The Croonian Lectures

ON

TRYPANOSOMES CAUSING DISEASE IN MAN AND DOMESTIC ANIMALS IN CENTRAL AFRICA.

DELIVERED BEFORE THE ROYAL COLLEGE OF PHYSICIANS OF LONDON,

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LECTURE I.—CLASSIFICATION OF TRYPANOSOMES: TSETSE FLY.

I INTEND to confine myself in these lectures to a consideration of the trypanosomes causing disease in man and domestic animals in Central and Southern Africa, since it is only in Zululand, Uganda, and Nyasaland that I have had the opportunity of studying these parasites in the field and under natural conditions. The conditions, however, which obtain on the East and West Coasts of Africa between 20 deg. N. and 30 deg. S. latitude are much the same as those which are found in the central parts, and it is probable that the same trypanosome species are found throughout. So that in describing the species found in our own colonies, it may be assumed that all the important pathological species found in Central Africa are being dealt with, although in other places they may be known by other names.

The central region—the tropical or equatorial—corresponds with the distribution of the tsetse flies, and the trypanosomes causing disease in this region are carried from sick to healthy animals by various species of this genus of flies. In the north of Africa, outside the range of the tsetse flies, two trypanosome diseases are found, one of the horse (*dourine*) and another of camels (*surra*), the former conveyed from sick to healthy horses by contagion, the latter almost certainly by large biting flies, the so-called horse flies, or *Tabanidae*.

GEOGRAPHICAL DISTRIBUTON.

The wide distribution of these haematozoa is shown in the accompanying map of the world.

It will be seen that these blood parasites are found in most parts of the world—from South America through Africa, Southern Europe and Persia to India, Burma, China, and the Philippines.

In Africa there is a broad equatorial band, representing the trypanosome diseases carried by tsetse flies. It shows the distribution of *Trypanosoma gambiense*, the cause of the ordinary or Congo sleeping sickness, and of *Trypanosoma brucei*, the cause of nagana in animals and the Rhodesian and Nyasaland form of sleeping sickness or trypanosome disease in man.

Further north out of the tsetse fly region we have *Trypanosoma equiperdum*, the cause of dourine, which is widely distributed in Europe.

The area of *Trypanosoma evansi* or surra spreads out of Africa along the camel caravan routes into India and China.

It is unnecessary for me to give an account of the early history of these parasites—it can be found in most textbooks; suffice it to say that the first trypanosomes were discovered some seventy years ago in the blood of fish and frogs. These were of interest to zoologists, but having no obvious bearing on the causation of disease did not attract the attention of medical men. Thirty years afterwards, in 1877, Surgeon Timothy Lewis, of the Army Medical Department, discovered in Bombay the trypanosome of the common rat, which was afterwards named after him—*Trypanosoma lewisi*. Three years later, in 1880, Dr. Evans, chief of the veterinary staff in Madras, discovered what were supposed for a long time to be similar flagellates in the blood of horses suffering from surra. It is from these two discoveries that we must date the beginning of the study of trypanosome diseases.

CLASSIFICATION OF THE AFRICAN TRYPANOSOMES.

In classifying the African trypanosomes I have striven for simplicity, as it seems better for practical purposes to divide them into a few well defined groups and species rather than to try, with our present incomplete knowledge, to subdivide them more minutely.

The three characters mainly relied upon in this classification of trypanosomes are, in the first place, their morphology; secondly, their pathogenic action on animals; and, thirdly, their mode of development in the tsetse flies.

At one time it was hoped that cultivation on artificial media might be used as an aid to classification, but up to the present this hope has not been fulfilled. Other methods, such as cross-inoculation experiments and serum diagnosis, I do not consider of much use in making a simple, workable, practical classification such as will be found of use for diagnosis in the field. If we admit them into our methods, the multiplication of species must proceed to an unmanageable degree.

I. *Morphology*.

The morphology is of great importance in classification. The trypanosomes are first examined in a fresh living condition, to ascertain their general appearance and kind of movement. There is a good deal of difference in the range of movement in different species. Whereas many vibrate about one spot and show little power of wider movement, others are capable of hurling themselves from one point to another with great power and rapidity.

But, of course, it is after the trypanosomes have been fixed and stained that their characteristics can be made out best. For example, the length and breadth of a fixed and stained trypanosome may be very useful in identifying it, and, in addition to the length and breadth, a description of the contents of the cell, the nucleus, micronucleus, undulating membrane, and flagellum, may all help in the differentiation of species.

II. *Susceptibility of Animals*.

The second means at our disposal for the classification or differentiation of trypanosomes—namely, their pathogenic effect on various experimental animals—must be used with caution.

For example, it is well known that their passage through a series of animals of the same species usually exalts their virulence towards that animal. For instance, the nagana parasite after passage through white rats for many generations kills the rats in as short a time as two days; whereas the naturally wild strain of nagana only kills the same animals on an average in twenty or thirty days.

On the other hand, another species of trypanosome in its natural wild state kills monkeys in a few days. But if it is first passed through a goat, and the attempt is made to infect a monkey with the goat's blood, the experiment always fails. Passage through the goat has lowered the virulence of the parasite towards the monkey.

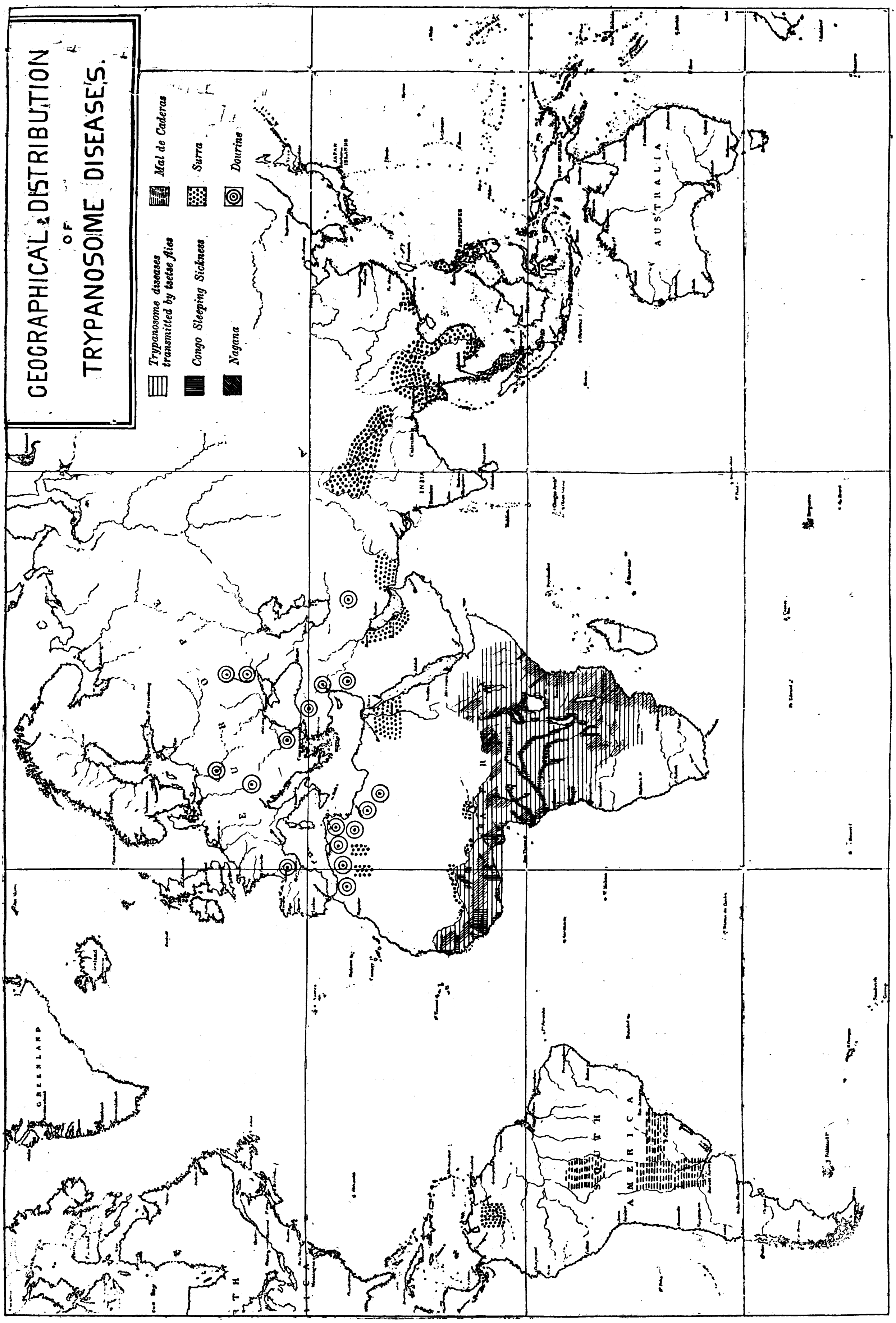
But if a trypanosome is caught in its wild state and straightway put through a series of experimental animals, the result is, in my opinion, of some use in differentiating species. For example, *Trypanosoma brucei* will be found to be much more virulent and kill off more quickly the various laboratory animals than *Trypanosoma gambiense*.

Some trypanosomes are deadly to horses and cattle and harmless to other animals, such as dogs, monkeys, and rabbits, while others show a marked preference for some particular species of animal, such as the domestic pig.

III. *The Development in the Invertebrate Host*.

The third character of use in classification is the mode of development in the tsetse fly or invertebrate host.

As already mentioned, all the pathogenic trypanosomes of man and his domestic animals found in Africa, with the exception of the two northern species, pass through a specific cycle of development in the tsetse flies. The mode of development in the tsetse flies is different for different species of trypanosomes, and this may be made use of in classification, and, in truth, is one of the best means at our disposal. It is not known whether the North African species are capable of developing in tsetse flies. It is possible that, on account of disuse, they have lost the faculty, if they ever had it. It would certainly be an interesting experiment to try to pass them through the "fly."


GEOGRAPHICAL DISTRIBUTION OF TRYPANOSOME DISEASES.
Trypanosome diseases transmitted by tsetse flies
Congo Sleeping Sickness
Nagana
Mal de Caderas
Surra
Dourine
AUSTRALIA
INDIA
GREENLAND
SOUTH AMERICA

CLASSIFICATION INTO THREE GROUPS.

The chief Central African pathogenic trypanosomes may be divided into three groups:

Group A.—*Trypanosoma brucei* group.
1. *Trypanosoma brucei.*
2. *Trypanosoma gambiense.*
3. *Trypanosoma evansi.*
4. *Trypanosoma equiperdum.*

Group B.—*Trypanosoma pecorum* group.
1. *Trypanosoma pecorum.*
2. *Trypanosoma simiae.*

Group C.—*Trypanosoma vivax* group.
1. *Trypanosoma vivax.*
2. *Trypanosoma caprae.*
3. *Trypanosoma uniforme.*

These names probably represent most of the principal pathogenic trypanosomes discovered up to the present time in Central Africa. For the sake of completeness I have placed the northern species, *Trypanosoma evansi* and *Trypanosoma equiperdum*, in the first group, as they seem by morphology and their action on animals to belong there. Each group is distinguishable or separable by well-defined characters.

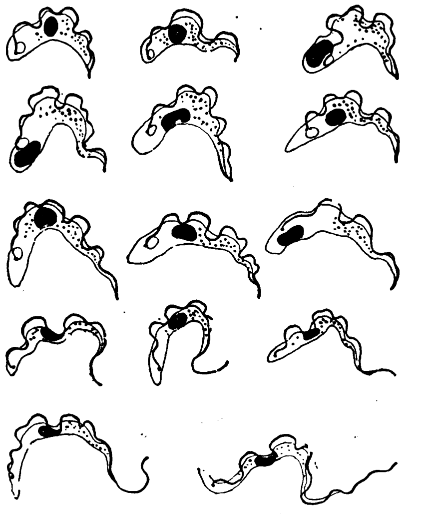


Fig. 1.—*Trypanosoma brucei*; Zululand, 1913.

GROUP A.
The Trypanosoma Brucei Group.

The species forming this group are all more or less polymorphic, varying in size and shape from short and stumpy forms without free flagella, to long and slender forms with free flagella (Fig. 1). The cytoplasm contains numerous dark staining granules. The micronucleus or kinetonucleus is small, and is situated, as a rule, some distance from the posterior extremity. The undulating membrane is well developed and thrown into bold folds. *Trypanosoma gambiense* is another member of the same group (Fig. 2).

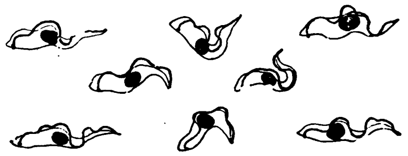


Fig. 3.—*Trypanosoma pecorum.*

The members of this group may be said generally to affect many different species of animals, as, for example, man, horses, cattle, dogs, and most of the smaller experimental animals.

The two Central African members of the group, *Trypanosoma brucei* and *Trypanosoma gambiense*, develop in the tsetse flies in the same way. At first the development takes place in the intestine, afterwards the parasites pass into the salivary glands, by way probably of the proboscis, and there complete their development into infective forms. This is the only group in which the salivary glands are invaded.

This group can be separated from the other groups by shape alone.

GROUP B.—*The Trypanosoma Pecorum Group.*

The trypanosomes are small and monomorphic (Fig. 3). The cytoplasm is non-granular. The micronucleus is prominent, subterminal, and often seems to project beyond the margin. The undulating membrane is fairly well developed.

Trypanosoma simiae is another example of this group (Fig. 4).

In Group B the cycle of development in the tsetse fly begins in the intestinal tract; afterwards the flagellates pass forward into the proboscis of the fly, and finally reach the salivary duct or hypopharynx, where they complete their development and become infective. The difference between Group A and Group B is that in the latter the salivary glands are never invaded. There are only two species at present included in this group—*Trypanosoma pecorum* and *Trypanosoma simiae*. The former gives rise to the most important trypanosome disease of cattle in Africa, while the latter is remarkable for the rapidity with which it kills the domestic pig.

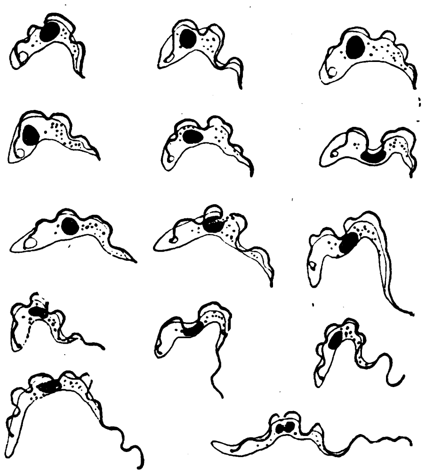


Fig. 2.—*Trypanosoma gambiense*, Dutton.

GROUP C.—*The Trypanosoma Vivax Group.*

The species making up this group are monomorphic, and remarkable for the extreme rapidity of their movements. The posterior extremity is enlarged. The cytoplasm is clear and hyaline. The micronucleus is large and terminal, and the undulating membrane is little developed and simple. This species only affects horses, cattle, goats, and sheep. Monkeys, dogs, rabbits, guinea-pigs, and rats are refractory.

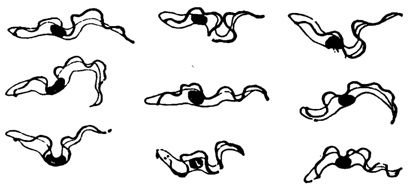


Fig. 4.—*Trypanasoma simiae.*

The cycle of development in Group C differs from that in Groups A and B in that it takes place at first only in the labial cavity of the proboscis, and later in the salivary duct or hypopharynx. No part of the cycle takes place in the intestinal tract or in the salivary glands. In addition to *T. vivax* (Fig. 5) this group contains *Trypanosoma caprae* (Fig. 6) and *Trypanosoma uniforme* (Fig. 7).

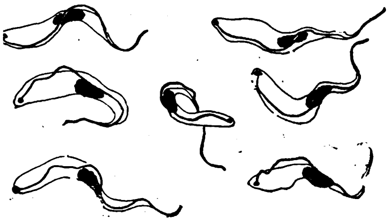


Fig. 5.—*Trypanosoma vivax*, Ziemann.

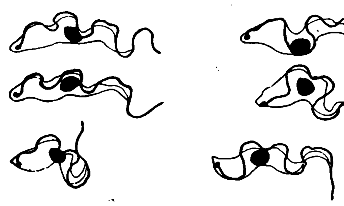


Fig. 6.—*Trypanosoma caprae*, Kleine.

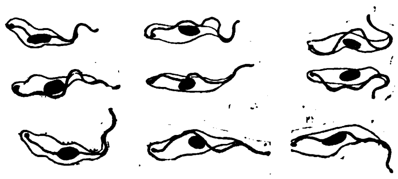


Fig. 7.—*Trypanosoma uniforme.*

These three groups are well marked, and it is fairly easy by microscopic examination alone to name what group a trypanosome belongs to when seen in the blood of the vertebrate host or even in the tsetse fly.

DESCRIPTION OF THE TSETSE FLIES.

Having now noticed the various types of pathogenic trypanosomes found in Central Africa, I will describe briefly the most important of the tsetse flies of the same region. In tropical Africa these flies play as important a part in the causation of these diseases as the parasites themselves, and therefore, before proceeding to describe

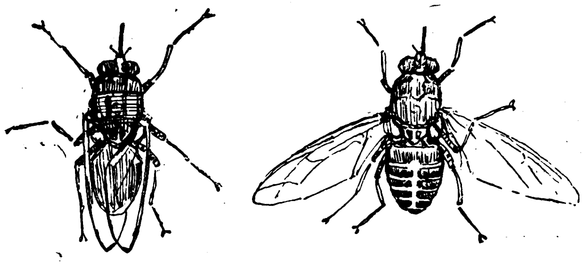


Fig. 8.—*Glossina morsitans*. (× 2.)

any of the individual species of trypanosomes, it will be well to have a general idea of the tsetse flies and their habits.

General Characters.

Glossina morsitans is a dull coloured, ordinary looking fly (Fig. 8), about half an inch in length. The strong proboscis stands out horizontally in front (Fig. 9). The wings are long and closed over each other, like the blades of a pair of scissors, when the fly is at rest. The dorsal aspect of the abdomen is marked by five more or less distinct transverse bands.

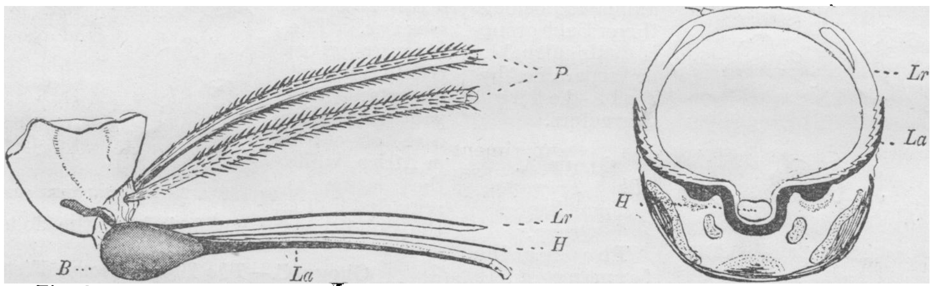


Fig. 9.—Mouth parts of *Glossina*. B, Bulb; P, palpi; Lr, labrum; La, labium; H, hypopharynx.

It is important to understand the structure of the proboscis, as this plays an important part in the development of Groups B and C. In Fig. 9 a side view and a transverse section of the mouth parts are given. In the side view the mouth parts—the labrum, the labium, and the hypopharynx or terminal duct of the salivary glands—are separated. This is done by slipping the point of a fine needle between them at the base and running it towards the tip. This separates the labrum from the labium, and as a rule the hypopharynx springs up from the hollow of the labium and appears between the two parts as an extremely delicate transparent tube.

In the transverse section the parts are seen in position, the labrum and labium joined together form a tube through which the blood is drawn in the act of sucking, and known as the labial cavity; and the delicate terminal duct of the salivary glands or hypopharynx lying in the hollow of the labium, and opening near the tip of the proboscis. The salivary glands are long convoluted organs lying chiefly in the abdominal segment of the fly.

When I arrived in Zululand in 1894 there was only one species of tsetse fly—*Glossina morsitans*. At the present time some fourteen or more different species have been named.

These are divided into four groups by Austen—the *Glossina palpalis* group, the *Glossina morsitans* group, the *Glossina fusca* group, and the *Glossina brevipalpis* group. For our purpose it will be sufficient to describe the principal species from the first two groups, *Glossina morsitans*, the carrier of *Trypanosoma brucei*, and *Glossina palpalis*, the carrier of *Trypanosoma gambiense*. But I may mention here that probably all the tsetse flies are capable of acting as carriers of all the pathogenic trypanosomes—at least, in laboratory experiments. What makes one species of fly the especial carrier of a particular trypanosome is probably bound up in the natural history, the habits, and distribution of the fly.

Natural size.

I. *Glossina morsitans*.

Glossina morsitans is the most widely distributed of the tsetse flies; its range extends from Senegambia, 16 degrees north latitude, in the north-west, to Southern Kordofan, 12 degrees north, and Southern Abyssinia in the north-east, and thence southward to Bechuanaland, the North-eastern Transvaal, and Zululand.

Habitat.

Glossina morsitans is the species made familiar by the writings of travellers like Livingstone and hunters like Gordon Cumming. Until a few years ago it was the only species of tsetse fly of which the habits had been studied, and when the word "tsetse" was used it was this species which was referred to.

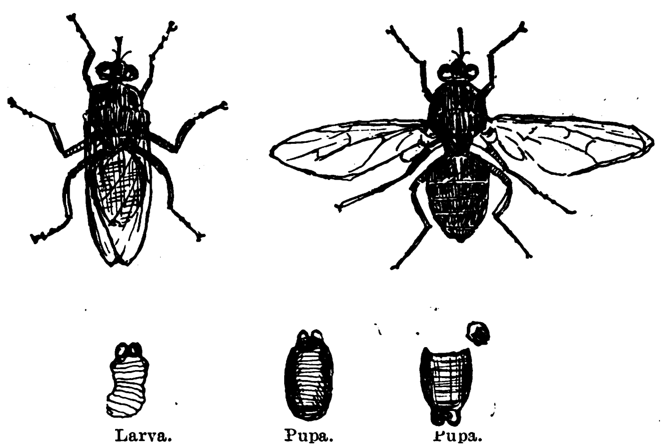
Its habitat is the dry thorny scrub which covers extensive areas of tropical Africa. It is not found along the banks of lakes or rivers, and, in fact, seems to have a distaste for water. It requires shade, however, and is never found on open plains where it would be exposed to the heat of the tropical sun. In the "fly country" there are thickets, undergrowth, and trees which supply the requisite shade (Fig. 10).

Fig. 10.—Habitat of *Glossina morsitans*.

Habits.

These flies begin to bite soon after sunrise if the day is fine, but usually disappear during the hottest hours, coming out again towards evening when it becomes cooler. About sunset they are often particularly active.

Moving objects, such as a motor cycle, seem to attract them greatly, and the cyclist is often surrounded by a cloud of them attacking him like an angry swarm of bees. In these circumstances there is often a great disparity in the numbers of the sexes, the males predominating. This is probably due to the males attaching themselves to a moving object in order to mate with the females, which are attracted by the same movement, and come out of their cover to feed. Tsetse flies are very sensitive to weather conditions. If it rains or blows they usually remain hidden. They diminish in numbers during the dry season, and are most numerous towards the end of the rains.



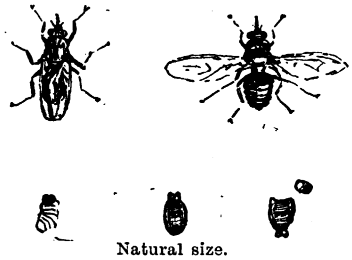


Fig. 11.—*Glossina palpalis.* (× 2.)

Food.

As to the food of *Glossina morsitans*, in one experiment 500 flies were caught in the sleeping sickness area, Nyasaland, and examined as to the contents of their intestine. It was found that 288 (or 57.6 per cent.) contained mammalian blood in a recognizable state. Measurements of these were made and the small type of blood cell was found to predominate, such as occur in the hartebeeste, waterbuck, and other antelope. In only 3 cases were nucleated red blood corpuscles found. From this experiment it appears that, at least in that district, the principal food of *Glossina morsitans* is the blood of antelope. Tsetse flies never suck the juices of fruits or vegetables, as is usual in the case of other biting flies such as mosquitos.

The question is sometimes asked as to how often tsetse flies feed under natural conditions. From experiments with flies in the laboratory it was found that blood is recognizable in stained specimens for two and even three days after a feed, but not beyond the third day. This means, roughly, that half the 500 flies examined had fed within at most three days of their capture; it results that flies feed under natural conditions once in five or six days.

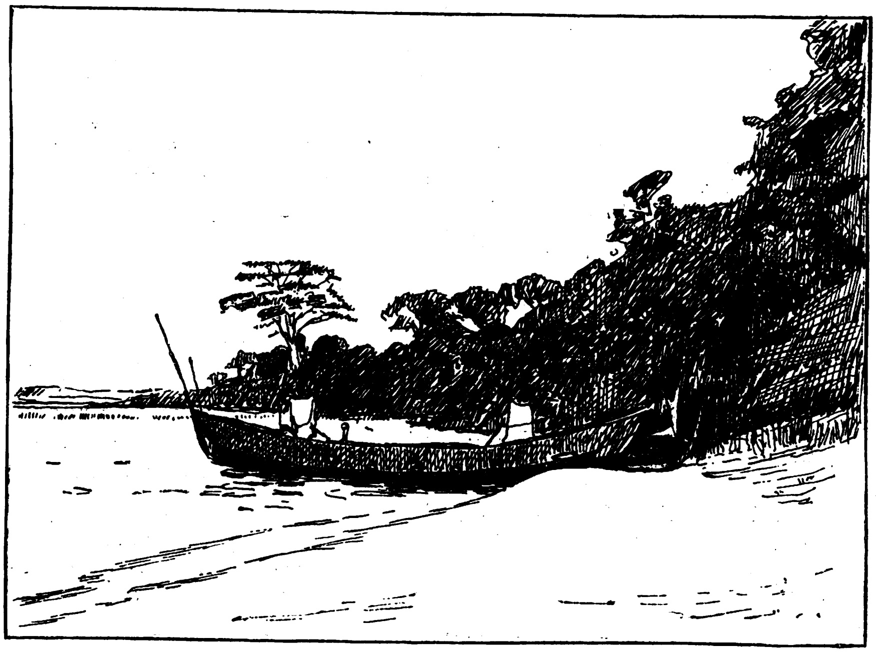


Fig. 12.—Habitat of *Glossina palpalis.*

It is rather an interesting point as to whether these flies could maintain an existence on the smaller mammals and birds if the big game disappeared. They are quite ready to feed on the smaller animals, but most of these, such as monkeys, shrews, the mongoose, and rats, are usually too quick, and snap up the fly before it has had time to feed. Another curious fact seems to be that when rinderpest sweeps through a country, the tsetse flies disappear with the game, and only return when the game has again multiplied to a fair extent. The curious point is that by no means all the game is killed off by the rinderpest; there is usually some species left which one would think would suffice to keep numbers of flies in food. It was thought at one time that this mortality among the flies might be due to some poisonous quality in rinderpest blood, but when flies were fed on rinderpest animals nothing untoward happened.

Act of Feeding.

As a rule the fly feeds very rapidly, fully distending itself in half to one minute after it has punctured the skin.

Reproduction.

Lastly, we come to the most important function of the tsetse flies—that of reproduction. The genus *Glossina* is distinguished from most of the other diptera in being pupiparous.

The female produces a single whitish or yellowish coloured larva about once in ten days. This is retained in the oviduct until it is fully grown. As soon as the larva is born it creeps at once into the soil, or into a crack in the earth, or among leaves and débris, and within a few hours has changed into a hard black puparium. The length of time the pupa remains quiescent depends mainly on the temperature. At a temperature of 85° F. the fly will emerge in twenty-three days, at 70° F. in thirty-five days, and at 65° F. in about sixty days.

These larvae are deposited all over the "fly" country, but there are certain positions and conditions which are favoured. The female usually chooses a spot with plenty of shade. A favourite site for her to take up her abode is on the under surface of a tree which has fallen, and which remains at places some inches above the ground. Another condition is that the breeding place is near a native or game path. This may be to enable the mother to procure her food easily, or it may be some instinct in favour of the offspring which enables the newly-emerged fly to obtain a feed within twenty-four hours of birth.

II. *Glossina palpalis.*

This is also a widely distributed species. It ranges on the west coast from the Senegal River—16 deg. N. latitude—to Angola. It extends eastwards into the Bahr el Ghazal. The eastern boundary of the species follows the valley of the Nile, the eastern shores of Lake Victoria and Lake Tanganyika. From the south end of the latter lake the boundary follows the frontier between the Congo Free State and Rhodesia in a south-westerly direction to Angola.

Habitat.

This species (Fig. 11) differs from *Glossina morsitans* in being a lover of water. It frequents the edge of lakes and rivers, where there is clear water and plenty of shade; this shade consists of thick jungle with high trees and dense undergrowth, which grows to within a few feet or yards of the water's edge (Fig. 12). This tsetse fly is never found on open beaches backed by grass plains, even although there may be some low scrub near the water. It is not found in reed beds or papyrus swamps, and as most of the Uganda river valleys are choked with reeds, it follows that the tsetse fly does not ascend these rivers; in short, it is never found away from water, except in cases where it has followed man or animals for a mile or so for the purpose of feeding. In this way it is brought much more into contact with man than *Glossina morsitans*, which is a dweller in desert places, the haunt of wild animals.

Glossina palpalis is a frequenter of watering places on lakes or rivers, or water holes in the bush, where the natives come for water, and where as a rule, on account of the density of the human population, there is little game.

Habits.

Glossina palpalis has the same habit of biting by daylight as *Glossina morsitans*. It is not seen until the sun is up. On a fine morning the flies may be active as early as seven or half-past seven. On dark shady spots on the lake shore they did not become troublesome until about ten o'clock, when the sun was well up. In cloudy or windy weather few flies are abroad. They have a rapid flight, and suddenly and silently flop on to the skin, causing so little sensation that the sharp stab of the proboscis is often the first thing felt. Sometimes they insert the proboscis so painlessly that the attention is not drawn to the spot. As a rule little or no irritation follows the bite, no itching or swelling or reddening of the skin. But if there have been many bites about the same place there is often a good deal of swelling, induration, redness, and irritation, which lasts for several days, and, as is natural, some individuals are much more affected than others.

Feeding.

As in *Glossina morsitans*, the time taken by the fly in filling itself with blood is short. Stuhlmann weighed the flies before and after feeding and found that they took in—the males one and a half times their own weight in blood, the females sometimes as much as two and a half times.

Food.

Under ordinary circumstances, in populous districts it may be assumed that man supplies most of its food, and it is considered by some that this fly thrives better on human blood than on any other. In captivity, however, it feeds readily on any warm-blooded animal, especially on birds, and, if hungry, may even be induced to feed on cold-blooded animals, such as the lizard or frog.

But, in the absence of man, it may be remarked that as this species is a dweller on the banks of rivers and lakes, it comes much more in contact with large birds—cormorants and other water-fowl—and reptiles—crocodiles and monitors—than *Glossina morsitans*.

In Uganda before the natives were removed from the lake shore and islands they doubtless formed the chief supply of blood for the fly. On the other hand, after the natives had been removed inland or in places where there was no native population, the fly had to adapt its diet to the available supply. One experiment made by us in Uganda in such a place showed that only 17 per cent. of the flies had fed on mammalian blood, and 83 per cent. on avian or reptilian, and as far as could be made out twice as many of the flies had fed on reptiles as on birds. In such places the large lizard or monitor seems to be a favourite dish with this species.

Reproduction.

I need not go fully into the reproduction of *Glossina palpalis*, as the process is the same as in *Glossina morsitans*. One point may be mentioned, however, and that is that *Glossina palpalis* in Uganda has a marked predilection for certain breeding grounds; these are sandy beaches along the lake shore backed by a belt of vegetation.

The breeding grounds on Damba Island, for example, where some thousands of pupae were collected every month for the laboratory, is formed of coarse sand and pebbles. It is four or five feet above the level of the lake, and the bush comes to within a few yards of the water's edge. The pupae are found as a rule an inch or two below the surface of the sand at or near the edge of the belt of vegetation. For a long time we failed to find any wild pupae, although days were spent in turning over soil and decaying vegetable matter in those places where the fly most abounds. At last their breeding haunts were discovered by Captain Fraser, R.A.M.C., to be in patches of sand at the edge of the lake. After this secret was discovered there was no lack. Our natives found them in large numbers. One day they brought up as many as 7,000, and as I had promised a cent for each pupa brought up to the laboratory I had to pay out nearly five pounds for them.

These wild pupae proved to be much healthier than those obtained from flies in captivity. The flies bred from pupae born in the laboratory rarely showed any marked vitality. On the other hand, the flies hatched out from the wild pupae found on the lake shore were fairly strong and vigorous, and lived in captivity for a couple of months or more. In conducting experiments with flies it is very necessary to work with laboratory-bred ones, as the wild flies may be naturally infected with several species of trypanosomes. Numerous experiments went to show that there is no hereditary transmission of trypanosomes in tsetse flies, and there is no reason to believe that flies become infected by contact with infected flies or from fouled cages. Any trypanosomes found in laboratory-bred flies may therefore be considered to be derived from the infected animal they were fed upon.

It will be seen, then, that the two principal groups of tsetse flies—the *morsitans* group and the *palpalis* group—differ from each other in well-marked characters, the former living in wild, unpopulated districts, and trusting to the wild game for their food; the latter along rivers and lakes which are usually thickly populated, and trusting to man for a food supply, or, in his absence, living on the large reptiles, birds, and antelope which frequent these places.

LENGTH OF LIFE OF TSETSE FLIES.

The duration of life of tsetse flies is an important question, but one difficult to answer. The importance comes in when we have to consider how long a place may remain dangerous from the presence of infected flies.

I remember at a meeting of the Royal Society, when the question came up, that the late Lord Avebury said he did not see any reason why a tsetse fly should not live for years. He had himself known an ant which lived in captivity for some fifteen years.

Carpenter tried to solve the question by marking large numbers in a given spot, and finding out how long the marked flies could be recaptured. One male was caught 199 days after it had been marked. Kleine states that he kept a fly in captivity for 227 days. If we put the life of the fly, then, at anything up to a year we may not be far out.

IT is intended shortly to add to the Oxford Medical Publications a serious of Oxford War Primers of medicine and surgery, and it is hoped that the earliest will appear within the next few weeks. They will be written mainly by members of the medical profession holding commissions in the naval and military services. Probably among the first will be volumes by Mr. Robert Jones of Liverpool, Major, R.A.M.C.(T.), and Mr. D'Arcy Power, Lieutenant-Colonel, R.A.M.C.(T.), who will edit the series.