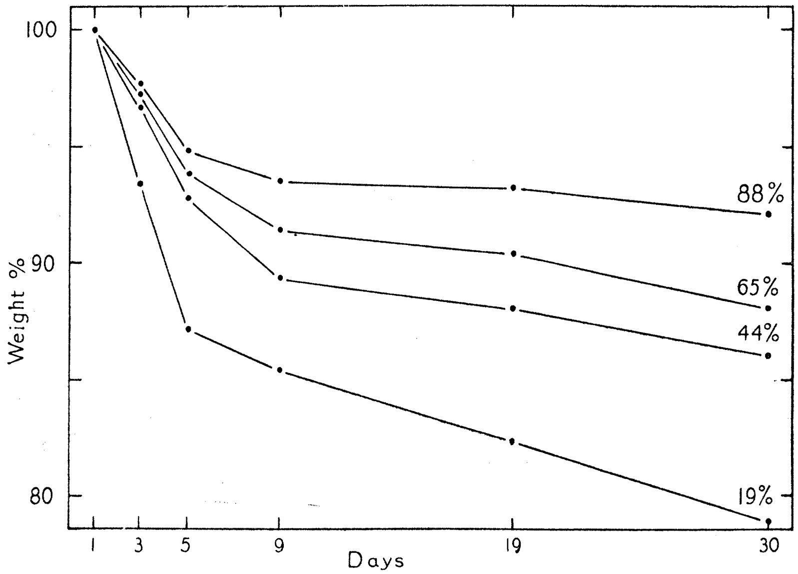

Fig. 10—Curves showing the changes in percentage weight of groups of puparia of *Glossina submorsitans* kept at 24° C (75·2° F) and four humidities. Further particulars are given in Table XXI.

obtained for *Glossina submorsitans* at 30° C and for *tachinoides* at both temperatures. The final weights are shown in Table XXI. It is seen that in *Glossina submorsitans* the loss of weight at different humidities at 24° C, for example, ranged from 7·8 to 21·3% of the original weight. In nearly every case the loss per cent is slightly greater in *tachinoides* than in *submorsitans*.

In fig. 11 the final percentage weights of the same puparia (*submorsitans*) at each temperature are plotted against the saturation deficiency of the air in which they were kept. The lines produced are nearly straight at either temperature, showing that the loss of weight is almost proportional to saturation deficiency and therefore due to evaporation. It is also apparent that more weight is lost at 24° than at 30° C. This is probably due to the fact that the weights were taken at the end of puparial life, on the 31st day in the case of those puparia at 24° and on the 20th day in those at 30° C. It is possible to allow for the differences in time by calculating the product of

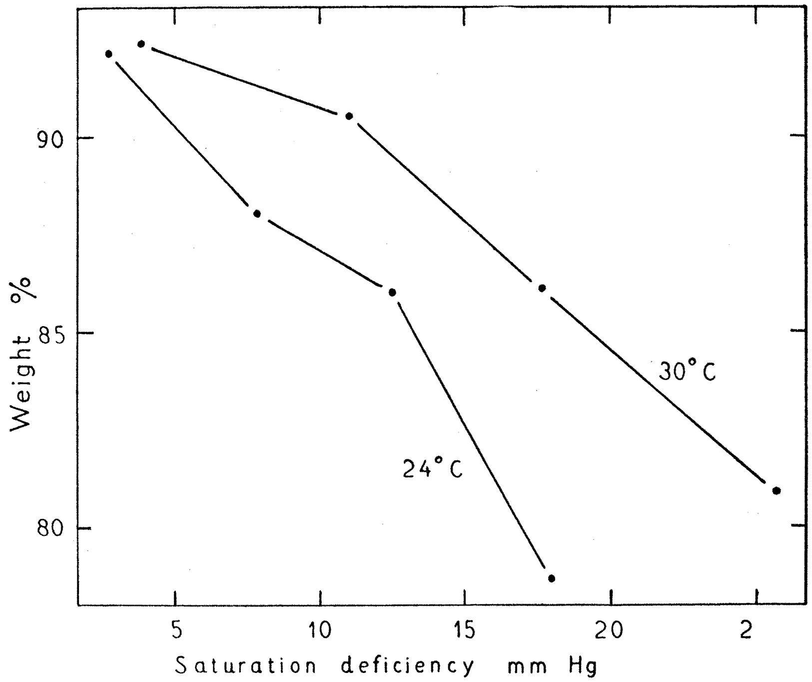

FIG. 11—The weights of puparia of *Glossina submorsitans* at the end of puparial life in relation to the saturation deficiency of the air in which they were kept. The puparia were kept at 24° C (75·2° F) and 30° C (86·0° F) and at four different humidities. Further particulars are given in Table XXI.

$$\frac{\text{loss of weight}}{\text{sat. deficiency}} \times \frac{1}{\text{time}}$$

This expression gives a " standardized loss." Reference to Table XXI shows that for *Glossina submorsitans* the value of this is nearly the same at both temperatures and all humidities except the highest. The figures for *tachinoides* do not show this, but the numbers are less. The conclusion may be drawn that differences in loss of weight are closely proportional to saturation deficiency except at high humidities. The standardized loss is nearly always higher in *tachinoides* than in *submorsitans* under the same conditions.

TABLE XXI—Giving the final weights of puparia of *Glossina tachinoides* and *submorsitans* kept at 24° and 30° C at different humidities. The weights were determined on the 31st day of puparial life at 24° and on the 20th day at 30° C, and are expressed as percentages of the weights of the puparia when less than 24 hours old.

				tachinoides				*submorsitans*		
	R. h., %	98	88	65	44	19	88	65	44	19
Temp.	Sat. def., mm	0·6	3·8	11·0	17·7	25·6	3·8	11·0	17·7	25·6
°C 30	No. of puparia	11	12	6	5	4	11	12	10	10
	Final weight as % of original weight, mean	90·8	89·9	82·4	80·5	77·2	92·4	90·5	86·1	80·9
	Standardized loss of weight	0·807	0·140	0·084	0·058	0·043	0·105	0·046	0·041	0·040
	Sat. def., mm		2·6	7·8	12·5		2·6	7·8	12·5	18·1
24	No. of puparia	—	15	8	6	—	15	12	9	14
	Final weight as % of original weight, mean	—	92·3	83·9	83·5	—	92·2	88·0	86·0	78·7
	Standardized loss of weight	—	0·099	0·068	0·044	—	0·100	0·051	0·037	0·042

Under natural conditions there may be a high mortality if the temperature is low and the air in the soil is not saturated ; for the puparial period may be extended, for example, to 50 days or more (NASH, 1933, *a*, p. 133). Throughout this period, the weight steadily continues to diminish. It is possible that this might cause high mortality at low temperatures. As to higher temperatures, it has been shown that both in *tachinoides* and *submorsitans* the mortality is higher at 30° than at 24° C ; 24° may therefore lie in the region of the optimum temperature, while there is increased mortality at either higher or lower temperatures.

It was next proposed to make a study of the composition of puparia. *Glossina tachinoides* puparia were kept for different periods at 24° C and killed. Some were kept at 19% relative humidity and others at 88% ; they were weighed on the first day. Some were killed half way through puparial life, after 15 days, others just before the end of puparial life, after 30 days, and others, as controls, were killed on the first day. All puparia were dried to constant weight at 105° C and weighed, and subsequently the ether-soluble fat was extracted.

The results are shown in Table XXII. Taking first the data for those killed after 15 days, we see that those at the high humidity lost about 8% of their weight and those at the low about 18%. This loss consisted of water, the dry weight, considered as a percentage of the original weight, being practically the same at both humidities as in the controls. Probably a loss occurred which was so small that it was obscured by the variability of the material. In puparia killed when 15 days old, there is no evidence that water of metabolism has been produced to compensate for increased evaporation. At the lower humidity the proportion of fat remaining is actually greater than at the higher. At both humidities, then, the puparia have become drier, the proportion of water being 64·2 and 60·2% as compared with 67·0% in the controls. It is perhaps surprising that the alteration in the proportion of water did not cause death ; possibly the water may have been lost from the rectum or from some other space outside the tissues.

In puparia killed on the 30th day, it was found that the dry weight had fallen. Nearly all the loss of dry material was of fat, more than half of which had been used. At both humidities there was a slight relative increase in water. Humidity, even by the end of puparial life, had no measurable effect on the consumption of dry material.

If we compare the changes in composition of the puparium of *Glossina*, as far as they are known, with those of another Muscid, *Calliphora vomitoria* (Weinland, 1906), we find several points in common. In both the loss of weight consists almost entirely of water and is more rapid at the beginning than in the middle of puparial life. In *Calliphora* about half the petrol-ether extract is used during the life of the puparium. Needham (1929) points out that changes in the intensity of the respiratory exchange of insect pupæ indicate two periods of increased metabolic activity with a less active intermediate period. In *Glossina* we find no evidence of the first of these periods, but the insect may have passed through that stage during larval life, for Evans (1932) emphasizes the fact that in the blow-fly, *Lucilia sericata*, the prepupa is "morphologically similar to the larval stage but physiologically belonging to the pupal stage." The second period of increased activity accounts for the loss of dry weight in the last part of pupal life, fat being used to meet the needs of the developing adult.

Table XXII—Showing the mean and percentage weights of puparia of *Glossina tachinoides* kept at 24° C at two humidities and killed, some on the 15th and others on the 30th day. The composition is shown as dry matter, including ether-soluble "fat," and water. Controls were killed within 24 hours of pupation.

Age when killed, days		0 (control)	15		30	
R. h., %			19	88	19	88
Sat. def., mm			18·1	2·6	18·1	2·6
No.		53	51	48	37	44
Mean weights, mg	Original	14·9189	14·8353	15·5167	15·6616	14·8318
	Final		12·1530	14·2729	11·7676	13·5432
	Dry	4·9623	4·8431	5·1042	4·4865	4·2659
	Fat	1·4528	1·471	1·292	0·676	0·598
	Water	9·9566	7·3099	9·1687	7·2811	9·2773
Weights as % of original weight	Final	100	81·9	92·0	75·1	91·3
	Dry matter	33·0	32·6	32·9	28·7	28·8
	Water	67·0	49·3	59·1	46·5	62·6
	Fat	9·7	9·9	8·3	4·3	4·0
Water as % of final weight			60·2	64·2	62·7	68·5

The weight of adults : if one takes a series of puparia which has been uniformly treated, and compares the weights of freshly emerged adults with the original weights of the puparia, the correlation appears to be linear or nearly so, heavier puparia producing heavier adults. The weights of the adults vary from about 60 to 80% of those of the puparia on the first day.

The following example gives the mean weights of puparia and adults of *submorsitans* kept at 24° C.

R. h.	No.	Puparium 1 day old	Puparium 30 days old	Adult 1 day old
%		mg	mg	mg
88	23	24·30	22·41	18·64
19	12	23·31	18·23	13·43

By adding the weight of a puparial case (2·36 mg) to the adult weight, we see that 6% of the original weight at the high and 11% at the low humidity were lost at about the time of emergence. In *tachinoides* this loss is somewhat greater. But though the adults from puparia kept at 19% humidity weigh less than the others, there is no difference in their dry weights.

VII—CLIMATE OF GADAU

Temperature

The main objective was to determine the effect of climatic factors upon *Glossina* exposed to controlled conditions in the laboratory ; but we desired also some knowledge of the climate round Gadau, particularly in the thickets or " kurimis " in which *Glossina submorsitans* and *tachinoides* find their permanent haunts. It was no part of our purpose to accumulate a large mass of meteorological data, but rather to explore the situation and discover which climatic factors should be further studied and recorded. For purposes of comparison a Stevenson's screen was erected outside the laboratory at Gadau in a good meteorological exposure, and readings were taken under standard conditions throughout the period March to July, 1933. Gadau was originally in the dry plain in which baobab and thorny trees are characteristic ; it is now the centre of an artificial clearing about 1½ miles in diameter. Maximum and minimum thermometers (with National Physical Laboratory certificates) and a thermohygrograph were exposed. The recording instrument was checked twice daily. The daily soil maximum at a depth of one inch was also recorded ; the area where this was done was kept free of weeds. It was necessary to lift the thermometer and re-bury it each day, so that the figures do not relate to undisturbed soil. In addition to collecting data at Gadau, we selected April as typical of the hot dry weather and July as about the wettest month of the year, and made a more detailed study of the climate in and near the flies' haunts during these two months. Instruments were exposed in screens, and checked daily ; each screen contained maximum and minimum thermometers and a thermohygrograph. The map, fig. 12, shows the position of the place at which this work was done. The distance from Gadau to the river is about five miles, and there is little difference in altitude between the two areas. Screen No. 1 was in a sheltered position under a tree on the edge of a small stream-bed ; the channel was completely dry in April but full of water in July. The records published by TAYLOR (1932*a*) were taken in March and April on the bank of the same stream a few hundred yards from our Screen 1. Screen 2 was in use for a short time during April, but in the middle of the month the instruments were moved to Screen 4, which was in a very dense thicket under a large tree swathed in creepers, *C*, Plate 19. This screen was on the ground at a place where puparia of *Glossina tachinoides* and *submorsitans* were abundant. Screen 1, 2, and 4 were all in " fringing forest " or " river bank " association of LLOYD, LESTER, TAYLOR, and THORNEWILL (1933, p. 234). They were all shaded by trees, many of them evergreen, beneath which grew dense bushes and creepers. In contrast with those positions, Screen 3 was placed in open country about 900 yards from the river, and four feet from the ground, *A* and *B*, Plate 19. It was in the open plain which covers so large a part of the country (" baobab and thorn " of the above authors). Apart from baobab (*Adansonia*) the commonest trees are *Balanites*, *Zizyphus*, and occasional *Acacia* spp.

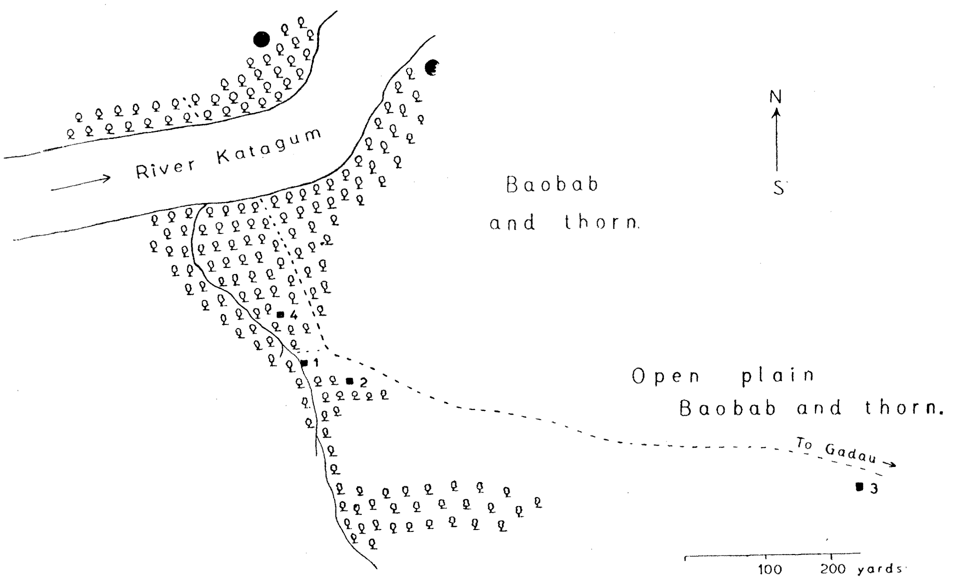

FIG. 12—Map of area near Gadau, showing positions (1–4) at which climatological readings were taken.

The temperature readings obtained in these places can be compared in Table XXIII. From it the following deductions appear to be justifiable.

TABLE XXIII—Temperature records for April and July, outside the laboratory at Gadau, and at three points in or near the permanent home of *Glossina*. Records from Screen 4 are only available for part of April. Readings from all places for that period are therefore given for purposes of comparison. Table shows shade maximum and minimum and soil maximum at one inch, and for each of these the mean (° C and ° F), and the highest and lowest readings observed during the period (° C).

	Gadau				Screen 3				Screen 1				Screen 4			
	Mean		High	Low	Mean		High	Low	Mean		High	Low	Mean		High	Low
	°C	°F	°C	°C	°C	°F	°C	°C	°C	°F	°C	°C	°C	°F	°C	°C
1–30 April																
Shade max. . .	39·61	103·30	41·7	35·0	39·95	103·91	42·0	35·0	38·87	101·97	41·0	34·1	No readings			
„ min. . .	23·39	74·10	26·8	17·5	22·95	73·31	26·1	15·6	20·11	68·20	24·2	13·4	„	„		
Soil max. . . .	51·02	123·84	55·4	40·4	51·41	124·54	58·0	41·6	32·98	91·36	36·2	30·3	„	„		
17–30 April																
Shade max. . .	39·91	103·84	41·7	35·0	40·28	104·50	41·5	35·0	39·28	102·70	41·0	34·1	38·64	101·55	40·0	32·8
„ min. . .	23·74	74·73	26·0	18·2	22·75	72·95	26·1	16·5	20·65	69·17	24·2	14·0	23·66	74·59	27·0	17·4
Soil max. . . .	50·81	123·46	55·4	40·4	51·52	124·74	58·0	41·6	34·23	93·61	36·2	31·0	29·47	85·05	33·0	28·3
1–31 July																
Shade max. . .	31·17	88·11	33·6	26·5	30·95	87·71	33·5	26·3	30·17	86·31	32·5	24·0	29·71	85·48	32·0	25·5
„ min. . .	21·66	70·99	23·9	18·9	21·15	70·07	23·0	19·5	20·97	69·75	22·9	19·0	23·03	73·45	24·5	21·0
Soil max. . . .	39·22	102·60	46·8	29·4	39·55	103·19	45·6	30·0	27·76	81·97	31·0	24·4	25·75	78·35	27·2	23·6

Season—The range of temperature is much greater in April than in July ; the following figures give the mean daily range (° C) in the four places :—

	Gadau	No. 3	No. 1	No. 4
April	16·2	17·0	18·8	15·0
July	9·5	9·8	9·2	6·7

The fact that the range is less in July is due in the main to a lower maximum. For instance, at Gadau the maximum in July is 8·5° lower than in April, whereas the minimum is only 1·7° lower.

Local differences—The temperature of Gadau differs very little from that at Screen 3 in the open country. This can be seen from the mean maximum and minimum, and also the absolute readings. It seems unnecessary to pursue the matter further and to work out the mean temperatures from two-hourly readings of the thermographs, and it is probably sufficient to assume that temperature records taken in the clearing at Gadau can be applied to the whole of the surrounding country except the actual thickets. When the readings for Gadau or for Screen 3 are compared with those taken in the thicket, Screens 1 and 4, the general resemblance is still surprising. A few points of difference will be noted ; for instance, in April the minimum in Screen 3 by the stream is distinctly lower than elsewhere. We did not observe the high maxima which Taylor records from a point very close to this ; one may perhaps conclude that the differences are extremely local. The figures for the mean range of temperature in the two months have already been given ; they show that the range is definitely less in the thickest place, Screen 4, than elsewhere. But on the whole the differences in shade temperature between the open country and the thicket are much less than we expected. This is perhaps not surprising in April, when many of the trees are leafless and when there is no grass ; but in July, when the kurimi is fringed with grass six or eight feet high and all the trees are in full leaf, a much greater difference in shade temperature would surely have been expected.

Shade maxima—From the data we have given in Table XXIII, it is clear that the shade maxima approach the temperature which is lethal to both species of *Glossina*. It will be remembered (p. 180) that on an hour's exposure, 43° C is critical, and 44° C (111·2° F) fatal. On a three hours' exposure, temperatures just below 40° C kill some flies, and 40·4° C (104·7° F) kills all. But in order to discover whether the temperatures recorded in nature are likely to prove fatal to the fly, one requires some knowledge of the length of time during which the temperature passed certain limits. The shade maximum hardly gives the information required. Making use of the corrected thermohygrograph records, we have tabulated the number of days on which the temperature passed 38° C or 40° C for periods up to one hour, from one to two hours, from two to three hours, etc. This has been done for Gadau for the period from March to July (Table XXIV). The table shows that during March, April, and May, the temperature frequently passed 38° C and even 40° C ; moreover, it frequently did so for a considerable period. For instance, in April there were 13 days on which the temperature passed 40° C, for a period of three to four hours on six occasions, and for a period of under one hour or one to two hours on only two occasions. In Table XXV the results of similar analysis of thermograph records taken in four places are presented. The figures relate only to April, for during July the temperatures never approached lethal limits. It seems clear that in the open country or in partial shelter, Screen 1, the temperature is frequently high for periods sufficient to kill *Glossina* during the hot dry months. It is only in the thickest places, Screen 4, that this does not occur. It seems improbable that the fly is more resistant to high temperature in nature than in the laboratory. It could hardly be so unless it has access to very considerable quantities of water (perhaps in the form of blood), by evaporating which it could lower its own body temperature. It seems more probable that *Glossina* avoids prolonged exposure to heat by resorting to places where the temperature is lower. It is possible that flies do this for short periods and so avoid *continuous* exposure to conditions which would otherwise be fatal. This suggestion is only a hypothesis, but there is recent work showing that micro-climatic conditions are often extremely localized and quite unlike the conditions which can be measured in meteorological screens ; many insects avail themselves of micro-climates when they are favourable.

TABLE XXIV—Showing number of days in each month on which shade temperature exceeded a certain limit for periods of under one hour, under two hours, etc. Data from screen outside laboratory at Gadau.

Month	Temp. limit		Hours							
	°C	°F	Nil	0–1	1–2	2–3	3–4	4–5	5–6	Over 6
March	38	100·4	18	0	0	3	4	2	2	2
	40	104·0	27	3	0	0	0	1	0	0
April	38	100·4	4	0	3	2	0	4	8	9
	40	104·0	17	2	2	2	6	0	1	0
May	38	100·4	17	0	0	0	0	3	4	7
	40	104·0	22	1	3	2	1	1	1	0
June	38	100·4	30	0	0	0	0	0	0	0
	40	104·0	30	0	0	0	0	0	0	0
July	38	100·4	31	0	0	0	0	0	0	0
	40	104·0	31	0	0	0	0	0	0	0

TABLE XXV—Showing number of days in April on which shade temperature exceeded a certain limit for periods of under one hour, under two hours, etc. Data from Gadau and from three screens in and near permanent home of *Glossina*.

Place	Temp. limit		Hours							
	°C	°F	Nil	0–1	1–2	2–3	3–4	4–5	5–6	Over 6
Gadau	38	100·4	4	0	3	2	0	4	8	9
	40	104·0	17	2	2	2	6	0	1	0
Screen 3	38	100·4	4	0	1	4	0	4	8	9
	40	104·0	14	2	1	6	3	3	1	0
Screen 1	38	100·4	6	2	1	6	9	3	2	1
	40	104·0	25	1	3	1	0	0	0	0
Screen 4*	38	100·4	3	1	1	0	1	3	4	1
	40	104·0	14	0	0	0	0	0	0	0

* Data for 17th to 30th April only.

Soil maximum—It will be remembered that the age and species of a *Glossina* puparium have some effect on the temperature which is lethal to it, but 45° C for one hour was invariably fatal in our experiments (p. 213). No information exists about the temperature which is lethal on longer exposures, except that 37° C is fatal in a few days. The data relating to soil temperatures were collected with a maximum thermometer, the bulb of which was at a depth of one inch. We have, therefore, no knowledge of the length of time during which the soil was at any particular temperature ; but we may assume that changes of temperature at one inch are gradual, and that if the maximum went to 45° C the temperature was at or near that figure long enough to kill all puparia. It is also assumed that if the maximum lay between 40° and 45° C, the exposure may have been long enough to kill some puparia though not all. The daily maxima have been tabulated under three groups to show the number of days on which the maximum was below 40°, between 40° and 45°, and over 45° C. The results are shown in Table XXVI, in which the soil maxima recorded outside the laboratory at Gadau for the months from March to July have been tabulated. The figures show that in the rainless and nearly cloudless months of March and April, conditions were lethal on nine days out of ten. May is intermediate, and June and July may be grouped together, for the rainfall and cloudiness were considerable. During these two months the soil maximum only exceeded 45° C on one day in six, but that is sufficient to kill the puparia whose life extends to many days. In the course of tabulating the facts, it was also observed that in the dry season the soil maximum at one inch was invariably above the shade maximum, frequently by many degrees, but during the rainy season it was frequently below the shade maximum.

TABLE XXVI—Showing number of days per month on which soil maximum (° C) at one inch below surface, at Gadau, was below 40° C, between 40° and 45° C, or above 45° C.

Temperature	March	April	May	June	July
Below 40° C (104·0° F)	1	0	4	6	18
40°–45° C (104·0°–113·0° F) . .	3	2	10	17	10
Above 45° C (113·0° F)	27	28	17	7	3

Soil maxima close to Screens 3, 1, and 4 were tabulated in the same way. At Screen 3 in the open, the readings were not unlike those taken at Gadau. The following figures were obtained :—

	April days	July days
Under 40° C (104·0° F)	0	14
40°–45° C (104·0°–113·0° F) . .	1	13
Over 45° C (113·0° F)	29	3

It follows that conditions in this position, and no doubt in the open country in general, would be rapidly fatal in April and fatal on about half the days in July.

But it would be rash to conclude that *Glossina* cannot breed in the open country. It is probable that there are many small spots, sheltered from the sun, in which it can do so. The soil maxima close to Screens 1 and 4 never passed 40° C either in April or July. The means and the highest and lowest values are given in Table XXIII.

With the instruments at our disposal, we could only study soil maxima. Continuous records from a distance thermograph would be valuable, for it is shown elsewhere (p. 213) that a constant exposure to 30° C, which is far below the "thermal death point," causes considerable mortality among puparia. It is possible that in the hot dry months the mean temperature in the soil, even in the densest shade, may be sufficiently high to cause the death of many puparia, and that even a *very little* clearing might cause a great increase in these deaths.

Humidity

It has been known for years, and it is abundantly shown in our experimental work, that humidity is of very great importance to *Glossina*. But it is not at all clear how to collect the necessary information, or to present it compactly and in the most useful manner. It is certain that readings taken once or twice in the 24 hours are useless, and that a current record of changes in humidity is required. But no type of hygrograph is entirely reliable, particularly under the conditions which prevail in the dry season, when the humidity frequently falls below 20% by day, and occasionally rises to 90% at night. The instruments which we used were operated by human hair ; they were checked daily, and generally twice daily. The only material errors are in the region above 90% humidity.

The following conclusions may be drawn from the records for April and July, at Gadau and at Screens 1, 3, and 4.

April—As a preliminary, the mean relative humidity at 6, 12, 18, and 24* hours was worked out for April, for Gadau and the three screens in the bush. Even when Gadau was compared with Screen 4 (on the ground in dense thicket) the difference was very slight ; in general the bush screens were 10% moister by night, and the differences during the day were slight and irregular. We already know that temperature is nearly the same at Gadau and in the bush during April, Table XXIII ; it seems, therefore, that the conditions of temperature and humidity during April show unimportant differences, and it is sufficient at the moment to concentrate attention on the records collected at Gadau. The hygrographs record relative humidity, but we already know that saturation deficiency is a more appropriate measurement in insect physiology : but in order to convert the readings to that scale the temperature must be known. Two-hourly means of temperature and relative humidity have therefore been worked out, for April at Gadau. For each interval of two hours, the mean of temperature and humidity is spotted on fig. 13, and when the points are joined they form curve A. Lines of equal saturation deficiency are also calculated and added to the graph. It is apparent that the mean

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* Times are quoted on this system, because it is simpler to show on a graph. Noon is 12, and midnight 24 hours.

conditions by day and by night are very different. Between midnight and dawn (24 to 6 hours), the temperature is a little over 25° C and the saturation deficiency 10 mm or slightly higher. From noon till about sunset the mean temperature is over 35° C and the saturation deficiency approaches 40 mm. The mean of day and night (derived from the two-hourly means) is 31·72° C with relative humidity 39·5%, *i.e.*, a saturation deficiency of 21·5 mm.

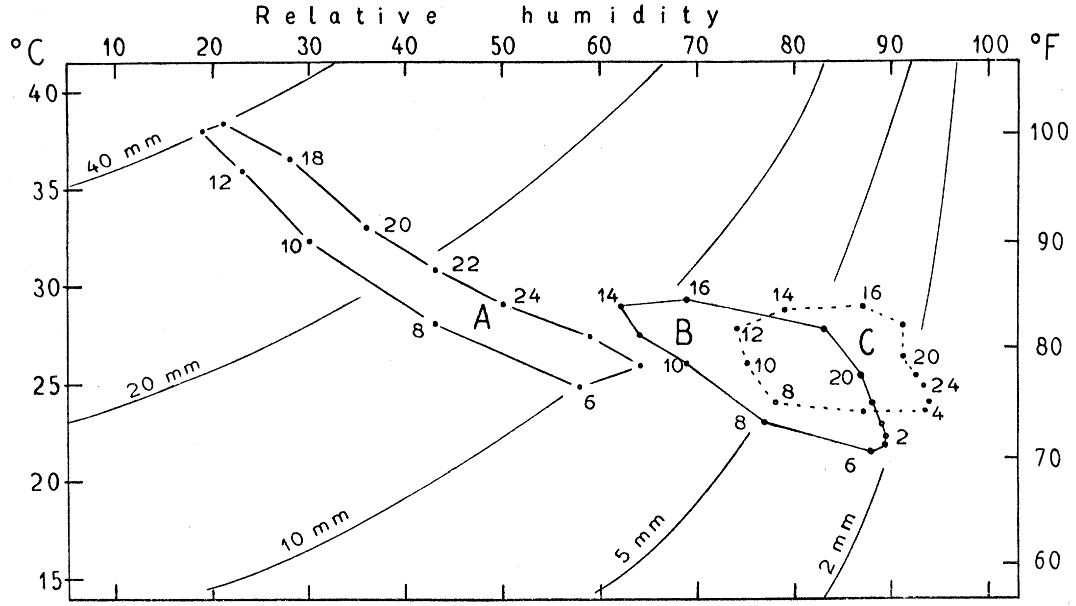

FIG. 13—Graph showing two-hourly mean values of shade temperature and relative humidity. A refers to Gadau in April and B in July ; C refers to Screen 4 (dense bush, Plate 19) in July. Lines of equal saturation deficiency (" 2 mm " etc.) are also shown.

July—Two-hourly means of temperature and humidity for Gadau were extracted from the records, and plotted ; the result is curve B in fig. 13. The great contrast between the two months is self-evident. In July temperature is lower, and humidity higher ; moreover, the difference between day and night is much reduced. If humidity is referred to the saturation deficiency scale, it is seen that at night the deficiency is just over 2 mm and by day about 10 mm. In fact, if we compare the day in July with the night in April, it is seen that the mean saturation deficiency is the same, and that the difference in temperature is slight. The mean values for the 24 hours are 25·04° C, relative humidity 79·3%, saturation deficiency 4·9 mm.

Conditions of temperature and humidity, during July, on the ground in the thicket, Screen 4, have been plotted in a similar way, and the result is C. The figure shows that the climate here is more damp, and more equable than that of Gadau. The mean saturation deficiency at night is less than 2 mm and by day rises to about 7 mm. Combining the two-hourly values, the means for the 24 hours are found to be 25·95° C, relative humidity 86·4%, and saturation deficiency 3·4 mm.

Further study of fig. 13 shows another matter of considerable interest. Gadau in April (area A) is at about 30° C at 9 and 23 hours. But the mean relative humidity at these times is 37 and 47. This difference prevails consistently between day and night, and corresponds to a difference of 2 to 3 mm of vapour pressure. Furthermore, if lines of equal vapour pressure were drawn on fig. 13, it would be seen that the absolute humidity rises steadily from sunset to dawn, and falls throughout the day. There is, no doubt, a slight instrumental error due to hysteresis, but it would give a lower relative humidity by night than by day so that the real difference is greater than that shown in the diagram. Moreover, the difference is much greater in July, either at Gadau or in the thicket, as curves B and C show. In both of them the relative humidity is about 15% higher at night than it is by day at the same temperature (a difference of 3—4 mm on the absolute scale). It must therefore be accepted that the amount of water (the absolute humidity) in the air is greater by night than by day, and that this is more pronounced in July than in April. The cause is not known, but we suggest that evaporation from the soil and vegetation is always tending to raise the absolute humidity of the air near the ground, except when it is saturated, an event which never occurs in April. This effect is counteracted during the daytime, owing to sunshine which causes convection currents and turbulence ; owing to this, the air near the ground, moistened by evaporation, is diluted by drier air from a higher level. For the same reason the effect is less in July which is cloudy and wet, than in April which is dry and bright. We have had the advantage of discussing the point with Dr. C. E. P. Brooks of the Meteorological Office, and he accepts this explanation as probably correct.

It appears that such a diagram as fig. 13 gives much information compactly, and justifies the considerable labour spent on extracting and tabulating the data. But it gives only the means, and a mass of possibly valuable information is lost. The occasional extremes are not shown, but perhaps they matter little. But one wishes that a way could be found of showing the combinations of temperature and humidity which are rather common. To obtain this it would probably be necessary to take all the humidity readings which occurred (irrespective of hour) at a particular temperature, and to work out the mean and perhaps the quartiles. This would have to be done independently at each temperature, though it might be possible to combine it subsequently in one diagram. But the labour would hardly be justified, until it is clear that that type of information is needed. Information of quite a different type is also lost in fig. 13. It is possible that a spell of abnormal weather might endure several days, and perhaps have a very definite effect on the population of *Glossina*. Such spells actually occurred ; for 2½ days from noon on 13th April the humidity was always below 30%, and for two days from noon on 28th it was always above 30%.

We have only been able to study shade temperature and humidity, but it must be remembered that in nature many other ecological factors have their effect upon *Glossina*. The very great contrast in the vegetation between the rainy* season

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* Monthly rainfall for Gadau, 1933 : January to March, Nil ; April, 0·51 inch ; May, 5·28 ; June, 2·43 ; July, 10·80 ; August, 20·28 ; September, 2·79 ; October to December, Nil. Annual total, 42·09 inches. The April fall was on two days only, and the first heavy rain fell 16th to 20th May.

(mid-May to September) and the rainless dry season must be seen to be believed. Plate 20 illustrates this. It shows the same spot, rather at the edge of fringing forest, as it appeared in the dry season (17th March) and towards the end of the rains (12th August).

VIII—Discussion

The adult

The previous pages have been devoted mainly to facts, but the reader must have observed that different experimental procedures have frequently led to similar conclusions. If, for instance, *Glossina tachinoides* is exposed at 30° C (86·0° F) to a range of humidities, we find that 44% humidity is nearer the optimum than any of the others. At that humidity the adults feed well, Table VII ; the females live longest (fig. 3) ; the standardized birth-rate is highest, Table V. Lower humidities are less favourable, whether judged by appetite, survival or reproduction, and humidities of 65% and over are even more unfavourable. We are, therefore, perhaps justified in publishing such a diagram as fig. 14, provided it is accepted as tentative. For there are large areas in the graph for which no facts are available ; moreover, when knowledge is more complete, it will be found that the limits of temperature and humidity are not the same for different activities, so that it is scarcely legitimate to express appetite, length of life, and birth-rate in one figure. Using it with caution, fig. 14 shows a favourable zone round 30° C and 44% humidity extending downwards perhaps to 19% humidity ; that is to say, over a

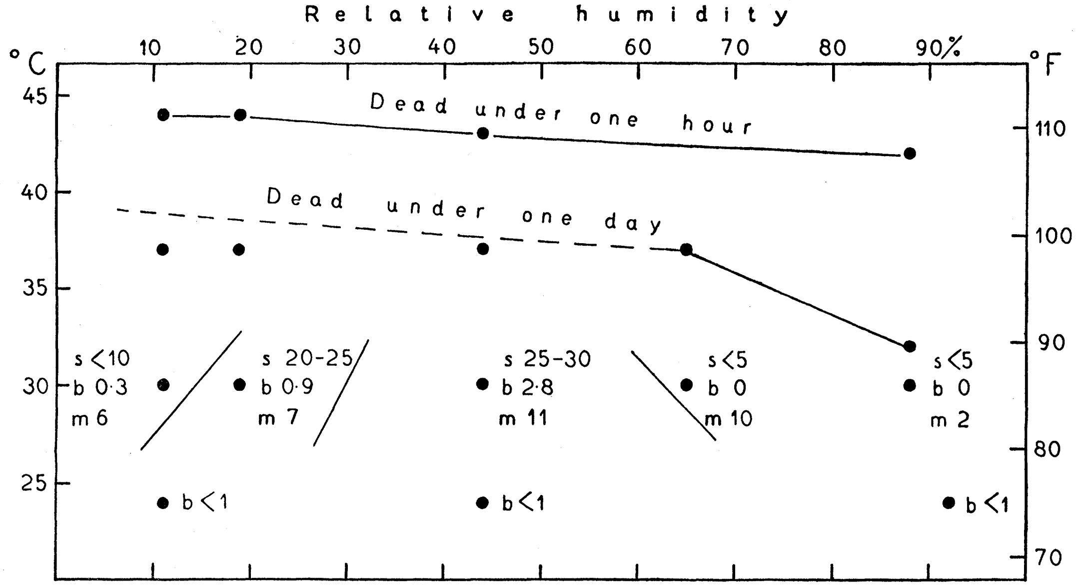

Fig. 14—Schematic diagram intended to define the effect of conditions of constant temperature and humidity upon *Glossina tachinoides*, offered food daily. *s* = mean survival of ♀ in days ; *b* = births per 100 ♀ days ; *m* = mean weight of meal in mg.

range from 6 to 14 mm of saturation deficiency. At higher temperatures, 37° C is most unfavourable under all conditions of humidity (p. 184), but here there is discrepancy between our data and those published by TAYLOR (1932*a*). It is much to be desired that studies should be made at about 32°—34° C, partly in relation to TAYLOR's discovery about enhanced parasitism with *Trypanosoma*, partly because at the hottest time of the year, the insects are exposed to such temperatures for comparatively long periods, for the true mean temperature of Gadau for April was 31·72° C (p. 229). It seems that there is a great difference between the effects of humidity at 30° and at 24° C, for at this lower temperature reproduction can occur at humidities ranging from 11 to 93%, though more facts would be welcome (p.192).

It is clear, then, that middle humidities are most favourable, at any rate at 30° C and higher temperatures ; this is true of both species, and of fed and also unfed flies (p 229). It is not yet possible to give a physiological explanation of this, but it is evident that the effect of humidity on *Glossina* is not direct, for none of the phenomena are proportional to it. It follows that the amount of water lost (which we believe to be determined by saturation deficiency) may be unfavourable because it is excessive or because it is too little.

One would naturally suppose that the unfavourable effect of high humidity is due to the accumulation of water in the insect. This is consistent with what is known for other insects (see BUXTON, 1932, p. 297). The general explanation is that at high temperatures, water of metabolism is produced rapidly and accumulates in the tissues unless the evaporative power of the air is considerable ; but at low temperatures the rate of metabolism, and therefore of production of water, is less so that insects can live normally over a much greater range of relative humidity. But there is no evidence that this water-logging occurs in *Glossina*, and the facts in Table XIII appear to show that it does not occur in unfed flies. The possibility, however, remains that water accumulates in some organ or tissue with unfavourable effects on the whole fly. It is also difficult to explain the unfavourable effect of very low humidity upon the flies' appetite, length of life, and birth-rate. We know that unfed flies burn more fat at lower humidities than at higher, and that in this way they produce water of metabolism which tends to compensate for excessive loss by evaporation, Tables XII and XIII. But in similar experiments with fed flies, the results appear to be contradictory (p. 206). The simple explanation that in very dry air the fly uses up its food to compensate for evaporation, and therefore has little material for reproduction, appears therefore to be untenable.

Fig. 14 summarizes much of what is known about *Glossina tachinoides* which are fed ; but we can compare the fed and the unfed insects. It has been found that the thermal death-point of young unfed flies is about 1° C lower than that of the fly which has been fed (p. 181), provided the insects are exposed for one hour. But it is probable that on longer exposures a greater difference would be found, owing to the unfed fly using its reserves rapidly at high temperatures. The duration of life at 37° C has been studied, and it appears that there is very little difference between fed and unfed flies at the high humidities ; but at lower humidities the fed flies live conspicuously longer, presumably because they have greater reserves either of water or of some solid material. At 30° C a similar difference can be observed, the fed flies resisting starvation for about twice as long as the unfed, at lower humidities, Table III. A partial study has also been made of the difference in chemical composition between the unfed and fed flies. The matter is extremely complex owing to rapidity of feeding and excretion, and size of meals. It goes without saying that a well-nourished fly is much heavier than a freshly-emerged unfed individual ; its dry weight is also greater ; but during starvation, a fed fly may survive after its weight is lower than that of one which has never been fed. We cannot, therefore, use the weight of wild flies to separate unfed newly-emerged individuals from the others. So far as present knowledge goes, the differences in chemical composition between newly-emerged and well-nourished flies are not very great. In both the mean proportion of dry matter is just over 30%, and in spite of the fact that these insects frequently take their own weight of blood at a meal, the proportion of dry matter to wet is adjusted in less than four hours after feeding (p. 204). The weight of "fat"—ether-soluble material—is about 1 mg in newly-emerged flies, and after they have fed several times, it rises to between 1·2 and 1·7 mg, Table XV. A similar rise has been observed in *Glossina submorsitans*.

The greater part of the work was carried out with *Glossina tachinoides*, but many of the experiments were repeated on *submorsitans*. As far as is known, the physiological differences between the two are not very great. The thermal death points are very similar, and both species can survive a rather higher temperature in drier air than in moister (p. 180). In the starvation tests, comparable data only exist for unfed individuals at 30° C. The following figures have been extracted from Table III :—

	Relative humidity %		
	88	44	11
Dead %, 1st day			
submorsitans	43	6	4
tachinoides	72	65	42
Mean life, days			
submorsitans	1·58	2·86	2·62
tachinoides	1·29	1·44	1·61

From these figures it appears that the death-rate for the first day is much less in *submorsitans* than in *tachinoides*, particularly at lower humidities. The mean duration of life in days shows a similar difference ; at 44% and 11% humidity, *submorsitans* lives almost twice as long as *tachinoides*. When adults were kept in groups in the damp cupboard and fed daily, no difference in the rate of survival could be discovered, Table IV. Under conditions of constant high humidity, at 30° C, both species died out quickly ; at 44% there seemed to be no difference in the survival of females, fig. 3 and p. 191. With regard to reproduction, it was found that the interval before the first birth occurred was slightly longer in *submorsitans* than in *tachinoides*. If the standardized birth-rates at 30° C are compared, Tables V and

VI, it is found that at the most favourable humidity, the figure is higher for *submorsitans* than for *tachinoides*. But one should be cautious in generalizing from such data ; it is possible that 30° C is close to the optimum temperature for one species and further from it for the other. As to chemical composition, we know little, but no great difference between the species has been found.

The puparium

In many respects the puparium and the adult *Glossina* are extremely different. The adult lives actively in an aerial environment, under climatic conditions which change continually ; at any rate at 30° C its optimum is in the middle of the range of humidity ; its weight and composition change rapidly, in response to external conditions and also because it feeds, digests, and excretes. The puparium lies inactive in the soil, where rapid changes of temperature and humidity do not generally occur ; its optimum humidity is high ; it neither feeds nor excretes.

A remarkable fact about the puparium is the great range in weight, among individuals weighed when they were under 24 hours old. The coefficient of variation of *Glossina tachinoides* was 19·2, of *submorsitans* 14·8. This is apparently an effect of captivity, though the precise explanation is unknown ; it is not simply that the births in the cages are premature, for the puparia are both larger and smaller than those produced in nature. But whatever the cause may be, the effect is far-reaching, for the weight of the puparium is closely related to that of the adult which it produces. One should therefore be cautious in applying observations made on flies bred in captivity to problems in the field.

A number of factors which might affect the weight of the puparium were studied. It is probable that sex is a factor, for we know that unfed males weigh significantly less than females, Tables IX—XI. But when we tabulated weights of puparia (on their first day), no relation to sex could be found. We also investigated the effect of the climate in which the parents lived upon the weight of puparia which they produced. It seemed that humidity had no effect on the weight of the puparia. But the effect of the temperature at which the parents lived almost certainly affected the puparia, those produced by adults kept at 30° C being less in weight than those at 24° C, though the difference was not statistically significant. If this can be shown to be so, it explains the observations, Tables VIII and IX, that freshly hatched adult *tachinoides* weigh less in March, April, and May than in February (which is colder) ; they weigh less, because their parents lived during the hot season, and therefore produced smaller puparia.

A number of observations were made during the life of the puparium. The effects of temperature and humidity can at present only be recorded ; no explanation seems possible. Within one sex and species, the duration of life at a particular temperature was very constant, indeed, it is one of the most constant characteristics yet known (coefficient of variation 3·2 — 4·6). Temperature not only determines the duration of life of the puparium, but also affects mortality, which is higher at 30° C than at 24° C even at the most favourable humidity. The upper thermal death point is not far from that of the adult, but the age of the puparium has a considerable effect. Humidity has no effect on the duration of life, but much on the mortality, the optimum being at or near saturation, at least for *tachinoides.* But individual puparia differ greatly from one another in this matter, two out of 15 emerging at 30° C and 11% relative humidity, *i.e.*, a saturation deficiency of 28·1 mm.

Physiological studies made on the puparium show that humidity affects the loss of weight, which is nearly proportional to saturation deficiency, even over a wide range of humidity and at two temperatures. The material which is lost is water, and the puparium does not compensate for high evaporation by burning fat or other reserves, and so producing water of metabolism. The high mortality observed in puparia of *tachinoides* at low humidities is therefore due to loss of water. The puparium, in fact, behaves very like a fasting *Cimex* (MELLANBY, 1932*b*).

The effect of experimental conditions is much influenced by the age of the puparium. The temperature which is fatal in middle life is some 2° C (3·6° F) higher than that which is fatal at the beginning or end, Table XVII. Puparia in middle life can also survive longest if submerged, Table XIX. It has also been observed, under a great variety of external conditions, that death (detected by sudden loss of weight) is commonest at the beginning or end of the puparial stage. It is clear, therefore, that experiments, if they are to yield precise or comparable results, must be made with puparia of known age.

It is a matter of some interest that the differences between the puparia of *Glossina tachinoides* and *submorsitans* are slight. But when they differ, that of *tachinoides* is the least resistant in nearly every respect. Its thermal death point (one hour) is 1° C below that of *submorsitans* ; it is much more affected by drier air at 30° C. If puparia are kept at identical temperature and humidity, the death-rate is nearly always higher in *tachinoides*, Table XVIII. If *Glossina tachinoides* are kept at 30° C and a favourable humidity, they produce puparia which die, but this does not apparently occur in *submorsitans.* There seems to be little evidence at present to show that a similar difference in mortality occurs in nature.

The relation of laboratory studies to field work and to control

The results of a few months' experimental work seem to be consistent and definite. But to what extent may such laboratory work be applied in the field, and what relation have these studies to the problem of control?

The experimental method has its own difficulties, limitations, and advantages. The difficulties are in the main due to the variability of adults and puparia ; much of this is inherent and not due to extrinsic causes and it seems to be unusually high in *Glossina.* Even if one weighs flies which are known to be under two hours old and which have emerged in the same cage, there are great differences in their weights and in the proportion of dry matter in their bodies (coefficients of variation 7·27 and 8·57, Table X). The differences are much greater among flies which are of any age up to 24 hours, greater still if they have been fed (coefficient of variation

11 to 20, Table XIV). Moreover, changes in weight and in composition are extremely rapid ; the flies may take more than their own weight of blood in the course of a few minutes, and lose that, or nearly as much, in 24 hours by excretion and evaporation. Puparia also differ greatly from one another, the coefficient of variation of weight on the first day being over 14 in both species. As to the limitations of the experimental method, they cannot be precisely defined, for we cannot say that life in a cage is " unnatural " because we are so ignorant of life in the field. But certain facts show that conditions in the laboratory are not unfavourable to puparia or adults. As to puparia, under favourable conditions 80% or so hatch, which is higher than the percentage of wild puparia which hatch in any month (TAYLOR, 1932*b*, Table I). With regard to adults, at 30° C and the most favourable humidity, half the female *Glossina tachinoides* lived 25 days, fig. 3 ; the duration of life of female *submorsitans* was approximately the same. In the damp cupboard life was longer, perhaps because the temperature was lower or because the conditions fluctuated ; here half the females lived for more than two months Table IV. We can also measure the suitability of the laboratory conditions by the birth-rate. We believe that under favourable conditions in nature, one pregnancy succeeds another with very short intervals. In certain months, JOHNSON and LLOYD (1923) found as many as 80% of females pregnant ; these females had approached man, and been captured. If we assume that they were a fair sample of all the females, it would indicate a very short interval between pregnancies, which must be difficult to diagnose during the first few days. What birth-rate might we expect in the laboratory at 30° C under the best conditions of humidity ? The duration of pregnancy at 30° C is not precisely known, but as puparia are occasionally deposited on the 13th day of the female's life, we estimate it at 12 days or a little less (p. 189). If one pregnancy immediately succeeds another in all females, and if the duration is 12 days, the standarized birth-rate would be 8·33. But, in fact, we obtained 2·85, the mean of three cages which varied between 7·10 and 1·58, Table V. It seems, therefore, that under our best conditions, the births are about one-third of what might be expected. The figures for *Glossina submorsitans* are not essentially different, and one notes that POTTS, working with *morsitans* at Kikori, Tanganyika, obtained birth-rates of two or a little over (POTTS, 1933, p. 294, para. *a*). It must therefore be admitted that, though *Glossina* appears to survive well, the birth-rate shows that cage conditions are not as good as they might be. It is possible that exposure to stable conditions of temperature and humidity is unfavourable to *Glossina*, and we need experimental data on that point. At the same time, it would be well to investigate the effect of light.

But in spite of difficulties and limitations, we claim certain advantages and successes for the experimental method. By it much that is new has been discovered ; for instance, the effect of season on the weight of flies, Tables VIII and IX ; the fact that flies will die of inanition rather than feed at high humidities, Table VII, fig. 3 ; the discovery that temperature in the field is close to the lethal, for adults, Tables XXIII and XXV, and for puparia, Table XXVI ; and the observations that if adults are kept at 30° C the resulting puparia have a high mortality (p. 216). Such knowledge, which is unobtainable in the field, contributes to our understanding of the fly in its natural environment.

The experimental method has begun to explain and systematize the field work. It has been observed in several parts of Africa that the proportion of female *Glossina* which are pregnant varies with season. For instance, JOHNSON and LLOYD (1923) found 20—40% of female *tachinoides* pregnant in the rains (May to October), 60—80% in the dry season (November to April). They also showed that during the rains the flies fed less, judged by examining the gut, and they were inclined to relate low fertility to difficulty of finding mammals' blood. This explanation may be correct, though it should be remembered that the temperature is low during the rains, and that this in itself would probably reduce feeding and reproduction. But in the laboratory it has been shown that even if temperature is high, 30° C, and daily opportunities of feeding are given, births are rare except within a circumscribed range of humidity (Tables V and VI). With a little more work one could discover whether this is due to nutrition, or the lethargy which is observed at constant high humidity and which may prevent copulation. It is already familiar that the number of flies caught is low in the rains. This has been attributed to their wide dispersion, and may be partly due to temperature. But the observation that they die rapidly at high humidities (fig. 3), and the chart of the July climate at Gadau (fig. 13), show that humidity alone might explain the field data.

In quite a different direction there is agreement between the field and the laboratory. Some years ago, NASH (1931) suggested that there was a relation between the rate of evaporation and the population of *Glossina morsitans*, as measured in his fly-counts ; also, that the flies were most numerous when evaporation was neither very high nor very low. Recently, NASH (1933*b*) has analysed a larger collection of data, and shown high and significant correlation between evaporation and the number of flies ; there is also high correlation between evaporation and the number of flies caught in the subsequent month, proving that the effect is not momentary on the activity of the fly, but that it affects the numbers of wild flies. His figures also show that the number of flies caught is more influenced by evaporation than by temperature. If it is permissible to compare NASH's work on *morsitans* in Tanganyika with our experiments on *tachinoides* and *submorsitans* in Nigeria, one finds an approximate coincidence between his discovery that a moderate evaporation is associated with higher fly-counts, and our observations that at 30° C and moderate humidity, 44%, the two species of *Glossina* live longest, eat most, and have the highest birth-rate.

The object of nearly all the work which has been done on *Glossina* is the control of that insect. It is certainly pertinent to ask whether the investigations, here described at considerable length, will help to achieve control, or whether they suggest modifications in existing practice. The question might be answered in this way. For many years attempts have been made to control *Glossina* by cutting and burning trees and bushes, particularly in the primary foci of these insects. These methods have met with considerable local success in Nigeria, Tanganyika, and perhaps elsewhere, but they lack precision and they are empirical. Moreover, the policy of clearing is much criticised by Forestry Officers and others. They say, with truth, that ruthless clearing destroys vegetation which is of great value (and it is to be remembered that the villager resorts to the woodland for timber, fruit, edible leaves and roots, cosmetics, medicine, fibres, honey, and a host of other products). In particular it is urged, and with good foundation, that in the north of Nigeria the destruction of thickets on river banks is dangerous, for the land is sandy and rather flat, and the " kurimi " is its main safeguard against erosion and dangerous floods. By the experiments described in this paper, the climatic conditions which are unfavourable or fatal to adult and puparium have been defined. It seems that conditions in the field at the end of the dry season (March to early May) are close to the lethal, and that they must kill a considerable proportion of the flies and their puparia. This seems to suggest that a modified form of clearing might be sufficient to exterminate the fly at that season, and that it might be less harmful to forest and river bank. We urge that this should be tried under natural conditions, and that at the same time a more intense study of climatology in relation to these insects should be made.

IX—SUMMARY

It is already known from work in the field that the numbers of tsetse-flies (*Glossina*) are much affected by climatic conditions, but it is difficult to distinguish the effect of particular factors because natural conditions are continually changing. The matter is of great practical importance, because the most effective method of controlling these insects is by producing alterations in the vegetation, and thereby in the micro-climate.

The subject seems to demand study by an entirely new technique ; we have subjected adults and puparia of *Glossina tachinoides* (and to some extent *submorsitans*) to controlled conditions of temperature and humidity. It seems that the effect of temperature both on puparium and adult is relatively simple. The upper and lower limits which *Glossina* can tolerate have been defined, and a little is known of the effect of raised temperature in quickening puparial development. It was also shown that, if females are kept at high temperature, they deposit puparia which are below the average in weight and produce small adults. This produces seasonal changes in the weight of newly emerged adults ; this has perhaps a number of far-reaching consequences.

The effects of humidity are more complex and unexpected. For instance, adult flies were kept at 30° C (roughly the mean annual temperature of the area in which the work was done) and offered food daily, groups of them being kept at different humidities ; 44% humidity was near the optimum, the flies feeding and taking blood nine days out of ten, whereas at humidities above or below this figure, they

fed less frequently, and at 88% humidity they nearly always refused to feed and died of inanition. It was also observed that adults lived longest and bred best at 44% humidity. If the birth-rate was standardized to allow for differences in adult mortality, a figure of 2·85 was obtained at 44% humidity, 0·90 at 19% and still lower figures at higher and at lower humidities. At higher temperatures the effect of humidity is important, for in drier air the insects can survive for a short period temperatures (42°—43° C) which are lethal in moist air. It is not possible to provide physiological explanations of most of these observations ; this can only be done when we know more of the metabolism and particularly of the excretion of this insect. But it has been shown that, in very dry air, the unfed fly burns additional fat, presumably in order to produce water of metabolism to compensate for the excessive evaporation.

The puparium is found in soil in shady places in a very stable environment ; the contrast between its life and that of the adult is very great. It was shown experimentally that the optimum humidity is close to saturation. This seems surprising, for there are large numbers of living puparia in the soil at the end of the dry season, when atmospheric humidity is very low and no rain has fallen for months. It is possible that the atmosphere in the soil spaces is nearly saturated owing to diffusion of water vapour from the deeper layers of the soil.

Detailed studies under controlled conditions in the laboratory mean little unless they are considered in relation to the insect's natural life. We chose one month in the dry and one month in the wet season, and made observations on temperature and humidity with recording instruments both in open country and in the thickets which are the permanent home of the fly. Observations in the field and in the laboratory support one another in a satisfactory manner. It is, for instance, a matter of observation that at the end of the dry season adults of *Glossina tachinoides* are abundant and that a high proportion of the females are pregnant, and it was found that at that season the climatic conditions were close to the optimum as defined in the laboratory. Similarly, during the wet season it has been observed that very few of the females are pregnant. Working in the laboratory, it was shown that high humidity alone, even at a temperature known to be favourable and with frequent opportunities for feeding, caused the flies to die quickly ; in these circumstances the birth-rate is negligible. In one point there is an inconsistency between work in the field and in the laboratory. At the end of the dry season the shade temperature is very high, indeed it often passes 40° C for three or four consecutive hours. Under laboratory conditions such an exposure would kill all the flies ; one may suppose that under natural conditions the lethal temperature is a little higher than it is in an experimental vessel, but it is possible that the flies avoid continuous exposure to high temperatures by resorting from time to time to cooler spots, in animals' holes or among green leaves. Even so, the temperature at this season must be close to the lethal, and it seems likely that clearing undergrowth might kill large numbers of flies by exposing them to radiant heat at a time when temperature was high.

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A *B* *C*

Positions at which climatological readings were taken. *A* on open plain (Screen 3), grass in foreground, *Zizyphus*, &c., in background; taken 22nd June, four weeks after first rain. In dry season open ground is quite without living vegetation. *B* same place, 12th August (shelter has been removed). *C* Screen 4 in thicket, at base of large tree surrounded by creepers.

The same spot, photographed at end of dry season (17th March) and in the middle of the rains (12th August). The trees are *Combretum*, &c., lying between thicket, *C*, Plate 19, and open plain with thorn and baobab, *A* and *B*, Plate 19.

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