THE EFFECT OF AGE, SEX AND STARVATION OF *GLOSSINA MORSITANS MORSITANS* ON THE TRANSMISSION OF *TRYPANOSOMA CONGOLENSE* AND *T. CONGOLENSE/T.B. BRUCEI* MIXED INFECTIONS

By

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D E C L A R A T I O N

This thesis is my original work and it has not been presented for a degree in any other University.

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LIST OF ABBREVIATIONS

EEC	-	European Economic Community
FAO	-	Food and Agricultural Organization of the United Nations
IAEA	-	International Atomic Energy Agency
IBAR	-	International Bureau for Animal Resources
ICIPE	-	International Centre of Insect Physiology and Ecology
ILCA	-	International Livestock Centre for Africa
ILRAD	-	International Laboratory for Research on Animal Diseases
OAU	-	Organization of African Unit
UNEP	-	United Nations Environmental Programme
WHO	-	World Health Organization of the United Nations
EATRO	-	East African Trypanosomiasis Research Organization
ARPPIS	-	African Regional Postgraduate Programme in Insect Science

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SUMMARY

Transmission of trypanosomes (Genus Trypanosoma) by tsetse flies (Genus Glossina) is a process influenced by several factors. Such factors may arise from the trypanosomes, tsetse flies, hosts upon which flies feed and environmental conditions such as temperature. Investigations of these variables have a direct bearing on tsetse/trypanosomiasis control measures as well as providing information on the disease situation. In this study, three factors were investigated under laboratory conditions; these comprised age, sex and period of starvation of G.m. morsitans on the effect of uptake and development of Trypanosoma congolense and T. congolense/ T.b. brucei mixed infections.

In the first study to investigate fly age, sex and period of starvation on the development of T. congolense, a total of 5 groups of flies were used comprising 1,739 specimens. Following infection with T. congolense all flies were maintained on rabbits for a period of 30 days before each was dissected and the infection assessed.

Mature trypanosome infections were highest in 16 hour old and 2 day old (48 hours starved) flies, neither of which had previously had a blood meal. The other groups comprising 2 day old fed flies, 7 day old fed flies and 7 day old (48 hours starved) flies, had significantly lower infection levels and, furthermore, did not differ significantly ($P > 0.05$) from each other.

Within both 2 day old fly groups, the group starved for 48 hours had significantly higher levels of infection than the group which had been allowed to feed on rabbits prior to infection. Additionally, within the two 7 day old fly groups, a period of 48 hours starvation did not produce any enhancement of infection levels over the non-starved group which were maintained on rabbit blood for 5 days prior to infection.

The results indicate that the age of the fly alone, is not a significant factor controlling the level of trypanosome infections. Rather, it may be concluded that trypanosome infections are elevated significantly when the first blood meal that an adult fly receives, is an infective one. From this laboratory derived data it could therefore be postulated that in the field situation, young flies which take their first blood meals from an infected host animal, are most likely to have the highest levels of trypanosome infection in the case of T. congolense and this species of vector.

A sex-linked factor was also noted in this study in that immature infections were most frequently recorded in female flies whilst males displayed a preponderance of mature infections. Whether trypanosome maturation is inhibited in females or enhanced in males, remains unknown.

In the second study, to determine the effect of mixed trypanosome (T. congolense/T.b. brucei) infections on levels of infection rates in flies, 5 groups of flies were again

employed comprising 571 specimens. Following maintenance of flies on rabbits for a period of 30 days, infected fly organs were inoculated into Swiss mice to determine the species of trypanosomes. One out of 46 (2.2%) males of the flies that fed on a mixed trypanosome infected meal developed double mature infections of T. congolense and T.b. brucei. It seems possible that even under natural conditions a single fly can transmit such mixed infections to a susceptible host.

Labral infections in the group of flies that fed on only T. congolense infected meal through the silicone membrane were observed in 6 out of 51 (11.8%) males and 5 out of 59 (8.5%) females. These infection levels were statistically comparable to labral infections in the group of flies that fed on mixed T. congolense/T.b. brucei infected meal for which results were, 4 out of 46 (8.7%) males and 10 out of 71 (14.1%) females. The latter group, however, showed significantly lower ($X^2 = 6.4$, $p < 0.05$) hypopharyngeal infections in females, 2 out of 71 (2.8%) than the former group, 9 out of 59 (15.3%), respectively. Similar comparisons of mature infections between groups that had fed on T.b. brucei infected meal alone and on mixed infections did not reveal any significant differences. It seems that within the fly system, development of T. congolense might be inhibited by T.b. brucei in experimentally infected flies.

Defibrination of infected blood for silicone membrane feeding lowered salivary gland infections in the case of

T.b. brucei as compared to a control group that fed directly on the same donor rats. This was not the case for T. congolense infections in which infections in flies that fed through the membrane did not differ significantly with a control group of flies that fed directly on infected rats.

CHAPTER I

1.0 INTRODUCTION

The role played by tsetse flies in the transmission of African trypanosomiasis has been of veterinary and medical concern since 1894 when David Bruce incriminated both the tsetse fly and the trypanosome as the cause of animal trypanosomiasis (Nash, 1969; Hoare, 1972).

Transmission of trypanosomes by tsetse flies has been the subject of extensive study, speculation and considerable controversy. The process of transmission involves a complex of events which, for convenience, may be broadly divided into three parts: the interaction between uninfected tsetse flies and infected reservoir hosts; the development of trypanosomes in the flies; and the interaction between infected flies and susceptible hosts. All these three areas are subject to variation in relation to the species of tsetse flies in question, sex of the fly, age of the fly at the time of the infective feed, physiological state of the fly, hosts used by flies, the availability of these hosts, environmental conditions such as temperature, trypanosome species and strains and concurrent bacterial and viral infections in the flies.

Much information has been gathered on trypanosome infection rates of tsetse flies and the factors that influence them. However, until quite recently much of this information

was based largely on field observations where factors vary from locality to locality and season to season, and not on laboratory studies where such factors could be standardised. Different results and hypotheses regarding the effect of these factors on infection rates have been advanced by various workers, and quite often these have been conflicting.

Part of the discrepancies in results and conclusions reached by different workers accrued from the host of interacting factors which, in themselves, vary in the way they affect transmission in nature. In part, different results have been arrived at due to the use of different methods employed to sample wild tsetse fly populations. It is now known that the various methods of sampling tsetse flies all tend to pick out only a fraction of a population which is not wholy representative of that population (Harley, 1967; Jordan, 1974; Ryan, et al. 1982; Rogers, 1983). Tsetse fly samples do not reflect actual infection rates which are influenced by a number of factors (Fairbairn and Culwick, 1950; Buxton, 1955; Jordan, 1964, 1974, 1976; Lambretch, 1980; Moloo, 1980; Molyneux, 1977, 1980).

Trypanosomiasis as a disease, presents one of the most urgent medical and veterinary problems facing the African continent today. Several thousand people die of this disease and about 35 million are estimated to be at risk (FAO, 1979). Livestock and agricultural production are affected too, creating an impediment to the much needed food production programmes in many African countries. As long as this problem persists,

further research will be necessary not only to understand how the disease spreads but also to find ways of controlling it.

The gravity of the situation is reflected by the programmes of national, subregional and international organisations researching and contributing to the control of this disease. Such organisations include United Nations agencies: UNEP, WHO, FAO and IAEA; international centres like ILRAD, ICIPE and ILCA; and numerous intergovernmental bodies such as the EEC, OAU and IBAR. In addition, there are numerous bodies at the national level in some countries working on this problem. A recent report from WHO (1983) showed that the epidemiology of sleeping sickness due to T.b. rhodesiense is still far from being clear. Further research is needed to understand the risk factors involved in this disease. In some areas control efforts have broken down and trypanosomiasis is on the increase calling for a more active approach to the disease situation.

Further examples of the effects of trypanosomiasis on the economy in Africa may be cited from work reported by Wilson, et al. (1963) and FAO (1979). Wilson, et al. (1963) pointed that problems presented by tsetse flies are not only those of disease but that human and social issues are also involved. They estimated that if the then tsetse fly infested area (approximately 10,400,000 km^2) was cleared of tsetse fly, the potential cattle population to be raised in this area would be 125,000,000 head. At the time cattle

population in Africa was estimated at 114,000,000 head. There would be an increase of 11,000,000 - an increase which would improve the human diet that lacks in proteins. They reported that forestry and fertility of the soil in terms of manure from cattle were also adversely affected by trypanosomiasis. About one and half decade later, FAO (1979) confirmed these findings and showed that animal trypanosomiasis affects directly and indirectly; human health, livestock production, agricultural production, rural and national economies. An area estimated at 7 million km^2 was still tsetse infested and if cleared of this scourge could support 140 million head of cattle.

Trypanosomiasis control programmes are generally based on the biology of the parasite and the vector. Methods used against tsetse flies include: use of traps and impregnated screens, insecticides, biological control, sterilization, clearing of vegetation and game elimination. Of these insecticides have been most widely used but in the light of side effects that they cause on the environment, safer methods are now advocated. Against the parasite is the use of trypanocidal drugs and of late, research is being intensified to produce a vaccine. Another approach is the development and use of trypanotolerant animals in tsetse infested areas.

Each individual method has its own limitations. While research to improve some of the control methods is continuing, research on the biology of the parasite and vector should not be

ignored. In this connection entomological investigations throw some light on the epidemiology and epizootiology of the disease and have a direct bearing on present and future control measures.

1.1 Objectives of the study

In this study three of the factors that may influence transmission of trypanosomes by tsetse flies were singled out and investigated in the laboratory under standardised conditions. These comprised: sex of the fly, age of the fly at the time of the infective feed, period of starvation of the fly, in addition to the transmission of mixed trypanosome infections. The importance of these variables are difficult to assess with sufficient accuracy under field conditions because of inherent variation.

T. congolense was chosen for the greater part of the study because published work on how these factors affect its transmission is scanty as compared to T. vivax and T. brucei type trypanosomes. Yet, in East Africa and probably elsewhere in Africa T. congolense causes one of the most serious forms of cattle trypanosomiasis. Information provided by a study of this kind is useful to workers carrying out studies on trypanosomiasis challenge models as well as those interested in the epidemiology and epizootiology of trypanosomiasis. The following objectives were borne in mind:

1. To determine the effect of age of G.m. morsitans on infection with T. congolense.

2. Find out whether susceptibility to *T. congolense* infections differs in the sexes of *G.m. morsitans*.

3. Determine the effect of period of starvation of *G.m. morsitans* on the transmission of *T. congolense*.

4. Determine whether mixed *T. congolense*/*T.b. brucei* infections will influence transmission of these trypanosomes by tsetse flies.

CHAPTER II

2.0 LITERATURE REVIEW

2.1 Tsetse flies

Details of tsetse evolution and systematics are discussed by various authorities including Buxton (1955), Glasgow (in Mulligan and Potts, 1970), Potts (1970), Laird (1977) and recently Pollock (1980). While some authorities place the genus Glossina under the family Muscidae of the Order Diptera, together with the subfamily Stomoxyinae, others place it in a monogeneric family the Glossinidae. Most authors agree that there are 22 species of tsetse flies which are recognized today. Some of these species are divided into subspecies, bringing the total number of species and subspecies to 30. A list of these species and subspecies as cited from Glasgow (in Mulligan and Potts, 1970) and Jordan (1974) is given in Table 1.

On the basis of the characters of the male and female genitalia, the genus is subdivided into three groups: the fusca, palpalis and morsitans groups. In a review of the recent developments in the ecology and methods of control of tsetse flies, Jordan (1974) reported that these subdivisions, initially based on morphological characters and habitat preferences, have been confirmed by genetic studies conducted by various workers. Accounts of each of these groups in relation to climate and vegetation, and their medical and veterinary

Table 1: The genus Glossina
(from Glasgow, in Mulligan and Potts, 1970)

SPECIES/SUBSPECIES		AUTHORITY	YEAR
FUSCA GROUP			
Glossina	fusca fusca	Walker	1849
G.	fusca congolensis	Newstead and Evans	1921
G.	tabaniformis	Westwood	1850
G.	longipennis	Corti	1895
G.	brevipalpis	Newstead	1910
G.	nigrofusca nigrofusca	Newstead	1910
G.	nigrofusca hopkinsi	Van Emden	1944
G.	fuscipleuris	Austen	1911
G.	medicorum	Austen	1911
G.	severini	Newstead	1913
G.	schwetzi	Newstead and Evans	1921
G.	haningtoni	Newstead and Evans	1922
G.	vanhoofi	Henrard	1952
G.	nashi	Potts	1955
PALPALIS GROUP			
G.	palpalis palpalis	Robinean-Desvoidy	1830
G.	palpalis gambiensis	Vanderplank	1949
G.	tachinoides	Westwood	1850
G.	pallicera pallicera	Bigot	1891
G.	pallicera newsteadi	Austen	1929
G.	fuscipes fuscipes	Newstead	1910
G.	fuscipes martinii	Zumpt	1933
G.	fuscipes quanzensis	Pires	1948
G.	caliginea	Austen	1911

Table 1: Continued..

MORSITANS GROUP

SPECIES/SUBSPECIES		AUTHORITY	YEAR
Glossina	longipalpis	Wiedemann	1830
G.	morsitans morsitans	Westwood	1850
G.	morsitans submorsitans	Newstead	1910
G.	morsitans centralis	Machado	1970
G.	pallidipes	Austen	1903
G.	austeni	Newstead	1912
G.	swynnertoni	Austen	1923

importance, are given in detail by Buxton (1955) and Nash (1969).

The fusca group species are large flies measuring 9-13.5 mm in length from the front of the head, excluding the proboscis, to the tip of the abdomen. All species in this group except G. longipennis which has a lighter shade of body colour, are brown to very dark brown. They are forest species and seldom use man as a host for feeding. According to Nash (1969) none of these has been observed to transmit human trypanosomiasis although all probably do transmit animal trypanosomiasis. They appear to be of little importance in disease transmission.

The palpalis group comprises species of medium size ranging from 6.5-11 mm. The colour of the dorsal part of the abdomen varies from black bands on a yellowish background to black bands on a dark background. These are riverine and lacustrine species. However, among them G. tachinoides may venture into drier areas following the course of streams. The group comprises some species of medical importance notably; G. palpalis and G. tachinoides, which are associated with the transmission of T.b. gambiense. Palpalis group species are also known to transmit animal trypanosomiasis (T. vivax, T. congolense and T.b. brucei).

The third group, the morsitans group, also consists of species of medium size ranging from 7.5-11 mm. They have brownish or blackish bands on a yellow to organge-coloured background of the dorsal part of the abdomen. Members of this

group abound in savannah woodlands which support most of Africa's wildlife. They feed mainly on game animals, hence the name "game tsetse". This group constitutes a very important group, medically and veterinary, in that among its members are major vectors of both human (mainly due to T.b. rhodesiense) and animal trypanosomiases.

Besides body size and the characters of the genitalia, taxonomists use other morphological features to distinguish between individual tsetse species. Among others, these include the shape and proportion of the antenna, colour of underside the thecal bulb, size of hairs on the side of the thorax and colour of the tarsal segments of the legs.

2.2 Distribution of tsetse flies

Although fossil tsetse of about 40 million years ago have been found in North America in the Oligocene shales of Florissant, Colorado (Buxton, 1955; Nash, 1969; Ford, 1970, 1971) tsetse flies are now found only in sub Saharan Africa. On a continental scale, the first edition of tsetse distribution maps was that prepared by Potts in 1953/4. Ford and Katondo (1977) updated these and compiled the second edition which included information available up to 1973. More recently, Katondo (1984; personal communications) revised the second edition of tsetse distribution maps. He pointed out that the general tsetse distribution is quite well known particularly of the morsitans and palpalis groups. That of the fusca group

is still lacking in some areas. Tsetse distribution is a changing phenomenon and should be treated with caution as there are always changes taking place in infested areas due to a number of variables like control measures and extensions and retractions of fly belts. Challier (1982) and Katondo (1984), for instance, reported that the recent droughts have modified the distribution of some species along their northern distribution limits and that there are advances and extensions of tsetse populations in Namibia, Zambia, Botswana, Mozambique, Tanzania, Togo, Niger, Mali, Upper Volta and Ivory Coast.

The distribution of Glossina is shown in Figure 1. The infested area generally falls between latitudes $15^{o}N$ and $29^{o}S$ representing a surface area of approximately 10 million km^2. Distribution is limited by climatic and ecological factors such as vegetation and availability of hosts upon which flies feed. The northern limit is the Sahara desert with an annual rainfall of less than 500 mm, creating arid conditions which are unfavourable to tsetse existence. In the south the Kalahari desert forms the limit. Further south and south east temperatures become too cold for flies to exist.

2.3 Trypanosomes

A comprehensive classification of trypanosomes is dealt with by Hoare (1972). There are about 125 species of trypanosomes distributed in about 400 species of mammalian hosts that include rodents, bats, ungulates, primates including man, carnivores, endentates, marsupials, insectivores, lagomorphs,

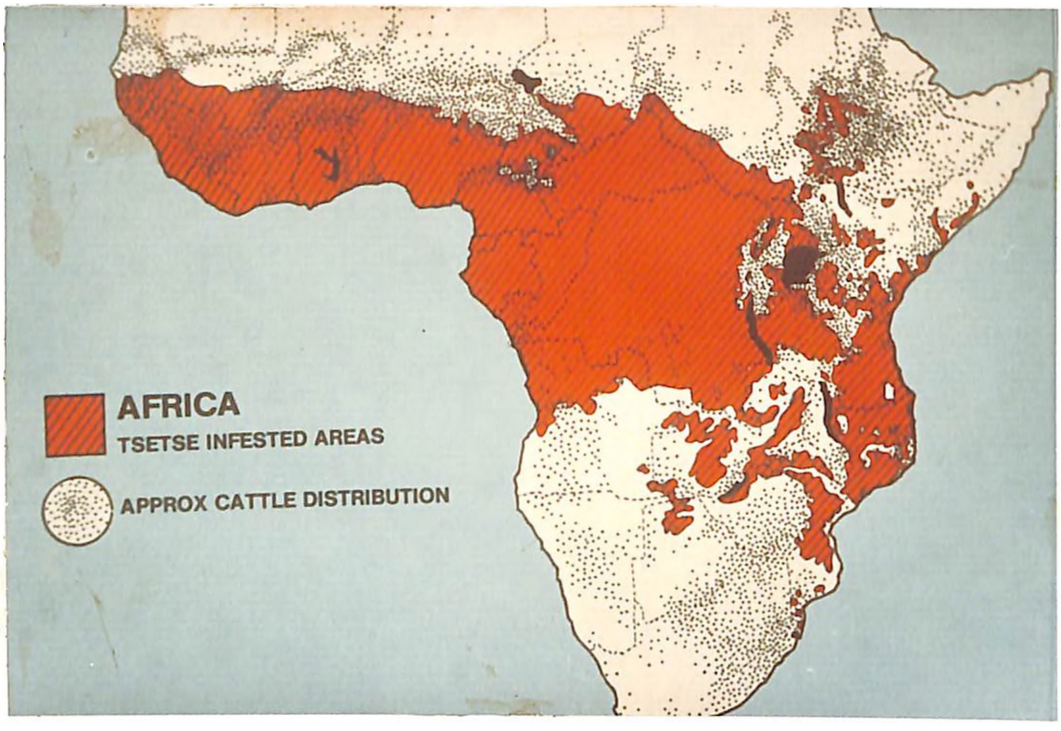

Fig. 1: Map of Africa showing tsetse and cattle distribution. (derived from FAO, 1985).

elephants and monotremes. Of prime importance to the African situation, and of interest in this study are those that are tsetse-borne and fall under the subgenera: Duttonella, Nannomonas and Trypanozoon. The classification of trypanosomes according to Hoare (1972) is abridged in Table 2.

The subdivision of the genus Trypanosoma into sections Stercoraria and Salivaria is based on the site of development of the trypanosomes in the vector and on their reproductive strategy in the mammalian host. Trypanosomes transmitted through the faecal medium and with a discontinuous type of reproduction in the mammal at the amastigote and epimastigote stages are grouped under section Stercoraria (Hoare, 1972). On the other hand, those transmitted through the proboscis in saliva of the vector, and which divide repeatedly in the mammal are referred to as salivarian trypanosomes.

In this study two species of trypanosomes were employed; Trypanosoma (Nannomonas) congolense Broden 1904 and Trypanosoma (Trypanozoon) brucei brucei Plimmer and Bradford 1899. Hoare (1972) described the subgenus Nannomonas as, "those trypanosomes in which a free flagellum is typically absent, the kinetoplast is of medium size and is marginal". The development of these trypanosomes in the tsetse fly takes place in the midgut and proboscis. Trypanosomes of this subgenus are among the most important pathogenic parasites affecting domestic animals in Africa.

Table 2: Classification of trypanosomes to the subgenus level. (from Hoare, 1972).

Classification		Authority	Year
Phylum	Protozoa	Goldfuss	1818
		emend. Siebold	1845
Subphylum	Sarcomastigophora	Honiberg and Balamuth	1963
Superclass	Mastigophora	Diesing	1866
Class	Zoomastigophorea	Calkins	1909
Order	Kinetoplastida	Honiberg	1963
Suborder	Trypanosomatina	Kent	1880
Family	Trypanosomatidae	Doflein	1901
		emend. Grobben	1905
Genus	Leptomonas	Kent	1880
"	Herpetomonas	Kent	1880
"	Blastocrithidia	Laird	1959
"	Phytomonas	Donovan	1909
"	Leishmania	Ross	1903
"	Endotrypanum	Mesnil and Brimont	1908
"	Trypanosoma	Gruby	1843
Section	Stercoraria		
Subgenus	Megatrypanum	Hoare	1964
"	Herpetosoma	Doflein	1901
"	Schizotrypanum	Chagas	1909
Section	Salivaria		
Subgenus	Duttonella	Chalmers	1918
"	Nannomonas	Hoare	1964
"	Trypanozoon	Lühe	1906
"	Pycomonas	Hoare	1964

The subgenus Trypanozoon, according to Hoare (1972), comprises pleomorphic forms (the term pleomorphic taken to mean slender, intermediate and stumpy forms of the developmental stages of the parasite) with or without free flagellum, and with a small kinetoplast that is subterminal. In T. equinum, however, the kinetoplast is invisible. The brucei-complex trypanosomes (T.b. brucei, T.b. rhodesiense and T.b. gambiense) develop in the midgut, proboscis and salivary glands of tsetse flies. Members of this group are responsible for trypanosomiases in man (T.b. rhodesiense and T.b. gambiense) and domestic animals (T.b. brucei) such as cattle, horses, sheep, goats, camels and dogs.

2.4 Distribution of trypanosomes

While tsetse distribution may be mapped out with considerable accuracy, it is less easier to map out the distribution of tsetse-borne trypanosomes. According to Ford (1971) the greater part of these are found in the wild animal population. Surveys to show trypanosome distribution in wild animals are not easy to carry out and have yet to be improved upon. Human trypanosomiases, on the other hand, have been comparatively easier to map out.

Generally, the distribution of pathogenic trypanosomes follows the distribution of their vectors but there are exceptions. The subgenera Duttonella, as shown by T. vivax occurs outside tsetse fly belts such as in South America, West Indies and Mauritius (Hoare, 1972), where transmission is maintained

mechanically by other biting flies like those of the family Tabanidae and probably subfamily Stomoxyinae. T. evansi and T. equiperdum although closely related to the brucei-complex are not cyclically transmitted by tsetse flies. T. evansi (including T. equinum) is transmitted mechanically by Tabanids while T. equiperdum is transmitted sexually in equines. The distribution of these trypanosomes obviously does not conform to tsetse distribution.

The distribution of trypanosomes affecting cattle is fairly known. Ford (1971) showed the geography of trypanosome distribution in cattle in East and West Africa. He reported that in surveyed areas the proportions of infections due to T. vivax, T. congolense and T.b. brucei were influenced by proximity to fly belts and frequency of drug treatment given to cattle. T. vivax predominated with an increase in distance from fly belts in West Africa. The proportion of trypanosome infections in West Africa in decreasing order were: T. vivax, T. congolense and T.b. brucei while the picture in East Africa was in the decreasing order: T. congolense, T. vivax and T.b. brucei. Mwambu and Mayende (1973) confirmed these proportions in Ikoma, Tanzania.

The distribution of human trypanosomiasis, as reported by Ford (1971) and Pollock (1980) shows sleeping sickness due to T.b. rhodesiense occuring in small scattered foci ranging from the Okovango delta in Botswana, spreading north-east to the Zambezi valley into Mozambique and through Zambia

and western Tanzania to the northern corner of Lake Victoria. Ford (1971) and Baker (1974) reported the disease to have reached as far north as western Ethiopia. The T.b. gambiense form of the disease occurs in small isolated pockets from north of Lake Victoria stretching westwards into the greater part of West Africa incorporating Uganda, Zaire, Gabon, Cameroun, Chad, Nigeria, Ghana, Upper Volta, Mali, Gambia Senegal, Guinea and Ivory Coast.

2.5 Hosts of tsetse flies and infection rates of tsetse flies

It has been established that tsetse flies locate their hosts by both visual and chemotactic response (Buxton, 1955). Particular animal hosts form regular blood feeding hosts because of their availability and attractiveness. Lloyd et al. (1924) studied the hosts of G. morsitans and G. tachinoides in northern Nigeria on the basis of identification of erythrocytes in preparations made from the flies' stomach contents. Employing this method they demonstrated that G. tachinoides took most of its feeds from mammalian hosts (54.5%), followed by reptilian (40.8%) and avian (4.9%) hosts. G. morsitans fed mainly on mammalian hosts (93.8%), but a few feeds were also derived from avian hosts (7%).

The development of more sophisticated methods (Weitz, 1956) of identifying the source of blood meals by immuno-precipitation taken by arthropods greatly improved the knowledge of the animal hosts used by tsetse flies. Thus,

Jordan et al. (1961) and Jordan et al. (1962) studied the animal hosts used by various Glossina species in Nigeria and southern Cameroun. These included G. palpalis, G. pallicera, G. longipalpis, G. medicorum, G. tabaniformis, G. haningtoni, G. fusca, G. nigrofusca, G. tachinoides and G. morsitans. They pointed that the availability of animal hosts and their attractiveness to flies affected the extent to which they were used by various species of tsetse flies. Duickers (Cephalophus rufilatus), for example, were observed to be rarely fed upon despite being the most abundant antelopes in the fauna of the study area. In addition, man as a host of flies, was shown to be unattractive to species of flies other than G. palpalis, G. longipalpis and G. tachinoides. Ostensibly, because of their close contact with man, G. palpalis and G. tachinoides are the most important vectors of sleeping sickness in West Africa. In northern Nigeria where cattle come into close contact with G.m. submorsitans, this species forms the major vector of cattle trypanosomiasis.

Weitz (in Mulligan and Potts, 1970) summarized the results of over 25,000 blood meals of 15 species and subspecies of Glossina collected from various parts of Africa. On the basis of blood meal analysis he grouped tsetse species and their sources of feeds as shown in Figure 2. Suids provided the majority of the feeds of G. swynnertoni, G. austeni, G. tabaniformis and G. fuscipleuris. Bovids and suids were equally used by G.m. morsitans, G.m. submorsitans and

GLOSSINA	PRIMATES	SUIDS	BOVIDS	OTHER MAMMALS	BIRDS	REPTILES
swynnertoni	4	65	23	7	1	
austeni	5	58	36	2		
fuscipleuris	1	64	20	1		
tabaniformis		70	10	21		
m. morsitans	10	36	45	7		
m. submorsitans	18	46	22	8	5	
m. orientalis	7	36	41	15	1	
pallidipes	3	13	80	4		
longipalpis	2	4	90	2		
fusca		14	74	13		
longipennis	1	1	18	73	7	
brevipalpis	1	39	23	36		
fuscipes	18	3	38	5	1	34
palpalis	39	6	22	4	2	27
tachinoides	43	2	30	16		9

Fig. 2: Summary of the feeding habits of 15 species of Glossina obtained from the identification of approximately 25,000 feeds. The figures indicate the percentage of feeds of each species of Glossina derived from each category of host.

(derived from Weitz, in Mulligan and Potts, 1970).

G.m. orientalis. Following the taxonomic revision of G. morsitans subspecies by Machado as cited in literature (e.g. Jordan, 1974; Ford, 1971), the G.m. morsitans and G.m. orientalis refered to by Weitz (in Mulligan and Potts, 1970) should refer to the presently known G.m. centralis and G.m. morsitans, respectively. Bovids alone formed a major source of feeds for G. pallidipes, G. longipalpis and G. fusca. Mammals other than pigs or bovids were used by G. longipennis and G. brevipalpis. Lastly, G. palpalis, G. fuscipes and G. tachinoides were observed to be opportunist feeders, taking their feeds from man and the most available hosts. These results are in keeping with the list compiled by Pollock (1980) although, as has been stated earlier, variation in hosts used by the same species of tsetse might occur in different localities and seasons.

As a factor that may influence infection rates of tsetse flies, animal hosts were first referred to by Lloyd et al. (1924). They reported that G. morsitans which relied heavily on antelopes for its feeds had higher proboscis infections (28.1% of the 6,314 specimens) than G. tachinoides (7.9% of the 11,500 specimens) which had about 40% of its feeds from reptilian hosts. Fairbairn and Burtt (1946) indicated that transmissibility of a strain of T.b. rhodesiense they had cyclically passaged for several years in different animals was influenced by the type of host. A line passed through Bohor reedbuck (Redunca redunca) was more transmissible than two other lines passed through Thomson's gazelle and monkey. Further studies by Ashcroft (1959) demonstrated that some

game animals are better reservoirs of trypanosomes than others. Suids were less efficient trypanosome reservoirs than a number of species of bovids. This work was followed up by Allsop (1972), who found in Lambwe Valley, Kenya, that 9 out of the 10 bushbuck he examined were infected with trypanosomes while the 3 bushpigs caught in the survey did not show infection.

Infection rates of G. morsitans in three study areas in Nigeria reflected the extent to which this species fed on bovids (Jordan, 1964). Similar results were latter reported in other species of tsetse (Jordan, 1965) in Nigeria and elsewhere (Harley, 1966a; Clarke, 1969). As a general rule, species of tsetse flies that feed mainly on bovids exhibit higher levels of infection rates than species that feed on less efficient reservoirs. Jordan (1965, 1974) suggested that this phenomenon arose due to higher levels of T. vivax infections in bovids compared to suids. The incidence of T. congolense infections was relatively constant in all Glossina populations because T. congolense occurred in both bovids and suids. Geigy et al. (1971b) postulated that it is not the individual but the species of mammals from which blood meals are derived that induce differences in infection rates.

2.6 Life cycle of T. vivax, T. congolense and T. brucei type trypanosomes

The migratory pattern of trypanosomes in the tsetse fly is diagnostic of their identity. When ingested with blood by a tsetse fly, T. vivax

trypanosomes attach themselves in the proboscis, between the labrum-epipharynx and the labium. They multiply in this site giving rise to infective forms, metacyclics, which are later also found in the hypopharynx. Those which are ingested and pass to the midgut with the blood meal degenerate (Buxton, 1955; Hoare, 1972). The period taken to complete the cycle in fly varies with temperature. It may take as short as 2 days or as long as 13 days according to Hoare (1972). Pollock (1980) reported an average of 10 days but temperature was not specified.

The period is longer with T. congolense trypanosomes than the T. vivax group. The route followed as described by Kaddu (1978) starts in the proboscis to the oesophagus, crop and through the proventriculus to the midgut. In the midgut trypanosomes are initially found in the endoperitrophic space but later pass through the open end of the peritrophic membrane into the ectoperitrophic space. From here they migrate to the proventriculus where they pass across the freshly secreted peritrophic membrane into the lumen of the oesophagus and finally reach the labrum. Development is completed in the hypopharynx, the site in which metacyclics are formed. The shortest period of this developmental cycle was reported by Kaddu (1978) to be 10 days in G. pallidipes. His results were in agreement with results obtained earlier by Elce (1971). Harley and Wilson (1968) in a different study involving G. morsitans, G. pallidipes and G. fuscipes concluded that 14 days was the shortest developmental period

and 28 days as the longest. Methods used, strains of trypanosomes and differences in temperature under which these studies were conducted could perhaps explain the different periods of time for the completion of the cycle.

Robertson (1913, cited by Mshelbwala, 1972) described the life cycle of brucei group trypanosomes. Similar studies were reported by Taylor (1932), Yorke et al. (1933) and Fairbairn (1958). The route taken from the proboscis to the midgut and back to the proventriculus appears to be the same as that taken by congolense group trypanosomes described above. In brucei group trypanosomes the cycle is more complicated in that from the proventriculus and oesophagus, trypanosomes pass to the hypopharynx and in some cases labrum, before migrating to the salivary glands where the cycle is completed. The period of development to completion of the cycle within the fly is influenced by temperature as reported already and the strain of trypanosome. It may take 12-40 days or longer from the time of ingestion of trypanosomes to the time metacyclics are formed in the salivary glands (Taylor, 1932). Pollock (1980) indicated 20-30 days as the average time the cycle would be completed.

The classically described route followed by brucei group trypanosomes has been questioned by various workers such as Mshelbwala (1972) and Otieno (1973). Mshelbwala (1972) showed that in G. tachinoides, G. morsitans and G. palpalis, infective forms of T.b. brucei may occur in the

haemocoel. He suggested that trypanosomes could have gained entry into the haemocoel by passing across the gut wall and, furthermore, questioned whether they could enter the lumen of proboscis and be transmitted without going through the salivary glands. Otieno *et al.* (1976) demonstrated that *T.b. brucei* inoculated into the haemolymph was able to invade the midgut and proboscis. In one case, the host on which such flies were maintained developed *T.b. brucei* infection. They did not, however, observe any salivary gland infections. Studies by Ward and Bell (1971), Dipeolu and Adam (1974) and Otieno (1983) have shown that transmission of *T.b. brucei* may take place without the salivary glands of the fly being involved visibly on dissection. Further studies to ascertain whether there is a direct route other than the classically described one that confines the trypanosome to within the alimentary canal are required. Furthermore, it remains to be shown whether such a route would apply to other species of trypanosomes other than the one reported.

2.7 Age of fly and trypanosome infection rates

Van hoof *et al.* (1937) showed that *G.p. palpalis* was readily infected with *T.b. gambiense* when flies were one day old and less so when they were a few days old. They also pointed out that infection rates were higher when the first blood meal was the infective one. This latter finding was in disagreement with the work of Duke (1935) who considered that a preliminary feed or two by newly hatched *G.p. palpalis*

and G. morsitans on clean blood before they fed on an animal infected with T.b. rhodesiense did not make any difference in infection rates. In his discussion of the factors in individual tsetse that may influence infection rates, Buxton (1955) concluded that age is an important factor. He deduced from the then available information that flies would readily be infected with the brucei sub-group trypanosomes if they took the infected meal on the first and second day of emergence from pupae. Wijers (1958) confirmed results earlier obtained by Van hoof et al. (1937) and conclusions reached by Buxton (1955) when he demonstrated that to achieve higher infection in G.p. palpalis with T.b. gambiense, the first blood meal must be taken from an infected host. He noted that total infection in the first-day flies was 7.6%, in the second day 1.9%, third day 0.7% and older flies 0%.

From field results in Uganda, Harley (1966a, 1966b) observed a positive correlation between age of G. pallidipes, G.p. fuscipes and G. brevipalpis and infections due to T. vivax and T. congolense. Similar results from field studies were obtained by Moloo et al. (1973) in Ikoma, Tanzania, for T. vivax and T. congolense and again by Willemse et al. (unpublished) in G.m. morsitans and G. pallidipes in Luangwa valley, Zambia. Harley (1966a, 1966b) and Moloo et al. (1973) suggested that flies could become infected at any age with T. vivax and T. congolense. Infections due to T. brucei group trypanosomes were

encountered only in older flies. On the other hand Clarke (1966, 1969) observed that while T. vivax infections in wild populations of G. morsitans in Zambia were acquired at any age, those due to T. congolense appeared to occur if an infective feed was taken early in life of the adult fly.

Laboratory colonization of tsetse flies and mass rearing procedures (Nash et al., 1966; Nash and Jordan, 1970; Laird, 1977) made it possible to carry out transmission studies involving large numbers of flies of known history and age. Ward (1968) showed with T.b. brucei and G. austen bred in the laboratory, that age of the fly at the time of the infective meal was the most important physiological condition of the vector for successful laboratory infections. He found that one day old flies had midgut infection rates of 27% while older flies had less than 1%. The hypothesis to explain this phenomenon as suggested by Willett (1966) was that the peritrophic membrane was not fully developed in young flies of about 24 hours old but was developed in older flies. This permitted trypanosomes to reach the ectoperitrophic space around the region of the proventriculus or easily penetrate the freshly secreted membrane. In older flies the membrane was well developed and only a few trypanosomes could reach the ectoperitrophic space via the midgut and hindgut where they could circumnavigate the peritrophic membrane and pass on to the proventricular region.

Elce (1974) demonstrated that G. morsitans reached a maximum infectivity of 5.25% at 21-24 hours after emergence for T.b. rhodesiense, with minimal infectivity after 27 hours. He observed maximum infectivity for T. congolense to be 8.5% at 24-27 hours but still observed quite a high infectivity of 6.15% in 54-61 day old flies. Prior to these findings, Harley (1971a) stated that age was not actually a critical factor as had been supposed by previous workers like Wijers (1958). In his studies (Harley, 1971a) with G. fuscipes and T.b. rhodesience, he noted that while infection varied with age, old flies could still become infected. The decline in susceptibility to infection was extremely rapid over the first two days and thereafter very slow. Ward and Bell (1971) reported similar results in G. austeni infected with T.b. brucei.

The importance attached to the knowledge of infection rates of wild populations of flies in relation to the epidemiology and epizootiology of trypanosomiasis can not be over-emphasized. In this respect studies have been conducted on a variety of tsetse species to determine their vectorial capacity. Thus, Ryan et al. (1982) showed that the acquisition of trypanosomes by wild tsetse populations of G. longipalpis, G.f. fuscipes, G.p. palpalis, G.p. gambiense, G. brevipalpis, G. longipennis, G. swynnertoni, G.m. morsitans, G.m. centralis and G.m. submorsitans increased with age for T. vivax, T. congolense and T.b. brucei. They found that in Ivory Coast, G. longipalpis acquired infections more rapidly than G.m. submorsitans and

other species studied by previous workers. However, their results were not in accordance with the work of Distelmans et al. (1982) who in a laboratory study involving G.p. palpalis and T. congolense, found that flies could not be infected with trypanosomes in the proventriculus and proboscis after 32 hours of emergence. Older flies of 2-25 days of age were infected only in the gut without proboscis involvement. Recently, studies by Gingrich et al. (1982), contrary to the general hypothesis that it is only the young flies which can be readily infected with T. brucei group trypanosomes showed that even older flies can be readily infected if starved. Their study indicated that G. morsitans of 21-25 days old could be infected with T.b. rhodesiense in proportions comparable to teneral flies of less than 24 hours of age, if starved for a period of 4 days. How valid then is the hypothesis that only teneral flies will be infected with trypanosomes, particularly on T. congolense for which information appears to be scanty?

2.8 Fly sex and trypanosome infection rates

Duke (1930) compared the sexes of G.p. palpalis infected with T.b. gambiense and T.b. rhodesiense. He reported that 4.5% of the 12,737 males and 5.5% of the 11,772 females were infected. Salivary gland infections were 1.3% in males and 1.5% in females respectively and he concluded that there was not a significant difference in infection rates between the sexes. Later, Duke (1933c) re-examined his records and found that actually, more females were infected than males and the

difference was highly significant. Corson (1935, cited by Fairbairn and Culwick, 1950), and Burtt (1946b), on the other hand, reported male G. morsitans to be more susceptible to infection with T.b. rhodesiense than females. Van hoof (1947), who carried out studies on G.p. palpalis and T.b. gambiense, reported similar results obtained earlier by Corson (1935) and Burtt (1946b). In his study Van hoof (1947) observed salivary gland infections in 32.4% of the males and 21.4% of the females. Studies by Fairbairn and Culwick (1950) also indicated males, not females as earlier considered by Nash (1948) on the basis of their longevity, to have higher infection rates. They found that under laboratory conditions, infection with T.b. rhodesiense in male G. morsitans was largely influenced by temperature at which pupae were maintained whereas, in the females both pupal and temperature of maintenance of adults were influential. However, Baker and Robertson (1957) studied infectivity of G.m. morsitans to T.b. rhodesiense and T.b. brucei under laboratory conditions and did not find significant differences in infection rates between the sexes.

In a study of infection rates in the mouthparts of tsetse flies in Zambia, Clarke (1969) observed that males were more frequently infected than females. He acknowledged that his results could have had an age bias caused by sampling techniques. Field studies conducted in Uganda by Harley (1966b) on G. pallidipes, G.p. fuscipes and G. brevipalpis and Moloo et al. (1973) in Tanzania on G. swynnertoni and G. pallidipes, revealed that females had higher infections

than males. Both these workers also acknowledged the effect of sampling techniques on their results. The wing fray method has been shown to overestimate the age of females than males thereby introducing a bias in the results.

Harley (1971a) conducted laboratory studies on infection of G. fuscipes with T.b. rhodesiense and noted that infection rates did not differ significantly between the sexes, but in another study Harley (1971b) reported males to have higher infection rates than females of G. pallidipes, G. morsitans and G. fuscipes infected with T.b. rhodesiense.

Moloo (1981) reported male and female G.m. morsitans maintained on different hosts after infection with T. vivax, T. congolense and T.b. brucei to be equally infected in the case of T. vivax infections, inconsistent in T. congolense infections, and that males showed higher infection rates than females with T.b. brucei infections. Distelmans et al. (1982) in a laboratory study found males (pooled infection of 13.15%) to be more infected than females (pooled infection of 9.15%). Their results were based on one day old flies which showed gut and proboscis infections. In another study Otieno et al. (1983) subjected 3 groups of flies to temperatures of $5^{o}C$, $25^{o}C$ and 29^{o} C and observed a propenderance of infection in males over females in all the treatments. They suggested that higher T.b. brucei infection in males could have been due to the possibility that females digested their blood meal faster, thereby subjecting the ingested trypanosomes to the

unfavourable environment arising from the faster release of proteolytic enzymes. Moloo and Kutuza (1970) had earlier shown that the rate of crop emptying in the females was generally faster than in males. Clearly, this subject needs further studies if we are to understand the biology of the parasite and the vector.

2.9 Transmission of mixed trypanosome infections

The occurence of mixed trypanosome infections involving T. vivax, T. congolense and T.b. brucei in domestic and wild animals has been reported (Ashcroft, 1959; Godfrey et al., 1965; Geigy et al., 1971a; Geigy and Kauffman, 1973 ; Mwambu and Mayende, 1973). In tsetse flies, multiple infections were referred to early in the century by Johnson and Lloyd (1923) in Nigeria. Lloyd et al. (1924) also in Nigeria reported mixed T. congolense/T. brucei infections in G. tachinoides and G. morsitans. Multiple infections in flies were confirmed by Duke (1933a) in wild caught G. swynnertoni infected with both T. congolense and T. brucei in Uganda. He observed that, "......certain individuals among the tsetse populations are specially fitted to act as intermediate hosts of the pathogenic trypanosomes, and that the peculier endowment appeals to at least two or probably to all three groups of trypanosomes." The questionthen was that of calculating infection rates, on whether a single fly with double infection was to be considered as one or two separate infections. Since then various workers in different parts of Africa have reported

mixed infections in wild tsetse populations. However, studies on transmission of mixed infections by tsetse flies are scanty. The only published work on this subject appears to be of Moloo et al. (1982) who showed that T. vivax, T. congolense and T.b. brucei could all infect a fly concurrently, and that such mixed infections could be transmitted to a susceptible host. In their study they showed that although a fly could be infected with double or tripple infections, only one of the species might be transmitted to mice or goats. Of the groups of flies they infected in vivo, one group of 8 flies showed metacyclics in the salivary probes. When fed on mice, two became infected with mixed T.b. brucei/T. congolense infections while the other 6 had T.b. brucei alone. When the 8 flies were later dissected, two of them had T.b. brucei/T. congolense infections while the other 6 had only T.b. brucei. In another group, 12 flies showed metacyclics in the saliva and they all transmitted infections to mice. Six mice displayed T.b. brucei/T. vivax mixed infections while the other 6 showed only T.b. brucei. In yet another group of flies which were fed through the in vitro system, Moloo et al. (1982) obtained upon dissection of the 148 surviving flies that had fed on a meal infected with T. vivax, T. congolense and T.b. brucei, the following mature infections: 10.5% T.b. brucei, 1.3% T. congolense and 0.7% T. vivax. The flies which had T.b. brucei/T. vivax mixed infection upon dissection transmitted only T.b. brucei infection to mice by inoculation of macerated fly organs. These findings

contrast with evidence from the field indicating that T.b. brucei group infections are rare in flies. For example results reported by Duke (1933a), Buxton (1955), Jordan (1964, 1965, 1974, 1976), Moloo et al. (1973) and Otieno (1983) and others confirmed the rarity of T. brucei group trypanosomes in comparison to T. congolense and T. vivax. What appears intriguing is the predominance of T.b. brucei infections in relation to T. vivax and T. congolense infections in laboratory infected flies.

2.10 Genetic factors: the individual and species of fly

Duke (1933a, 1933b) considered that G. morsitans was a more efficient vector of trypanosomes than G. palpalis. In one of his comparative studies Duke (1933b) observed that 6.6% of 2,238 G. morsitans were infected while only 3% of 2,355 G. palpalis were infected. He confirmed this finding in a later study (Duke, 1936) with G. morsitans and G. palpalis infected with T. congolense and T.b. brucei. More information on this subject was provided by studies of Harley and Wilson (1968) who, in a comparative study, showed that G. fuscipes was a poor vector with an infection of 2.9% than G. morsitans (11.6%) and G. pallidipes (13.2%). Harley (1971b) compared infection with T.b. rhodesiense of G. pallidipes, G. morsitans, G. fuscipes and G. brevipalpis and found that none of the 202 G. brevipalpis developed salivary gland infections. Male and female salivary gland infection rates in G. morsitans were 10.3% and 2.6%; in G. pallidipes 24.1% and 9.5% and in G. fuscipes 20.6% and 14.6%, respectively. Similar comparative

studies on tsetse species were carried out by Roberts and Gray (1972), Wilson et al. (1972) and Janssen and Wijers (1974). Maudlin (1982) reported that susceptibility of G. morsitans to T. congolense was determined by maternal phenotype. Whether this can be extended to other trypanosome species remains to be shown. Jordan (1976) considered that due to the readiness with which T. vivax infects Glossina species, genetic factors may be limited to T. congolense and T. brucei infections.

2.11 Temperature and trypanosome infection rates of tsetse flies

The available literature shows that earliest observations on the effect of temperature on the development of trypanosomes in tsetse flies were made by Kinghorn et al. (1913) as cited among others by Taylor (1932), Duke (1933d) and Buxton (1955). Kinghorn et al. (1913) reported that infection rates of G. morsitans in Northern Rhodesia (now Zambia) with T.b. rhodesiense were two and a half times higher in the Luangwa valley where the climate was hotter (about 23.9°C-28.9°C) than in the neighbouring plateau with temperatures of about 15°C-18.3°C. They observed that two batches of G. morsitans infected experimentally with T.b. rhodesiense and maintained in the laboratory at a temperature of about 15.6°C failed to infect a monkey after 36 and 57 days, respectively. But when these two batches were placed in the incubator for 6-8 days at 23.9°C-29.4°C the life cycle of

trypanosomes was completed in both batches. They concluded that higher temperatures of about 23.9°C - 29.4°C were necessary if T.b. rhodesiense was to complete its life cycle in G. morsitans.

Taylor (1932) considered that temperature seemed to be the climatic factor most likely to influence the development of T.b. gambiense in G. tachinoides. He postulated that since trypanosomes spend the greater part of their exogenous cycle in the ectoperitrophic space of the midgut, in the proventriculus and in the salivary glands, they are well protected from the influence of atmospheric humidity. T. vivax, which develops in the proboscis would be probably an exception. He pointed out that atmospheric temperature influences the body fluids of insects. Therefore, raising the temperature of maintenance of the tsetse fly would affect the behaviour of the trypanosome in the fly. By incubating G. tachinoides for 24 hours at 37°C after an infected meal with T.b. gambiense, Taylor (1932) raised infection rates from 10.5% to 77.8%. In comparison his control flies kept at room temperature had 0-10.5%. Furthermore, he recorded 100% infection rates in several individual batches of incubated flies.

Duke (1933d) followed up this work. He infected paired groups of G. morsitans with T.b. brucei, T.b. rhodesiense and T.b. gambiense at laboratory temperature and at raised temperatures of about 30.0° C - 31.1°C and 35.0°C - 36.7°C.

His results indicated that flies incubated at 35.0°C - 36.7°C had higher infection rates than those kept at laboratory temperature. He suggested that temperature acted not only on the trypanosome in the fly but possibly also on the physiology of the fly in a way favourable to the trypanosome. He considered that although species of tsetse flies differed in their vectorial capacities there was a minimum temperature below which the life cycle of the trypanosomes could not be completed in Glossina. Above this minimum temperature species of flies were affected differently by higher temperatures.

Burtt (1946a), as well as Fairbairn and Burtt (1946), observed that incubation of G. morsitans pupae at 30°C raised transmission rates in the resultant adults. The cycle of trypanosomes in adults from the incubated pupae was significantly shortened compared to flies which emerged from normally maintained pupae. Fairbairn and Culwick (1950), as stated earlier, also showed that pupal temperatures of up to 30°C raised infection rates in male flies but that in females, both a raised pupal temperature and temperature of fly maintenance were required. These workers also observed that there was a tendency for elevated temperatures of fly maintenance to shorten the cycle of T.b. rhodesiense in male G. morsitans. Similar observations were later reported by Fairbairn and Watson (1955) who demonstrated that G. palpalis, which emerged from pupae incubated at 28°C - 29°C, and maintained at various temperatures ranging from 23°C - 30°C had higher T. vivax infection rates than those which emerged

from pupae incubated at a lower temperature of 24°C - 26°C. By increasing pupal temperatures from 24°C - 26°C infection rates were raised irrespective of the temperature at which adult flies were maintained. Pupal temperatures of about 30°C proved too high for effective transmission of T. vivax by G. palpalis while below 23°C it was too cool for transmission.

Buxton (1955) who reviewed the factors that influenced infection rates in the individual tsetse fly reported that the effect due to temperature was complex. He stated that increased infection rates might be due to increased activity or vigour of the trypanosomes or to the effect of temperature on a particular tissue or process in the fly. While the effect of raised temperature on the puparium might be physiological it was complex because it gave rise to three aspects: a tendency to produce high infection rates in flies; a rapid development of trypanosomes in the fly; and greater multiplication of trypanosomes in the fly. Buxton (1955) noted that mature infection rates due to T. vivax, T. congolense and T.b. rhodesiense were recorded more frequently in hotter months than cool ones by various workers. He concluded that infections in flies depended on a seasonal factor which probably could be due to temperature acting either on the adult fly, or puparium, or both.

Ford and Leggate (1961) reported a positive correlation between the total incidence of trypanosomes in the morsitans group and temperature. This correlation was mainly due to

T. vivax type trypanosomes and to a lesser extent *T. congolense*. On the basis of geographical distribution of *Glossina*, they plotted a median line which they referred to as the "*Glossina* equator" at 7°S. They indicated that there was a trend for fly infection rates to increase with distance away from the "*Glossina* equator". This increase corresponded with an increase in the mean annual temperature before falling off towards the temperate regions. Ford and Leggate (1961) reached the conclusion that although temperature controlled the general level of infection of *Glossina* population, local variations may result from differences in the game population utilized by tsetse flies.

Dipeolu and Adam (1974) confirmed earlier findings on the acceleration of development of trypanosomes and increased proportions of flies that become infected when temperature is raised. They observed that incubating *G. morsitans* pupae at 31°C and maintaining the flies at 26°C - 31°C significantly increased infection rates. When temperature was lowered to 20°C, fewer flies became infected and the development of trypanosomes was delayed. More recently, Otieno *et al.* (1983) on the other hand, did not find temperature of fly maintenance to affect infection rates of *G.m. morsitans* with *T.b. brucei* They found that flies subjected to temperatures of 5°C, 25°C and 29°C developed similar levels of salivary gland infection of 16.3%, 16.4% and 14.6%, respectively.

C H A P T E R III

3.0 MATERIALS AND METHODS

3.1 Reagents

3.1.1 Physiological saline

Physiological saline was prepared by dissolving 9 grams of sodium chloride in 1 litre of distilled water to make a 0.9% solution (Baker, in Mulligan and Potts, 1970; Murray et al., 1983).

3.1.2 Phosphate Buffered Saline

This was prepared by mixing two stock solutions of M/15 sodium phosphate dibasic (Na_2HPO_4) and M/15 possium acid phosphate (KH_2PO_4) as described by Murray et al. (1983). The sodium phosphate dibasic stock solution was prepared by dissolving 9.47 g of Na_2HPO_4 in 1 litre of distilled water. Similarly, 9.07 of KH_2PO_4 was dissolved in 1 litre of distilled water to prepare the stock solution of potassium acid phosphate. The final solution, Phosphate Buffered Saline, was prepared by mixing 90 ml of sodium phosphate dibasic with 10 ml of potassium acid phosphate and was stored at $4^{o}C$. The pH of the solution was 7.7.

3.1.3 Giemsa stain

The stock solution of Giemsa stain was prepared by dissolving 3.6 g of Giemsa powder (standard stain of BDH chemicals, England) in 250 ml methanol (analar) and 250 ml glycerine, similar to the method reported by Lynch et al.

(1969) and Baker (in Mulligan and Potts, 1970). Rickman (personal communications) confirmed these proportions to produce good results for trypanosome staining. Twenty to thirty glass beads were placed in a dark bottle cleaned and rinsed with 70% methanol and then 3.6 g of Giemsa powder, 250 ml of methanol and 250 ml of glycerine were added. This mixture was shaken thoroughly for about 15 minutes and stored at room temperature. To stain blood smears, the stock solution of Giemsa stain was diluted in the ratio 1:10 in distilled water. The resultant dilution was used to stain blood smears for about 20-30 minutes.

3.1.4 Anaesthesia

Sagatal (sodium pentabarbitone) was used to anaesthetize rats before flies fed on them. Rats were inoculated intraperitoneally with 0.22 ml of Sagatal per kilogram body weight. Chloroform or ether was used to kill flies before dissection.

3.1.5 *Anticoagulant*

Heparin was used as an anticoagulant during serial passages. A small amount of heparin, enough to wet the syringe, was used.

3.1.6 Disinfectants

To sterilize bottles, instruments and for swabbing animals before bleeding them, 70% ethanol or 70% industrial methylated spirit was used.

3.2 Laboratory animals and trypanosome parasites

3.2.1 Trypanosome strains

Trypanosoma (Nannomonas) congolense: the strain used in these experiments was obtained from Dr. J.H.P. Nyeko, an ARPPIS Ph.D student with ICIPE. This strain designated as Moruma, was originally isolated from a cow in the Coast Province of Kenya on 6th July 1983, by the Kabete Chemotrypanosomiasis Project Team. From the time of isolation to the time of use in these experiments, the strain had been syringe passaged 17 times in mice and rats. Nyeko (personal communications) reports that this strain is resistant to normal doses of samorin.

Trypanosoma (Trypanozoon) brucei brucei: EATRO 1969 was obtained from Dr. L.H. Otieno of ICIPE. It was isolated from a naturally infected female hyaena (Crocuta crocuta) in the Serengeti National Park in 1971 by Professor R. Geigy. This strain was first stabilated as EATRO 1857 after a short maintenance by serial passages in several mice and rats. It was tested for infectivity to man by inoculation of a volunteer who showed no signs of trypanosome infection. On this basis it was classified as T.(T.) brucei.

3.2.2 Tsetse flies

Tsetse flies used, Glossina morsitans morsitans Westwood 1850 were obtained from the insectary of ICIPE. The colony at ICIPE originated from flies obtained from

Langford, Bristol, England, in 1968. The colony at Langford in turn originated from Zimbabwe. The flies were bred at a temperature of 25 ± 0.5°C and 70-80% relative humidity. Both sexes were used but flies were kept in batches according to sex and age. The cages used were made from polyvinyl material and nylon mesh.

3.2.3 Rats

Swiss Wistar rats (*Rattus norvegicus*) were obtained from the animal house of the Zoology Department at Chiromo campus. These were supplemented by rats of the same species obtained from ICIPE. Rats used weighed between 160-240 g. They were all kept in plastic cages covered with stainless steel wire tops and all the cages were kept in an isolated fly-screened room in the University departmental animal house. They were fed on commercial rodent pellets and water was supplied *ad libitum*.

3.2.4 Mice

Balb C mice, bred in the departmental animal house of the University of Nairobi, were used in serial passages for *T. congolense*. Swiss mice were obtained from ICIPE and the University animal houses. These were used in *T. congolense*/ *T.b. brucei* mixed infection experiments.

3.2.5 Rabbits

These were obtained from the animal house of the University of Nairobi. The rabbits were of the Californian white breed obtained originally from Muguga in 1974. They were fed on rabbit pellets, cabbage, carrots and water.

3.3 Experiment 1: Effect of age, sex and starvation of *G.m. morsitans* on infection with *T. congolense*

3.3.1 Inoculation of rats with *T. congolense*

Experiment 1 was carried out from August 1984 to January 1985. Initially, two Wistar rats were inoculated intraperitoneally with 0.2 ml of infected blood from a mouse. From the third day following inoculation, the rats were bled from the tail daily, and the blood was examined as wet blood films and thin smears under light microscopy at x 400 magnification. At first peak of parasitaemia, 15 days later, one of the rats was anaesthetized with chloroform and bled from the heart. The infected blood so collected was used for making stabilates. These stabilates were preserved in dry ice (solid carbon dioxide) in the ICIPE trypanosome bank for later use. The remaining blood was used to infect groups of rats from which flies were infected. On day 19, post inoculation, the remaining rat was used to infect batches of flies for the preliminary experiment. Rats were infected in groups of six. A group of rats was inoculated

with the same amount of inoculum varying from 0.1-0.2 ml depending on the level of parasitaemia in the infected blood (Fig. 3). The number of parasites in the inoculum was estimated with a haemacytometer.

3.3.2 Age categories of flies used

Five age groups of flies were used as follows:

i) 7 day old flies starved for 48 hours before infection.

ii) 7 day old flies fed daily for 6 consecutive days.

iii) 2 day old flies starved for 48 hours.

iv) 2 day old flies fed once prior to infection.

v) 16 hour old flies not offered any blood meal prior to infection

The first four replicates of the experiment consisted of four age groups only: the 7 day old fed, 2 day old starved, 2 day old fed and the 16 hour old flies. For the sixth to the tenth replicates, a fifth group of flies: 7 day old 48 hours starved was added to the four groups to investigate further, the effect of starvation on infection rates in older flies.

Since all the groups were to be infected at the same time and on the same infected rat, the arrangement was that the 7 day old flies were obtained first. These were obtained as

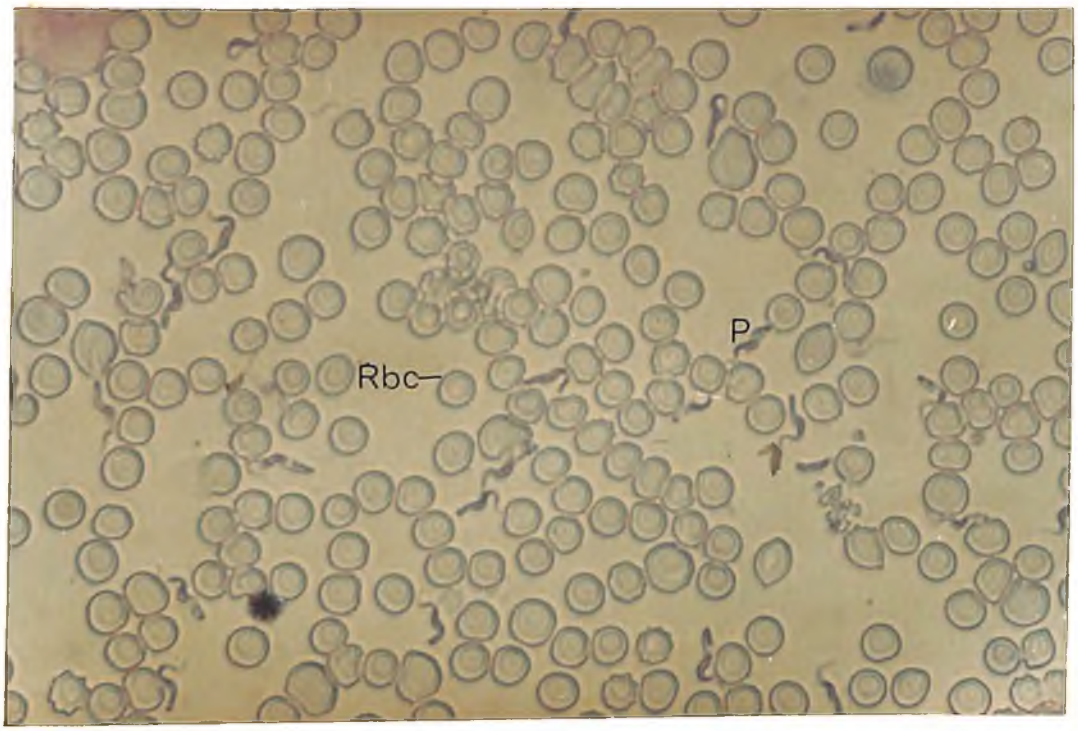

Fig. 3: Trypanosoma congolense infection in rat.
P = Parasite; Rbc = Red blood cell
(magnification x 250).

one day old flies from the insectary at 10.00 hours in the morning. One group was fed daily on a rabbit for 6 days prior to infection while another group was fed only on the first 4 days, being starved on the fifth and sixth day. On the sixth day after obtaining the first groups of flies, the 2 day old flies were obtained as one day old flies from the insectary at 10.00 hrs. One group was offered a blood meal on a rabbit while the second was not. The 16 hour old flies were picked up as young flies emerging from the pupae in the insectary 16 hours before exposure to infection. In all groups, male and female flies were kept separately in batches of 25 flies per cage.

3.3.3 Infecting tsetse flies from rats infected with *T. congolense*

Flies were fed only on parasitaemic rats. These were selected by first examining their tail blood for the presence of trypanosome infection. If parasitaemia was high, about 100 trypanosomes per field of wet blood film at x 400 magnification, the rat was anaesthetized with sodium pentabarbitone. Flies were fed on its belly, one batch after the other, each batch being given about 10 minutes (Fig. 4). All the batches constituting a replicate (10 cages of flies altogether) were fed on the same infected rat. Only fed flies which showed red or black distended abdomens were used, unfed ones were discarded (Fig. 5). The infected blood meal was offered only once to the flies.

Fig. 4: Infecting G.m. morsitans from a rat infected with T. congolense.

Note anaesthetised rat resting on a cage containing flies.

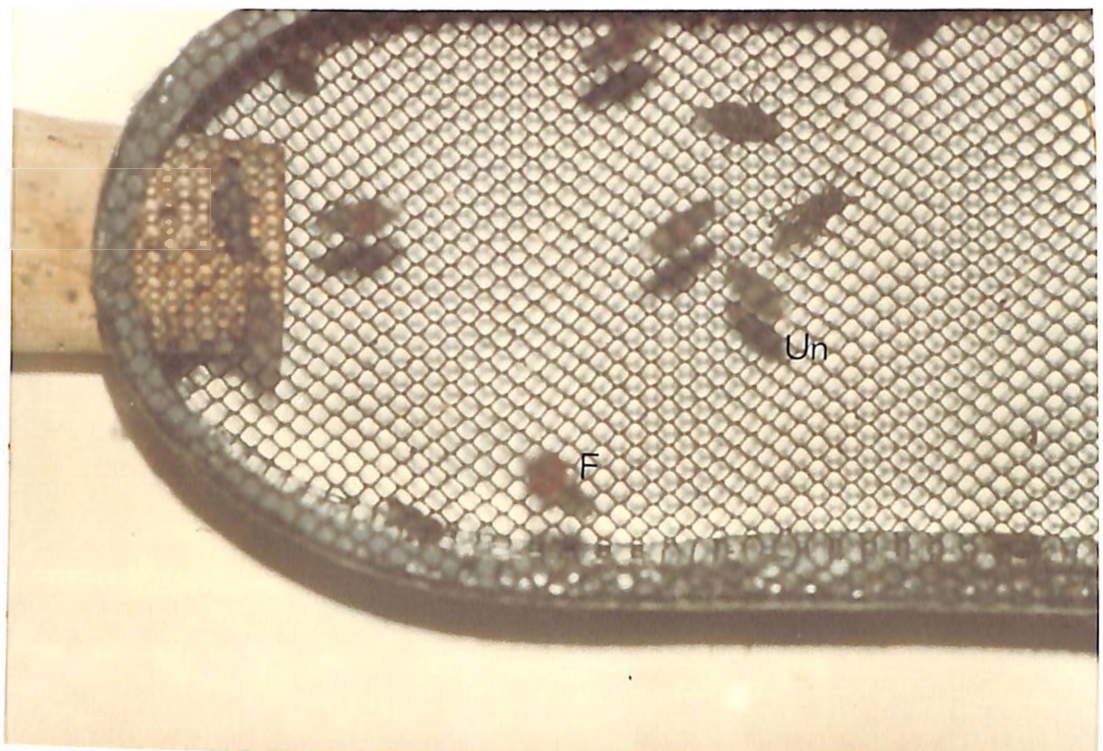

Fig. 5: Fed and unfed G.m. morsitans

F = Fed ; Un = Unfed.

3.3.4 Maintenance of tsetse flies on rabbits

Rearing of tsetse flies in the laboratory is reported by Laird (1977) and Leegwater (1983) to have started as early as 1917. From this early date to the present, methods of maintaining tsetse flies in the laboratory have been improved. The method used was similar to that reported by Nash et al. (1966), Nash and Jordan (in Mulligan and Potts, 1970) and Jordan et al. (1967). This method involves the use of rabbits to maintain flies in the laboratory. Recently, Moloo (1981) confirmed rabbits to be ideal for maintaining infected flies.

After feeding on infected rats, flies were fed on ears of rabbits daily except Sundays (Fig. 6). Each batch of flies was given 10-15 minutes to feed. Flies were then kept in the fly room where the temperature was 25 $\pm$ 3^{o}C and relative humidity of 60-80%. A daily record of each cage was kept. Flies which died were removed and dissected if they were still fresh to check for trypanosome infection.

Rabbits upon which flies fed were examined weekly for trypanosome infection by bleeding them from the ears. Both wet blood films and the microhaematocrit centrifugation test were used to examine for trypanosome infection. If found positive the rabbits were replaced with uninfected ones. Infected ones were killed with chloroform. As a general precaution rabbits were changed after 2-3 weeks from the first day of infected flies feeding on them.

Fig. 6: Maintenance of flies on a rabbit.

Fig. 7: A rabit infected with T.b. brucei.
Note ocular lesions.

3.3.5 Dissection of flies

At the end of 30 days, flies were starved for 1-2 days before dissection so that the gut contents did not conceal trypanosomes. Flies were killed while still in cages with chloroform or ether in a plastic bag. After removal, the wings and legs were removed with a pair of scissors and the midgut labrum and hypopharynx dissected in 0.9% saline under a binocular dissecting microscope at x 20 - x 40 magnification. These organs were then examined for trypanosome infection under the light microscope at x 400 magnification.

The dissection procedure involved the removal of the head from the rest of the body with fine forceps, to avoid contamination of the proboscis with trypanosomes from the gut. Two drops of saline were placed at the ends of a slide. The hypopharynx and labrum were dissected on one drop and the midgut on the other. The forceps were wiped with a clean tissue paper each time the midgut was dissected before moving on to the proboscis. Trypanosomes were recorded as being absent or present after examination of each organ (Figures 8, 9 and 10).

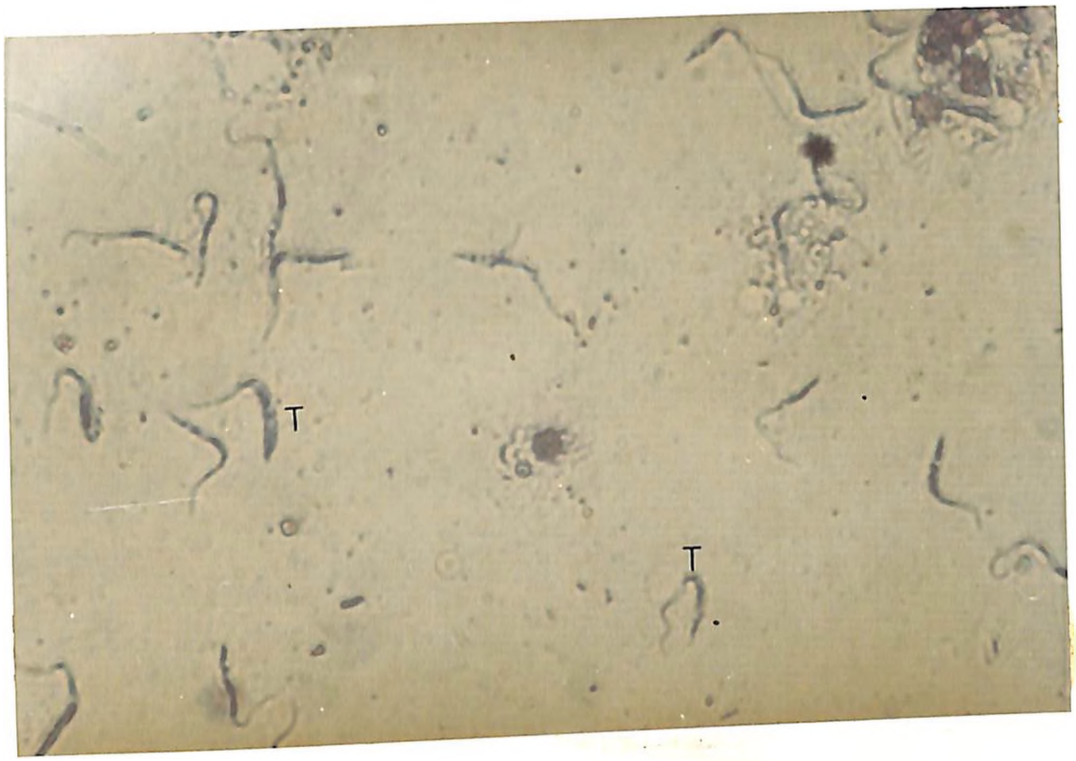

Fig. 8: Gut-forms (T. congolense) in fly.
T = Trypanosome (magnification x 400)

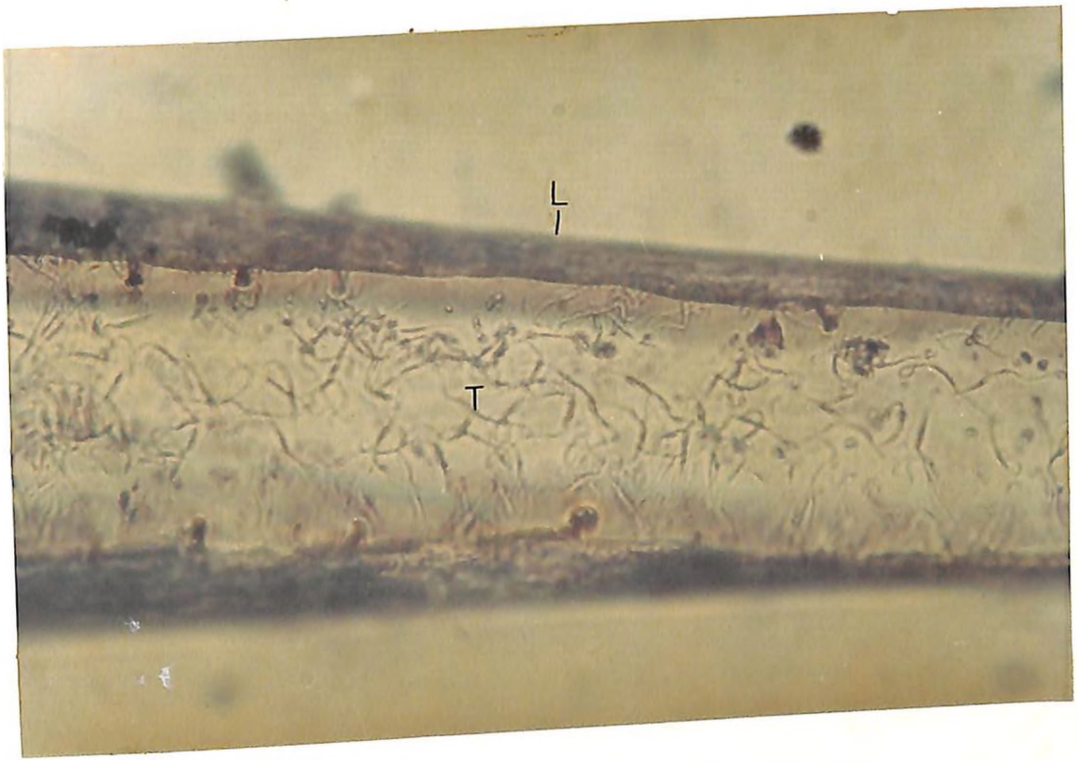

Fig. 9: Labrum infections (T. congolense) in fly
T = Trypanosome; L = Labrum
(magnification x 400)

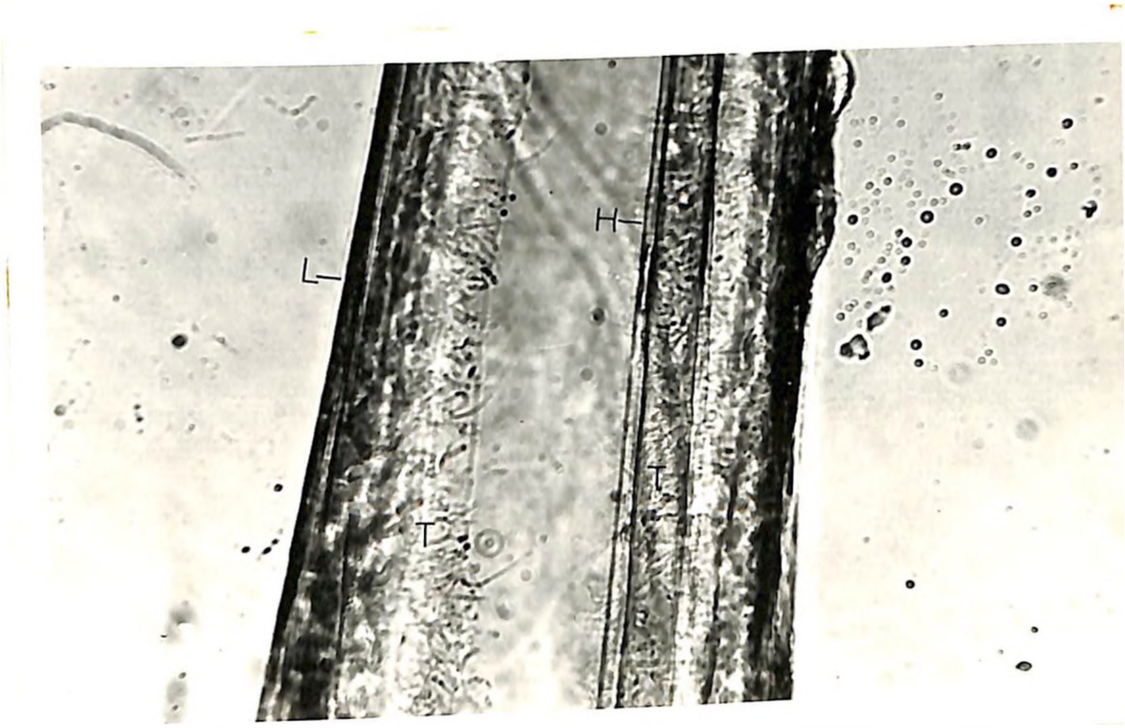

Fig. 10: Labrum and hypopharynx infections (T. congolense) in fly.

L = Labrum; H = Hypopharynx; T = Trypanosome

(magnification x 250)

3.4 Experiment 2: Transmission of *T. congolense*/*T.b. brucei* mixed infections

3.4.1 Inoculation of rats with trypanosomes

Experiment 2 was carried out between February 1985 and May 1985. Two groups consisting of six rats each were inoculated intraperitoneally with 0.1-0.2 ml of infected blood, one group with blood containing *T. congolense* and the other *T.b. brucei*. As parasitaemia was observed to take a longer period to reach the peak in rats infected with *T. congolense* than with those infected with *T.b. brucei*, inoculation of trypanosomes was such that rats with *T. congolense* were inoculated and left for about 10 days while those with *T.b. brucei* were left for about 5 days before selecting the rats with the highest parasitaemia from each group.

3.4.2 Infecting tsetse flies

In this experiment, 48 hour old starved male and female flies were used. It was observed in the first experiment that this group of flies was as susceptible to infection with *T. congolense* as 16 hour old flies. Infection in these two groups was higher than in other groups of flies. On this basis 2 day old, 48 hour old starved flies were chosen as these were easier to arrange than the very young flies which had to be picked as they emerged from the pupae. Males and females, in batches of 15 per cage, were exposed to infection starting at 10.00 hrs each day when they reached the age of 2 days. Infection was carried out as follows:-

i) 15 males and 15 females fed on a rat infected with *T. congolense* at peak parasitaemia.

ii) 15 males and 15 females fed on a rat infected with *T.b. brucei* at peak parasitaemia.

When these two groups of flies had fed, the rats upon which they fed were bled from the heart and the blood was defibrinated separately.

iii) 15 males and 15 females fed on defibrinated blood with *T. congolense* infection from the first rat through the silicone membrane.

iv) 15 males and 15 females fed on defibrinated blood with *T.b. brucei* infection from the second rat through the silicone membrane (see section 3.4.2.2).

v) Another group of 15 males and 15 females fed on mixed *T. congolense*/*T.b. brucei* defibrinated blood from the two rats through the silicone membrane.

Five such replicates were carried out. The figure of 15 flies per cage was chosen because it was observed that too many flies in a cage (25 or more) resulted in crowding and flies did not feed well.

3.4.2.1 Defibrination of infected blood

The selected infected rat was dosed with Sagatal. It was laid on a clean tissue on the bench with the belly facing upwards. The belly was swabbed with 70% ethanol before removing the skin with a pair of scissors and forceps. The diaphragm was opened up and the heart bled with a syringe. In the case of heart puncture a Pasteur pipette was used to collect blood from the chest cavity. The blood collected was immediately transfered to a universal bottle containing 10-15 glass beads. It was gently stirred, this process being performed continuously until enough blood was collected. Up to 8 ml of blood per rat was defibrinated in this way.

3.4.2.2 Infecting flies using the silicone membrane

The membrane used and the procedure followed is that described by Bauer and Wetzel (1976). The chronology of events regarding the development of membrane feeding is discussed in depth by Laird (1977) and Bauer and Wetzel (1976).

Prior to feeding flies, the silicone membrane, the accompanying grooved glass plate and the aluminium cases, were thoroughly washed in water and sterilized with 70% industrial methylated spirit or 70% ethanol. These were left under ultra-violet light overnight to dry and to maintain sterility. To feed flies, the grooved glass plate was placed on a hot plate with a temperature of about 32^{o}C. The hot plate and grooved glass plate were flamed twice for a few seconds with a gas

burner for sterilization. Defibrinated blood from the T. congolense and T.b. brucei infected rats and the mixture of the two were poured on the glass plate in three separate pools in the ratio by volume; one part of T. congolense infected blood, one part of T.b. brucei and two parts of T. congolense/ T.b. brucei mixed blood. The silicone membrane was spread over the pools of blood on the glass plate ensuring that the three pools of blood never touched. Cages of flies were placed over the membrane and flies were left to feed for about 20 minutes. The apparatus is shown in Figure 11. At the end of feeding, both fed and unfed flies were counted and those that did not feed were discarded. Motility of trypanosomes in the remaining pools of blood was checked under the light microscope as wet blood films immediately feeding was over. The flies were kept in the fly room and maintained on rabbits.

3.4.3 Maintenance of flies on rabbits

The five groups of flies were maintained on rabbits. They were fed daily except Sundays as previously reported. Flies which had their infective feed on blood with T. congolense were fed on one rabbit, those with T.b. brucei infection on a second rabbit and flies fed on T. congolense/ T.b. brucei mixed infections on another rabbit. Rabbits were replaced when they showed parasitaemia on examination of blood from the ears. Infected ones were killed with chloroform (Figure 7).

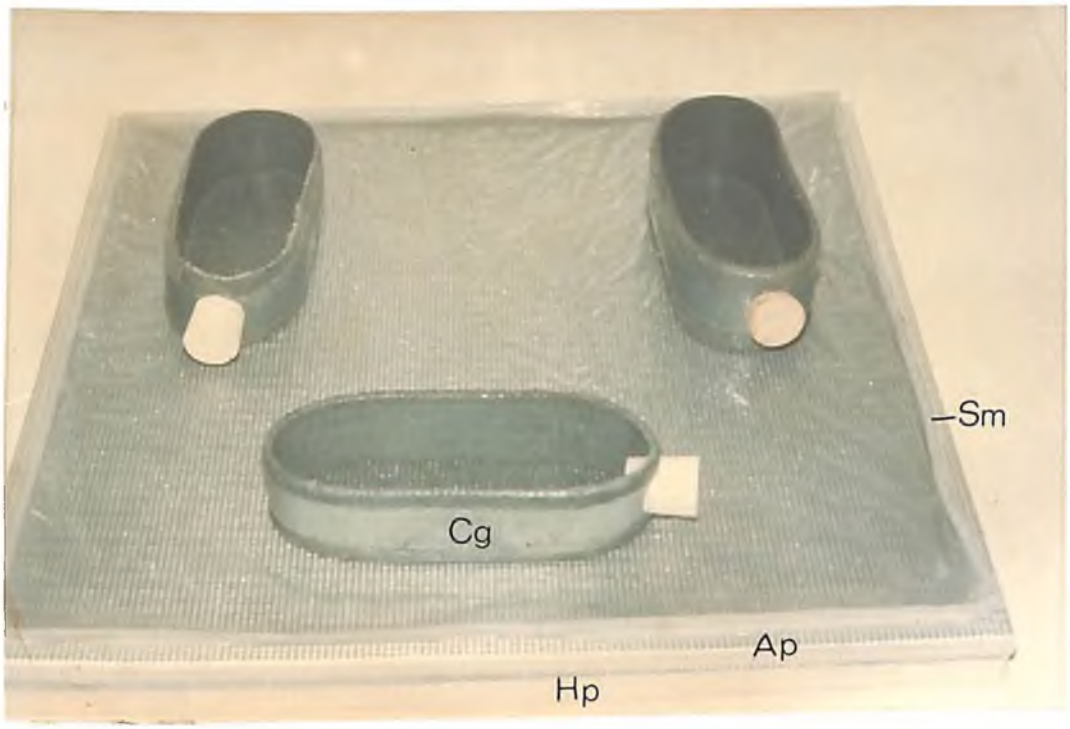

Fig. 11: Apparatus used for membrane feeding of flies.

Cg = Cage containing flies; Sm = Silicone membrane;
Ap = Aluminium plate; Hp = Hot plate

3.4.4 Dissection of flies and inoculation of fly organs into mice

After maintaining flies on rabbits for 30 days they were not fed for 1-2 days before dissection. For T. congolense infected flies, two drops of saline were placed at the ends of a slide. The hypopharynx and labrum were dissected together on one drop while the midgut was squashed on the other. These organs were examined under the light microscope at x 400 magnification. Flies with T.b. brucei only and T. congolense/ T.b. brucei mixed infections were, in addition to the midgut and proboscis, examined for trypanosome infection in the salivary glands. Salivary glands were teased out gently together with the head from the abdomen through the thorax. If a section remained, the abdomen was opened up and it was removed near the region of the thorax. As in the case of the midgut and hypopharynx and labrum, the salivary glands were examined at x 400 magnification (Figure 12).

If a fly showed infection in the proboscis, in the case of T. congolense/T.b. brucei mixed infections, the midgut, labrum and hypopharynx and salivary glands were macerated in saline separately and inoculated into mice. Gut only infections were not inoculated into mice, only when the proboscis or salivary glands were involved was the gut also inoculated into mice to check if midgut and proventricular forms of the trypanosome would be infective. Mice inoculated with fly organs were examined for the presence of trypanosome infection for 30 days by bleeding them from the tail.

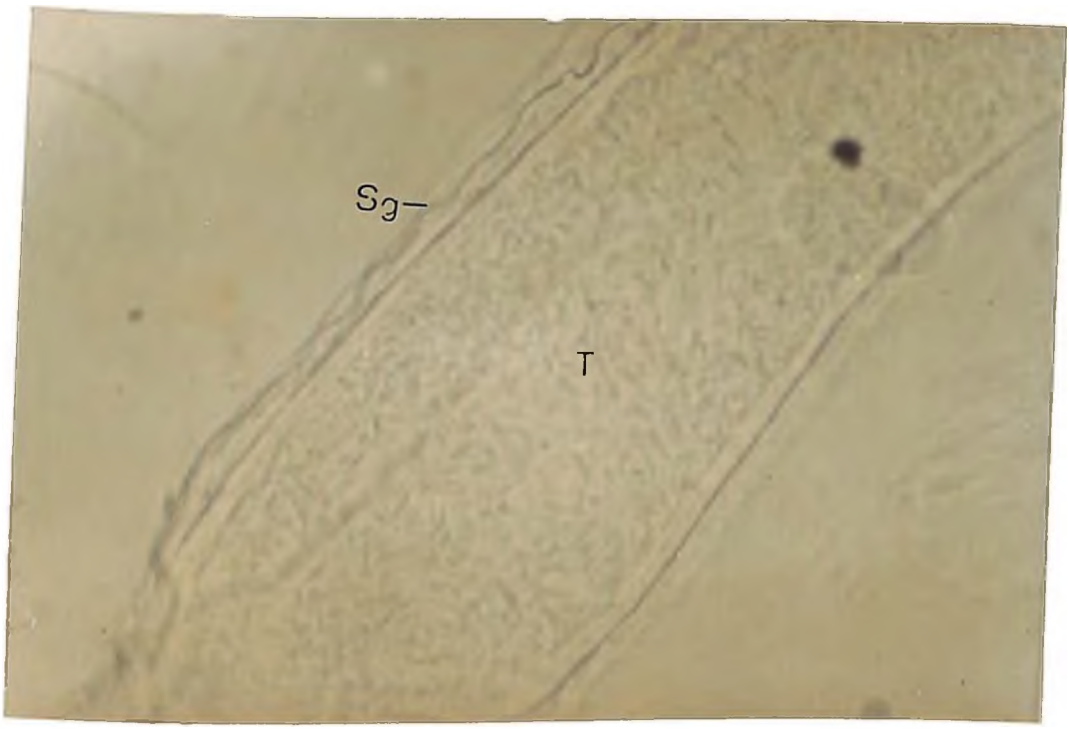

Fig. 12: A section of salivary gland of fly infected with T.b. brucei.
Sg = Salivary gland; T = Trypanosomes.
(Magnification x 400)

3.5 Maintenance of trypanosomes in rats and mice

The strains of trypanosomes used; Maruma and EATRO 1969, were separately maintained in rats by syringe passage. Of the rats used to infect flies some were selected at peak parasitaemia for passaging. Passages were carried out as described by Lumsden et al. (1973). The rat from which passage was to be made was heavily anaesthetized with chloroform. Its belly was swabbed with 70% ethanol, the heart felt with a finger and bled directly from the heart. About 3 ml of blood was bled out for inoculation into 6 additional rats as well as for use in the calculation of the number of trypanosomes in the inoculum.

3.5.1 Calculation of number of trypanosomes in the inoculum

The method used was similar to that described by Lumsden et al. (1973) and Murray et al. (1983) involving the use of the improved Neubaer haemacytometer. Infected blood was obtained by cardiac puncture as described above. The number of trypanosomes per ml was calculated using the following formula:

$$N \times V.F. \times D.F. \times 1000 = \text{Number of trypanosomes/ml.}$$

Where, N = the number of trypanosomes counted per mm^2.

V.F.= the volume factor due to the depth of the cell of the haemacytometer (0.1 mm).

D.F.= the dilution factor of the infected blood.

1000 = the constant to work out trypanosomes per mm^3.

Mathematical details of this formula are given by Macinnis and Voge (1970), Lumsden et al. (1973) and Murray

et al. (1983). The infected blood was diluted with Phosphate Buffered Saline according to the level of parasitaemia. A small amount of this suspension was poured onto one of the counting chambers of the Neubaer haemacytometer using a Pasteur pipette. Trypanosomes were counted under the microscope at x 400 magnification in each of the four corner squares, each subdivided into 16 squares. The average number of the trypanosomes in the four squares was taken to calculate the number of trypanosomes per square (Figure 13). No effort was made to calculate the number of trypanosomes using the haemacytometer in the infected blood immediately before flies were given an infected feed. Instead parasitaemias in rats were examined in wet blood smears under the light microscope at x 400 magnification.

3.5.2 Wet blood films

Wet blood films were prepared by cutting the tip of the tail of a rat with a pair of scissors or puncturing a vein on the ear of a rabbit with a sterilized hypodermic needle. A small drop of blood from the puncture was collected onto a slide and a coverslip placed over it, pressing the latter gently with a forceps to make the smear uniform before examination under the microscope. Up to 50 microscope fields were examined before declaring a slide negative.

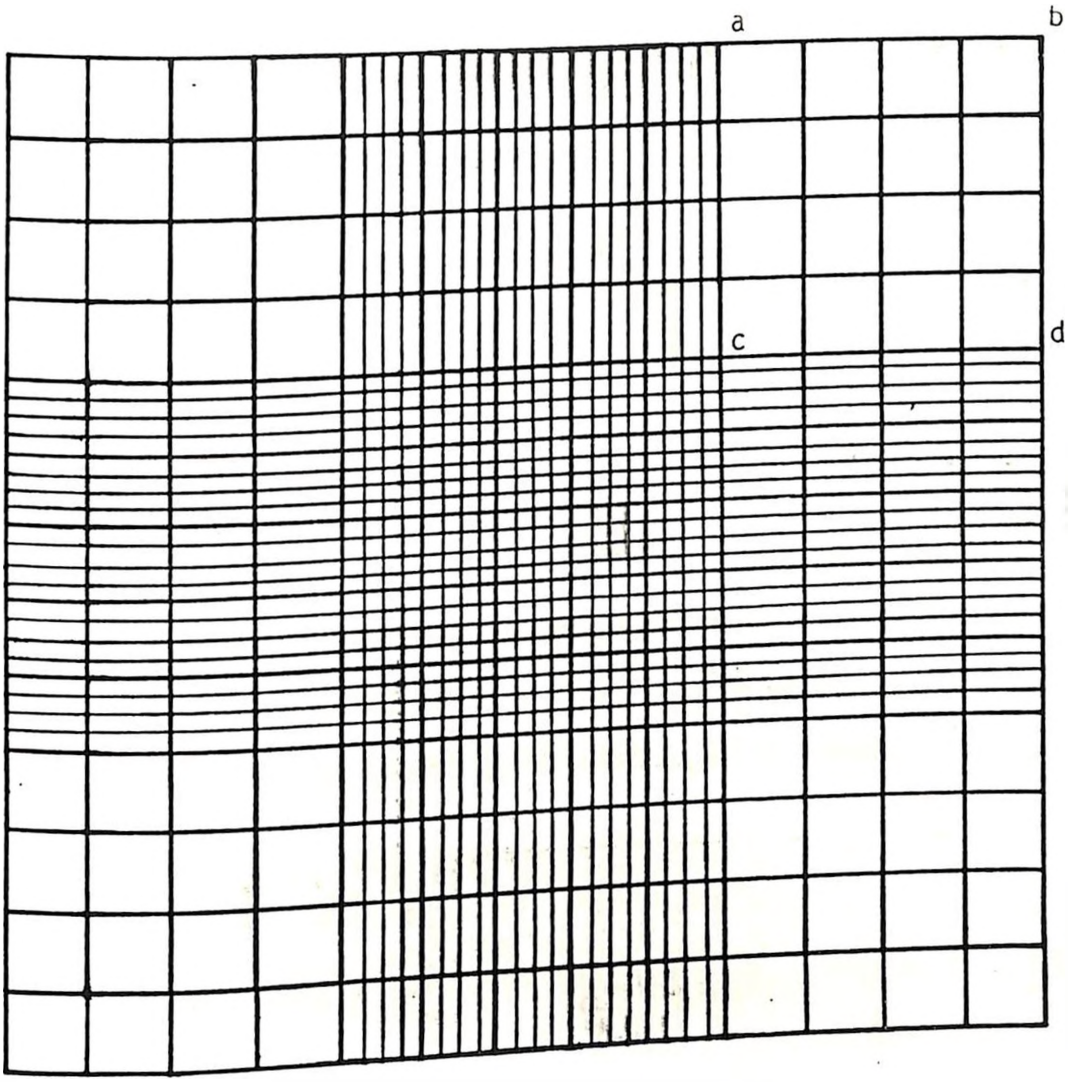

Fig. 13: A diagram of the improved Neubaer haemacytometer used to estimate the number of trypanosomes in a suspension. One of these squares is marked a b c d.

3.5.3 Thin blood smears

Thin smears were taken to confirm infection in rats, mice and rabbits. A small drop of blood was collected as described above onto a clean slide. The edge of a second slide was touched to the first one which was placed horizontally. The second slide was drawn to the drop of blood which formed a smear along its edge. At about 30^{o} from the first slide, the second slide was gently drawn forward leaving a trail of blood. The smear was left to dry, fixed with absolute methanol for 30 seconds and stained in Giemsa for 20-30 minutes. Miscroscopic examination of slides was carried out at x 400 and x 1000 magnifications.

3.5.4 Microhaematocrit centrifugation

To ascertain infection in rabbits as early as possible so that they could be replaced if infected, the microhaematocrit centrifugation technique was used, as described by Woo (1969, 1971a, 1971b). Heparinized capillary tubes were filled with blood from an ear puncture to the two-thirds level. They were sealed with cristaceal and centrifuged at 12,000 g for 5 minutes. The capillaries were mounted on a slide and examined at x 100 magnification through distilled water. Trypanosomes, when present, were seen near the buffy coat layer. It is possible to observe densities as low as 39.8 trypanosomes/ml using this method (Lumsden et al. 1973).

C H A P T E R IV

4.0 RESULTS

4.1 Experiment 1: The effect of age, sex and starvation of G.m. morsitans on infection with T. congolense

4.1.1 Dose of inoculum used and infection in flies

The dose of inoculum and the ensuing infection in flies are shown in Table 3. The infection in flies could not be correlated with the dose of inoculum because flies were exposed to infection at different periods after inoculation of trypanosomes into rats. However, mature infections involving the hypopharynx derived from these inoculations, showed a fairly small range in variation of between 10-21.5% as assessed by fly dissection.

4.1.2 Mortality of flies maintained on rabbits

The number of flies that died during the 30 days of maintenance on rabbits for each age group is summarized in Table 4. Of these 5 age groups of flies used, relatively higher mortality was recorded in the 7 day old fed males (17.4%) and 2 day old fed females (15.1%). Mortality of flies in other groups was fairly low falling generally below 5%.

Table 3: Dose of inoculum in rats and *T. congolense* infection in flies.

Replicate	Rat No.	Trypanosomes/mm^3	% Flies infected (All groups)		
			Mg	Mg + L	Mg + L + H
1	P 2	Not determined	29.6	19.1	13.8
2	A 4	1.00×10^6	47.1	26.4	20.6
3	A 5	1.00×10^6	33.8	17.9	15.2
4	A 6	1.00×10^6	36.1	26.0	20.9
5	B 4	3.45×10^7	41.4	21.3	11.4
6	B 1	3.45×10^7	43.2	26.3	10.0
7	B 2	3.45×10^7	41.0	23.4	11.2
8	C 3	7.85×10^6	26.6	25.5	17.2
9	C 2	7.85×10^6	35.5	33.9	18.0
10	D 1	4.85×10^6	36.7	31.0	21.5

Mg = Midgut; Mg + L = Midgut and Labrum;

Mg + L + H = Midgut, Labrum and Hypopharynx.

Table 4: Mortality of flies fed on rats infected with *T. congolense* and maintained on rabbits.

	7 day old starved		7 day old fed		2 day old starved		2 day old fed		16 hour old	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Number initially fed	125	118	190	203	214	212	173	159	221	240
Number died by day 30	4	5	33	8	4	10	12	24	10	6
Number dissected	121	113	157	195	210	202	161	135	211	234
% mortality	3.2	4.2	17.4	3.9	1.9	4.7	6.9	15.1	4.5	2.5

The relatively higher mortality in 7 day old fed males and 2 day old fed females was attributed to possible contamination of some cages during cleaning. In one cage containing 7 day old fed males, 10 flies (half the number in the cage) had died by the thirteenth day of maintenance. When surviving flies were transferred to another cage mortality was greatly reduced. In three other cages containing 2 day old fed females, most of them died on the first night before exposure to infection. The few that survived were transfered to clean cages and mortality was again reduced.

4.1.3 Infection rates in flies

Results of trypanosome infection rates of flies are shown in Tables 5, 6, 7, 8 and 9. Infection was recorded at three levels: in the gut only; gut and labrum, and gut, labrum and hypopharynx. A few flies (Table 9) showed gut and hypopharynx infection only, without labrum involvement indicating that either trypanosomes in the labrum had all migrated to the hypopharynx in these flies or they were extremely few and therefore missed when examined with the light microscope at x 400 magnification.

In column 2 (Tables 6, 7, 8 and 9) the first four replicates for the 7 day old 'starved' flies were not done. This group of flies was included from replicate 5 to 10 in order to investigate further the effect of starvation in these older flies. Out of 1,739 flies, consisting of 860 males and 879 females that were dissected, 191 (11.0%)

were found to have trypanosomes established only in the gut. Of these 57 (29.8%) were males and 134 (70.2%) were females. Gut and labrum infections were recorded in 180 (10.4%) of the total flies dissected. Of these 77 (42.8%) were males and 103 (57.2%) were females. Mature infections in which the gut, labrum and hypopharynx were all involved were found in 260 (15.0%) flies. The majority of these 160 (61.5%) were males while 100 (38.5%) were females. Infection involving the gut and hypopharynx only was recorded in 6 males (0.7% of the total males dissected) and in 11 females (1.3% of the total females dissected). Tables 8 and 9 show the details. When all groups were considered overall infection indicated that females had significantly higher infections than males ($X^2 = 4.12$, $P < 0.05$). However, mature infections were more common in males as compared with females ($X = 14.46$, $P < 0.001$).

Table 5: Overall infection of G.m. morsitans infected with T. congolense. Infection is separated into: gut only; gut and labrum; and gut, labrum and hypopharynx.

Location in fly	Male	Female	Total
Number dissected	860	879	1,739
Gut only	57	134	191
% infected	6.6	15.2	11.0
Gut and labrum	77	103	180
% infected	9.0	11.8	10.4
Gut, labrum and hypopharynx	160	100	260
% infected	18.6	11.4	15.0
Gut and hypopharynx	6	11	17
% infected	0.7	1.3	1.0
Total infected	300	348	648
% infected	34.9	39.6	37.3

Table 6: Gut only infections in the five groups of G.m. morsitans infected with T. congolense.

Replicates (10)	7 day old starved		7 day old fed		2 day old starved		2 day old fed		16 hour old	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Number dissected	121	113	157	195	210	202	161	135	211	234
Number infected	3	23	10	26	19	40	7	12	18	23
% infected	2.5	20.4	6.4	13.3	9.1	19.8	4.4	8.9	8.5	14.1

Table 7: Gut and labrum only infections in the five groups of G.m. morsitans infected with T. congolense

Replicates (10)	7 day old starved		7 day old fed		2 day old starved		2 day old fed		16 hour old	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Number dissected	121	113	157	195	210	202	161	135	211	234
Number infected	11	2	11	15	20	32	8	16	27	38
% infected	9.1	1.8	7.0	7.7	9.5	15.8	5.0	11.9	12.8	16.2

Table 8: Gut, labrum and hypopharynx infection in the five groups of G.m. morsitans infected with T. congolense.

Replicates (10)	7 day old starved		7 day old fed		2 day old starved		2 day old fed		16 hour old	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Number dissected	121	113	157	195	210	202	161	135	211	234
Number infected	17	9	20	12	50	27	20	13	53	39
% infected	14.1	8.0	12.7	6.2	23.8	13.4	12.4	9.6	25.1	16.7

Table 9: Gut and hypopharynx only infections in the five groups of G.m. morsitans infected with T. congolense.

Replicates (10)	7 day old starved		7 day old fed		2 day old starved		2 day old fed		16 hour old	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Number dissected	121	113	157	195	210	202	161	135	211	234
Number infected	0	0	0	2	2	1	1	1	3	7
% infected	0	0	0	1.0	1.0	0.5	0.6	0.7	1.4	3.0

4.1.3.1 The effect of age of fly on infection rates

Analysis of Variance and the Least Significant Difference (LSD) statistical tests were used to analyse the data presented in Table 6, 7, 8 and 9. The data was considered as a single factor with 5 levels corresponding to the 5 groups of flies. Since in some replicates, some flies had died, the data was considered as unequal number of replicates versus treatment. The percentage values of infected flies were transformed by the angular transformation (Fisher and Yates, 1963) and the means analysed (Tables 10, 11, 12, 13, 14 and 15). Analysis of variance of treatment means indicated significant differences ($P < 0.05$) in females with gut and labrum infections (F ratio = 4.68; degrees of freedom = 4,39) and in both males (F ratio = 2.98; degrees of freedom = 4,40) and females (F ratio = 3.32; degree of freedom = 4,39) with mature infections (Table 16). Infections in the gut only in both males (F ratio = 0.86) and females (F ratio = 0.89) and gut and labrum in males (F ratio = 1.34) were not significantly different ($P > 0.05$) among the groups of flies. On the basis of the above results only infections that showed significant differences among the groups of flies were further analysed with the LSD method.

Comparison of means of gut and labrum infections in females indicated that 16 hour and 2 day old starved flies had the highest infection, with transformed mean infections of 21.77 and 21.14 respectively, followed by 2 day old fed

flies (19.30) and 7 day old fed flies (13.36), then 7 day old starved flies (3.24). The 16 hour old flies were not significantly different from 2 day old starved flies and the 2 day fed flies were also not significantly different from the 7 day old flies that had been fed before infection (Table 13).

Mature infections in males showed that 16 hour old flies with mean infection of 30.39 and 2 day old starved flies with mean infection of 29.12 had the highest infection among the groups of flies used. These two groups were not significantly different in infection rates from each other and were followed by 7 day old starved flies (21.14), 7 day old fed (19.20) and 2 day old fed flies (18.13). The last three groups were not significantly different in infection rates. Mature infections in females showed that 16 hour old flies had the highest infection rates (26.02), followed by 2 day old starved flies (20.36). The difference between these two groups was significant at $P < 0.05$. The 2 day old fed flies (15.72), 7 day old fed (13.63) and 7 day old starved (13.08) showed no significant differences in infection rates among them and had the lowest infection rates.

The results indicate that although age of the fly has an influence on infection it is not the single most determining factor. The 16 hour old flies were statistically as susceptible to infection as the 2 day old starved flies.

Table 10: Angular transformed data of gut only (*T. congolense*) infections in male flies.

Replicates	Fly age group				
	1	2	3	4	5
1		16.74	34.02	0.00	27.13
2		17.56	20.27	24.88	11.09
3		16.74	12.92	20.70	18.91
4		0.00	0.00	0.00	22.79
5	0.00	0.00	21.13	17.95	26.57
6	0.00	28.11	25.25	-	12.38
7	17.95	14.54	20.70	13.69	17.15
8	0.00	0.00	0.00	0.00	0.00
9	0.00	0.00	0.00	0.00	0.00
10	12.38	0.00	0.00	0.00	0.00
$\bar{X}$	5.06	9.37	13.43	8.58	13.60

Table 11: Angular transformed data of gut only (*T. congolense*) infections in female flies.

Replicate	Fly age group				
	1	2	3	4	5
1		0.00	17.56	0.00	11.83
2		23.58	48.45	30.00	0.00
3		12.11	31.11	15.45	34.33
4		20.70	19.46	22.22	26.57
5	40.45	28.45	28.11	-	30.00
6	28.11	35.24	30.00	20.70	23.58
7	42.42	34.02	21.64	-	27.13
8	14.06	0.00	0.00	13.69	11.83
9	0.00	0.00	12.11	0.00	16.43
10	0.00	14.54	32.83	0.00	12.92
$\bar{X}$	20.84	16.86	24.13	12.76	19.46

Table 12: Angular transformed data of gut and labrum only (*T. congolense*) infections in male flies.

Replicate	Fly age group				
	1	2	3	4	5
1		0.00	0.00	0.00	27.13
2		12.38	11.54	14.06	11.09
3		16.74	12.92	20.70	18.91
4		0.00	17.15	0.00	22.79
5	15.45	28.11	27.76	12.66	12.92
6	23.58	13.69	21.64	-	31.50
7	22.22	14.54	20.70	19.46	24.65
8	0.00	16.11	12.92	19.46	12.66
9	12.92	19.46	17.95	0.00	20.27
10	17.46	25.25	20.70	17.56	18.43
$\bar{X}$	15.27	14.63	16.33	11.54	20.04

Table 13: Angular transformed data of gut and labrum only (*T. congolense*) infections in female flies.

Replicate	Fly age group 1	2	3	4	5
1		0.00	21.64	13.69	11.83
2		23.58	11.54	20.70	29.00
3		12.11	0.00	15.45	0.00
4		0.00	19.46	12.66	20.27
5	0.00	0.00	24.12	-	27.56
6	19.46	22.22	30.00	14.54	31.95
7	0.00	20.70	21.64	-	30.00
8	0.00	12.92	21.64	28.11	16.74
9	0.00	27.56	41.27	25.70	31.95
10	0.00	14.54	20.09	27.56	18.43
$\bar{X}$	3.24	13.36	21.14	19.80	21.77

Table 14: Angular transformed data of gut, labrum and hypopharynx (*T. congolense*) infections in male flies.

Replicate	Fly age group				
	1	2	3	4	5
1		0.00	14.54	0.00	11.83
2		21.64	26.57	36.45	41.78
3		27.13	22.79	0.00	30.85
4		21.13	43.74	17.15	33.21
5	27.56	19.46	24.65	17.95	18.43
6	23.58	13.69	25.25	-	17.56
7	17.95	14.54	20.70	19.46	30.72
8	13.31	28.73	36.27	24.12	35.24
9	12.92	28.11	35.24	22.79	39.23
10	31.50	17.56	41.44	25.25	45.00
$\bar{X}$	21.14	19.20	29.12	18.13	30.39

Table 15: Angular transformed data of gut, labrum and hypopharynx (*T. congolense*) infections in female flies.

Replicate	Fly age group				
	1	2	3	4	5
1		15.45	31.50	28.11	35.24
2		23.58	11.54	20.70	24.88
3		17.15	26.57	0.00	25.25
4		16.74	31.82	12.66	31.95
5	0.00	0.00	24.12	-	25.03
6	19.46	12.66	0.00	20.70	16.43
7	12.38	14.54	17.56	-	20.70
8	0.00	0.00	25.25	28.11	24.12
9	20.70	15.45	21.13	0.00	26.57
10	25.91	20.70	14.06	15.45	30.00
$\bar{X}$	13.08	13.63	20.36	15.72	26.02

In Tables 10, 11, 12, 13, 14, 15 and 16;

Fly age group 1 = 7 day old (48 hours starved) flies

" 2 = 7 day old fed flies

" 3 = 2 day old (48 hours starved) flies

" 4 = 2 day old fed flies

" 5 = 16 hour old flies not fed prior to infection.

In Table 16;

SE = Standard error.

a,b,c and d = Significance levels. Means with the same letters are not significantly different from each other.

DF = Degrees of freedom.

CV = Coefficient of variation.

Table 16: Comparison of infection rates of flies using the Least Significant Difference test.

a) *T. congolense* infections in the gut and labrum of female *G.m. morsitans*.

Fly age group	Number of replicates	Transformed mean	SE	Significance
5	10	21.77	3.04	a
3	10	21.14	3.04	ab
4	8	16.70	3.40	bc
2	10	13.36	3.04	c
1	6	3.24	3.92	d

CV = 57%; F ratio = 4.68; DF = 4,39

b) *T. congolense* infections in the gut, labrum and hypopharynx of male *G.m. morsitans*

5	10	30.39	3.18	a
3	10	29.12	3.18	a
1	6	21.14	4.10	b
2	10	19.80	3.18	b
4	9	18.13	3.34	b

CV = 42%; F ratio = 2.98; DF = 4,40

c) *T. congolense* infections in the gut, labrum and hypopharynx of female *G.m. morsitans*

5	10	26.02	2.84	a
3	10	20.36	2.84	b
4	8	15.72	3.18	c
2	10	13.64	2.84	c
1	6	13.08	3.67	c

CV = 49%; F ratio = 3.32; DF = 4,39

Table 16: Comparison of infection rates of flies using the Least Significant Difference test.

a) *T. congolense* infections in the gut and labrum of female *G.m. morsitans*.

Fly age group	Number of replicates	Transformed mean	SE	Significance
5	10	21.77	3.04	a
3	10	21.14	3.04	ab
4	8	16.70	3.40	bc
2	10	13.36	3.04	c
1	6	3.24	3.92	d

CV = 57%; F ratio = 4.68; DF = 4,39

b) *T. congolense* infections in the gut, labrum and hypopharynx of male *G.m. morsitans*

5	10	30.39	3.18	a
3	10	29.12	3.18	a
1	6	21.14	4.10	b
2	10	19.80	3.18	b
4	9	18.13	3.34	b

CV = 42%; F ratio = 2.98; DF = 4,40

c) *T. congolense* infections in the gut, labrum and hypopharynx of female *G.m. morsitans*

5	10	26.02	2.84	a
3	10	20.36	2.84	b
4	8	15.72	3.18	c
2	10	13.64	2.84	c
1	6	13.08	3.67	c

CV = 49%; F ratio = 3.32; DF = 4,39

4.1.3.2 The effect of starvation of fly on infection rates

Results (Tables 6, 7, 8, 9 and 16) show that 16 hour old flies did not differ significantly from 2 day old starved flies and that these two groups had higher infection rates than 2 day old fed, 7 day old starved and 7 day old fed flies. The difference in infection rates between the first two groups and the rest of the groups is attributed to the effect of the first blood meal. Those groups (16 hour old and 2 day old starved) which were not offered a blood meal prior to infection had higher infection rates than those (7 day old groups and the 2 day old fed) which initially had a blood meal. Indeed, 2 day old starved flies with mature infection rates of 23.8% in males and 13.4% in females differed from 2 day old fed flies with 12.4% and 9.6%, respectively (Table 16). The 7 day old flies which were starved for 48 hours after feeding for 4 days did not show significant difference from flies of the same age which were fed consecutively for 6 days. Infection rates for the 7 day old starved flies were however slightly higher (14.1% for males and 8.0% for females) than the 7 day old fed flies (12.7% and 6.2%, respectively).

4.1.3.3 The effect of fly sex on infection rates

The number of males and females infected with trypanosomes changed with the course of infection from the gut to the hypopharynx. Females were more infected in the gut than males in all the age groups (Figure 14). Chi-square

analysis of data showed that gut only infections between males and females were significantly different in most of the groups (Table 17).

Although females were generally found to be more infected than males in the gut and labrum the majority of the groups indicated that the difference was not significant (Figure 15 and Table 17). The overall infection between males and females was not significantly different (X^2 = 3.58, $P > 0.05$).

The pattern of infection between the sexes changed in mature infections involving the hypopharynx (Figure 16). Overall infection showed a preponderance of males over females (X^2 = 14.46, $P < 0.001$). However, within individual groups the difference was not significant in three groups (Table 17).

Of the total females dissected establishment of trypanosomes in the gut only occured in 134 (15.2%) and in the gut and labrum in 103 (11.8%). In comparison, infection was found to be lower in males with 57 (6.6%) gut only and 77 (9.0%) gut and labrum infections. A bigger proportion of trypanosomes in the gut and labrum in female flies failed to reach the hypopharynx. Of the 348 females that were infected, trypanosomes reached the hypopharynx in only 111 (31.9%). In comparison, a total of 300 males were infected and of these trypanosomes reached the hypopharynx in 166 (55.3%). The overall infection in the gut was higher in females than males (X^2 = 33.02; $P < 0.001$) but more males became infected in the hypopharynx than females (X^2 = 14.46, $P < 0.001$).

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Although females were generally found to be more infected than males in the gut and labrum the majority of the groups indicated that the difference was not significant (Figure 15 and Table 17). The overall infection between males and females was not significantly different ($X^2 = 3.58$, $P > 0.05$).

The pattern of infection between the sexes changed in mature infections involving the hypopharynx (Figure 16). Overall infection showed a preponderance of males over females ($X^2 = 14.46$, $P < 0.001$). However, within individual groups the difference was not significant in three groups (Table 17).

Of the total females dissected establishment of trypanosomes in the gut only occured in 134 (15.2%) and in the gut and labrum in 103 (11.8%). In comparison, infection was found to be lower in males with 57 (6.6%) gut only and 77 (9.0%) gut and labrum infections. A bigger proportion of trypanosomes in the gut and labrum in female flies failed to reach the hypopharynx. Of the 348 females that were infected, trypanosomes reached the hypopharynx in only 111 (31.9%). In comparison, a total of 300 males were infected and of these trypanosomes reached the hypopharynx in 166 (55.3%). The overall infection in the gut was higher in females than males ($X^2 = 33.02$; $P < 0.001$) but more males became infected in the hypopharynx than females ($X^2 = 14.46$, $P < 0.001$).

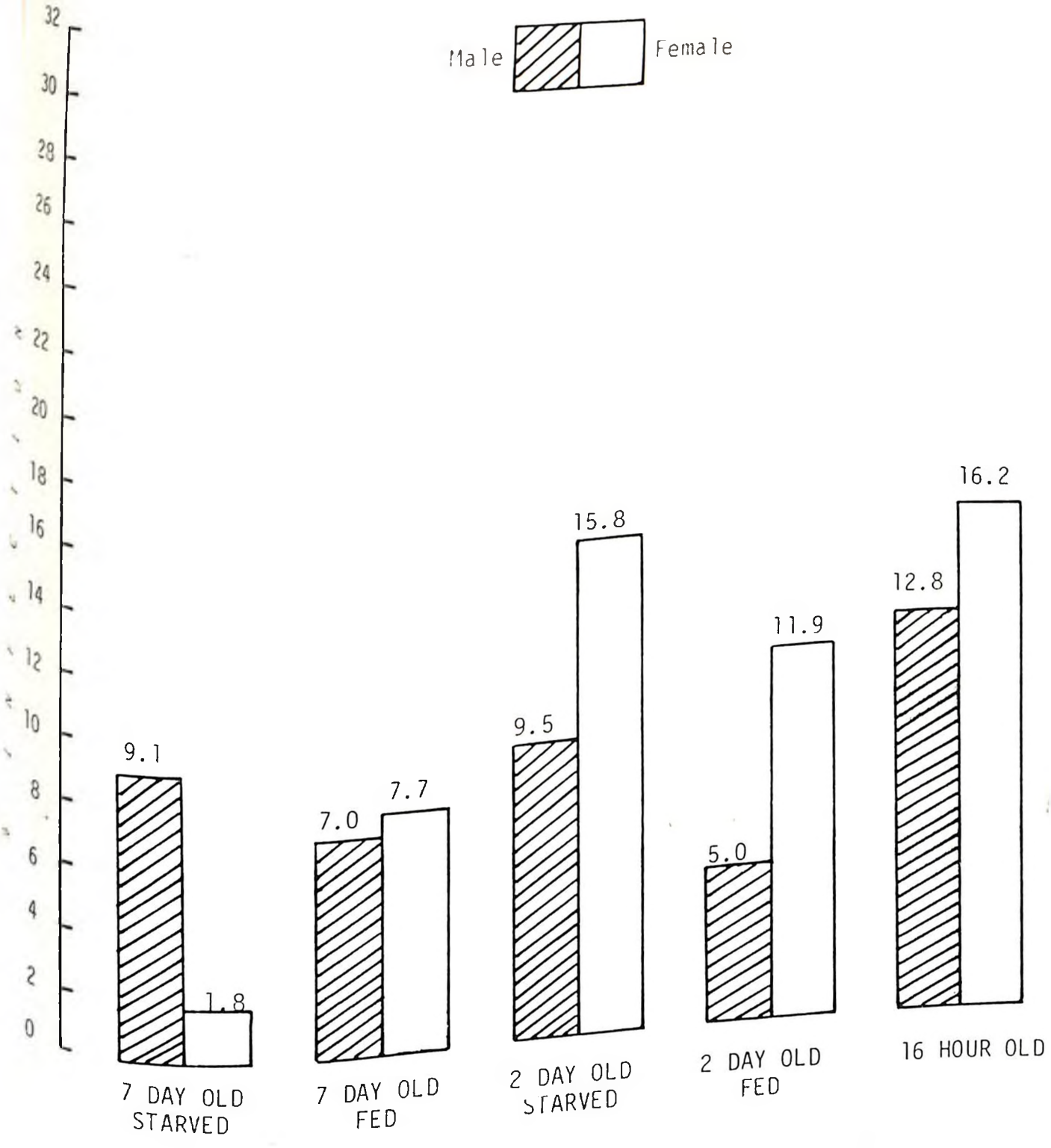

Fig. 15: Gut and labrum only infections in five groups of G.m. morsitans infected with T. congolense

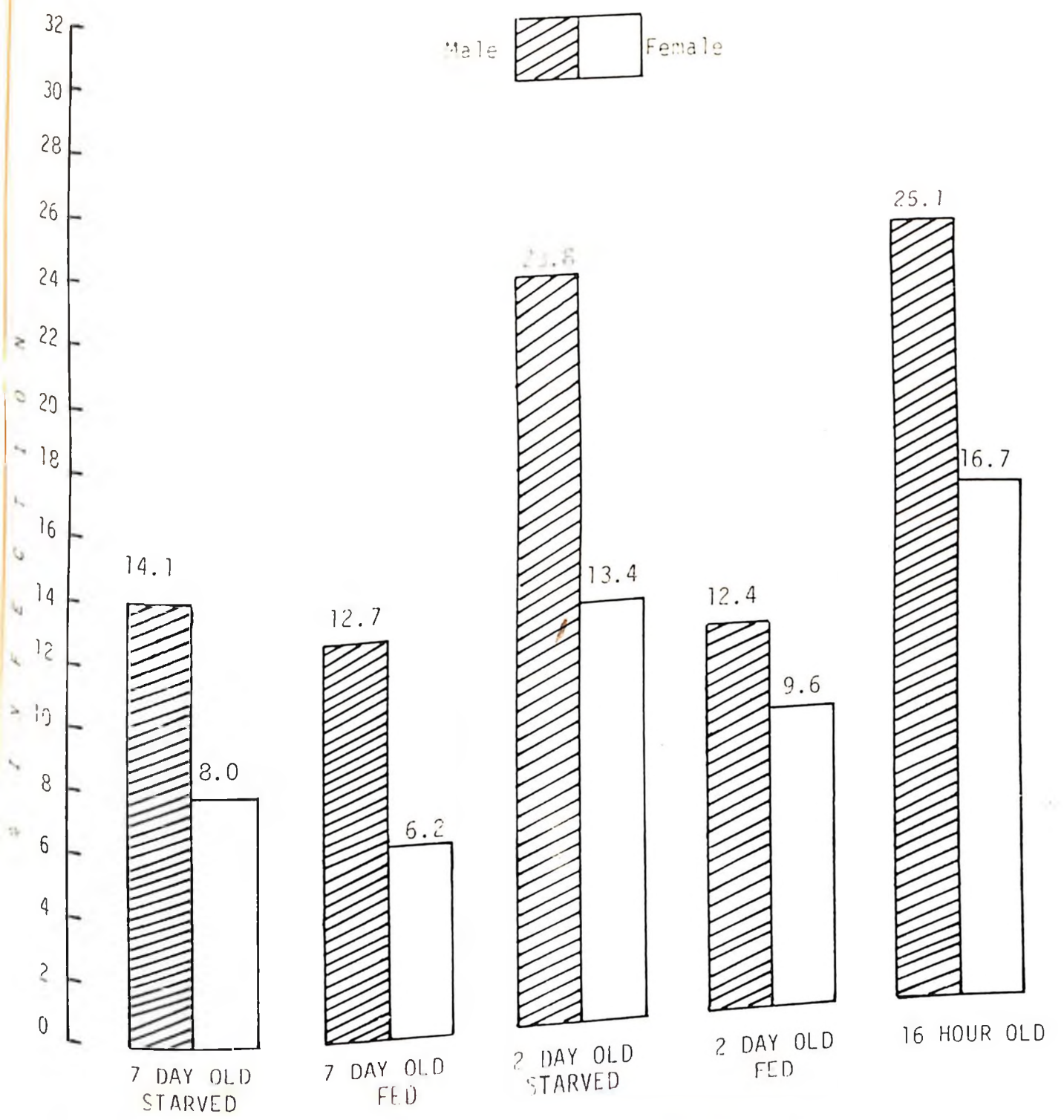

Fig. 16: Gut, labrum and hypopharynx infections in five groups of G.m. morsitans infected with T. congolense

Table 17: Comparison of trypanosome infection in male and female flies within the different age groups using the Chi-square test.

Fly group	% infection rate Male	% infection rate Female	Calculated χ^2 value	Table χ^2 value	Probability
Gut only infection					
7 day old starved*	2.5	20.4	18.11	6.64	0.001
7 day old fed*	6.4	13.3	4.59	3.84	0.05
2 day old starved*	9.1	19.8	9.71	6.64	0.01
2 day old fed	4.4	8.9	2.52	3.84	0.05
16 hour old	8.5	14.1	2.29	3.84	0.05
Gut and labrum only infection					
7 day old starved*	9.1	1.8	5.97	3.84	0.02
7 day old fed	7.0	7.7	0.06	3.84	0.05
2 day old starved	9.5	15.8	3.73	3.84	0.05
2 day old fed*	5.0	11.9	4.67	3.84	0.05
16 hour old	12.8	16.2	1.05	3.84	0.05
Gut, labrum and hypopharynx infection					
7 day old starved	14.1	8.0	2.19	3.84	0.05
7 day old fed*	12.7	6.2	4.56	3.84	0.05
2 day old starved*	24.8	13.8	7.82	6.64	0.01
2 day old fed	13.0	10.3	0.50	3.84	0.05
16 hour old	26.5	19.7	2.97	3.84	0.05

*Indicates significant difference between male and female flies of the given fly age group.

4.2 Experiment 2: Transmission of *T. congolense*/*T.b. brucei* mixed infections by *G.m. morsitans*

4.2.1 Trypanosome inoculations

Table 18 shows the estimation of the number of trypanosomes in the inoculum initially administered into rats and the volumes of blood that were later used to infect flies using the membrane feeding technique. The parasitaemias were consistently heavier for *T.b. brucei* than for *T. congolense*.

4.2.2 Feeding and mortality of flies

A greater number of flies fed when offered a blood meal directly from rats (*in vivo*) than from the silicone membrane (*in vitro*) (Table 19). More males (100%) and females (92.4%) took the *T. congolense* infected blood meal directly from rats compared to the silicone membrane for which figures for males were (86.7%) and females (80.0%). The difference was found to be significant for males ($X^2 = 8.16$, $P < 0.05$) and for females ($X^2 = 5.13$, $P < 0.05$). In the case of *T.b. brucei* infection the number of males that fed directly on rats (94.9%) did not differ significantly from those that fed through the silicone membrane (85.7%). Females differed significantly ($X^2 = 6.97$, $P < 0.01$), those that fed on rats had a higher figure (96.1%) than the silicone membrane (83.1%).

The number of flies that died during the 30 days of maintenance is given in Table 19. A higher number of flies

Table 18: Dose of inoculum initially administered into rats and the volumes of infected blood offered to flies.

Replicate	Trypanosomes species	Rat Number	Trypanosome/mm^3 (inoculum)	Volume of blood (ml)
1	T. congolense	CA3	1.235×10^7	1.0
	T.b. brucei	BA3	4.400×10^7	1.0
	T. congolense/T.b. brucei	CA3/BA3	mixed	2.0
2	T. congolense	CA5	1.235×10^7	2.0
	T.b. brucei	B32	4.400×10^7	2.0
	T. congolense/T.b. brucei	CA5/BB2	mixed	4.0
3	T. congolense	CA4	1.235×10^7	1.5
	T.b. brucei	BC3	8.680×10^7	1.5
	T. congolense/T.b. brucei	CA4/BC3	mixed	3.0
4	T. congolense	CB5	8.000×10^5	1.5
	T.b. brucei	BC2	8.680×10^7	1.5
	T. congolense/T.b. brucei	CB5/BC2	mixed	3.0
5	T. congolense	CB3	8.000×10^5	1.5
	T.b. brucei	BC1	8.680×10^7	1.5
	T. congolense/T.b. brucei	CB3/BC1	mixed	3.0

Table 19: Number of flies that fed directly on rats and through the silicone membrane and mortality during maintenance on rabbits.

Total number of flies = 571; Male = 245; Females = 326.

	T. congolense in vivo		*T. congolense* in vitro		*T.b. brucei* in vivo		*T.b. brucei* in vitro		*T. congolense*/ *T.b. brucei* in vitro	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Number of flies before feeding	57	79	60	80	59	77	56	77	57	76
Number fed	57	73	52	64	56	74	48	64	47	72
% fed	100	92.4	86.7	80.0	94.9	96.1	85.7	83.1	82.5	94.7
Number died	5	6	1	5	6	6	2	3	1	1
% died	8.8	8.2	1.9	7.8	10.7	8.1	4.2	4.7	2.1	1.4
Number dissected	52	67	51	59	50	68	46	61	46	71

died from the *in vivo* feeding method as compared to the *in vitro* feeding procedure but overall the difference was not significant.

4.2.3 Infection rates of flies

Results of infections in flies after 30 days of maintenance on rabbits are summarized in Table 20.

4.2.3.1 *T. congolense* infections

Infection rates of flies that fed directly on rats were compared with infection rates of flies resulting from the silicone membrane feeding using the Chi-square test. In both immature and mature infections, the differences were not significant ($P > 0.05$). Gut only infections were 5.8% and 4.5% for males and females that fed on rats (*in vivo*) and 11.8% and 6.8%, respectively, for those that fed through the silicone membrane (*in vitro*). Hypopharynx infections were 15.4% for males and 19.4% for females that were infected *in vivo* and 23.5% and 15.3% *in vitro*. The *in vitro* feeding method performed as well as in the case of infected rat.

4.2.3.2 *T.b. brucei* infections

T.b. brucei infections resulting from *in vivo* and *in vitro* feeding were also compared using the Chi-square test. For gut only infections the differences were not significant (Table 20). The two groups were highly significantly different ($X^2 = 6.64$, $P < 0.01$) in females infected in the

salivary glands. Females which fed on rats showed a higher salivary gland infection (12.3%) than those that fed from the silicone membrane where none developed salivary gland infection. Infection was lower in males fed through the membrane (2.2%) than in males fed on rats (12.0%) though the difference was not significant ($X^2 = 3.42$, $P > 0.05$).

Table 20: Comparison of *T. congolense* and *T.b. brucei* infections with *T. congolense*/*T.b. brucei* mixed infections in *G.m. morsitans*

Location in fly	*T. congolense* in vivo		*T. congolense* in vitro		*T.b. brucei* in vivo		*T.b. brucei* in vitro		*T. congolense*/*T.b. brucei* in vitro	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Number dissected	52	67	51	59	50	68	46	61	46	71
Gut only	3	3	6	4	9	30	9	27	6	14
% infected	5.8	4.5	11.8	6.8	18.0	44.1	19.6	44.3	13.0	19.7
Gut and labrum	9	6	6	5	0	0	0	0	4	10
% infected	17.3	9.0	11.8	8.5	0	0	0	0	8.7	14.0
Gut, labrum and hypopharynx	8	13	12	9	0	0	0	0	7	2
% infected	15.4	19.4	23.5	15.3	0	0	0	0	15.2	2.8
Salivary glands	-		-		6	7	1	0	3*	1
% infected					12.0	12.3	2.2	0	6.5	1.4

*One of the three male flies with salivary gland infections (*T.b. brucei*) also had *T. congolense* in the proboscis as revealed by mice inoculations.

4.2.3.3 *T. congolense/T.b. brucei* mixed infections

It was not possible to distinguish between *T. congolense* and *T.b. brucei* in gut only infections. However, the overall gut only infections in males infected with *T. congolense/T.b. brucei* mixed infections were statistically comparable to males infected with *T. congolense* and *T.b. brucei* separately (Table 20 and Figure 17). Females infected with mixed *T. congolense* and *T.b. brucei* were significantly higher than those infected with *T. congolense* ($P < 0.05$) and lower than those with *T.b. brucei* ($P < 0.01$).

Gut and labrum infections from *T. congolense/T.b. brucei* mixed infections were 8.7% in males and 14.0% in females and were comparable to flies infected with *T. congolense in vivo* (males, 17.3% and females, 9.0%) and *in vitro* (males, 11.8% and females, 8.5%) (Figure 18). When hypopharynx infections were considered, it was found that females (2.8%) infected with mixed *T. congolense* and *T.b. brucei* were significantly lower than females (19.4%) infected with *T. congolense in vivo* ($X^2 = 9.79$, $P < 0.01$) and females (15.3%) infected *in vitro* ($X^2 = 6.43$, $P < 0.05$) (Figure 19). In males the difference was not significant.

When *T.b. brucei* infections in the salivary glands of flies fed *in vivo* and *in vitro* were compared to salivary gland infections in flies that fed on *T. congolense/T.b. brucei* infections it was found that the differences were not significant ($P > 0.05$) in males of all the three groups (Figure 20).

On the other hand, comparison of females showed that *in vitro* feeding resulted in lower salivary gland infections than *in vivo* feeding. Infections in flies that fed on *T.b. brucei* infected blood *in vitro* were comparable to those that fed *in vitro* on *T. congolense*/*T.b. brucei* infected meal. This shows that the lower infections in the mixed infections as compared to *in vivo* feeding is by no means due to influence of *T. congolense*, rather this appears to be due to factors associated with the use of the silicone membrane.

One male fly (2.2%) of the 3 males with salivary gland infections (*T.b. brucei*) was also found to have *T. congolense* infections in the hypopharynx as revealed by inoculation of macerated salivary glands and probosces into mice. It is likely that more than one fly could have carried mixed *T. congolense*/*T.b. brucei* infections but since the Swiss mice used might have been refractory to *T. congolense* infection the latter species could not be detected easily. All positive salivary glands from 4 flies inoculated into mice became positive while only 2 hypopharynx were observed in mice. Results obtained from mice inoculations are given in Table 21. Note that replicate 1 flies were not inoculated into mice after dissection.

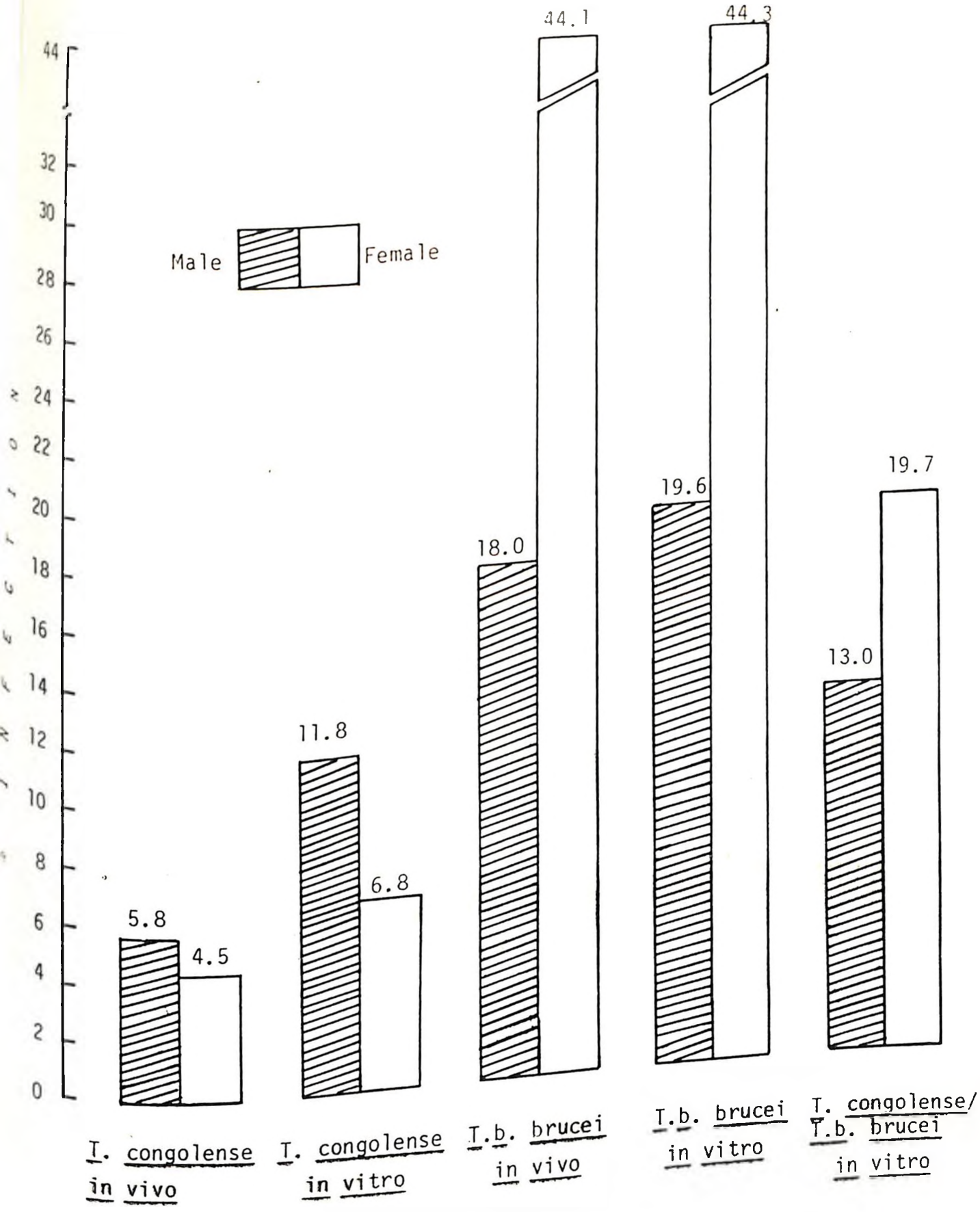

Fig. 17: Gut only infections in G.m. morsitans infected with T. congolense, T.b. brucei and T. congolense/T.b. brucei infections.

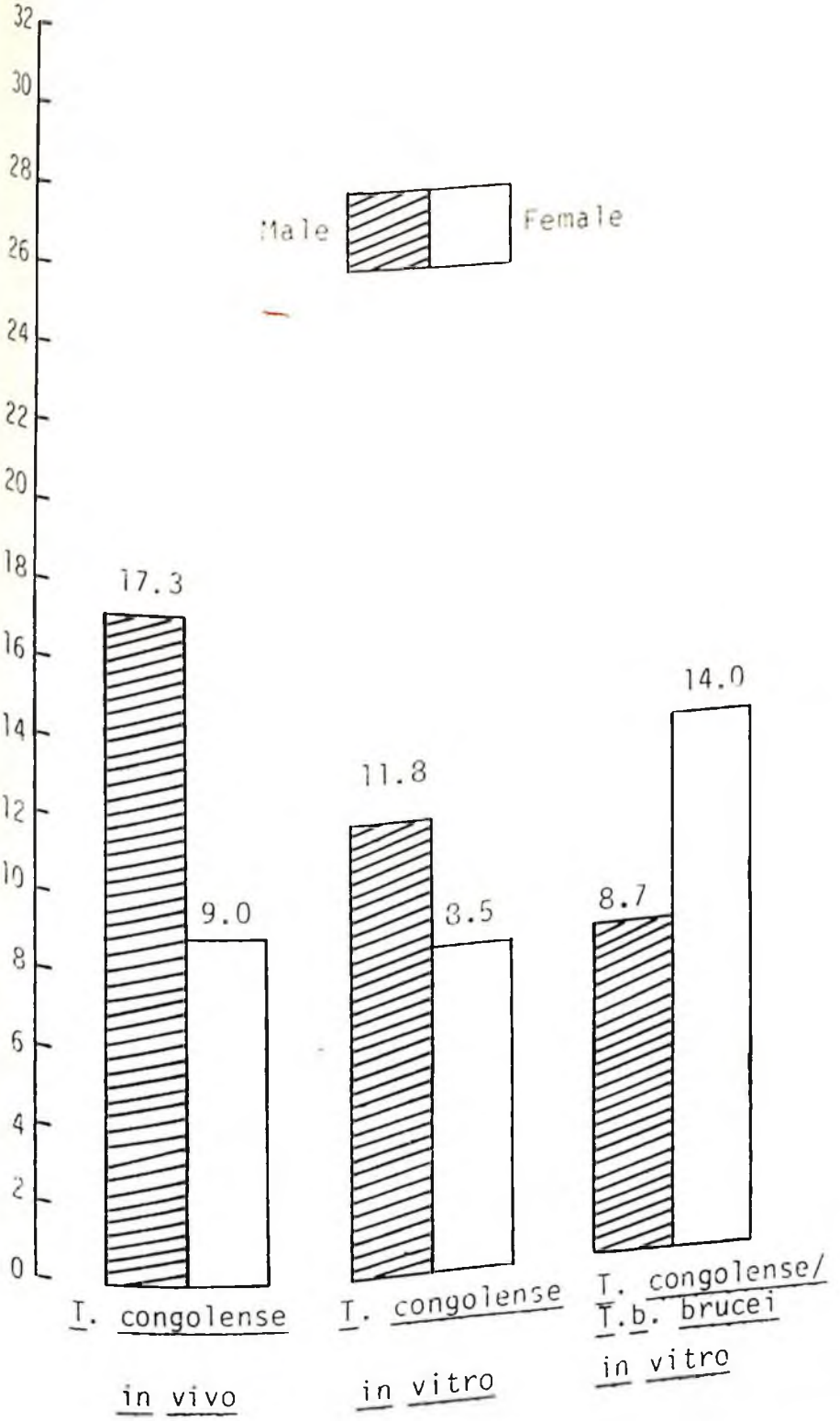

Fig. 18: Gut and labrum infections in G.m. morsitans infected with T. congolense and T. congolense/T.b. brucei mixed infections.

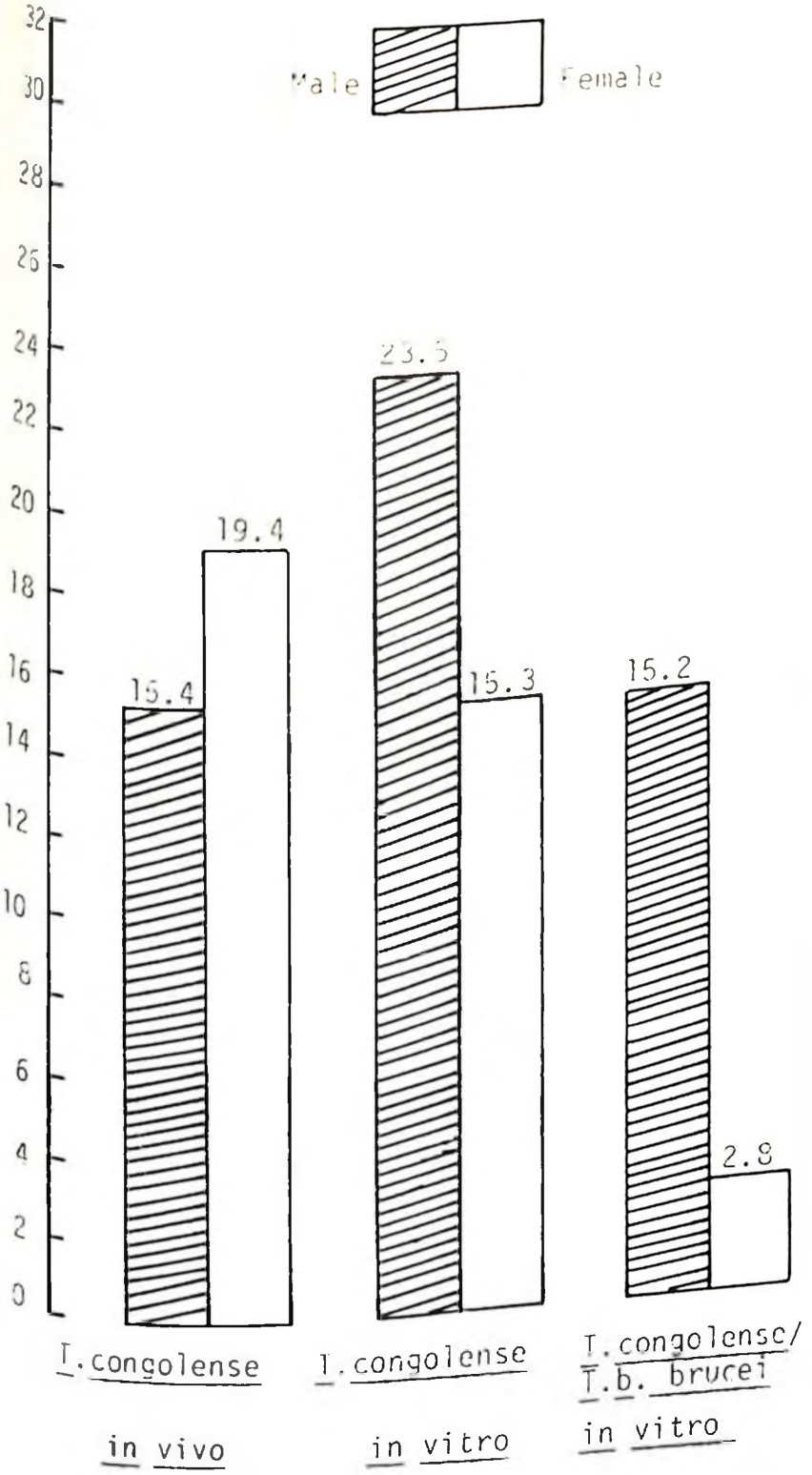

Fig. 19: Gut, labrum and hypopharynx infections in G.m. morsitans infected with I. congolense and I. congolense/T.b. brucei.

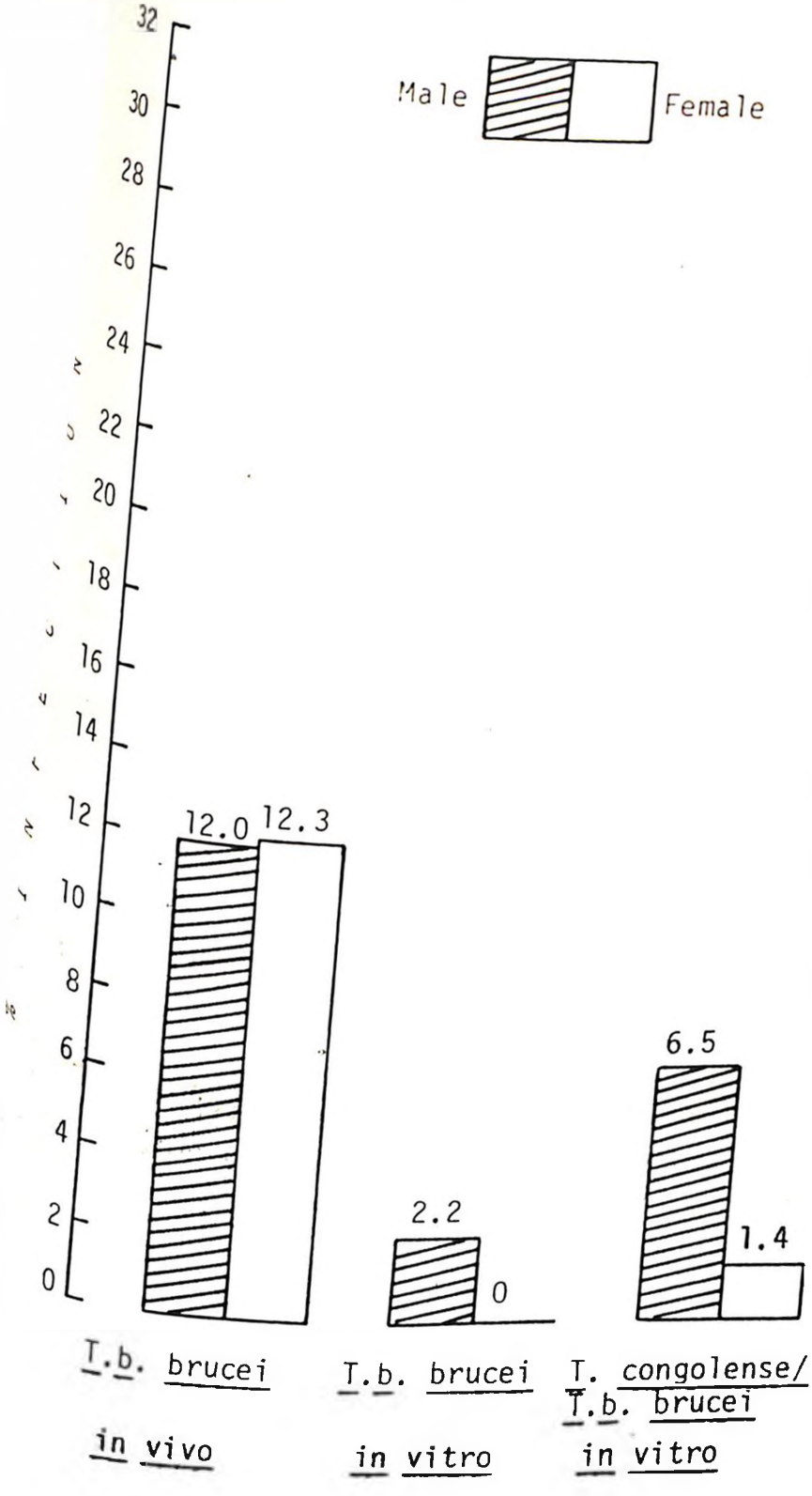

Fig. 20: Salivary gland infections in G.m. morsitans infected with T.b. brucei and T. congolense/T.b. brucei mixed infections.

Table 21: Results obtained from inoculations of mice with macerated gut, proboscis and salivary glands of infected flies.

Replicate/Fly number	Organ of fly infected				Result in mice
	Mg	L	H	Sg	
Replicate 2					
M 3	+	+	+	-	T. congolense
M 9	+	+	-	-	-
F 2	+	+	-	-	-
F 6	+	+	-	-	-
F 9	+	+	-	-	-
F 11	+	+	-	-	-
Replicate 3					
M 2	+	+	+	-	-
M 5	+	+	+	-	-
M 11	+	+	+	-	-
Replicate 4					
M 1	+	+	-	-	-
M 5	+	+	+	-	-
M 7	+	+	-	-	-
M 10	+	+	-	-	-
M 11	+	+	+	-	-
Replicate 5					
M 2	+	+	+	+	T. congolense/ T.b. brucei
M 4	+	+	+	-	-
M 10	+	+	+	+	T.b. brucei
M 12	+	-	+	+	T.b. brucei
F 2	+	+	-	-	-
F 8	+	-	+	+	T.b. brucei
F 11	+	+	+	-	-

Mg = Midgut L = Labrum H = Hypopharynx Sg = Salivary glands

4.3 Maintenance of trypanosomes in rats and mice

4.3.1 *T. congolense* (Maruma strain)

From 23rd August, 1984 to 17th April, 1985 12 syringe passages involving 11 rats and 1 mouse were performed to maintain this strain (Figure 21). After the seventh passage the strain was maintained in a fly for 34 days after which the proboscis was macerated and inoculated into a rat. The strain passed through a mouse only once during this study. Altogether the strain was maintained for 226 days. As can be seen from Figure 21 the prepatent period in rats varied from 4-7 days and so did the dose of the inoculum.

4.3.2 *T.b. brucei* (EATRO 1969)

EATRO 1969 was maintained exclusively on rats from 8th February, 1985 when the strain was obtained from ICIPE to 17th April, 1985 when these studies came to an end. It was passaged 5 times in 6 rats and this was a period of 57 days (Figure 22). The prepatent period was 3 days.

Figure 21: Maintenance of Maruma strain in rats, mouse and fly.

Date	Inoculum (ml)	Animal	Number of passage	Prepatent period (days)
		MOUSE ▽		
23.8.84	0.2	RAT 4A	1	7
		(14 days) ↓		
7.9.84	Not estimated	MOUSE	2	Not estimated
		(10 days) ↓		
18.9.84	0.5	RAT 4B	3	Not estimated
		(24 days) ↓		
13.10.84	Not estimated	RAT 1C	4	7
		(19 days) ↓		
2.11.84	0.2	RAT 2D	5	Not estimated
		(11 days) ↓		
14.11.84	0.2	RAT 6E	6	4
		(9 days) ↓		
23.11.84	Not estimated	RAT 2F	7	4
		(11 days) ↓		
5.12.84	Not estimated	FLY	8	-
		(34 days) ↓		
9.1.85	0.2	RAT 4G	9	Not estimated
		(26 days) ↓		
5.2.85	0.1	RAT 1H	10	6
		(14 days) ↓		
20.2.85	0.2	RAT 1I	11	6
		(13 days) ↓		
6.3.85	From tail	RAT 3J	12	7
		(18 days) ↓		
25.3.85	From tail	RAT 2 K		Not estimated
		↓		
17.4.85		END		

Figure 22: Maintenance of EATRO 1969 in rats.

Date	Inoculum (ml)	Animal	Number of passage	Prepatent period (days)
		RAT		
8.2.85	0.2	RAT 5AA	1	3
		(9 days)		
18.2.85	0.1	RAT 4 AB	2	3
		(7 days)		
26.2.85	0.1	RAT 4AC	3	3
		(7 days)		
6.3.85	From tail	RAT 2AD	4	Not estimated
		(12 days)		
19.3.85	From tail	RAT 3AE	5	Not estimated
		(9 days)		
		RAT 1 AF		
		(23 days)		
		END		
14.4.85				

CHAPTER V

5.0 DISCUSSION AND CONCLUSION

In this study flies were infected in sets, each consisting of a representative number of age groups. For this reason, 10 infected rats were employed in the first part of the study and another 5 in the second part to infect 10 and 5 sets of flies, respectively. It was not expected that different parasitaemias in the rats would bias the overall results since all the representative fly age groups were fed on the same infected rat at a time. Results of the respective sets of flies were pooled. Otieno et al. (1983) observed that five groups of G. morsitans that fed on varying trypanosome concentrations of 10, 10^2, 10^3, 10^4 and 10^6 per ml developed salivary gland infections of 8.7%, 5.9%, 9.5%, 10.6% and 12.2% which were not significantly different. They did, however, observe a tendency for the flies which had fed on the highest trypanosome concentrations to show the highest incidence of immature infections.

Flies were offered the infected blood meal only once. Duke (1935) who worked with G. palpalis and various strains of T.b. gambiense and T.b. rhodesiense observed that feeding flies on an infected animal several times did not result in significantly higher infection rates than when the flies were fed only once. After the infective meal, the flies were maintained on rabbits for 30 days. Although rabbits were replaced after every 2-3 weeks from the first day of infected flies feeding on them, and that they were examined for

trypanosome infection every week, there is a possibility that some flies could have been reinfected from them. When considering infection rates, it was assumed that all the flies were infected on the day the infective feed was offered.

How frequently flies feed in the field appears to be a matter of speculation. The availability of hosts, nature of these hosts, prevailing climatic conditions and species of fly being investigated will undoubtedly determine the frequency of feeding. Buxton (1955) reported that given a daily opportunity to feed, G. tachinoides will do so at least 4 days out of 5 at 30°C. There was no significant difference in the frequency of feeding between the sexes. Based on field observations from Tanzania, Buxton (1955) reported that the hunger cycle in G. swynnertoni and G. morsitans was about 4-5 days. In G. morsitans this period could extend to 8-10 days in the cold season. Jackson (1954) who also studied the hunger cycle of G. morsitans and G. swynnertoni in Tanzania during the mid-dry season estimated the hunger cycle for the former species to be between 3-4 days and for the latter to fall between 2½ and 3 days at a mean temperature of about 28.8°C.

In this study a period of 48 hours of flies being withheld from feeding, was considered as starvation. Earlier observations showed that flies that did not feed for 48 hours, fed more readily when later offered an opportunity to feed than flies of the same age which were fed daily at

a temperature ranging between 20°C-30°C. Under favourable field conditions, most flies will have probably taken their first blood meal by the first week (Gatehouse, 1972; Jackson, 1946). On the basis of these observations, the age groups of flies employed in this study should simulate what may happen in nature.

Infection in flies were considered at three levels: gut only, gut and labrum, and gut, labrum and hypopharynx. The results presented indicate that gut only infections do not differ significantly among all the age groups in both sexes. It has been reported by various workers (Yorke et al., 1933; Duke and Mellanby, 1936; Dipeolu and Adams, 1972; Harley, 1971b), that in some flies, trypanosomes may fail to develop cyclically. They may be only localized in the crop or gut without reaching the proboscis or salivary glands.

Results for mature infections indicate that in both sexes of flies, the groups (16 hour old and 2 day old 48 hours starved) that did not feed prior to the infective feed, developed higher infection rates than those (2 day old fed, 7 day old fed and 7 day old 48 hours starved flies) that had fed previously. While younger flies were generally more susceptible to infection than older flies, a clean blood meal when offered early to young flies, lowered infection rates. Once fed, starvation of flies for a period of 48 hours did not significantly raise infection rates in older flies.

It seems reasonable to conclude that it is the very young flies that are most susceptible to infection with T. congolense. Older flies will develop infection but at proportions lower than younger flies. These results, therefor, corroborate earlier findings by various workers (Van hoof et al., 1937; Wijers, 1958; Elce, 1971; Harley, 1971b; Otieno et al., 1983). Starvation is a fly age related factor in that the longer the period of starvation the older the fly becomes. As a factor determining infection rates in flies, starvation elevates trypanosome infection in young flies.

The observation that once fed, the 2 day old flies developed infection rates similar to 7 day old flies points to the development of the peritrophic membrane in the fly. Early studies on the formation of this membrane in the midgut of the fly (Wigglesworth, 1929) shed some light on the understanding of trypanosome migration in the fly. Willett (1966) demonstrated that the peritrophic membrane in G. morsitans, G. palpalis and G. austeni was completely formed after the first blood meal. He determined that the membrane increased in length by 1 mm per hour in the first 24 hours after emergence, and thereafter more slowly until it reached the hindgut in 3-4 days if the fly remained unfed. Willett (1966) further suggested that as long as the peritrophic membrane had not reached the hindgut, or ruptured, its posterior end was completely closed forming an unbroken sac. In younger flies, the membrane stretched more rapidly and extensively when they took their first blood meal. This rapid stretch creates an area of membrane

which is still semi-fluid through which trypanosomes can gain entry into the ectoperitrophic space where they would multiply and later, migrate to the proboscis or salivary glands. In older flies the membrane is fully formed and extends to the hindgut. Entry into the ectoperitrophic space in this case is by passing round the end of the membrane in the hindgut, and this will occur in only a small proportion of flies.

Harmsen (1973) advanced another hypothesis in an attempt to explain why only a few flies develop salivary glands infections. He suggested that when trypanosomes of the brucei group are ingested by a fly, they undergo a period of transformation inside the crop varying from 45-60 minutes in order for them to survive in the midgut and thereafter develop into the typical fly-gut forms. He postulated that such a transformation will take place mostly in younger flies of less than 24 hours old because these have an incompletely formed peritrophic membrane which results in a reduced rate of blood flow. In older flies (over 30 hours of age) the peritrophic membrane is well developed and extends farther into the midgut. In such flies the blood is completely transfered from the crop to the midgut within 20 minutes, thereby reducing the period of trypanosome transformation into forms that will survive in the fly.

Whether Willet's (1966) or Harmsen's (1973) hypothesis or both are accepted, the fact still remains that the growth of the peritrophic membrane is a function of fly age, that the

rate at which this grows is influenced by feeding activity. Fly age and feeding activity should therefore be considered as factors that will determine trypanosome infection. The data presented clearly show that fly age alone is not the principal determining factor.

More recently, Gingrich et al. (1982) showed that starvation of male G. morsitans of 21-25 days old for 4 days raised T.b. rhodesiense infections to proportions comparable to young flies of less than 24 hours old. This finding conflicts with the hypotheses advanced by Willett (1966) and Harmsen (1973) and results presented in this study. It remains to be demonstrated whether prolonged periods of starvation produce effects on the peritrophic membrane of old flies in a similar way to that which happens in young flies.

It was originally postulated (Taylor, 1932; Yorke et al., 1933; Fairbain, 1958) that trypanosomes passed from the endoperitrophic into the ectoperitrophic space at the free end of the peritrophic membrane in the hindgut. Recently, Freeman (1970a, 1970b, 1973) considered that there are 3 possible ways in which trypanosomes can reach the ectro-peritrophic space, notably:

a) trypanosomes can travel along the length of the gut and pass round the open, distal end of the peritrophic membrane within 24 hours after emergence of the fly.

b) trypanosomes can cross this membrane if it is ruptured, in the anterior mid or hind gut regions, during engorgement.

c) trypanosomes might also reach the ectoperitrophic space by forcing their way through the peritrophic membrane at the anterior end where it is soft and freshly secreted.

Of these routes, he considered that the third (c) was most likely. The first route (a) was ruled out because conditions in the hindgut, with a pH of 5.8, were considered lethal to trypanosomes. In the midgut, pH was estimated at 7.2. The second route was not considered likely either because it is rare that the peritrophic membrane has been observed to be ruptured. Moloo et al. (1970), however, disagreed with this suggestion for the reason that trypanosomes were unlikely to cross the peritrophic membrane unless they possessed enzymatic means of breaking down the membrane. They suggested that the observations made by Freeman (1970a, 1970b) could have been due to the fact that the trypanosomes were merely enclosed in a membrane fold. Evans and Ellis (1975, 1977, 1978) later demonstrated that T.b. rhodesiense could actually penetrate midgut cells of G.m. morsitans.

Regarding the susceptibility of the fly sexes to trypanosome infection, observations presented in this study show that there are differences in infection rates

between the sexes at different levels of infection. Individual differences among the age groups existed but if we are to consider overall infections in all the groups employed it is clear that at the gut only level, females displayed significantly higher infections than males. At the gut and labrum level, overall infections between the sexes were not significantly different. However, mature infections involving the hypopharynx were recorded more frequently in males than females. Depending on the level of infection in the fly there appears to be differences between the sexes. This might explain the discrepancies found in literature and might also explain, for instance, why Duke (1930) initially concluded that there was no significant difference in infection rates between the sexes of G. palpalis but later, Duke (1933c) on examination of his records found that females were actually more infected than males.

In this study the overall infection of all groups indicated that females had significantly higher infection than males. Mature infections were however fewer in females than males. This again corroborates earlier findings by Otieno et al. (1983) who reported fewer mature infections in female G.m. morsitans infected with T.b. brucei than males and suggested that it was probably because females digested the blood meal faster thereby subjecting ingested trypanosomes more rapidly to unfavourable conditions in the gut. It is important to note that females seem to present a stronger barrier to trypanosome development than males. Further

studies on this line to identify and possibly isolate the underlying variables in the females may well have some control implications.

The second part of this study involved infection of some groups of flies directly from rats as control groups and others on defibrinated blood from a silicone membrane as experimental groups. It was observed that a greater number of flies fed when offered a blood meal directly on rats than from the silicone membrane. The lower number of flies that fed through the membrane could have been due to the fact that the membrane does not have some attractants that rats possess. Bauer and Wetzel (1976) operated their silicone membrane at a temperature of 37^{o}C and observed that G. morsitans engorged fastest through the silicone membrane compared to either the agar/parafilm membrane or rabbit ears.

In the case of T.b. brucei infection, females which fed on rats showed significantly higher salivary gland infections than the group that was fed from the membrane. It is possible that the mechanical process of defibrinating blood could have affected the ability of the trypanosomes to reach the salivary glands.

Hypopharynx infections in females fed on mixed T. congolense/T.b. brucei were significantly lower than in females which fed on a meal infected only with T. congolense. Since most of the hypopharynx infection may be attributed to T. congolense it is suggested that this drastic drop

observed in hypopharynx infections of flies fed on mixed infections was due to inhibition of T. congolense by T.b. brucei. This drop occured despite the fact that at the labrum level of infection, females fed on a single T. congolense infected meal had statistically similar levels of infection as those that fed on mixed infections. It is interesting to note that although competetion between T. congolense and T.b. brucei might have occured, T. congolense was never observed to reach as far as the salivary glands. Competetion in favour of T.b. brucei in experimentally infected flies might explain why Moloo et al. (1982) observed T.b. brucei more frequently than either T. vivax or T. congolense in mixed infections.

SCOPE FOR FURTHER WORK

1. It will be necessary to carry out further studies on the formation and constitution of the peritrophic membrane in starved flies to establish whether there are any changes that might be associated with increased trypanosome penetration of the membrane. It is possible that prolonged periods of starvation might affect the secretion of the membrane in a way that will permit increased trypanosome penetration.

2. Further studies are required to determine the variables that lower mature infections in females since this may open an application for use in control methodology.

3. It would be interesting to examine further why T.b. brucei predominates in mixed infections in the laboratory when this species is rarely encountered in the field.

6.0 REFERENCES

ALLSOP, R. (1972). The role of game animals in the maintenance of endemic and enzootic trypanosomiases in the Lambwe valley, South Nyanza District, Kenya.
Bull. Wld. Hlth. Org., 47: 735-746.

ASHCROFT, M.T. (1959). The importance of African wild animals as reservoirs of trypanosomiasis.
E. Afr. med. J., 36: 289-297.

BAKER, J.R. and ROBERTSON, D.H.H. (1957). An experiment on the infectivity to Glossina morsitans of a strain of Trypanosoma rhodesiense and a strain of T. brucei with some observations on the longevity of infected flies.
Ann. trop. Med. Parasit., 51: 121-135.

BAKER, J.R. (1970). Techniques for the detection of trypanosome infections. pp. 67-88. In the African Trypanosomiases.
Mulligan, H.W. and Potts, W.H. (Eds).
Allen and Unwin, London, 950 pp.

BAKER, J.R. (1974). Epimediology of African sleeping sickness. pp. 29-50. In Trypanosomiasis and Leishmaniasis with special reference to Chaga's disease.
Ciba Foundation symposium 20, (ed.).
Elsevier, Excerpta Media, Armsterdam, 353 pp.

BAUER, B. and WETZEL, R. (1976). A new technique for feeding Glossina morsitans West. (Diptera : Glossinidae). Bull. ent. Res., 65: 563-565.

BURTT, E. (1946a). Incubation of tsetse pupae; increased transmission rate of Trypanosoma rhodesiense in Glossina morsitans. Ann. trop. Med. Parasit., 40: 18-28.

BURTT, E. (1946b). The sex ratio of infected flies found in transmission experiments with Glossina morsitans and Trypanosoma rhodesiense. Ann. trop. Med. Parasit., 40: 74-79.

BUXTON, P.A. (1955). The natural history of tsetse flies. An account of the biology of the genus Glossina (Diptera). Mem. Lond. Sch. Hyg. trop. Med., No. 10: 816 pp.

CHALLIER, A. (1982). The ecology of tsetse (Glossina spp.) (Diptera : Glossinidae) - a review, 1970-1981. Insect Scie. Appli., 3: 97-143.

CLARKE, J.E. (1966). Trypanosome infections in the mouthparts of Glossina morsitans Westwood: variations in frequency and extent of labral infections with age. Ann. trop. Med. Parasit., 60: 223-229.

CLARKE, [illegible] (1960). Trypanosome infection rates in the mouth-parts of Zambian tsetse flies.
Ann. trop. Med. Parasit., 63: 15-34.

CORSON, J.F. (1935). A high rate of salivary gland infection of Glossina morsitans with Trypanosoma rhodesiense.
Trans. R. Soc. trop. Med. Hyg., 28: 501.

DIPEOLU, O.O. and ADAM, K.M.G. (1974). On the use of membrane feeding to study the development of Trypanosoma brucei in Glossina.
Acta trop., 31: 185-201.

DISTELMANS, W., D'HAESELEAR, F., KAUFMAN, L. and ROUSSEUW, P. (1982). The susceptibility of Glossina palpalis palpalis at different ages to infection with Trypanosoma congolense.
Ann. Soc. belge Med. trop., 62: 41-47.

DUKE, H.L. (1930). On the susceptibility of the two sexes of Glossina palpalis to infection with Trypanosoma gambiense and T. rhodesiense.
Ann. trop. Med. Parasit., 24: 95-96.

DUKE, H.L. (1933a). Studies on the factors that may influence the transission of the polymorphic trypanosomes by tsetse I: A review of existing knowledge on this subject with general observations.
Ann. trop. Med. Parasit., 27: 99-118.

DUKE, H.L. (1933b). Studies on the factors that may influence the transmission of the polymorphic trypanosomes by tsetse III: Glossina morsitans versus G. palpalis as a transmitter of the polymorphic group of trypanosomes.
Ann. trop. Med. Parasit., 27: 123-130.

DUKE, H.L. (1933c). Relative susceptibility of the sexes of Glossina to infection with trypanosomes.
Ann. trop. Med. Parasit., 27: 355-356.

DUKE, H.L. (1933d). Studies on the factors that may influence the transmission of the polymorphic trypanosomes by tsetse V: On the effect of temperature.
Ann. trop. Med. Parasit., 27: 437-450.

DUKE, H.L. (1935). Studies on the factors that may influence the transmission of the polymorphic trypanosomes by tsetse IX: On the infectivity to Glossina of the trypanosomes in the blood of the mammal.
Ann. trop. Med. Parasit., 29: 131-143.

DUKE, H.L. (1936). On the power of Glossina morsitans and Glossina palpalis to transmit the trypanosomes of the brucei group.
Ann. trop. Med. Parasit., 30: 37-38.

DUKE, H.L. and MELLANBY, K. (1936). A note on the infectivity of Trypanosoma rhodesiense in the crop of Glossina palpalis.
Ann. trop. Med. Parasit., 30: 39.

ELCE, B.J. (1971). The transmission of Trypanosoma congolense through Glossina morsitans and the white mouse.
Trans. R. Soc. trop. Med. Hyg., 65: 239.

ELCE, B.J. (1974). The development of salivarian trypansomes in Glossina morsitans and small laboratory mammals.
Trans. R. Soc. trop. Med. Hyg., 68: 162.

EVANS, D.A. and ELLIS, D.S. (1975). Penetration of midgut cells of Glossina morsitans morsitans by Trypanosoma brucei rhodesiense.
Nature, Lond., 258: 231-233.

EVANS, D.A. and ELLIS, D.S. (1977). Passage of Trypanosoma brucei rhodesiense through the peritrophic membrane of Glossina morsitans morsitans.
Nature, Lond., 267: 834-835.

EVANS, D.A. and ELLIS, D.S. (1978). The penetrative ability of sleeping sickness trypanosomes.
Trans. R. Soc. trop. Med. Hyg., 72: 653-655.

FAIRBRAIRN, H. and BURTT, E. (1946). The infectivity to man of a strain of Trypanosoma rhodesiense transmitted cyclically by Glossina morsitans through sheep and antelope: evidence that man requires a minimum infective dose of metacyclic trypanosomes.
Ann. trop. Med. Parasit., 40: 270-313.

FAIRBAIRN, H. and CULWICK, A.T. (1950). The transmission of polymorphic trypanosomes.
Acta trop., 7: 19-44.

FAIRBAIRN, H. and WATSON, H.J.C. (1955). The transmission of Trypanosoma vivax by Glossina palpalis.
Ann. trop. Med. Parasit., 49: 250-259.

FAIRBAIRN, H. (1958). The penetration of Trypanosoma rhodesiense through the peritrophic membrane of Glossina palpalis
Ann. trop. Med. Parasit., 52: 18-19.

FISHER, R.A. and YATES, F. (1953). Statistical tables for biological, agricultural and medical research.
Longman, 146 pp.

FOOD AND AGRICULTURE ORGANIZATION, (1979). Report of the second consultation on the programme for the control of African trypanosomiasis held from 5-7 December, 1978, Lusaka, Zambia.
FAO, Rome, 75 pp.

FORD, J. and LEGGATE, B.M. (1951). The geographical and climatic distribution of trypanosome infection rates in Glossina morsitans group of tsetse flies (Glossina Wied: Diptera).
Trans. R. Soc. trop. Med. Hyg., 55: 383-397.

FORD, J. (1970). The geographical distribution of Glossina. pp 274-297. In the African trypanosomiases.
Mulligan, H.W. and Potts, W.H. (Eds).
Allen and Unwin, Lond, 950 pp.

FORD, J. (1971). The role of the trypanosomiases in African ecology: a study of the tsetse fly problem.
Clarendon, Oxford, 568 pp.

FORD, J. and KATONDO, K.M. (1977). Maps of tsetse flies (Glossina) distribution in Africa, 1973: according to subgeneric groups on scale of 1:5,000,000.
Bull. Anim. Hlth. Production, 25: 187-193.

FREEMAN, J.C. (1970a). The presence of trypanosomes in the ectoperitrophic space of tsetse flies, half an hour after ingestion of the infective blood meal.
Trans. R. Soc. trop. Med. Hyg., 64: 187-188.

FREEMAN, J.C. (1970b). The presence of trypanosomes in the ectoperitrophic space of tsetse flies, half an hour after ingestion of the infective blood meal.
Trans. R. Soc. trop. Med. Hyg., 64: 14.

FREEMAN, J.C. (1973). The penetration of the peritrophic membrane of the tsetse flies by trypanosomes. Acta trop., 30: 347-355.

GATEHOUSE, A.G. (1971). Host-finding behaviour of tsetse flies. pp 83-95. In the behavioural aspects of parasite transmission. Canning, E.U. and Wright, C.A. (Eds.). Supplement 1 to the Zoological Journal of the Linnean Society Vol. 51. Academic Press, 219 pp.

GEIGY, R. and KAUFFMANN, M. (1973). Sleeping sickness survey in the Serengeti Area (Tanzania) I. Examination of large mammals for trypanosomes. Acta trop., 30: 12-23.

GEIGY, R., MWAMBU, P.M. and KAUFFMANN, M. (1971a). Sleeping sickness survey in Musoma District, Tanzania IV. Examination of wild mammals as a potential reservoir for Trypanosoma rhodesiense. Acta trop., 28: 211-220.

GEIGY, R., KAUFFMANN, M., STEIGE, R. and BRUN, R. (1971b). Influence of blood meals from different donors on the infection rates of Trypanosoma brucei in Glossina. Acta trop. 28: 164-169.

GINGRICH, J.B., WARD, R.A., MACKEN, L.M. and ESSER, K.M. (1982). African sleeping sickness: new evidence that mature tsetse flies (Glossina morsitans) can become potent vectors.
Trans. R. Soc. trop. Med. Hyg., 76: 479-481.

GLASGOW, J.P. (1970). The genus Glossina: Introduction. pp 225-242. In the African trypanosomiases. Mulligan, H.W. and Potts, W.H. (Eds). Allen and Unwin, London, 950 pp.

GODFREY, D.G., KILLICK-KENDRICK, R. and FERGUSON, W. (1965). Bovine trypanosomiasis in Nigeria IV: Observations on cattle trekked along a trade-cattle route areas infested with tsetse fly.
Ann. trop. Med. Parasit., 59: 255-269.

HARLEY, J.M.B. (1966a). Studies on age and trypanosome infection rates in females of Glossina pallidipes Aust., G. palpalis fuscipes Newst. and G. brevipalpis Newst. in Uganda.
Bull. ent. Res., 57: 23-37.

HARLEY, J.M.B. (1966b). Further studies on age and trypanosome infection rates in Glossina pallidipes Aust., G. palpalis fuscipes Newst. and G. brevipalpis Newst. in Uganda.
Bull. ent. Res., 57: 459-477.

HARLEY, J.M.B. (1967). The influence of sampling method on the trypanosome infection of catches of Glossina pallidipes and G. fuscipes.
Entomologia exp. appl., 10: 240-252.

HARLEY, J.M.B. and WILSON, A.J. (1968). Comparison between Glossina morsitans, G. pallidipes and G. fuscipes as vectors of trypanosomes of the Trypanosoma congolense group: the proportions of infected experimentally and the number of infective organisms extruded during feeding.
Ann. trop. Med. Parasit., 62: 178-187.

HARLEY, J.M.B. (1971a). The influence of the age of the fly at the time of the infecting feed on infection of Glossina fuscipes with Trypanosoma rhodesiense.
Ann. trop. Med. Parasit., 65: 191-196.

HARLEY, J.M.B. (1971b). Comparison of the susceptibility to infection with Trypanosoma rhodesiense of Glossina pallidipes, G. morsitans, G. fuscipes and G. brevipalpis.
Ann. trop. Med. Parasit., 65: 185-189.

HARMSEN, R. (1973). The nature of the establishment barrier for Trypanosoma brucei in the gut of Glossina pallidipes.
Trans. R. Soc. trop. Med. Hyg., 67: 364-373.

HOARE, C.A. (1972). The trypanosomes of mammals. A zoological monograph.
Oxford, Edinburgh, 749 pp.

JACKSON, C.H.N. (1946). An artificially isolated generation of tsetse flies (Diptera).
Bull. ent. Res., 37: 291-299.

JACKSON, C.H.N. (1954). The hunger-cycle of Glossina morsitans Westwood and G. swynnertoni Austen.
J. Anim. Ecology., 23: 368-372.

JANSSEN, J.A.H.A. and WIJERS, D.J.B. (1974). Trypanosoma simiae at the Kenya Coast. A correlation between virulence and the transmitting species of Glossina.
Ann. trop. Med. Parasit., 68: 5-19.

JOHNSON, W.B. and LLOYD, L. (1923). First report of the tsetse fly investigation in the Northern Provinces of Nigeria.
Bull. ent. Res., 13: 373-396.

JORDAN, A.M., LEE-JONES, F. and WEITZ, B. (1961). The natural hosts of tsetse flies in the forest belt of Nigeria and Southern Cameroons.
Ann. trop. Med. Parasit., 55: 167-179.

JORDAN, A.M., LEE-JONES, F. and WEITZ, B. (1962). The natural hosts of tsetse flies in Northern Nigeria.
Ann. trop. Med. Parasit., 56: 430-442.

JORDAN, A.M. (1964). Trypanosome infection rates in Glossina morsitans submorsitans Newst. in nothern Nigeria.
Bull. ent. Res., 55: 219-231.

JORDAN, A.M. (1965). Bovine trypanosomiasis in Nigeria V. The tsetse-fly challenge to a herd of cattle trekked along a trade-cattle route.
Ann. trop. Med. Parasit., 59: 270-276.

JORDAN, A.M., NASH, T.A.M. and BOYLE, J.A. (1967). The rearing of Glossina austeni Newst. with lop eared rabbits as hosts I: efficacy of the method.
Ann. trop. Med. Parasit., 61: 182-188.

JORDAN, A.M. (1974). Recent developments in the ecology and methods of control of tsetse flies (Glossina spp: Diptera, Glossinidae) - a review.
Bull. ent. Res., 63: 361-399.

JORDAN, A.M. (1976). Tsetse flies as vectors of trypanosomes.
Vet. Parasit., 2: 143-152.

KADDU, J.B. (1978). Trypanosoma congolense infection in Glossina pallidipes.
Ph.D. Thesis, University of Nairobi.

KATONDO, K.M. (1984). Revision of second edition of tsetse distribution maps: an interim report.
Insect Scie. Appli., 5: 381-388.

KINGHORN, A., YORKE, W. and LLOYD, L. (1913). Final report of the Luangwa sleeping sickness commission of the British South African Company.
Ann. trop. Med. Parasit., 7: 183-302.

LAIRD, M. (1977). Tsetse: the future for biological methods in integrated control.
IDRC - 077e, Ottawa, 220 pp.

LAMBRECHT, F.L. (1980). Ecological and physiological factors in the cyclic transmission of African trypanosomiasis.
Insect Scie. Appli., 1: 47-54.

LEEGWATER van der LINDEN, M.E. (1983). The rearing of the tsetse fly Glossina pallidipes Austen.
Armsterdam, 138 pp.

LLOYD, L., JOHNSON, W.B., YOUNG, W.A. and MORRISON, H. (1924). Second report of the tsetse fly investigation in the Northern Provinces of Nigeria I.
Bull. ent. Res., 15: 1-27.

LUMSDEN, W.H.R., HERBERT, W.J. and MCNEILLAGE, G.J.C. (1973). Techniques with trypanosomes.
Churchill Livingstone, Edinburgh and London, 183 pp.

LYNCH, M.T., RAPHAEL, S.S., MELLOR, L.D., SPARE, P.D., INWOOD, M.J.H. (1969). Medical laboratory technology and clinical pathology.
Saunders, Philadelphia, London, Toronto, 1359 pp.

MACINNIS, A.J. and VOGE, M. (1970). Experiments and techniques in Parasitology.
Freeman, San Francisco, 232 pp.

MAUDLIN, I. (1982). Inheritance of susceptibility to Trypanosoma congolense infection in Glossina morsitans.
Ann. trop. Med. Parasit., 76: 225-227.

MOLOO, S.K. and KUTUZA, S.B. (1970). Feeding and crop emptying in Glossina brevipalpis Newstead.
Acta trop., 27: 356-377.

MOLOO, S.K., STEIGER, R.F. and HECKER, H. (1970). Ultra-structure of the peritrophic membrane formation in Glossina Wiedemann.
Acta trop., 27: 378-383.

MOLOO, S.K., STEIGER, R.F. and BRUN, R. (1973). Trypanosome infection rates in Glossina swynnertoni and G. palliaipes in Ikoma, Musoma District, Tanzania. Parasitology, 66: 259-267.

MOLOO, S.K. (1980). Interacting factors in the epidemiology of trypanosomiasis in an endemic/enzootic region of Uganda and its contiguous area of Kenya. Insect Scie. Appli., 1: 117-121.

MOLOO, S.K. (1981). Effects of maintaining Glossina morsistans morsitans on different hosts upon the vectors subsequent infection rates with pathogenic trypanosomes. Acta trop., 38: 125-136.

MOLOO, S.K., FAIQA, D. and KAMUNYA, G.W. (1982). The transmission of mixed infections of pathogenic trypanosoma species to susceptible hosts by Glossina morsitans morsitans. Acta trop., 39: 303-306.

MOLYNEUX, D.H. (1977). Vector relationships in the trypanosomatidae. Adv. Parasit., 15: 1-82.

MOLYNEUX, D.H. (1980). Host-trypanosome interactions in Glossina. Insect Scie. Appli., 1: 39-46.

MSHELBWALA, A.S. (1972). Trypanosoma brucei infections in the haemocoel of tsetse flies.
Trans. R. Soc. trop. Med. Hyg., 66: 637-643.

MURRAY, M., TRAIL, J.C.M., TURNER, D.A. and WISSICOQ, Y. (1983). Livestock productivity and trypanotolerance: network training manual.
ILCA, Addis Ababa, 198 pp.

MWAMBU, P.M. and MAYENDE, J.S.P. (1973). Salivarian trypanosome infections in cattle in Ikoma, South Mara District, Tanzania.
Parasitology, 66: 381-385.

NASH, T.A.M. (1948). A note on the effect of high temperature on the pupal stage of Glossina in relation to the transmission rate of trypanosomes.
Ann. trop. Med. Parasit., 42: 30-32.

NASH, T.A.M., JORDAN, A.M. and BOYLE, J.A. (1956). A promising method for rearing Glossina austeni Newst. on a scale based on the use of rabbits' ears for feeding.
Trans. R. Soc. trop. Med. Hyg., 60: 183-188.

NASH, T.A.M. (1969). Africa's bane: the tsetse fly.
Collins, London, 224 pp.

NASH, T.A.M. and JORDAN, A.M. (1970). Methods for rearing and maintaining Glossina in the laboratory. pp 441-452. In the African trypanosomiases. Mulligan, H.W. and Potts, W.H. (Eds). Allen and Unwin, London, 950 pp.

OTIENO, L.H. (1973). Trypanosoma (Trypanozoon) brucei in the haemolymph of experimentally infected young Glossina morsitans. Trans. R. Soc. trop. Med. Hyg., 67: 886-887.

OTIENO, L.H., DARJI, N. and ONYANGO, P. (1976). Development of Trypanosoma (Trypanozoon) brucei in Glossina morsitans inoculated into the tsetse haemocoel. Acta trop., 33: 143-150.

OTIENO, L.H., DARJI, N., ONYANGO, P. and MPANGA, E. (1983). Some observations on factors associated with the development of Trypanosoma brucei brucei infections in Glossina morsitans morsitans. Acta trop., 40: 113-120.

OTIENO, L.H. (1983). Inadequacy of the dissection method of estimating trypanosome infection rates. Ann. trop. Med. Parasit., 77: 329-330.

POLLOCK, J.N. (1980). (Ed.). FAO training manual for tsetse control personnel. Rome, FAO, Vol. 1-3.

POTTS, W.H. (1970). Systematics and identification of Glossina. pp 243-273. In the African trypanosomiases. Mulligan, H.W. and Potts, W.H. (Eds). Allen and Unwin, London, 950 pp.

ROBERTSON, M. (1913). Notes on the life history of Trypanosoma gambiense with a brief reference to the cycles of T. nanum and T. pecorum in Glossina palpalis. Reports of sleeping sickness Commission Roy. Soc., 13: 119.

ROBERTS, C.J. and GRAY, A,R. (1972). A comparison of Glossina morsitans submorsitans Newst. and G. tachinoides West. collected and maintained under similar conditions as vectors of Trypanosoma (Nannomonas) congolense, T. (N.) simiae and T. (Duttonella) vivax. Ann. trop. Med. Parasit., 66: 41-53.

ROGERS, D. (1983). Trypanosomiasis risk or challenge - a review with recommendations. In the Livestock productivity and trypanotolerance. Network training manual. Murray, M. Trail, J.C.M., Turner, D.A. and Wissocq, Y. (Eds). ILCA, Addis Ababa, Ethiopia, 198 pp.

RYAN, L., TOPPER, R., CROFT, S.L., MOLYNEUX, D.H. and CLAIR, M. (1982). Differences in rates of acquisition of trypanosome infections between Glossina species in the field.
Ann. Soc. belge Med. trop., 62: 291-300.

TAYLOR, A.W. (1932). The development of West African strains of Trypanosoma gambiense in Glossina tachinoides under normal laboratory conditions and at raised temperatures.
Parasitology, 24: 401-419.

VAN HOOF, L.M.J.J., HENRARD, C. and PEEL, E. (1937). Influences modificatrices de la transmissibite' cyclique du Trypanosoma gambiense par Glossina palpalis.
Ann. Soc. belge Med. trop., 17: 249-263.

VAN HOOF, L.M.J.J. (1947). Observations on trypanosomiasis in the Belgian Congo.
Trans. R. Soc. trop. Med. Hyg., 40: 728-761.

WARD, R.A. (1968). The susceptibility of Glossina austeni to infection with Trypanosoma brucei.
Trans. R. Soc. trop. Med. Hyg., 62: 672-678.

WARD, R.A. and BELL, L.H. (1971). Transmission of Trypanosoma brucei by colonized Glossina austeni and G. morsitans.
Trans. R. Soc. trop. Med. Hyg., 65: 236-237.

WEITZ, B.G.F. (1958). Identification of blood meals of blood sucking arthropods.
Bull. Wld. Hlth. Orgn., 15: 473-490.

WEITZ, B.G.F. (1970). Hosts of Glossina. pp 317-326. In the African trypanosomiases. Mulligan, H.W. and Potts, W.H. (Eds).
Allen and Unwin, London, 950 pp.

WIGGLESWORTH, V.B. (1929). Digestion in the tsetse fly.
Parasitology, 21: 288-321.

WIJERS, D.J.B. (1958). Factors that may influence the infection rate of Glossina palpalis with Trypanosoma gambiense I: The age of the fly at the time of the infected feed.
Ann. trop. Med. Parasit., 52: 385-390.

WILLEMSE, L.P.M., MWANGELWA, M.I. and MWANZA, L.M. (1983). A short term study on the ecology and trypanosome infection rates of a Glossina population in an endemic sleeping sickness focus in the Luangwa valley, Zambia.
Report made to the Tropical Diseases Research Centre Ndola, Zambia.

WILLETT, K.C. (1956). Development of the peritrophic membrane in Glossina (tsetse flies) and its relation to infection with trypanosomes.
Exp. Parasitol., 18: 290-295.

WILSON, S.G., MORRIS, K.R.S., LEWIS, I.J. and KROG, E. (1963). The effects of trypanosomiasis on rural economy with special reference to the Sudan, Bechuanaland and West Africa.
Bull. Wld. Hlth. Organ., 28: 595-613.

WILSON, A.J., DAR, F.K. and PARIS, J. (1972). A study on the transmission of salivarian trypanosomes isolated from wild tsetse flies.
Trop. Anim. Hlth. Prodn. Afri., 4: 14-22.

WOO, P.T.K. (1969). The haematocrit centrifuge for the detection of trypanosomes in the blood.
Canadian J. Zoology, 47: 921-923.

WOO, P.T.K. (1971a). Evaluation of the haematocrit centrifugation, techniques for the field diagnosis of human trypanosomiases and filariasis.
Acta trop., 28: 298-303.

WOO, P.T.K. (1971b). The haematocrit centrifuge technique for the detection of low virulent strains of trypanosomes of Trypanosoma congolense subgroup.
Acta trop., 28: 304-308.

WORLD HEALTH ORGANIZATION, (1983). Report of the steering committee of the scientific working group on epidemiology. July 1978 to June, 1983. TDR/EPID/SC. SWG(78-83)/83.3.

YORKE, W., MURGATROYD, F. and HAWKING, F. (1933). The relation of polymorphic trypanosomes, developing in the gut of Glossina to the peritrophic membrane. Ann. trop. Med. Parasit., 27: 347-354.