Growth, differentiation and the regulation of *Trypanosoma brucei* infections.

Nasreen Aslam B.Sc.

Laboratory for Biochemical Parasitology,

Department of Zoology,

University of Glasgow.

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ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

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Declaration

The results presented in this thesis are those of my own original work except where otherwise stated.

The results presented in Chapter 4 have been submitted to Parasitology Research: N. Aslam and C.M.R. Turner (1992)(in press) The relationship of variable antigen expression and population growth rates in *Trypanosoma brucei*. These results were also previously published as a meeting abstract: N. Aslam and C.M.R. Turner (1991). Does variable antigen expression influence growth rates in *Trypanosoma brucei* ?. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 85, 328.

Summary

The experiments described in this thesis were conducted to determine how trypanosome growth may regulate the course of an infection.

The question of whether cloned stocks of *Trypanosoma brucei* differ in the courses of infection that they produce in inbred mice was addressed. Accurate parasitaemic profiles were obtained for 6 cloned stocks of trypanosomes and from a comparison of these it was shown that the courses of infection differed between them. Infections with one of the cloned stocks were characterised by the occurence of a high parasitaemia and no remission of infection whilst infections with another were characterised by a low parasitaemia and several cycles of remission and recrudescence. Parasitaemic profiles obtained for the other four cloned stocks were intermediate between these two extremes. Parasitaemic profiles were also compared between three lines of trypanosomes derived from the same cloned stock but differing in the number of rapid syringe-passages undergone through mice. Parasitaemias were shown to increase as a result of rapid syringe-passaging.

Rates of trypanosome population growth, replication of slender forms and differentiation from slender to stumpy forms were compared between cloned stocks of trypanosomes known to differ in the courses of infection that they produced in inbred mice. The objective of this study was to determine whether the observed differences in the courses of infection could be explained by differences in these rates. When cloned stocks were compared a positive correlation was observed between the rate of growth, rate of replication and

course of infection: high parasitaemias were associated with faster rates of growth and replication. This correlation was not observed however, in the second comparison between the three trypanosome lines of the same genotype but with different passage histories. From these results it would appear that the basis of the variation in the courses of infection differs between trypanosomes in the laboratory and in the field. A relationship was not observed between rates of trypanosome differentiation and courses of infection in either of the two comparisons made.

The relationship between variable antigen type (VAT) expression and trypanosome growth rates was reinvestigated. Growth rates in mice were compared between pairs of cloned trypanosome populations each of which homogeneously expressed a different VAT. Two groups of VATs were compared and within each group, the lines expressing different VATs were of the same passage history. In a sensitive assay of relative growth, no significant differences were found in 4 of 6 experiments using the first group of VATs and in 1 of 3 experiments using the second group. In those experiments where a difference was observed the data were analysed further to determine the differences in population doubling times. These differences were less than 10% in all cases. It was concluded that variable antigen expression will exert no effect on rates of trypanosome population growth.

List of Abbreviations

EATRO - East African Trypanosomiasis Research Organisation

FITC - Fluoroscein isothiocyanate

GUP - Glasgow University Protozoology

GUTAR - Glasgow University *Trypanozoon* Antigen Repertoire

GUTat - Glasgow University *Trypanozoon* antigen type

HDL - High Density Lipoprotein

ILRAD - International Laboratory for Research on Animal Diseases

ILTat - International Laboratory for Research on Animal Diseases *Trypanozoon* antigen type

LouTat- Louisville *Trypanozoon* antigen type

MHC - Major Histocompatibility Complex

MHV - Mouse Hepatitis Virus

NIM - National Institute for Medical Research

PBS - Phosphate buffered saline pH. 8.0

PDT - Population doubling time

SE - Standard error

SHAM - Salicylhydroxamic acid

STIB - Swiss Tropical Institute Basel

TREU - Trypanosomiasis Research Edinburgh University

VAT - Variable antigen type

VSG - Variable surface glycoprotein

CHAPTER 1

GENERAL INTRODUCTION

1.1 GENERAL BIOLOGY OF THE AFRICAN TRYPANOSOMES

1.1.1 TAXONOMY

The African trypanosomes are flagellate protozoa of the order Kinetoplastida, family Trypanosomatidae and genus *Trypanosoma*. Trypanosomes of this genus are generally classified as salivarian or stercorarian according to the method of transmission by the insect vector and the African trypanosomes are members of the former category. The economically important species include those of the subgenera *Trypanozoon* (for example *Trypanosoma brucei* group), *Nannomonas* (for example *Trypanosoma congolense*) and *Duttonella* (for example *Trypanosoma vivax*). The *T. brucei* group consists of three morphologically identical subspecies, *T. b. brucei*, *T. b. rhodesiense* and *T. b. gambiense* (Hoare, 1972). Of the three, only trypanosomes of *T. b. brucei* are lysed by normal human serum and this difference forms the basis of the Blood Incubation Infectivity Test which is used to differentiate trypanosomes of *T. b. rhodesiense* from *T. b. brucei* and *T. b. gambiense* (Rickman & Robson, 1970). These divisions are not absolute as Tait *et al* (1984) demonstrated that on the basis of population genetic criteria, *T. b. gambiense* was distinct from both *T. b. brucei* and *T. b. rhodesiense* which were similar. Also the aforementioned ability of trypanosomes of *T. b. brucei* to be lysed by normal human serum is an unstable phenotypic trait (Tait *et al*, 1984)

1.1.2 THE INSECT VECTOR

The insect vectors of the salivarian trypanosomes are dipteran flies of the genus *Glossina*, more commonly known as

tsetse flies. There are several species of *Glossina* which act as vectors in a range of environmental habitats. The dry Savanna regions of Africa are generally populated by tsetse flies of the *morsitans* group (*Glossina morsitans morsitans, G. m. centralis* and *G. pallidipes*) which transmit *T. b. rhodesiense* and *T. b. brucei*. In contrast, areas of land alongside river systems and streams are generally populated by tsetse flies of the *palpalis* group (*G. palpalis, G. fuscipes* and *G. tachinoides*) which transmit *T. b. gambiense*. *T. vivax* and *T. congolense* are both transmitted by tsetse flies of the *morsitans* group. However, these divisions are not absolute. Both male and female flies are vectors and female flies require regular blood meals for larval development to be completed (Ford, 1971).

1.1.3 THE LIFE CYCLE

The life cycle of the salivarian trypanosomes is complex. There are two distinct parts to the developmental cycles of which one occurs in the mammalian host and the other in the tsetse fly, thus transmission is cyclical. Noncyclical transmission between host species has also been demonstrated (Duke *et al*, 1934). The general life cycle for *T. brucei* is described.

1.1.3.1 TRYPANOSOME DEVELOPMENT IN THE MAMMALIAN HOST

Infective metacyclics are deposited into the skin of the mammal when the tsetse fly takes a blood meal. A localised skin reaction, the "chancre", occurs at the site of inoculation. From the skin the metacyclics enter the bloodstream via the lymphatics. The metacyclics transform into slender forms and a multiplicative phase of exponential

growth ensues (reviewed in Vickerman, 1985). Division occurs by binary fission along the longitudinal plane of the trypanosome (Sherwin & Gull, 1989).

Slender forms are metabolically adapted to life in the mammal. The mitochondrion is inactive and uptake of host glucose, the main substrate for energy metabolism, occurs by active transport. Slender forms are covered with a replacable, antigenic glycoprotein coat, the variable surface glycoprotein (VSG). The process by which trypanosomes change their VSG coat is called antigenic variation and is described in section 1.3.2.3. The VSG coat can be visualised and identified by immunofluorescence using antibodies specific to the VSG, thus each distinct VSG is said to be of a particular variable antigen type (VAT). The VSG coat protects the trypanosome from both specific and nonspecific mechanisms of the host immune response (reviewed in Vickerman & Barry, 1982).

Some slender forms instead of undergoing division transform into stumpy forms (reviewed in Vickerman, 1985). The transformation process is not discrete but gradual with the appearance of intermediate morphological forms. Only stumpy forms are infective to the fly, (Wijers & Willet, 1960; Hoare, 1972). They are pre-adapted to survive in the tsetse fly because they possess a partially activated mitochondrion (reviewed in Vickerman, 1985).

1.1.3.2 TRYPANOSOME DEVELOPMENT IN THE TSETSE FLY VECTOR

Bloodstream trypanosomes are taken up by the tsetse fly with the bloodmeal but slender forms are rapidly killed. In the tsetse fly midgut a peritrophic membrane surrounds the bloodmeal within which stumpy forms shed their VSG coat,

acquire a coat of procyclin and transform into procyclic forms (reviewed in Roditi & Pearson, 1990). The function of the procyclin coat is not known.

Procyclic forms are elongate, divide by binary fission and are metabolically adapted to life in the tsetse fly vector in that they possess a fully functional mitochondrion and use the amino acid proline as their primary energy source. Populations of trypanosomes of procyclic morphology are generally established in the posterior midgut of the tsetse fly. In this region, division of procyclic forms occurs in both ecto- and endo-peritrophic spaces. Ecto-peritrophic trypanosomes which have moved forward to the proventriculus differentiate to non-dividing proventricular forms. From here they migrate to the salivary glands of the tsetse flies. In the salivary glands, the proventricular stage transform into the epimastigote form.

The epimastigotes divide attached by their flagella to the epithelium of the salivary glands. Transformation of the epimastigote to non-dividing metacyclic forms takes place in the salivary glands. The metacyclic forms are detached and lie free in the lumen of the salivary glands. This final stage in the developmental cycle of the trypanosomes in the tsetse fly is also metabolically adapted for survival in the mammal host. Metacyclic forms possess a partially functional mitochondrion and a VSG coat (reviewed in Vickerman, 1985).

The developmental cycles of both *T. congolense* and *T. vivax* in the mammalian host are essentially similar to that described for *T. brucei*. However in infections with these parasites the morphological distinction between trypanosomes

resembling the dividing slender form and those which resemble the vector infective stumpy form is not as apparent. The developmental cycle of *T. congolense* in the tsetse fly is similar to that for *T. brucei* except that there is no development of metacyclics in the salivary glands. The transformation of proventricular forms to epimastigote and that of epimastigote to metacyclic forms occurs in the proboscis and the hypopharynx. In contrast the entire developmental cycle of *T. vivax* in the tsetse fly is restricted to the proboscis (Hoare, 1972).

1.1.4 AFRICAN TRYPANOSOMIASIS

Tsetse flies of the genus *Glossina* are confined to an area of Africa known as the tsetse belt. This region extends over an estimated 11 million km^2 in which approximately 45 million people are at risk from infection with trypanosomes of *T. b. rhodesiense* and *T. b. gambiense* however only 10,000 cases are reported each year. Infections are generally restricted to sporadic outbreaks but have reached epidemic proportions in the past (Jordan, 1986).

Infections with *T. b. gambiense* are generally chronic lasting for several months to years whereas infections with *T. b. rhodesiense* are generally acute lasting for several weeks to months. Infections with both *T. b. gambiense* and *T. b. rhodesiense* are characterised by the invasion of the central nervous system (CNS) and other extravascular sites with the slender forms of the parasites, hence the name "sleeping sickness" (reviewed in Boreham, 1979; Poltera, 1985).

The animal trypanosomiases caused by *T. b. brucei*, *T. congolense* and *T. vivax* occur in a wide range of cattle,

domestic animals and all livestock and are more commonly known as Nagana (Hoare, 1972). The clinical symptoms of the diseases caused by these trypanosomes are virtually indistinguishable. Symptoms include fever, weight loss, anaemia and abortion is common amongst pregnant females (Jordan, 1986). Mixed infections of *T. b. brucei*, *T. congolense* and *T. vivax* in an individual host are not uncommon (Unsworth, 1953; Godfrey *et al*, 1963).

Not all cattle and livestock are equally susceptible to disease. Indigenous breeds of humpless cattle, the N'dama and Muturu (*Bos taurus*) are trypanotolerant. These breeds of cattle generally have lowered susceptibility to disease when compared with Zebu (*Bos indicus*) cattle which are highly susceptible. Infections generally produce mild symptoms and are characterised by a barely detectable parasitaemia. However, in areas heavily infested with tsetse flies (Murray *et al*, 1979) or under stress (Jordan, 1986) even trypanotolerant cattle will succumb to infection. Trypanotolerance has also been demonstrated in sheep and goats (reviewed in Griffin, 1978).

The importance of African trypanosomiasis lies in the limitations imposed upon land use. Within the tsetse belt vast areas of land with the potential for agriculture and rearing livestock are uninhabitable as a result of infestation with tsetse flies (Jordan, 1986). The situation is further complicated by the maintenance of trypanosome infections amongst reservoir hosts (Ashcroft *et al*, 1959). African trypanosomiasis is a debilitating disease resulting in loss of productivity in both human and cattle hosts which can have devastating effects in communities where

agriculture is cattle driven and dependent upon manual labour (Jordan, 1986).

1.2 THE COURSES OF INFECTION

The course of a trypanosome infection is characterised by the occurrence of several waves of parasitaemia. Each wave consists of an initial rise in parasite numbers which on reaching a peak of parasitaemia is followed by remission of the infection. Recrudescence of the infection initiates the second wave of parasitaemia (Fantham & Thompson, 1910, 1911; Ross & Thompson, 1910a, 1910b; Herbert & Parratt, 1979; Vickerman & Barry, 1982).

Previous studies have compared the courses of infection of strains derived from different species of trypanosome in a range of host species (Table 1.1), have compared trypanosome lines derived from a parent strain in one host species (Table 1.2) and have compared several strains derived from one species of trypanosome in a range of host species (Table 1.3). From the results of these studies, several characteristics of the courses of infection with trypanosomes are apparent. (1) Susceptibility to infection and the courses of subsequent infection differs between host species (Archibald & Riding, 1926; Ashcroft *et al*, 1959; Ashcroft, 1960; Goble *et al*, 1960; Godfrey, 1960; Barry, 1986), between individuals within a host species (Ashcroft *et al*, 1959; Goble *et al*, 1960; Godfrey, 1960) and between strains of a single species of host (Morrison *et al*, 1978; Levine & Mansfield, 1981; De Gee *et al*, 1982; Pinder, 1984; Roelants & Pinder, 1987). (2) The survival time of the host generally correlates with the parasitaemias observed

(Godfrey, 1960; Clayton, 1978; Inverso & Mansfield, 1983; Barry, 1986). (3) The course of infection is altered as a result of repeated passage of field isolates in laboratory rodents and other animals and that these differences are generally associated with decreased survival time and increased parasite load (Corson, 1936; Binns, 1938; Ashcroft, 1959a). (4) Lines of trypanosomes from different isolates differ in the courses of infection that they produce in a single host (Corson, 1936; Binns, 1938; Unsworth, 1953; Goble *et al*, 1960; Godfrey, 1960; Roelants & Pinder, 1987). On examining the studies shown in Tables 1.1-1.3 it can be seen that comparisons of the courses of infection were often made in outbred hosts and between uncloned stocks of trypanosomes.

1.3 FACTORS REGULATING THE COURSES OF INFECTION

The course of a trypanosome infection is regulated by a complex interaction between the host and the parasite (reviewed in Herbert & Parratt, 1979; Barry & Turner, 1991). External factors such as ambient temperature (reviewed in Herbert & Parratt, 1979; Otieno, 1972) and stress (Ferguson *et al*, 1970) have also been implicated but these are thought to act indirectly on infections by altering host physiology.

1.3.1 HOST DEPENDENT FACTORS REGULATING THE COURSES OF INFECTION

The influence of age and sex of host on the course of infection has been extensively investigated, with conflicting results (Ashcroft, 1959b; Lumsden *et al*, 1968; Greenblatt & Rosentreich, 1984; Pinder, 1984). However, the size of the host is recognised as an important factor in determining the course of infection (reviewed in Herbert &

Parratt, 1979). In a small rodent, for instance a potentially lethal level of parasitaemia may be attained before the induction of specific immune responses.

The genetic background of the host is also an important factor in regulating the course of infection. It is well known that the courses of infection differ amongst the diverse range of hosts which are susceptible to infection. A comprehensive list of these studies is given in Tables 1.1 and 1.2.

In the field, trypanotolerant and susceptible breeds of cattle differ in their ability to control trypanosome infections (Chandler, 1952; Desowitz, 1959). Previous studies attributed these differences to the genetic background of the host species (reviewed in Roelants, 1986).

In the laboratory, differences in the courses of infection are also apparent between strains of inbred mice (Table 1.2). The inheritance of susceptibility to infection has been extensively investigated in such strains of laboratory rodents. From these studies it would appear that susceptibility to infection is inherited as a recessive trait and appears not to be MHC regulated (Clayton, 1978; Morrison *et al*, 1978; Levine & Mansfield, 1981; Greenblatt *et al*, 1984; Pinder, 1984). It is not known whether inheritance of susceptibility in cattle is regulated by similar mechanisms.

The predominant host dependent factor in regulation of infection is the ability of the host to mount an effective humoral immune response (reviewed in Barry & Turner, 1991). Experimental studies in athymic nude mice have shown that control of *T. brucei* infection is primarily T-independent

(Campbell *et al*, 1978; Clayton *et al*, 1979). In an immunologically intact host the immune response is characterised by the production of VSG specific IgM antibodies against trypanosome VATs. Relatively little IgG antibody is produced. Trypanosome killing and clearance from the bloodstream is dependent upon VSG specific IgM causing complement-mediated lysis and phagocytosis of opsonised or complement-killed cells by macrophages(reviewed in Vickerman & Barry, 1982). Trypanosomes escape specific immune lysis and clearance by undergoing antigenic variation. Thus infections are chronic with a fluctuating parasitaemia (reviewed in Vickerman & Barry, 1982).

The ability to control parasitaemia is either reduced or absent in immunosuppressed hosts. The mechanisms of trypanosome induced suppression of the immune response have been extensively studied (reviewed in Vickerman & Barry, 1982). In chronic infections of *T. brucei*, IgG levels are suppressed almost completely whilst those of IgM are lowered but not to the same extent (Sacks & Askonas, 1980). In a study by Sacks *et al* (1980) it was also demonstrated that the degree of virulence is determined by the level of IgM suppression induced by a population of trypanosomes. However there is little evidence to suggest that the immunosuppression observed is specific to the VAT expressed by the trypanosomes (Dempsey & Mansfield, 1983; Diffley, 1983; Seed & Sechelski, 1988).

1.3.2 PARASITE DEPENDENT FACTORS REGULATING THE COURSES OF INFECTION

The principle parasite dependent factors regulating the courses of infection are, the rate of trypanosome

growth, the rate of differentiation from slender forms to stumpy forms and antigenic variation (reviewed in Barry & Turner, 1991).

1.3.2.1 TRYPANOSOME GROWTH

Growth of a population of trypanosomes is undoubtedly regulated by both intrinsic and extrinsic factors. Evidence for intrinsic regulation of growth comes from two sources. First, it has been demonstrated that populations of trypanosomes grow exponentially in mice until high densities are reached (Turner *et al*, 1986b). Second, parasite densities attained in a single species or strain of host can differ between lines of trypanosomes (Table 1.3).

The predominant extrinsic factor regulating trypanosome growth is that of the basic requirement for normal host serum (Desowitz & Watson, 1952; Lincicome & Hinnant, 1962; Greenblatt & Lincicome, 1966; De Gee *et al*, 1981). Also, Ballon-Landa *et al* (1985) and Turner & Barry (1989) observed that trypanosomes grown in chambers subcutaneously placed in mice grew at slower rates than those which were observed in the bloodstream populations. Several serum components have been identified to date, glucose and albumin (reviewed in Vickerman, 1985), epidermal growth factor (Hide *et al*, 1989), lipoproteins (Coppens et al, 1988; Black & Vanderweerd, 1989; Vanderweerd & Black, 1989) and transferrin (Schell, 1991). Other serum components, the production of which is induced by the host as a direct result of parasite infection, have also been shown to promote trypanosome growth. For example, tumour necrosis factor, a product of activated macrophages (Kongshavn *et al*, 1988) and interferon-γ, a product of

activated T cells (Bakhiet *et al*, 1990; reviewed in Olsson *et al*, 1992) have been shown to promote trypanosome growth in *T. musculi* and *T. brucei* infections of mice respectively. Trypanosome uptake of host serum components occurs by receptor mediated endocytosis via clathrin coated vesicles located in the membrane of the flagellar pocket (Vickerman, 1985; Coppens *et al*, 1988; Hide *et al*, 1989).

However, not all serum components promote trypanosome growth. The trypanolytic factor (TLF) of human serum is a high density lipoprotein (HDL) fraction found to occur in humans and ground dwelling primates endemic to the tsetse belt (Hajduk *et al*, 1989; Seed *et al*, 1990; Pierce *et al*, unpublished results cited in Hajduk *et al*, 1992). Lysis of *T. b. brucei* trypanosomes occurs as a direct result of fusion of HDL particles with lysosomes and preferentially kills slender form parasites (Rifkin, 1978; reviewed in Hajduk *et al*, 1992).

Trypanosome growth is also susceptible to density dependent effects. At high concentrations the parasites rapidly deplete the serum of host glucose thus limiting trypanosome growth (and killing the host) (Herbert *et al*, 1975). The rate of trypanosome growth has also been shown to decrease when parasitaemias greater than approximately 3 x 10^8 trypanosomes/ml of blood are attained (Diffley, 1987).

1.3.2.2 DIFFERENTIATION OF TRYPANOSOMES

During the rising phase of parasitaemia, slender forms predominate over stumpy forms. Remission of infections is characterised by the predominance of stumpy forms. When absolute (rather than relative) numbers of slender and stumpy forms are determined during the course of an

infection, it would appear that stumpy forms are produced at a constant rate during the exponential phase of trypanosome growth. Thus the observed dramatic increase in the prevalence of stumpy forms after the peak of parasitaemia could be attributed to a reduction in the numbers of slender forms present (Balber, 1972). The results of these studies showed that differentiation is intrinsically regulated and not induced by antibody (Balber, 1972).

To explain the reduction in numbers of slender forms at the peak of parasitaemia Black *et al* (1985) proposed that at a high parasite density, depletion of a host derived nutrient factor necessary for trypanosome replication, could induce transformation of slender forms to stumpy forms. As an alternative, Seed & Sechelski (1989) proposed that at a high parasite density cell-cell contact of trypanosomes could act as a signal for cessation of division and initiate the transformation process. However the results of recent studies by McLintock (1990) contradicted those of Black *et al* (1985) and Seed & Sechelski (1989). It was shown that there did not appear to be a marked reduction in rates of replication at high parasite densities and suggested that a reduction in the numbers of slender form trypanosomes may be caused by selective immune lysis and clearance of slender forms.

A number of compounds such as the anti-inflammatory agents indomethacin (Jack *et al*, 1984) and cortisone (Ashcroft, 1957), the immunosuppressive agent busulphan (Ormerod *et al*, 1974) and the polyamine inhibitor difluoromethylornithine (De Gee *et al*, 1984; Giffin *et al*, 1988) have been shown to promote differentiation.

Differentiation is inhibited by theophylline (Reed *et al*, 1985).

1.3.2.3 ANTIGENIC VARIATION

The predominant trypanosome dependent factor regulating the courses of infection is that of antigenic variation (reviewed in Turner, 1984; Vickerman, 1989; Barry & Turner, 1991). In the mammalian host, slender form parasites have the capacity to replace their VSG coat with that of an antigenically unrelated type. Non-dividing metacyclic and stumpy form trypanosomes also possess a VSG coat, however antigenic variation has not been demonstrated in these forms. The genetic and molecular basis of antigenic variation has been extensively studied (reviewed in Pays & Steinert, 1988; Borst, 1991). Each VSG coat is the expressed product of a single gene resident in an active expression site of which there are approximately 20 in the genome. An estimated 1000 VSG genes are thought to exist in *T. brucei* and, in one study, 101 different VATs were identified in a single infection (Capbern *et al*, 1977). Active expression sites are located at chromosome telomeres. Expression of a VSG gene can occur by one of two main mechanisms. VSG genes which are telomeric and located in an expression site may be activated *in situ*. VSG genes located internally within chromosomes, however, are activated by duplication of the gene and transposition of the expression linked copy formed to an active expression site. The order of expression of VSG genes is not random and occurs in a characteristic sequence in an infection. Telomeric VSG genes are expressed early in an infection, followed by nontelomeric (internal) genes.

Metacyclic VSG genes are all located on telomeres of large chromosomes, in expression sites which are structurally different from those used in bloodstream forms and all VSG genes are expressed *in situ* (Graham & Barry, 1991). Other differences in metacyclic VAT expression exist. Expression of the metacyclic VAT (M-VAT) repertoire is independently regulated (Turner *et al*, 1986a). Also the M-VAT repertoire is restricted in size, only a small subset (<27 VATs) of the bloodstream VAT repertoire is expressed (Turner *et al* 1988)

During the course of a *T. brucei* infection, VATs are expressed in a partially predictable manner (Gray, 1965a; Capbern *et al*, 1977). In cyclically transmitted infections the M-VATs are the first to be detected followed by the VAT which initiated the infection in the tsetse fly (Hajduk & Vickerman, 1981; Barry & Emery, 1984). Bloodstream VATs are then expressed in a "semi-predictable" sequence (reviewed in Turner, 1984; Vickerman, 1989; Barry & Turner, 1991). If the sequence of expression of VATs is interupted, whether by uptake of parasites by tsetse fly (Le Ray *et al*, 1977) or by syringe (Gray, 1965b) then transfer of trypanosomes to a new host 'restarts' the sequence of expression. Hierarchial expression of bloodstream VATs is not restricted to *T. brucei* and has also been shown to occur in infections with *T. congolense* (Nantulya & Doyle, 1980), *T. vivax* (Dar, 1972; De Gee *et al*, 1979; Barry, 1986) and *T. equiperdum* (Capbern *et al*, 1977).

Three mechanisms by which the ordered sequence of appearance of VATs could be generated have been proposed: (1) VAT related growth rates, (2) VAT specific switching

rates and (3) the influx of trypanosomes from extravascular sites (expressing novel VATs) into the bloodstream (reviewed in Turner, 1984; Vickerman, 1989; Barry & Turner, 1991).

Variable antigen expression has been directly linked with trypanosome growth rates (reviewed in Herbert & Parratt, 1979; Barry & Turner, 1991). Several studies have shown that when combinations of different VATs are inoculated into mice, one VAT grows faster and predominates over the slower growing VATs. Thus an order of sequence of appearance of VATs can be defined based on differences in growth rates (Van Meirvenne *et al*, 1975; Seed, 1978; Miller & Turner, 1981; Seed *et al*, 1984; Myler *et al*, 1985). More accurate comparisons of population doubling times, however, have given conflicting results (Barry *et al*, 1979; Myler *et al*, 1985). Also the use of mathematical models to predict the order of appearance of VATs on the basis of differences in growth rates alone, indicate that an ordered sequence of appearance of VATs is unlikely to be generated using this process (Kosinski, 1980; Agur *et al*, 1989).

The frequency with which trypanosomes replace their surface coat, the switching rate, has been estimated as 2.0-9.3 x 10^{-3} switches/cell/generation in fly-transmitted infections (Turner & Barry, 1989). Switching is a directed process in that there is a tendency for switching to occur to some VATs at higher rates than to others (Turner & Barry, 1989) and the overall rate probably remains fairly constant throughout the course of an infection (Barry & Turner, 1991). However, switching rates are decreased as a result of repeated syringe-passaging in laboratory rodents (Lamont *et al*, 1986; Turner, 1991 unpublished results). It can be

envisaged that in a population of trypanosomes of mixed VATs, highly directed rates of switching would result in some VATs predominating over others, these VATs would then be cleared from circulation by the immune response allowing other VATs to increase in their relative abundance (Barry & Turner, 1991; Turner & Barry, 1989). Thus an ordered sequence of appearance of VATs could be generated based on differences in VAT-specific switching rates.

Trypanosomes rapidly disseminate from the site of inoculation to the bloodstream via the lymphatics (Willet & Gordon, 1957; Barry & Emery, 1984). From the bloodstream some trypanosomes will disperse into a number of extravascular sites including the CNS (reviewed in Boreham, 1979; Poltera, 1985). Populations of trypanosomes established in extravascular sites have been shown using indirect methods to be antigenically different from those observed in the bloodstream (Seed & Effron, 1973; Tanner *et al*, 1980; Seed *et al*, 1984). On the basis of these results it has been suggested that an ordered sequence of appearance of VATs could be generated as a result of immune clearance of bloodstream trypanosomes and subsequent "reseeding" by trypanosomes expressing different VATs from extravascular sites (Seed *et al*, 1984). Evidence that this is unlikely was forwarded by Turner *et al* (1986) who, using more direct methods, demonstrated that trypanosome populations obtained from several extravascular sites were antigenically similar to each other and to the bloodstream populations.

1.4 AIMS OF THE STUDY

The aim of this study was to investigate how trypanosome growth may regulate the course of an infection.

Three studies were conducted each of which addressed a different aspect of trypanosome growth; these were

(1) To accurately determine and compare the courses of infection of a group of cloned lines of *T. brucei* in inbred mice.

(2) To compare growth and differentiation rates in cloned lines of *T. brucei* known to exhibit differences in the courses of infection produced in mice and to determine whether changes in rate values could explain these differences.

(3) To reexamine the relationship between variable antigen expression and trypanosome growth rates in *T. brucei* and to determine whether VAT expression influenced growth rates.

Table 1.1

Studies in which the courses of infection with a line of trypanosomes were compared in a range of host species. This list is incomplete in that only those studies where the main objective was to compare the courses of infection have been included.

a- For each study the method of detection of parasitaemia was designated as S for examination of stained bloodsmears, W for examination of wet blood films, H for haemocytometer counts and NS where the method of detection was not stated.

b- In those studies where the mean survival time was determined, this data is given as the number of days except where otherwise stated. The mean survival time determined after repeated passage of a line is shown in brackets. ND indicates those studies or animals where the mean survival time was not determined.

Information has also been included where possible as to whether the animals were killed by the infection, K, or not killed by the infection, NK, during the period in which they were monitored. Animals in which infections did not become established are indicated as NI.

c- For those studies where parasitaemia profiles were presented an attempt has been made to classify the overall parasite load, P, as + for those infections in which the the parasitaemia tended to remain below detectable levels, ++ for a relapsing parasitaemia which tended to remain below antilog 7.0 trypanosomes/ml of blood, +++ for a relapsing parasitaemia which tended to remain above antilog 8.0 trypanosomes/ml of blood and ++++ for those infections with no relapse of parasitaemia.

Table 1.1

Author	Method of detection[a]	Trypanosome species/strain	Host species	Mean survival time (days)[b]	P[c]
Fantham & Thompson, 1910, 1911	S	T. gambiense	rats	13.8	++
			guinea-pigs	111	+++
		T. rhodesiense	rats	11.3	++++/++
			rabbits	27	
			guinea-pigs	59	
Archibald & Riding, 1926	NS	T. rhodesiense			
		man 1	monkey	17	
			desert rat	10-44	
			rat	31	
			white rat	30-35	
			rabbit	42	
			guinea-pig	53	
			lizard *Varanus niloticus*	NI	
		man 2	dog	6	
			white rat	14	
			rabbit	15	
			monkey	18	
			gerbil	18-23	
			guinea-pig	34	
			goat	NK	
Carmichael, 1934	NS	T. brucei strain Kasinga	ntalaganya	19 months	
			reedbuck	NK	
			waterbuck	NK	
			bush-pig	NI	
			jackal	ND	
		T. congolense strain Mbarara	ntalaganya	NK	
			bush-pig	NI	
			oribi	"	
			reedbuck	"	
			waterbuck	"	

Table 1.1 cont.

Binns, 1938	W	*T. congolense*		
		strain Limbe	guinea-pig	38
			rat (3 of 6 NI)	67.3 (40.3)
			rabbit	NI
		strain Midima	guinea-pig	NI
			rabbit	"
			rat	"
		strain Chikwawa A	guinea-pig (1 of 2 NI)	39
			rat (6 of 8 NI)	69.5 (37)
		strain Lunzu A	rat (9 of 12 NI)	40
			guinea-pig (1 of 2 NI)	51
Corson, 1939	NS	*T. rhodesiense* fly transmitted	dikdik	3-21 weeks
			bushbuck	NK
			duiker	"
			eland	"
			impala (1 of 3 NI)	"
			reedbuck	"
			steinbock	"
			Thompson's gazelle (1 of 2 NI	"
Ashcroft *et al*, 1959	S	*T. brucei* strain Shinyanga I	steinbock	3 months
			Thompson's gazelle	4-16 months
			bohor reedbuck	NK
			monkeys	NK
			bush-pig	NI
			porcupine	NI
		T. rhodesiense strain Tinde	monkeys	65
			dikdik	46-148
			Thompson's gazelle	4-16 months
			bohor reedbuck	NK
			bush-pig	"
			common duiker	"
			eland	"
			impala	"
			porcupine	"
			wart-hog	"

Table 1.1 cont.

Ashcroft, 1960	H	T. rhodesiense Tinde		
		strain Kahama	rats	20-80
			rabbits	64.5
			monkeys	59.3
		strain Wellcome	rats	4-6
			monkeys	51.5
			rabbits	78
			guinea-pigs	NK
			sheep	NK
Goble et al, 1960	NS	T. brucei		
		strain Waghorn-C	dogs	5-20
			monkeys	19
		strain Waghorn-S	guinea-pigs	K
			dogs	NK
			rabbits	NK
			monkeys	NI
		T. gambiense		
		strain Wellcome TS	dogs	16-47
			monkeys	39-116
			rabbits	NK
		T. rhodesiense		
		strain Kahama	dogs	<14
			monkeys	27-49
		strain Wellcome CT-G	dogs	20-27
			monkeys	NK
		strain Wellcome CT-T	dogs	15-20
			monkeys	59

Table 1.1 cont.

Godfrey, 1960	W	T. congolense			
		strain Zaria	rats	30-62	++++
			sheep	NK	+
		strain Ekpoma	baboons	NI	
			cattle	NK	+
			dogs	"	+
			rats	"	+
			sheep	"	+
		strain Ibadan	rats	34	++++
			sheep (2 of 3 NK)	42	++
			cattle	NK	++
		strain Vom	rats	14-22	++++
			dogs	6-34	++
			sheep (35 of 50 NK)	13-59	++
			cattle	42	++
			baboons	NI	
		strain Donga	rats	9-18	++++
			cattle	42	
			sheep	NK	++
Barry, 1986	H	T. vivax strain Zaria Y486			
		ILDat 1.1	rats	8	+/++
			mice	14	+++
			goats	21.5	++/+++
			cows	NK	++
			rabbits	NK	+
		ILDat 1.9	mice (1 NK)	15	++/+++
			goats	31.5	++/+++
			rabbits	NK	+
		ILDat 1.23	mice (1 NK)	13	++/+++
			goats	29	++
			rabbits	NK	+
		ILRAD V28	rats	18.3	++/+++
			rabbits (2 of 3 NK	54	+/++
			cows	NK	++

Table 1.2

Studies in which the courses of infection with a line of trypanosomes were compared in different genetic strains of a single host species. This list is incomplete in that only those studies where the main objective was to compare the courses of infection have been included. For details, see legend to Table 1.1. Also: O = other method of detection of parasitaemia and

d- TREU- Trypanosomiasis Research Edinburgh University

NIM- National Institute for Medical Research

EATRO- East African Trypanosomiasis Research Organisation

ILRAD- International Laboratory for Research on Animal Diseases

ILDat- International Laboratory for Research on Animal Diseases antigen type

LouTat- Louisville *Trypanozoon* antigen type

Table 1.2

Author	Method of detection[a]	Trypanosome species/strain[d]	Host species/strain	Mean survival time (days)[b]	P[c]
Desowitz, 1959	W	T. vivax	cattle- Zebu	NK	++
			N'dama	NK	++
			Muturu	23	++++/++
Herbert & Lumsden, 1968	W	T. brucei TREU 164	mice- CERU	10.6±1.8	
			- TUCK	6.8±0.6	
Clayton, 1978	W	T. b. brucei Lister S42 NIM 6	inbred mice		
			CBA/H x C57Bl/6	28±3.8	+++/++
				30.9±5.1	++
				28±4.6	
			CBA/H	19.7±1.8	+++
				19.9±3.1	
				19.9±3	
			C3H/HE	15.4±3.0	+++/++++
			Balb/c	20.5±3.8	+++
			DBA/2	25.3±2.2	++
			C57Bl/6	29.6±3.4	++
Morrison et al, 1978	H	T. congolense EATRO 209 strain 5E-12	inbred mice		
			A/J	15.8	++++
			SWR/J	16.9	++
			129/J	22.6	++++
			DBA/1J	36.3	++
			Balb/c	49.5	++++
			C3H/HeJ	59.0	++
			AKR/A	81.7	++++
			C57Bl/6J	110.2	++
			C57Bl/6J x A/J	133.2	++

Table 1.2 cont.

Levine & Mansfield, 1981	O	T. rhodesiense EATRO 1886 LouTat 1.0	inbred mice		
			C3HeB/FeJ	18.6±2.6	
			Balb/cByJ	30.4±5.7	
			CBA/J	33.9±1.3	
			A/J	36.7±2.0	
			C57B1/6J	43.6±1.8	
			B10.BR/SgSn	51.3±2.6	
			B10.D2nSn	53.2±3.9	
			B10.A/SgSn	53.6±4.0	
			C57B1/10Sn	57.2±1.8	
De Gee et al 1982	H	T. vivax strain Zaria Y486	inbred mice		
			Balb/c	10,13	+++
			A/J	11	
			SWR	22	
			AKR	25	
			C57B1/10	31	++
			C57B1/6	32	
			B10.D2n	39	+++
Pinder, 1984	R	T. congolense Dinderesso/80/ CRTA/3	inbred mice		
			A/J	14.7±3.8	
			CBA/J	18.5±2.0	
			Balb/c	20.6±3.6	
			AKR/J	30.0±7.6	
			C57B1/6J	NK	
Roelants & Pinder, 1987	W	T. congolense Dinderesso/80/ CRTA/3 (DiNat 3.1)	inbred mice		
			Balb/c	ND	++++
			CBA/J	"	+++
			C57B1/6	"	++
		Samandeni/82/ CRTA/28 (DiNat 1.1)	Balb/c	"	++++
			C57B1/6	"	++++
			CBA/J	"	++++
		Karankasso/83/ CRTA/57 (DiNat 4.1)	CBA/J	"	++
			Balb/c	"	++
			C57B1/6	"	+

Table 1.3

Studies in which the courses of infection with several lines of one species of trypanosome were compared in a single host species. This list is incomplete in that only those studies where the main objective was to compare the courses of infection have been included. For details, see legend to Table 1.1.

Table 1.3

Author	Method of detection[a]	Trypanosome species/strain	Host species/strain	Mean survival time (days)[b]	P[c]
Blacklock & Yorke, 1913	NS	T. vivax			
		passage 35 in goat	rabbits	15	
		passage 38 in goat	rabbits	26	
Corson, 1936	NS	T. rhodesiense			
		strain Maswa 1	goat	36 (35)	
		" Maswa 3	"	39 (>51)	
		" Maswa 2	"	43 (53)	
		strain Maswa 4	rat	27 (15)	
		" Ushirombo 3	"	43 (9)	
		" Ushirombo 1	"	47 (17)	
		" Maswa 5	"	48 (23)	
		" Ushirombo 2	"	48 (15)	
		" Ushirombo 4	"	65 (15)	
Binns, 1938	W	T. congolense			
		strain Lisungwe	rat (2 of 6 NI)	28 (11)	
		" Lunzu A	" (9 of 12 NI)	40	
		" Lunzu B	" (4 of 12 NI)	43.6	
		" Port Herald	" (3 of 6 NI)	45.7 (25.5)	
		" Limbe	" (3 of 6 NI)	67.3 (40.3)	
		" Chikwawa A	" (6 of 8 NI)	69.5 (37)	
		" Chikwawa B	"	NI	
		" Chiromo	"	"	
		" Midima	"	"	
		" Ntalaganya	"	"	
		strain Limbe	guinea-pig	38	
		" Chikwawa A	" (1 of 2 NI)	39	
		" Lunzu A	guinea-pig (1 of 2 NI)	51	
		" Midima	guinea-pig	NI	
		strain Limbe	rabbit	NI	
		" Midima	"	"	

Table 1.3 cont.

Corson, 1939	NS	T. rhodesiense	sheep	214
		fly-sheep passage, passage number 28 from man	sheep	NK
		sheep-antelope line, passage number 16 from man	sheep	158(NK)
Unsworth, 1953	NS	T. vivax		
		area 8 Bokkos	Zebu	27
		" 7 Toro	"	34.7
		" 6 Mokwa	"	50.7
		" 1 Gitata	"	75.9
		" 2 Lumu	"	76.1
		" 4 Katabu	"	127.6
		" 5 Shendam	"	129.4
		" 3 Jema'a	"	<140
Ashcroft, 1959	NS	T. rhodesiense strain Tinde		
		line-monkey/sheep	rats	27 (62)
		- sheep	"	49 (43)
		- antelope	"	58 (34)
		"	"	62 (30)
Ashcroft, 1960	H	T. rhodesiense Tinde		
		strain Wellcome	rat	4-6
		" Kahama	"	20-80
		strain Kahama	rabbit	64.5
		" Wellcome	"	78
		strain Wellcome	monkey	51.5
		" Kahama	"	59.3

Table 1.3 cont.

Goble et al, 1960	NS	T. brucei			
		strain Waghorn-C	dog	5-20	
		" Waghorn-S	"	NK	
		T. gambiense			
		strain Wellcome TS	dog	16-47	
		T. rhodesiense			
		strain Kahama	dog	<14	
		" Wellcome CT-T	"	15-20	
		" Wellcome CT-G	"	20-27	
		T. brucei			
		strain Waghorn-C	monkey	19	
		" Waghorn-S	"	NI	
		T. gambiense			
		strain Wellcome TS	monkey	39-116	
		T. rhodesiense			
		strain Kahama	monkey	27-49	
		" Wellcome CT-T	"	59	
		" Wellcome CT-G	"	NK	
		T. brucei			
		strain Waghorn-S	rabbits	NK	
		T. gambiense			
		strain Wellcome TS	rabbits	NK	
Godfrey, 1960	W	T. congolense			
		strain Donga	rat	9-18	++++
		" Vom	"	14-22	++++
		" Zaria	"	30-62	++++
		" Ibadan	"	34	++++
		" Ekpoma	"	NK	+
		strain Vom	sheep (35 of 50 NK)	13-59	++
		" Ibadan	sheep (2 of 3 NK)	42	++
		" Donga	"	NK	++
		" Ekpoma	"	"	+
		" Zaria	"	"	+
		strain Donga	cattle	42	
		" Vom	"	42	++
		" Ibadan	"	NK	++
		" Ekpoma	"	NK	+
		strain Ekpoma	baboon	NI	
		" Vom	"	"	
		strain Vom	dog	6-34	++
		" Ekpoma	"	NI	+

Table 1.3 cont.

Herbert & Lumsden, 1968	W	T. brucei			
		TREU 87	mice- CERU	ND	
		" 164	"	10.6±1.8	
		TREU 87	mice- TUCK	ND	
		" 164	"	6.8±0.6	
McNeillage & Herbert, 1968	W	T. brucei			
		TREU 284 (ETat 2)	mice-	6.5	
		" 280 (" 3)	Swiss CD-TO	7.1	
		" 396 (" 5)		7.7	
		" 398 (" 6)		8.9	
		" 295 (" 4)		10.5	
		" 289 (" 1)		11.0	
Godfrey & Killick-Kendrick, 1967	W	T. brucei			
		Isolate 8/18	chimpanzee	ND	+++
		T. rhodesiense			
		Uganda			
		Isolate 8/8/105A	"	42	++
		T. gambiense			
		Nigeria	"	ND	++
Clayton, 1978	W	T. b. brucei			
		Lister S42 NIM 2	inbred mice		
			CBA/H x C57Bl/6	22±1.2	+++
		Lister S42 NIM 6	"	28±3.8	+++/++
			"	30.9±5.1	++
			"	28±4.6	
Inverso & Mansfield, 1983	H	T. rhodesiense			
		EATRO 1886			
		LouTat 1.5	inbred mice	28±0.9	+++
		LouTat 1.4	C57Bl/10 SnJ	30±1.3	+++
		LouTat 1.3		44±1.7	++
		LouTat 1.0		62±1.5	++

Table 1.3 cont.

Roelants & Pinder, 1987	W	*T. congolense* Dinderesso/80/ CRTA/3 (DiNat 3.1)	inbred mice Balb/c	ND	++++
		Samandeni/82/ CRTA/28 (DiNat 1.1)	Balb/c	"	++++
		Karankasso/83/ CRTA/57 (DiNat 4.1)	Balb/c	"	++
		DiNat 1.1	CBA/J	"	++++
		DiNat 3.1	"	"	+++
		DiNat 4.1	"	"	++
		DiNat 1.1	C57Bl/6	"	++++
		DiNat 3.1	"	"	++
		DiNat 4.1	"	"	+

CHAPTER 2

A COMPARISON OF THE COURSES OF INFECTION IN LINES OF *Trypanosoma brucei* IN MICE

2.1 INTRODUCTION

The courses of trypanosome infection have been shown to differ between strains and subspecies of *Trypanosoma brucei* (Fantham & Thompson, 1910,1911; Ashcroft *et al*, 1959; Ormerod, 1963; Godfrey & Killick-Kendrick, 1967), between strains of *Trypanosoma congolense* (Godfrey, 1960; Roelants & Pinder, 1987) and *Trypanosoma vivax* (Barry *et al*, 1986) (see Table 1.3). However, a problem with the interpretation of the results of these studies is that comparisons of the courses of infection were made between stocks that were uncloned and/or in outbred hosts. Therefore the differences in the courses of infection observed could not be entirely attributed to intrinsic differences between trypanosomes of different stocks. There have been no reported studies of a direct comparison of the courses of infection between cloned stocks of *T. brucei* in a single inbred host strain. The question of whether cloned lines of trypanosomes differ in the courses of infection that they produce will be addressed in this chapter.

The maintenance of field isolates by repeated syringe-passaging in laboratory rodents and other mammals has been shown to change the courses of infection observed with the field isolates (Corson, 1936; Ashcroft, 1959a, 1960; McNeillage & Herbert, 1968; Soltys & Woo, 1969; Barry *et al*, 1979; De Gee *et al*, 1982; Inverso & Mansfield, 1983; Diffley, 1985; Inverso *et al*, 1988). In these studies the observed changes in the courses of infection of the laboratory adapted strains were reported as changes in growth characteristics. It has also been shown that field isolates of trypanosomes can be manipulated in the

laboratory to produce trypanosome lines which differ with respect to degree of pleomorphism and homogeneity of VAT expression (Turner, 1990). No formal comparison has been made of these changes which arise as a result of repeated passage of field isolates.

The aims of this study were; (1) to accurately determine the parasitaemia profiles of six cloned stocks of *Trypanosoma brucei* in a single inbred strain of mouse, and (2) to accurately determine the parasitaemia profiles of two rapid passaged lines and compare the course of infection with that of the original cloned stock. The cloned stocks were chosen because they are all within 10 mouse passages either of the original isolate, or of transmission through tsetse flies. Any differences between the lines, therefore, were considered to have resulted from selection in the field before isolation rather than after it in the laboratory.

2.2 MATERIALS AND METHODS

2.2.1 TRYPANOSOMES

Trypanosomes of six cloned stocks were used in this study: these were EATRO (East African Trypanosomiasis Research Organisation) 2340, EATRO 1216, EATRO 795, STIB (Swiss Tropical Institute Basel) 386, STIB 247 and TREU (Trypanosomiasis Research Edinburgh University) 927/4. STIB 247 was syringe passaged three times a week in adult female CFLP or CD1 mice. Stabilates were made of trypanosomes at passage 49 (STIB 247/49) and passage 80 (STIB 247/80). The pedigrees of these various stocks and lines are given in the Appendix.

2.2.2 THE COURSES OF CHRONIC INFECTIONS

Trypanosomes of each line were grown from stabilate in adult female CFLP mice and trypanosome-infected blood was diluted in Hanks balanced salts solution (0.15M, pH 7.2) with Heparin (10 IU/ml). For each line, parasite densities were accurately determined using an improved Neubauer haemocytometer and trypanosome-infected blood appropriately diluted in 0.85% ammonium chloride solution. 1×10^6 trypanosomes were inoculated by intraperitoneal (i.p.) injection into each of 6 age matched adult (34-36 weeks) female Balb/c mice. Parasitaemias in subsequent infections were monitored 6 times a week by haemocytometer counts of trypanosomes in samples of tail blood until mice were removed from the study. The minimum level of detection was antilog 4.6 trypanosomes/ml of blood. For those mice in which the parasitaemia was not detectable it was assumed that antilog 4.5 trypanosomes/ml of blood were present for the purposes of analysing the data.

To assess the effects of the number of trypanosomes in an inoculum on the course of an infection one group of mice was also i.p. inoculated with 1×10^4 STIB 247 trypanosomes/mouse. The size of the inoculum is representative of the numbers of metacyclics ejected by the tsetse fly into the mammalian host during the course of a bloodmeal (Hoare, 1972; Barry *et al*, 1983; Harley, 1966).

All mice were monitored for signs of clinical disease associated with infection. All mice were examined 6 times a week for low levels of locomotor activity, difficulty in movement, laboured breathing, pallor of skin and membranes and scruffiness of coat. Any mice judged to be exhibiting

other than neglible or mild symptoms of disease were removed from the study. Mice with a parasitaemia greater than or equal to antilog 9.0 trypanosomes/ml of blood were also removed from the study. At this density the trypanosomes are competing with the host for blood glucose, resulting in severe hypoglycaemia and inevitable death of the host (Herbert *et al*, 1975).

2.3 RESULTS

2.3.1 A COMPARISON OF CLONED STOCKS

Parasitaemia profiles obtained for each cloned stock in individual mice are shown in Figs. 2.1-2.6. A comparison of these figures shows clear differences between cloned stocks. There is also variation in the courses of infection between individual mice within a cloned stock. Thus it was observed that expressing the mean parasitaemias of all mice within a cloned stock disguised some important features of the course of infection in individual mice. A comparison of Figs. 2.7(a) and 2.7(b) and Figs. 2.1-2.6 shows this clearly. It was for this reason that the results for all mice were included in Figs. 2.1-2.6.

2.3.1.1 THE COURSES OF INFECTION WITHIN CLONED STOCKS

The pattern of infection obtained with EATRO 2340 is shown in Fig. 2.1. In 5 of 6 mice there was no remission of infection which for the purposes of this comparative study was arbitrarily defined as a drop in parasitaemia greater than one order of magnitude between consecutive time points. The infections plateaux on day 4 post infection (p.i.) in mice m1, m2, m3 and m5 and on day 3 p.i in m4 and m6 with a

parasitaemia greater than antilog 8.0 trypanosomes/ml of blood in 4 of 5 mice. Remission of infection occurred in m4, the parasitaemia on remission dropped to antilog 7.1 trypanosomes/ml of blood, recrudescence of the infection occurred and the parasitaemia continued to rise until the host was removed from the study. The time from inoculation of the mice to the removal of those from the study ranged from 17 to 42 days.

The pattern of infection obtained with EATRO 1216 is shown in Fig. 2.2. The peak of the first parasitaemic wave was on day 6 p.i. in m2 and on day 4 p.i. in all other mice. The parasitaemia on the day of the first peak was between antilog 7.0-7.3 trypanosomes/ml of blood. In all mice this first peak was followed by remission of infection. On remission the parasitaemia dropped to between antilog 4.5-4.9 trypanosomes/ml of blood on days 7-8 p.i. Recrudescence of the infection occurred in all mice. In m2 and m4 recrudescence of the infection was followed by a continual rise in parasitaemia until the host was removed from the study. A second remission occurred in the four other mice. In these mice the parasitaemia on remission dropped to between antilog 6.3-7.3 trypanosomes/ml of blood. A third and fourth cycle of remission and recrudescence of infection occurred in m1, m5 and m6 and m1 and m5 respectively. The time from inoculation of the mice to the removal of those mice from the study ranged from 42 to 72 days p.i.

The pattern of infection obtained with STIB 247 is shown in Fig. 2.3. Infections in all mice reached the peak of the first parasitaemic wave on day 3 p.i. In 3 of 6 mice the parasitaemia at the first peak was not greater than

antilog 7.0 trypanosomes/ml of blood. With infections in m1, m4 and m6 the parasitaemia at the first peak was not greater than antilog 7.4 trypanosomes/ml of blood. Remission of the infection occurred in all mice. On remission the parasitaemia dropped to between antilog 4.5-4.6 trypanosomes/ml of blood on days 5-6 p.i. In m2, m3 and m6 the parasitaemia on recrudescence of the infection reached a density of antilog 7.1-8.0 trypanosomes/ml of blood and plateaux. A second cycle of remission and recrudescence of infection occurred in m1, m4 and m5, a third and fourth cycle occurred only in m1. The time from inoculation of the mice to the removal of those mice from the study ranged from 44 to 45 days.

The pattern of infection obtained with STIB 386 is shown in Fig. 2.4. Infections in all mice reached the first peak of parasitaemia on day 5 p.i., with a parasitaemia not greater than antilog 8.0 trypanosomes/ml of blood. Remission of the infection occurred in all mice and the parasitaemia on remission dropped to between antilog 4.5-5.2 trypanosomes/ml of blood on days 6-7 p.i. In m2 and m6 the parasitaemia on recrudescence of the infection continued to rise until the host was removed from the study. A second remission occurred in all other mice. The subsequent recrudescence of the infection was followed by a third remission in m1. The time from inoculation of the mice to the removal of those mice from the study ranged from 20 to 42 days.

The pattern of infection obtained with EATRO 795 is shown in Fig. 2.5. The peak of the first parasitaemic wave was on day 4 p.i. in all mice, with a parasitaemia greater

than antilog 8.0 trypanosomes/ml of blood in 5 of 6 mice. Remission of the infection occurred in all mice, the parasitaemia on remission dropped to between antilog 4.5-5.3 trypanosomes/ml of blood on day 7 p.i. The parasitaemia on recrudescence of the infections plateaux in all mice. The time from inoculation of the mice to the removal of those mice from the study ranged from 33 to 43 days.

The pattern of infection obtained with TREU 927/4 is shown in Fig. 2.6. The peak of the first parasitaemic wave was on day 5 p.i. with infections in m1, m3 and m6 and on day 4 p.i. in m2, m4 and m5. In all mice the parasitaemia at the first peak was not greater than antilog 8.2 trypanosomes/ml of blood. Remission of the infection occurred in 5 of 6 mice, the parasitaemia on remission dropped to between antilog 5.3-6.3 trypanosomes/ml of blood on days 6-26 p.i. A second remission occurred only in m2. The time from inoculation of the mice to the removal of those mice from the study ranged from 20 to 39 days.

2.3.1.2 DIFFERENCES IN THE COURSES OF INFECTION BETWEEN CLONED STOCKS

The mean parasitaemia profiles of the cloned stocks are shown in Figs. 2.7(a) and 2.7(b). The peak of the first parasitaemic wave of infection and the first trough are clearly visible in all cloned stocks. However it was observed that because of small differences in timing of troughs between mice within a cloned stock, subsequent troughs were apparent only as an increase in variance.

In order to compare the differences between the cloned stocks, therefore, a summary of data extracted from the results shown in Figs. 2.1-2.6 was constructed describing

the parasitaemias in terms of a representative set of characteristics (Table 2.1). Remissions of infections of an order of magnitude or greater between two consecutive time points (troughs) were a consistent feature of infection with the lines EATRO 1216, STIB 247, STIB 386, EATRO 795 and TREU 927/4, infections with each had 3, 2, 2, 1 and 1 median no. of troughs respectively. This was in contrast with EATRO 2340 where no troughs were observed in 5 of 6 mice.

The first peak of parasitaemia occured on days 3-5 p.i. in all cloned stocks. The prevalence at the peak of the first parasitaemic wave of infection was approximately antilog 7.0 trypanosomes/ml of blood in infection with EATRO 1216 and STIB 247 and was approximately one order of magnitude higher in the other cloned stocks.

The proportion of time for which the parasitaemia was greater than antilog 8.0 trypanosomes/ml of blood in the first 20 days of infection was highest in infections with EATRO 2340 and lowest in infections with EATRO 1216. The proportion of time for which the parasitaemia was less than antilog 7.0 trypanosomes/ml of blood in the first 20 days of infection was highest in infections with EATRO 1216 and lowest in infections with EATRO 2340 (Table 2.1). The proportion of time for which the parasitaemia was greater than antilog 8.0 trypanosomes/ml of blood and less than antilog 7.0 trypanosomes/ml of blood for the cloned stock STIB 247 was intermediate between EATRO 2340 and EATRO 1216. The proportion of time for which the parasitaemia was greater than antilog 8.0 trypanosomes/ml of blood and less than antilog 7.0 trypanosomes/ml of blood for the cloned

stocks STIB 386, EATRO 795 and TREU 927/4 were intermediate between EATRO 2340 and STIB 247.

The mean day of removal from the study is not a reliable statistic, it has been included since it is most directly comparable with the day of death or survival time. This latter statistic is the most widely used in comparative studies of trypanosome virulence, a comprehensive list of which is given in Tables 1.1-1.3. Mice infected with EATRO 1216 harboured mild infections for the longest period of time, up to 51 days. This was in contrast with TREU 927/4 and EATRO 2340 where infections were mild for only 29 and 31 days respectively. Other stocks produced values intermediate between these extremes.

On the basis of the results shown in Figs. 2.1-2.6 and Table 2.1, it was possible to rank the cloned stocks in increasing order of their overall parasite load which correlates with the "severity" of infection: EATRO 1216, STIB 247, STIB 386, EATRO 795, TREU 927/4 and EATRO 2340. Infections with EATRO 2340 had the highest parasite load. Infections were characterised by a parasitaemia with a high plateau and no remissions. The parasitaemia remained greater than antilog 8.0 trypanosomes/ml of blood for almost 50% of the total infection. In contrast, infections with EATRO 1216 were mild, characterised by the occurrence of several cycles of remission and recrudescence of parasitaemia. The parasitaemia tended not to rise above antilog 8.0 trypanosomes/ml of blood, and infections were chronic with mice harbouring mild infections for approximately 51 days.

Infections with the cloned stock STIB 247 had an overall parasite load intermediate between EATRO 2340 and

EATRO 1216. Infections were characterised by the occurrence of two cycles of remission and recrudescence of parasitaemia. The parasitaemia tended not to rise above antilog 8.0 trypanosomes/ml of blood, and infections were chronic with mice harbouring mild infections for approximately 45 days.

Infections with the cloned stocks EATRO 795, STIB 386 and TREU 927/4 had overall parasite loads which were intermediate between the extremes observed in infections with EATRO 2340 and STIB 247. The observed differences between the lines can be most clearly seen in Fig. 2.8.

2.3.2 THE EFFECT OF RAPID SYRINGE-PASSAGING ON THE COURSE OF INFECTION WITH THE CLONED STOCK STIB 247

The parasitaemia profiles obtained for the lines STIB 247/49 and STIB 247/80 are shown in Figs. 2.9 and 2.10 respectively and the mean parasitaemias for both lines are shown in Fig. 2.11.

In infections with STIB 247/49 the peak of the first parasitaemic wave of infections was on day 3 p.i. in m3 and m4 and on day 4 p.i. in all other mice. The parasitaemia at the peak of the first parasitaemic wave of infection was between antilog 8.4-8.6 trypanosomes/ml of blood in all mice. Infections with STIB 247/49 were characterised by a single remission with a trough on days 6-7 p.i. and the parasitaemia on remission dropped to between antilog 4.5-5.0 trypanosomes/ml of blood. On recrudescence of the infection the parasitaemia continued to rise until the host was removed from the study. Infections in all mice were mild for 16 days.

In infections with STIB 247/80 the peak of the first parasitaemic wave was on day 3 p.i. in m4 and m5, day 4 p.i. in m1, m3 and m6 and on day 5 p.i. in m2. Remission of the infection occurred in all mice but m3 (which was removed from the study) with troughs on day 6 p.i. in m1, m4, m5 and m6 and on day 7 p.i. in m2. The parasitaemia on remission dropped to between antilog 4.5-6.9 trypanosomes/ml of blood. In the four mice in which infections recrudesced, the parasitaemias continued to rise until the hosts were removed from the study. The time from inoculation of the mice to the removal of those mice from the study ranged from 4 to 18 days p.i.

A comparison of the parasitaemia profiles obtained for the syringe passaged lines with that of the cloned stock STIB 247 shows that the parasitaemia increased with passage number. This increase is apparent from a comparison of the characteristics of the three shown in a summary table of data extracted from Figs. 2.3, 2.9 and 2.10 (Table 2.2) and in a comparison of parasitaemia curves (Fig. 2.12).

Infections with STIB 247, STIB 247/49 and STIB 247/80 each had 2, 1 and 1 median no. of troughs respectively. The first peak of parasitaemia occurred on days 3-4 p.i. for the cloned stocks STIB 247 and the lines STIB 247/49 and STIB 247/80. The prevalence at the peak of the first parasitaemic wave of infection was antilog 8.9 trypanosomes/ml of blood in STIB 247/80, antilog 8.4 trypanosomes/ml of blood in STIB 247/49 and antilog 7.0 trypanosomes/ml of blood in STIB 247. The proportion of time for which the parasitaemia was greater than antilog 8.0 trypanosomes/ml of blood in the first 16 days of infection

was largest in STIB 247/49 and smallest in STIB 247. The proportion of time for which the parasitaemia was less than antilog 7.0 trypanosomes/ml of blood in the first 16 days of infection was largest in STIB 247 and smallest in STIB 247/49. The time from inoculation of the mice to the removal of those mice from the study was approximately 45 days in STIB 247, approximately 16 days in STIB 247/49 and approximately 13 days in STIB 247/80.

On the basis of these results it was possible to rank the lines in order of increasing severity of infection, STIB 247, 247/49 and 247/80 but with relatively little difference between the last two.

2.3.3 THE EFFECT OF INOCULUM DOSE ON THE COURSE OF INFECTION WITH THE CLONED STOCK STIB 247

In order to determine whether the size of the inoculum dose influenced the resultant parasitaemia, a comparison was made between infections resulting from an i.p. inoculation of 1×10^6 and 1×10^4 STIB 247 trypanosomes/mouse. The parasitaemia profiles obtained for each inoculum dose in individual mice are shown in Figs. 2.3 and 2.13. A comparison of these figures and of the results obtained from these figures shown in Table. 2.3 suggest that differences in inoculum dose of up to two orders of magnitude have relatively little influence on the overall parasite load of infection with the cloned stock STIB 247.

There was no difference in the median no. of troughs observed when the inoculum dose was reduced to 1×10^4 trypanosomes/mouse. The prevalence at the peak of the first parasitaemic wave of infection was antilog 7.8 trypanosomes/ml of blood when the inoculum dose was 1×10^4

trypanosomes/mouse and antilog 7.0 trypanosomes/ml of blood when the inoculum dose 1 x 10^6 trypanosomes/mouse. There was negligible difference between the two groups in the proportion of time for which the parasitaemia was greater than antilog 8.0 trypanosomes/ml of blood or less than antilog 7.0 trypanosomes/ml in the first 20 days of infection. Infections with both doses of inoculum were chronic. All mice harboured mild infections for approximately 45 days when the inoculum dose was 1 x 10^6 trypanosomes/mouse and approximately 51 days when the inoculum dose was 1 x 10^4 trypanosomes/mouse.

2.4 DISCUSSION

All of the cloned stocks had different parasitaemia profiles and differed in their "severity" of infection. The highest parasitaemias were produced by EATRO 2340 and the lowest by EATRO 1216. The other cloned stocks STIB 386, EATRO 795, TREU 927/4 and STIB 247 were intermediate between these two in the order: STIB 247, STIB 386, EATRO 795 and TREU 927/4. The expected pattern of undulating parasitaemia characteristic of chronic infections (Fantham & Thompson, 1910,1911; Ross & Thompson, 1910a, 1910b; reviewed in Herbert & Parratt, 1979; Vickerman & Barry, 1982) occurred on infection with the cloned stocks STIB 247 and EATRO 1216. This pattern of infection was generally not observed for the other cloned stocks. A possible explanation for this could be the mouse strain used in this study. Comparisons of the courses of infection between different mouse strains have shown Balb/c mice to be particularly susceptible to trypanosome infection (Clayton, 1978; Levine & Mansfield, 1981; Pinder, 1984; Roelants & Pinder, 1987). The overall

parasite load of infection with STIB 247 increased on rapid syringe-passage of this cloned stock. Infections resulting from these passaged lines had greater overall parasite loads than that which was observed on infection with the cloned stock EATRO 2340. These results will be discussed in greater detail in the next chapter.

In addition to the differences observed between cloned stocks and between lines of different passage history, more minor differences in the course of infection were also observed between individual mice within a line. Since age and sex matched inbred mice were used these differences are difficult to attribute to variation in the immunological status of the hosts. The prescence of mouse hepatitis virus (MHV) was demonstrated in the animal facility. MHV is a causative agent of liver necrosis and diarrhoea in mice (cited in Carthew *et al*, 1978). Thus the health status of mice infected with MHV may explain the differences in the course of infections observed between mice within a cloned stock. Although there is no evidence of MHV affecting trypanosomes either directly or by modulating immune responses. Alternatively, inbred mice are greater than 99 percent genetically identical (Poole, 1987) and the remaining small percentage difference in genotype may account for the differences in the courses of infection observed. Also random variation around the mean value inoculum dose of 1 x 10^6 trypanosomes may explain the differences in the courses of infection observed. However the results shown in Table 2.3 suggest that this last suggestion is unlikely.

The experimental design of this study had several advantages over previous studies. Parasitaemias were determined accurately and in inbred mice, overall parasite loads were determined directly from the parasitaemia profiles and all cloned stocks were within 10 mouse passages either of the original isolates or of transmission through tsetse flies, therefore it was possible to compare the lines directly.

Several previous studies have suggested that severity of infection is dependent upon the intrinsic growth rate of a population of trypanosomes (reviewed in Herbert & Parratt, 1979; Myler *et al*, 1985; Diffley *et al*, 1987), thus the role of trypanosome growth rates in determining the severity of infection will be investigated in the following chapter.

Figs. 2.1-2.6 The parasitaemia profiles obtained for the cloned stocks EATRO 2340, EATRO 1216, STIB 247, STIB 386, EATRO 795 and TREU 927/4 in individual mice. Parasite densities are expressed as the log. no. of trypanosomes/ml of blood, m refers to the mouse no.

Fig. 2.1 EATRO 2340

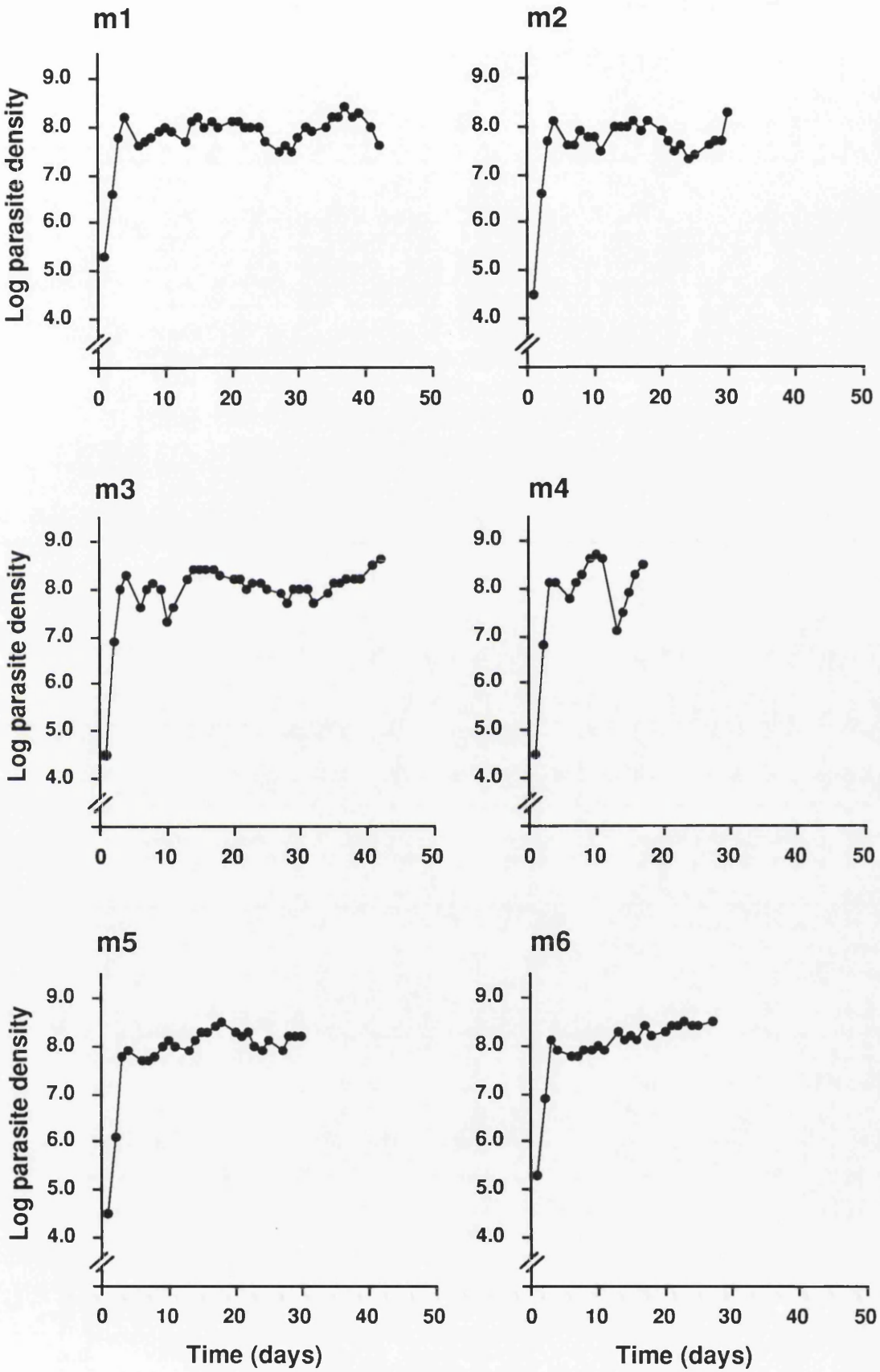


Fig. 2.2 EATRO 1216

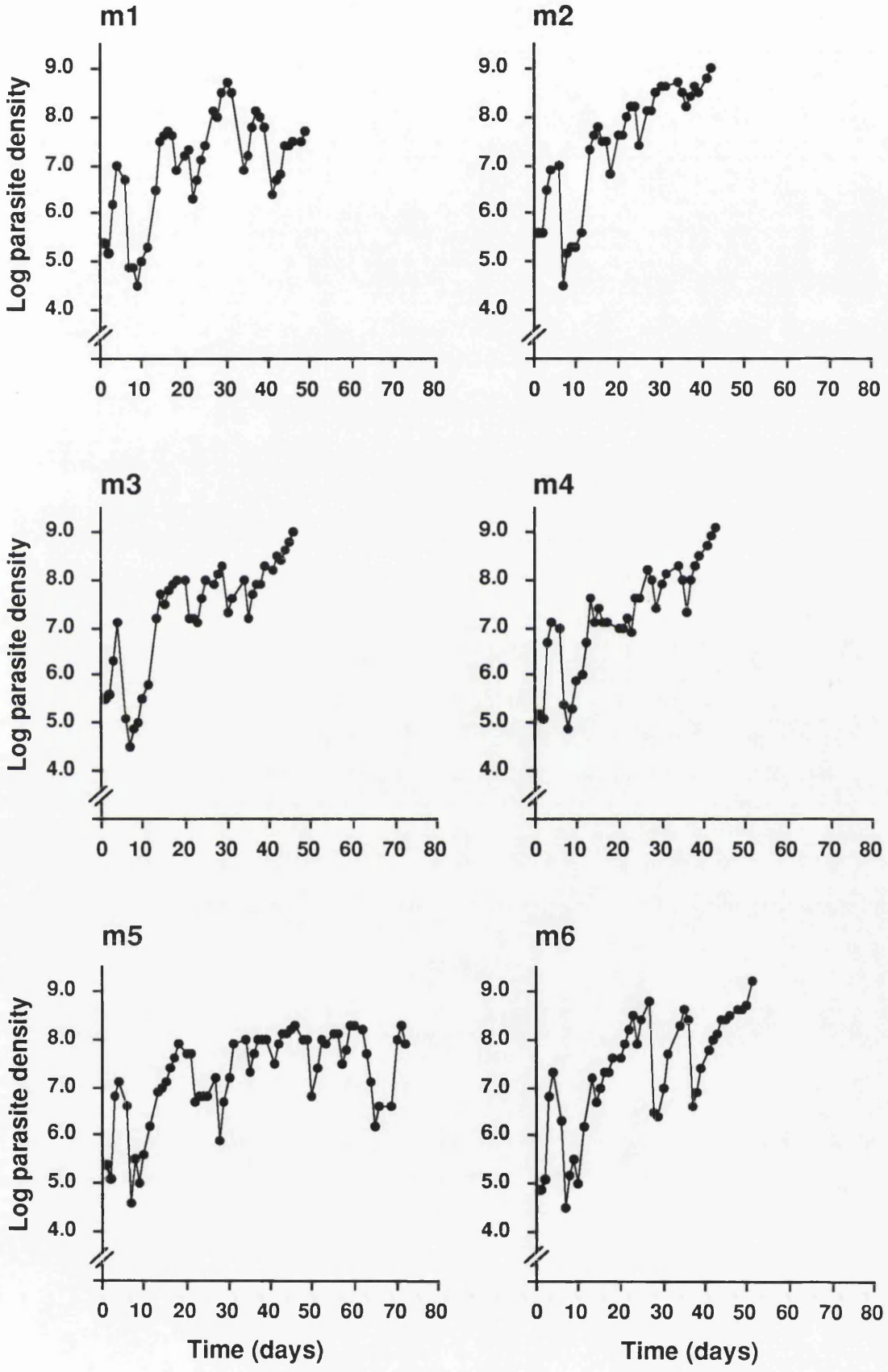


Fig. 2.3 STIB 247

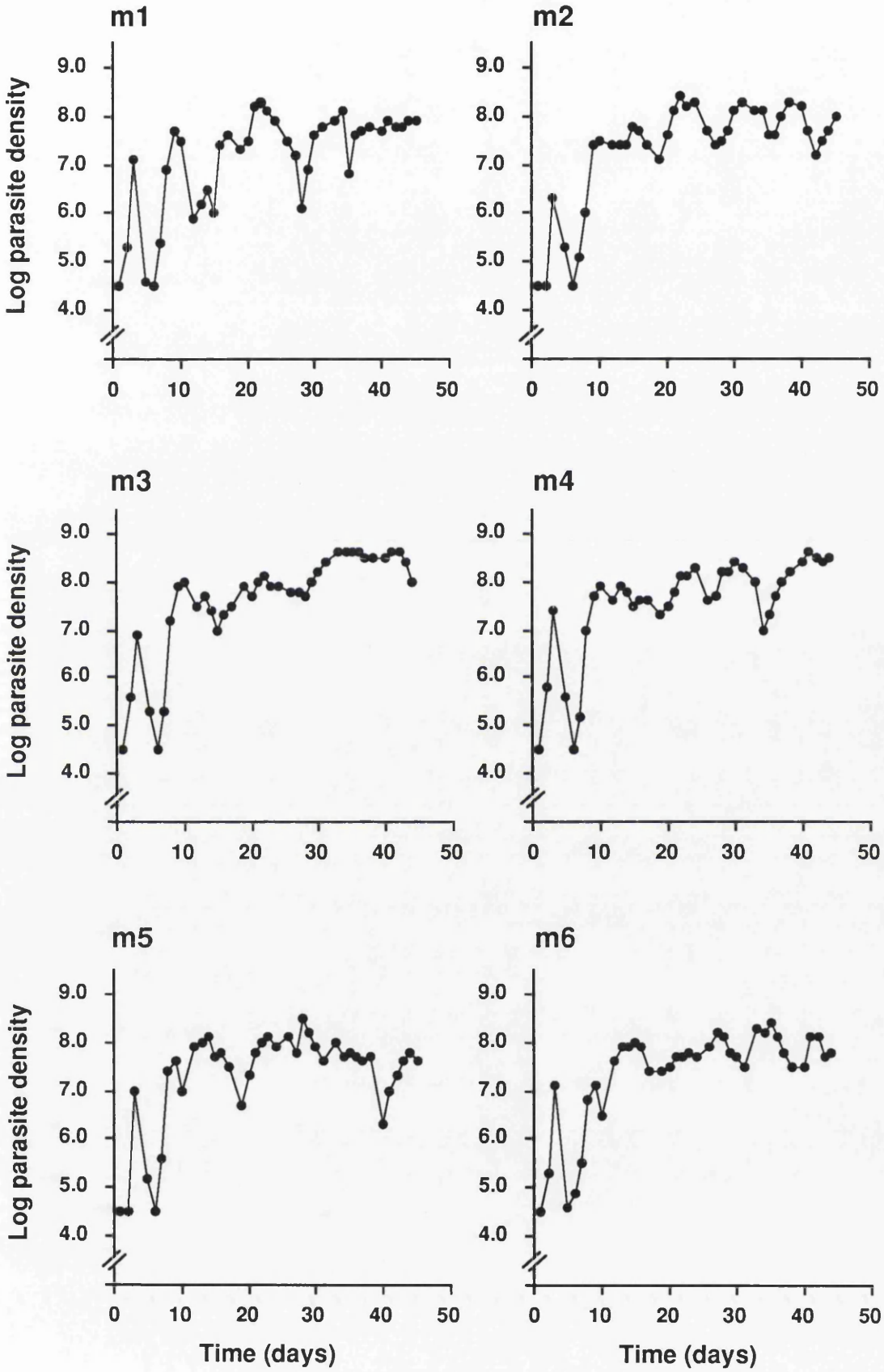


Fig. 2.4 STIB 386

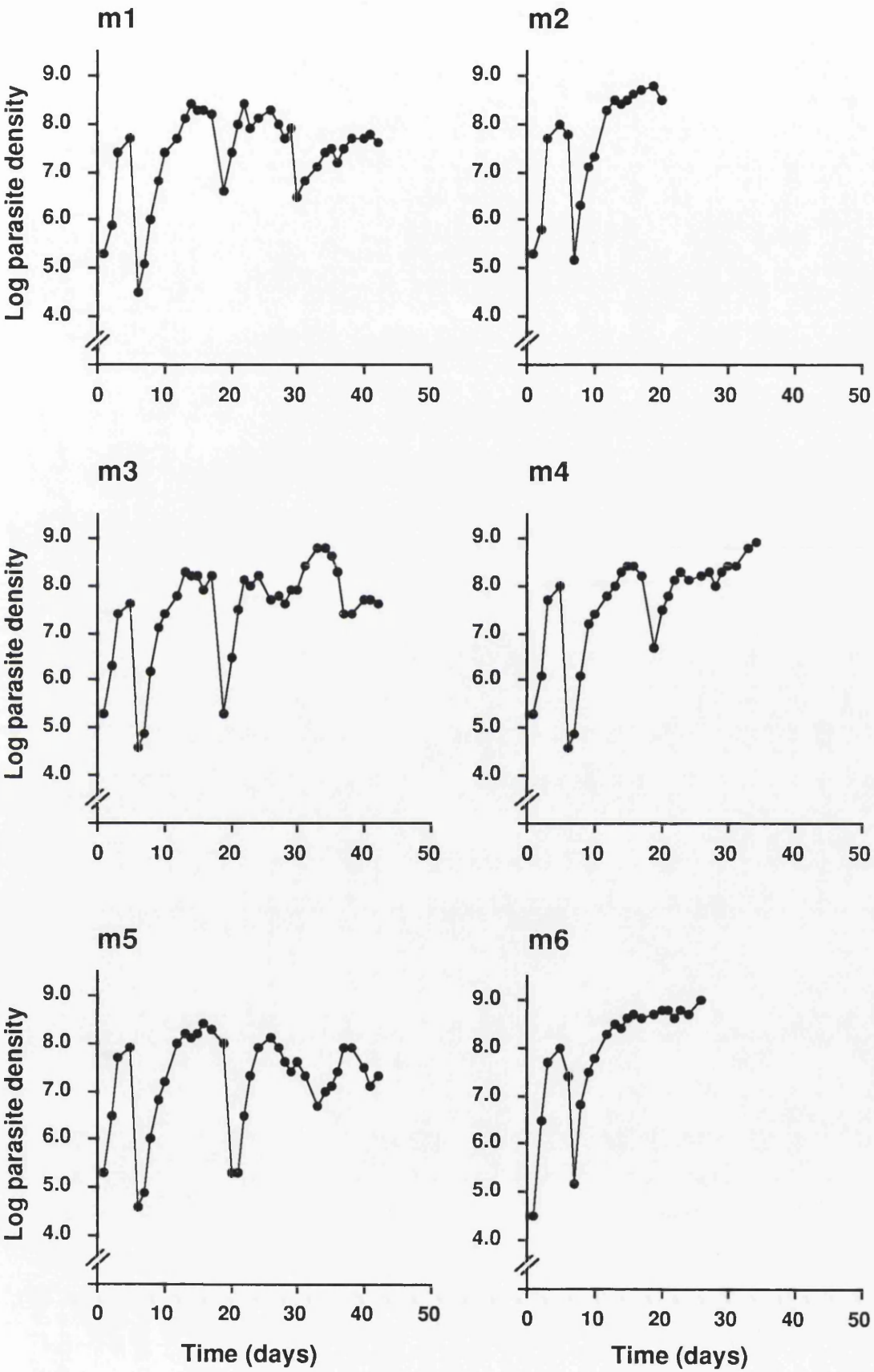


Fig. 2.5 EATRO 795

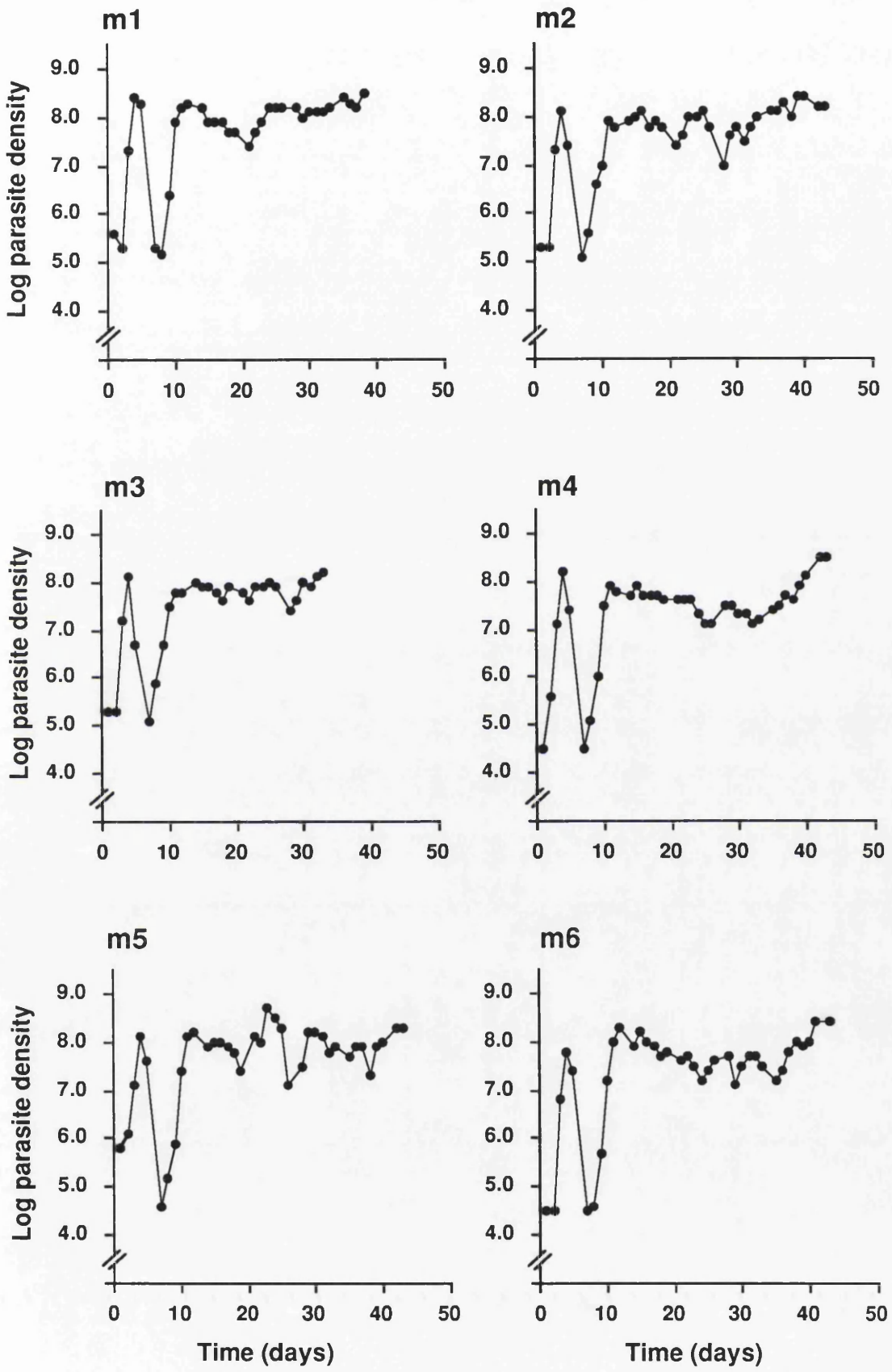
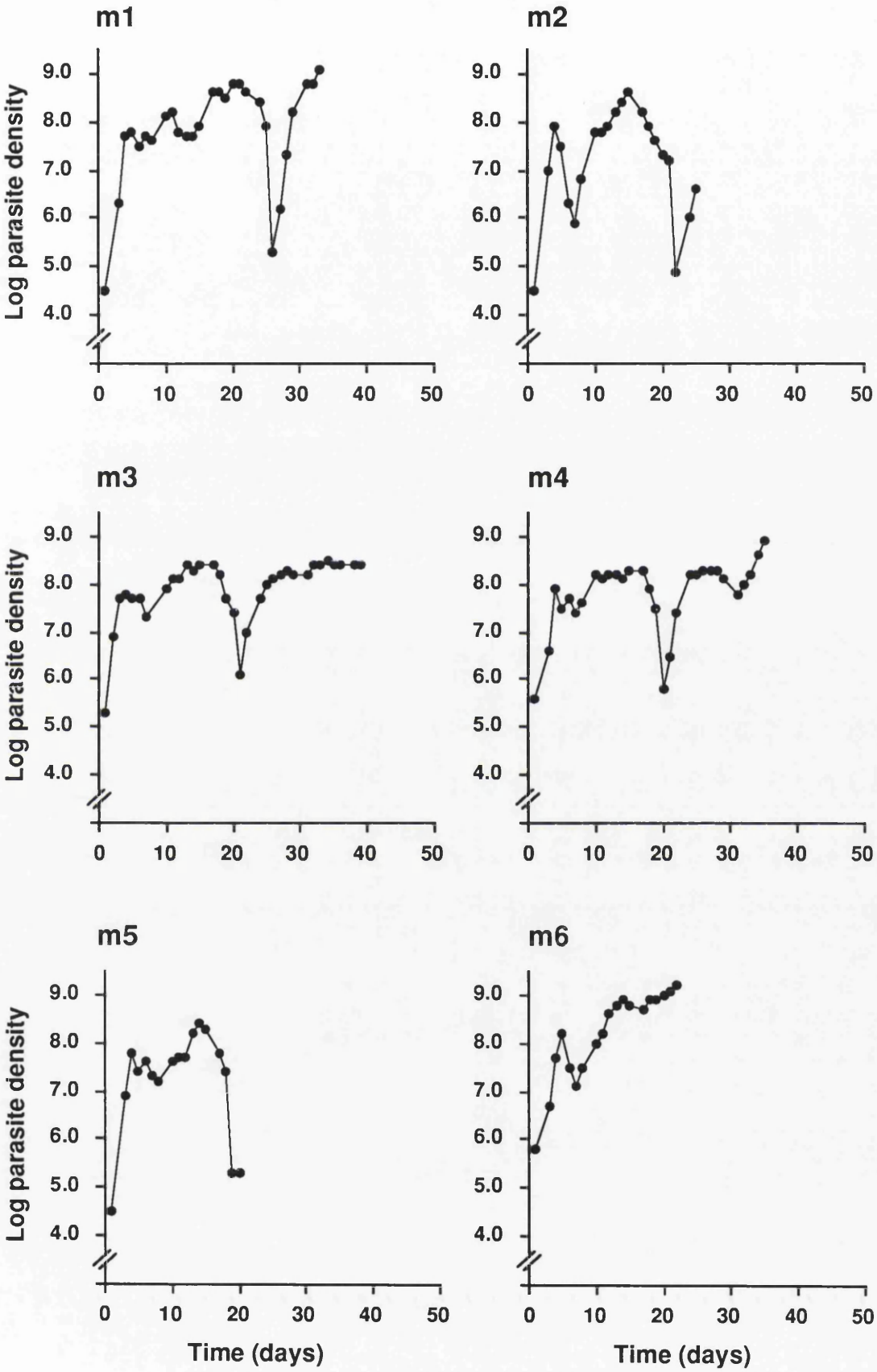


Fig. 2.6 TREU 927/4



Figs. 2.7(a) and **2.7(b)** The mean parasitaemia profiles of the cloned stocks EATRO 2340, EATRO 1216, STIB 247, STIB 386, EATRO 795 & TREU 927/4 in mice, n=6, geometric mean ± 2SE.

▼ denotes removal of a mouse from the study . On those days where more than one mouse was removed from the study the numbers of mice removed is indicated.

Fig. 2.7(a)

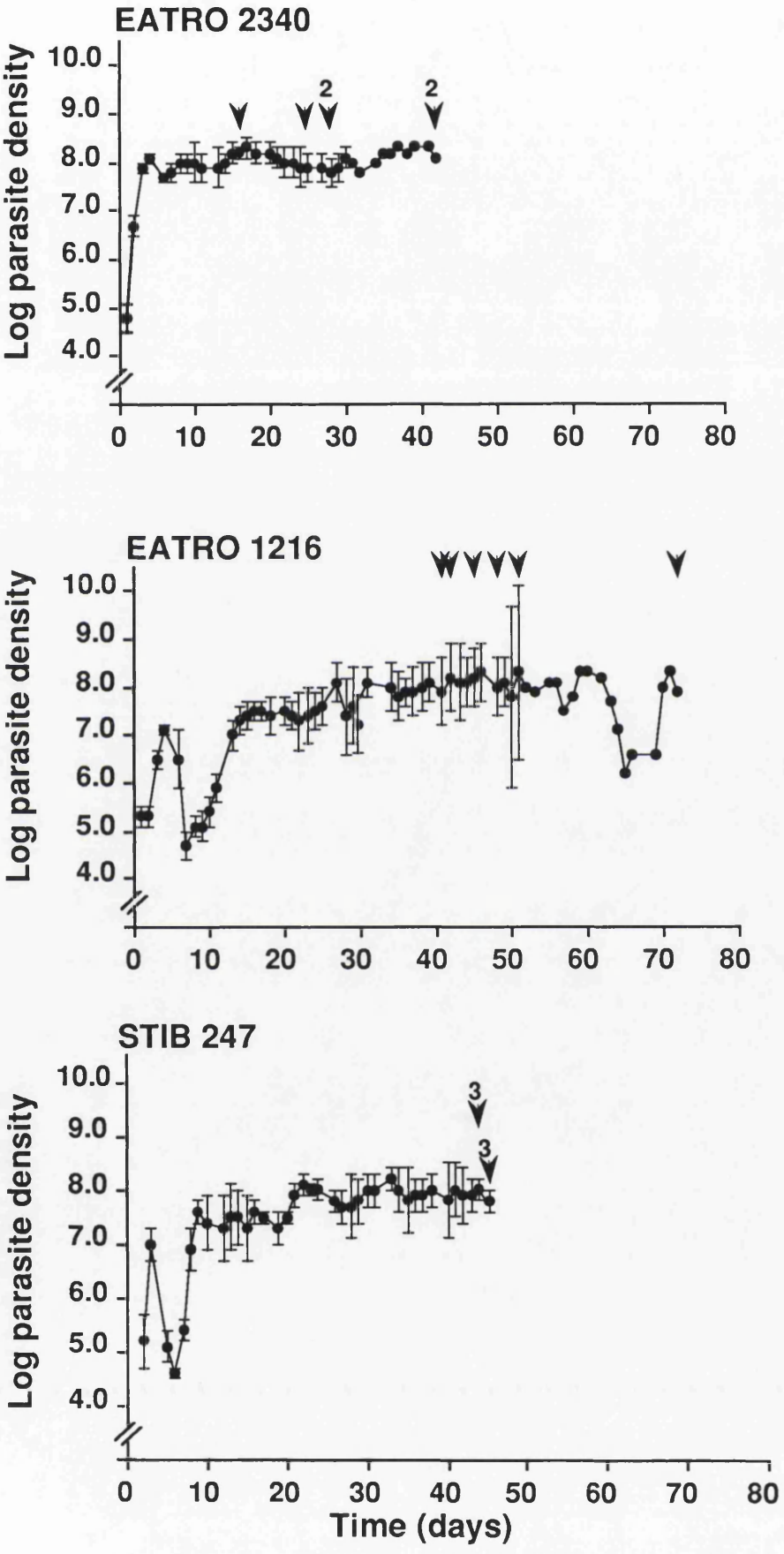

EATRO 2340
Log parasite density
10.0
9.0
8.0
7.0
6.0
5.0
4.0
2
2
0
10
20
30
40
50
60
70
80
EATRO 1216
Log parasite density
10.0
9.0
8.0
7.0
6.0
5.0
4.0
0
10
20
30
40
50
60
70
80
STIB 247
Log parasite density
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3
3
0
10
20
30
40
50
60
70
80
Time (days)

Fig. 2.7(b)

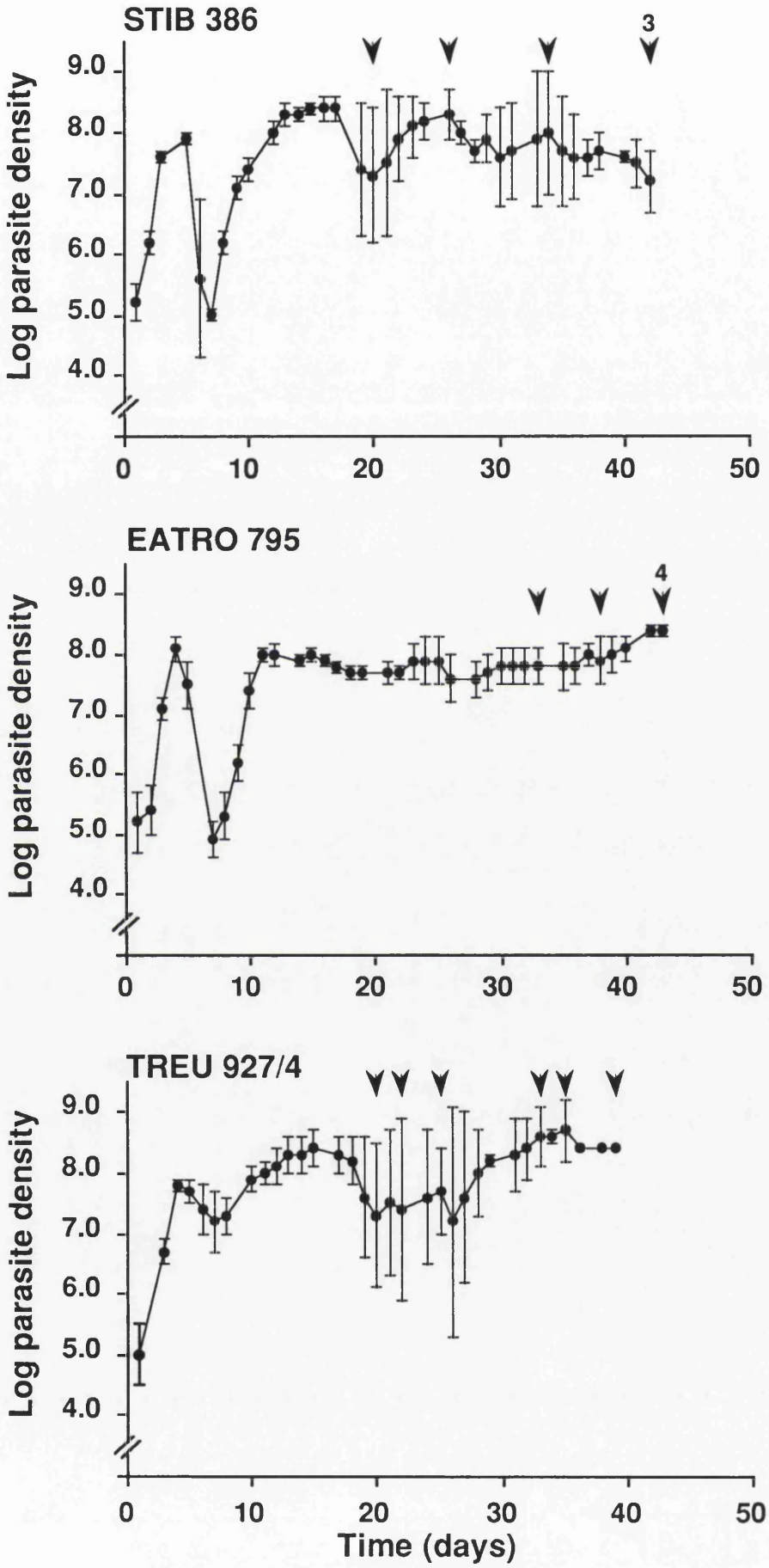

STIB 386
3
Log parasite density
9.0
8.0
7.0
6.0
5.0
4.0
0
10
20
30
40
50
EATRO 795
4
Log parasite density
9.0
8.0
7.0
6.0
5.0
4.0
0
10
20
30
40
50
TREU 927/4
Log parasite density
9.0
8.0
7.0
6.0
5.0
4.0
0
10
20
30
40
50
Time (days)

Table 2.1 Parasitaemia characteristics of six cloned stocks, determined from the results shown in Figs. 2.1-2.6.

a - A trough is arbritrarily defined as a drop in parasitaemia greater than one order of magnitude between adjacent points.

b - Geometric means of the parasitaemia on the peak of the first parasitaemic wave of infection with each stock.

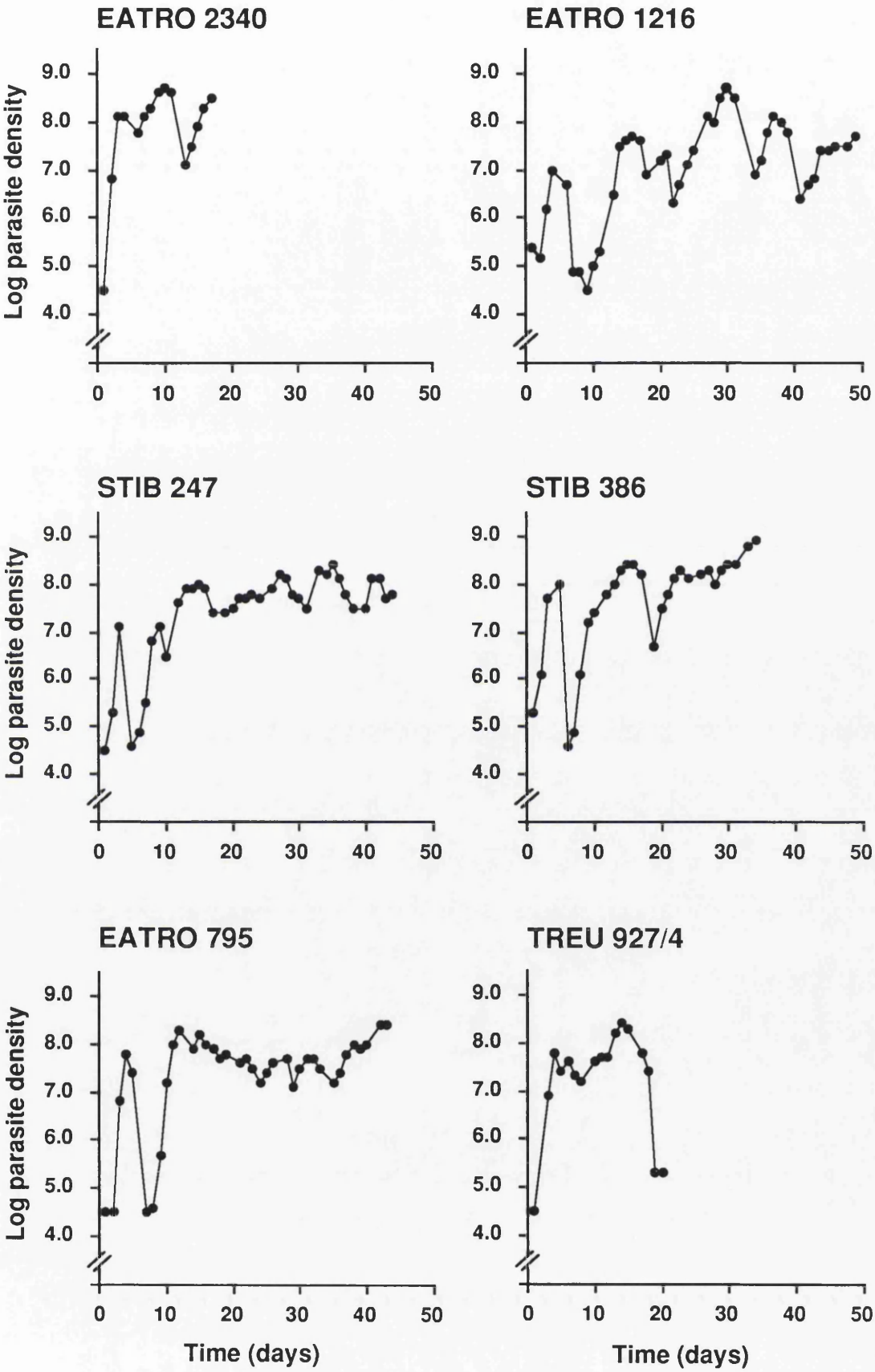
c - The mean no. of days on which the parasitaemia was greater than or equal to antilog 8.0 trypanosomes/ml of blood, and the no. of days on which the the parasitaemia was less than or equal to antilog 7.0 trypanosomes/ml of blood. These were determined over the first 20 days p.i. All mice except one (inoculated with EATRO 2340) survived for at least 20 days. It is for this reason that n=5 for EATRO 2340 and n=6 for other stocks. The data for the no. of days was also expressed as a percentage of the first 20 days p.i.

d - Mice were removed from the study when they were judged to be be exhibiting clinical symptoms of infection that were no longer considered to be only mild (see text for details).

Cloned stock	Median no. of troughs[a]	Prevalence at first peak[b]	No. of days $P \geqslant 8.0$[c] (percent)		No. of days $P \leqslant 7.0$[c] (percent)		mean day of removal from study[d]
EATRO 2340	0	8.1	8.8	(44)	2	(10)	31.3
EATRO 1216	3	7.1	0.3	(1.7)	10.8	(54.2)	50.5
STIB 247	2	7.0	0.7	(3.3)	7.5	(37.5)	44.5
STIB 386	2	7.9	6.7	(33.3)	5.8	(29.2)	34.3
EATRO 795	1	8.1	3.3	(16.7)	5.5	(27.5)	40.5
TREU 927/4	1	7.9	6.3	(31.7)	3	(15)	29

Fig. 2.8 Parasitaemia profiles of individual mice, each inoculated with a different stock of trypanosomes as shown to enable a direct comparison of the different lines. The mice were chosen from each group at random.

Fig. 2.8


EATRO 2340
EATRO 1216
STIB 247
STIB 386
EATRO 795
TREU 927/4
Log parasite density
9.0
8.0
7.0
6.0
5.0
4.0
0
10
20
30
40
50
Time (days)

Figs. 2.9 and 2.10 The parasitaemia profiles of individual mice inoculated with STIB 247/49 and STIB 247/80. Parasite densities are expressed as the log. no. of trypanosomes/ml of blood, m refers to mouse no.

Fig. 2.9 STIB 247/49

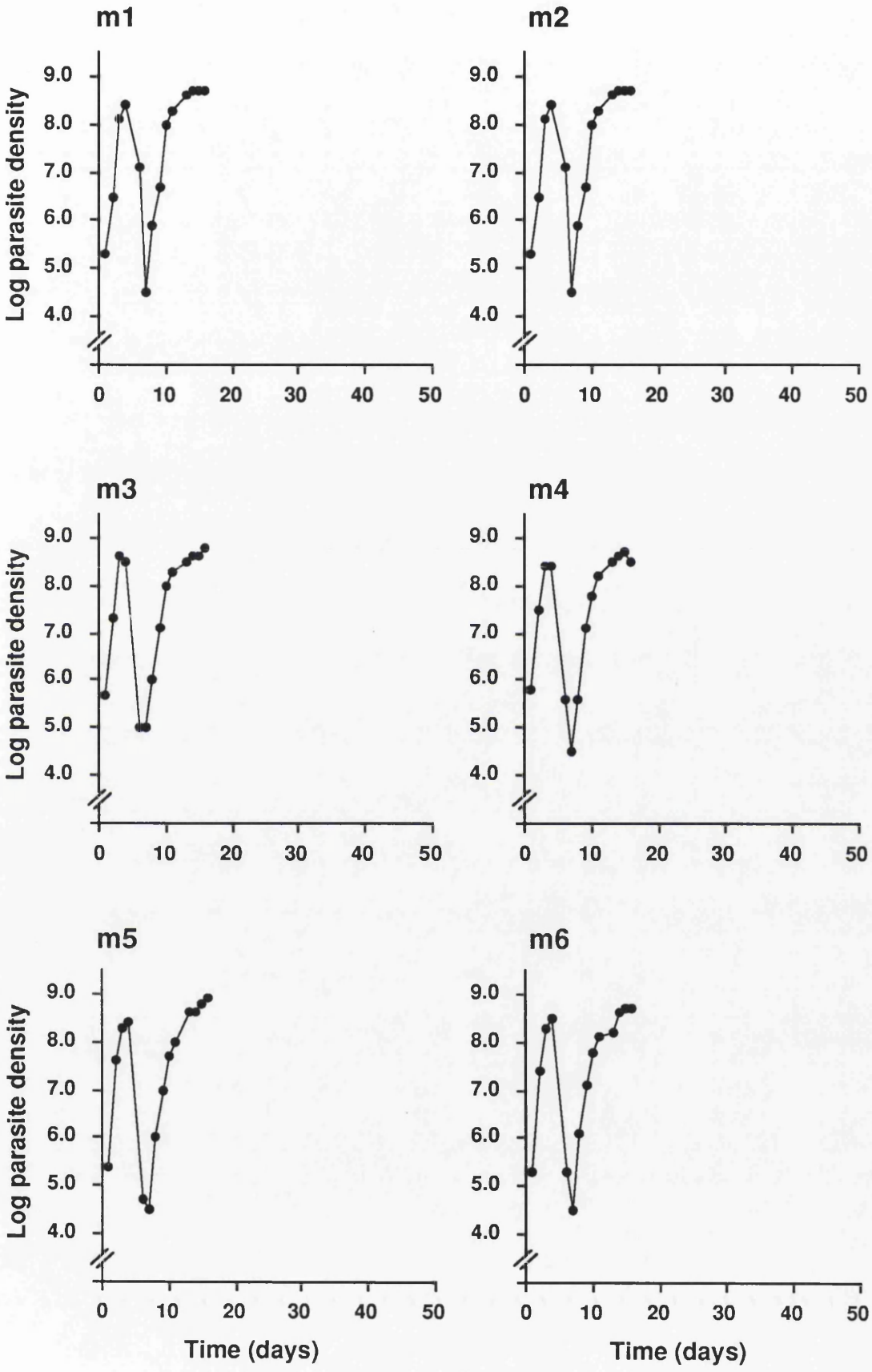


Fig. 2.10 STIB 247/80

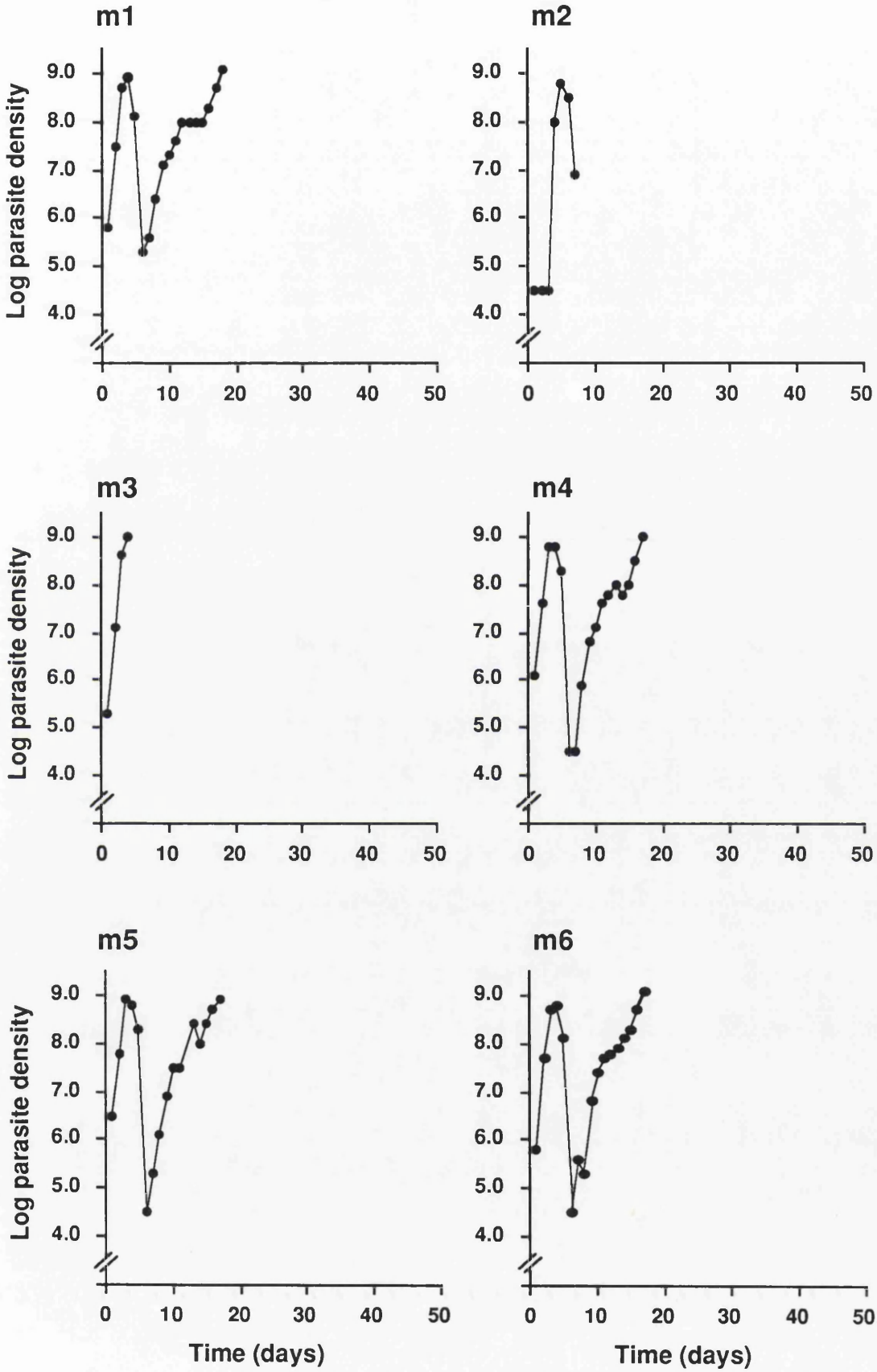
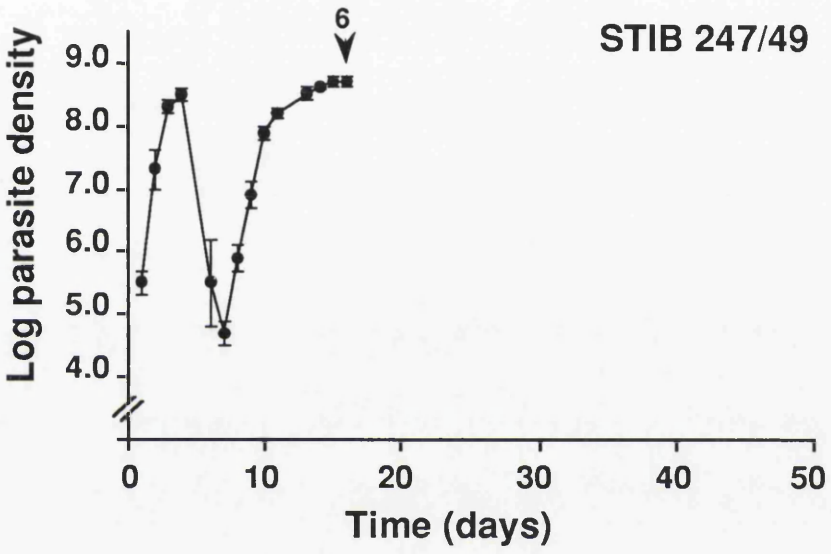


Fig. 2.11

The mean parasitaemia profiles of the lines STIB 247/49 and STIB 247/80 in mice. n = 6, geometric mean ± 2SE. ⮟ denotes removal of a mouse from the study. On those days where more than one mouse was removed from the study, the number of mice removed is indicated.



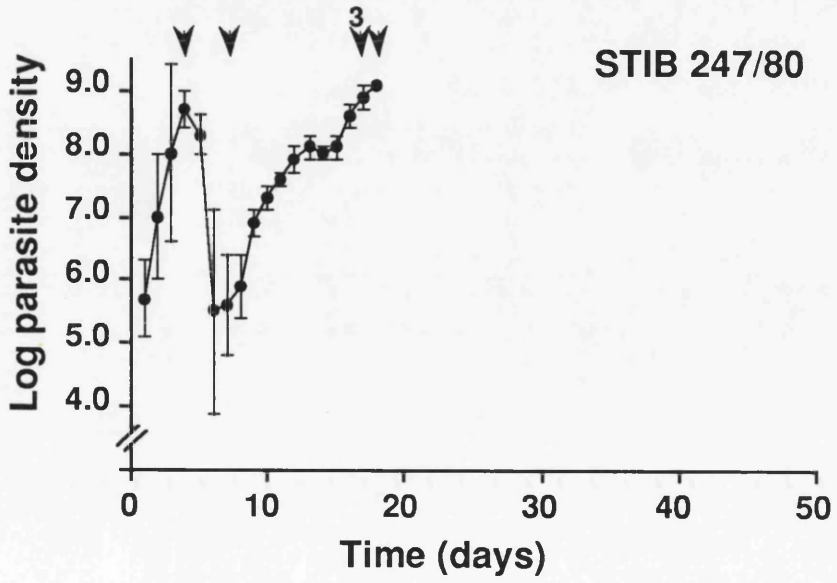


Table 2.2

Parasitaemia characteristics of the cloned stock STIB 247 and lines STIB 247/49 and STIB 247/80, determined from the results shown in Figs. 2.3, 2.10, 2.11.

a- A trough is arbitrarily defined as a drop in parasitaemia greater than one order of magnitude between adjacent points.

b- Geometric means of the parasitaemia on the peak of the first parasitaemic wave of infection with each line.

c- The mean no. of days on which the parasitaemia was greater than or equal to antilog 8.0 trypanosomes/ml of blood, and the no. of days on which the parasitaemia was less than or equal to antilog 7.0 trypanosomes/ml of blood.

In Table 2.2 these were determined over the first 16 days p.i. All mice except two (inoculated with STIB 247/80) survived for at least 16 days. It is for this reason that n=4 for STIB 247/80, n=6 for other lines. These data are also expressed as percentages.

d- Mice were removed from the study when they were judged to be exhibiting clinical symptoms of infection that were no longer considered to be only mild (see text for details).

Table 2.3

Parasitaemia characteristics of the cloned stock STIB 247 in mice inoculated with different numbers of trypanosomes. Values were determined from the results shown in Figs. 2.3 and 2.13. Details are as described for Table 2.2 except that for "c", values were determined over the first 20 days of infection to enable a direct comparison with the results shown in Table 2.1. These data are also expressed as percentages.

Table 2.2

Line	Median no. of troughs[a]	Prevalence at first peak[b]	No. of days P ⩾ 8.0[c] (percent)	No. of days P ⩽ 7.0[c] (percent)	Mean day of removal from study[d]
STIB 247	2	7.0	0.7 (4.2)	10.8 (45.9)	44.5
STIB 247/49	1	8.4	7.5 (46.9)	4.5 (28.2)	16
STIB 247/80	1	8.9	6.8 (42.2)	4.8 (29.8)	13.3

Table 2.3

Cloned stock	Inoculum dose	Median no. of troughs[a]	Prevalence at first peak[b]	No. of days P ⩾ 8.0[c] (percent)	No. of days P ⩽ 7.0[c] (percent)	Mean day of removal from study[d]
STIB 247	1E6	2	7.0	0.7 (3.3)	7.5 (37.5)	44.5
	1E4	2	7.8	0.8 (5.8)	9.8 (49.2)	51

Fig. 2.12

The parasitaemic profiles of three individual mice one inoculated with STIB 247, one with STIB 247/49 and one with STIB 247/80, to permit direct comparison of the lines. The mice were chosen at random.
○ = STIB 247, □ = STIB 247/49 and ● = STIB 247/80.

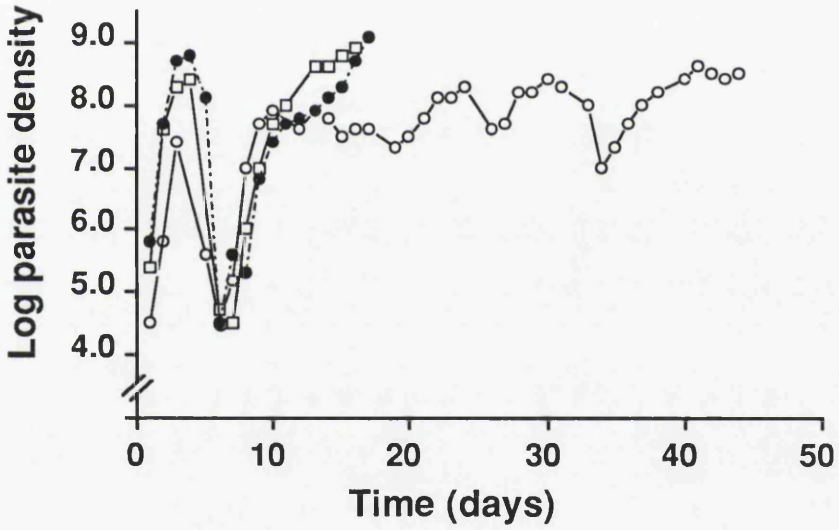
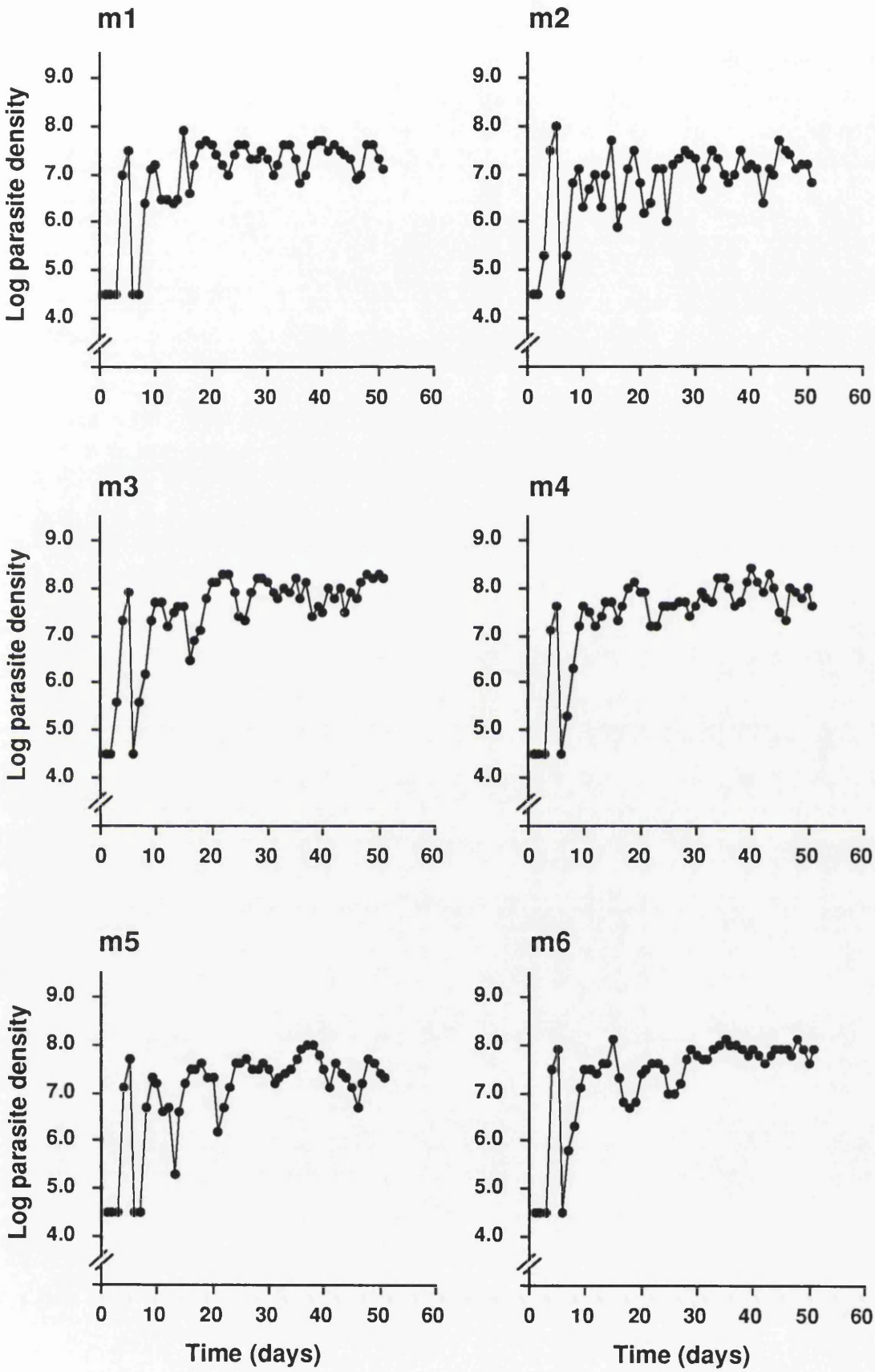


Fig. 2.13 The course of infection with STIB 247 in six individual mice, each mouse received 1 x 10^4 trypanosomes/ml of blood. Parasite densities are expressed as the log. no. of trypanosomes/ml of blood, m refers to the mouse no.

Fig. 2.13 STIB 247



CHAPTER 3

A COMPARISON OF GROWTH AND DIFFERENTIATION RATES IN LINES OF *Trypanosoma brucei* IN MICE

3.1 INTRODUCTION

A direct comparison of the courses of infection between six cloned stocks of trypanosomes was made in the previous chapter. It was shown that cloned stocks of trypanosomes of different genotype differed in the courses of infection that they produced in mice. Also rapid syringe-passaging of a cloned stock was observed to increase the parasitaemia. In this chapter, the question of whether these observed differences in the courses of infection could be directly attributed to differences in growth rates between the various lines of trypanosomes will be addressed.

There have been no reported studies of a direct comparison of growth rates between cloned stocks of different genotype. However comparisons of growth rates have been made between lines of different passage history (Herbert & Parratt, 1979; Barry *et al*, 1979; Inverso *et al*, 1988). The results of these studies showed that the differences in the courses of infection observed were not dependent upon the growth rates of the lines compared. In contrast the results of a study by Diffley (1987) suggested that the observed differences in the courses of infection did correlate with differences in growth rates.

In the aforementioned studies, growth rates were expressed as the time required for a population of trypanosomes to double in size - the population doubling time (PDT). The growth rate, however, is a composite of three biological processes: the rate of replication of slender form trypanosomes, the rate of differentiation of dividing slender form trypanosomes to non-dividing stumpy forms and the intrinsically regulated life expectancy of

stumpy form trypanosomes. Few, if any attempts have been made in previous studies to calculate rate values for these separate processess and to determine the contribution of these rate values to the overall growth rate and the differences between trypanosome populations in their courses of infection. In order to avoid confusion, throughout this chapter the terms growth, replication and differentiation are used to refer to the overall increase in the size of the trypanosome population, division of slender forms and the development from slender to stumpy forms respectively.

The aims of this study were to: (1) accurately determine the growth rates of several trypanosome lines, (2) accurately determine the rates of replication and differentiation in these lines, (3) determine whether these rate processes differed between lines and (4) determine if these differences correlated with the differences in parasitaemia between these lines. A comparison of growth, replication and differentiation rates was made between three lines of different genotype known to differ in the courses of infection that they produced in mice. A second comparison was made between lines of identical genotype but which differed in passage history.

3.2 MATERIALS AND METHODS

3.2.1 TRYPANOSOMES

The trypanosome lines used in this study were as described in section 2.2.1: these were EATRO 2340, EATRO 1216, STIB 247, STIB 247/49 and STIB 247/80.

3.2.2 EXPERIMENTAL DETERMINATION OF GROWTH RATES OF CLONED STOCKS AND LINES

For each line trypanosomes were grown from stabilate in adult female CD1, CFLP or Balb/c mice which had been cyclophosphamide treated (250 mg/kg bodyweight, Sigma Ltd) approximately 24 hours previously. Trypanosome infected blood was collected in Hanks-Heparin and parasite densities were determined as described in section 2.2.2. For each experiment, six replicate haemocytometer counts were made and 3×10^6 trypanosomes were inoculated i.p. into each of six age-matched adult (10 - 18 weeks) female Balb/c mice, except where stated below. When the infections became patent, parasite densities were accurately determined and bloodsmears were made from each mouse for subsequent Giemsa staining. This process was repeated at approximately 12 hourly intervals for up to four days. Four replicate experiments were conducted for the lines EATRO 2340 and STIB 247 and duplicate experiments were conducted for the lines EATRO 1216, STIB 247/49 and STIB 247/80. Growth rate experiments with the lines EATRO 1216, EATRO 2340 (experiment 4) and STIB 247 (experiment 4) were initiated with i.p. inoculations of 1×10^7, 5×10^6 and 6×10^6 trypanosomes/mouse respectively. The rate of growth of trypanosomes in each experiment, b, was calculated by least squares regression analysis and the PDT = ln 2/b.

3.2.3 GIEMSA STAINING BLOODSMEARS

Bloodsmears were fixed in methanol for 2 minutes, air dried and stained with 10% Giemsa's stain for 15 minutes.

3.2.4 DETERMINATION OF REPLICATION AND DIFFERENTIATION RATES OF CLONED STOCKS AND LINES

For one experiment from each line (chosen at random) trypanosomes from Giemsa stained bloodsmears were classified into 3 groups, slender, stumpy and intermediate according to the classification of Hoare (1972). 30 - 200 trypanosomes were counted/mouse at each time point for which the population density was determined during exponential growth. The number of trypanosomes of each form/ml of blood was calculated from the prevalence of that form and the determined population density. The rate of increase of slender forms, r, and the rate of increase of stumpy forms, d, was determined by least squares regression analysis. From these the rate of replication and the rate of differentiation were calculated. The rate of increase of stumpy forms is a loss rate from the slender form population which should be added to r to obtain a more accurate estimate of the replication rate, thus the rate of replication is calculated as r + d. This rate is expressed as population doubling time (PDT) where PDT = ln 2/(r+d). The differentiation rate is estimated as the rate of increase of stumpy forms and is based on the assumption that the life expectancy of stumpy forms is relatively high (see section 3.4). The rate of differentiation is expressed as a finite rate/generation (= $e^{d.PDT}$).

3.2.5 STATISTICAL ANALYSES OF DATA

The Minitab v7.1 statistics package was used in all data analyses except nested analysis of variance which was conducted using the SPSS package. In analyses of variance,

variance ratios (F values) were significant if the calculated F value was greater than the critical value of F (as determined from statistical tables, P = 0.05) for the required degrees of freedom (d.f.). Scheffés test (Klugh, 1974) was then used to determine which of the means being compared was significantly different.

3.3 RESULTS

3.3.1 A COMPARISON OF MORPHOLOGICAL PROFILES OF SEVERAL POPULATIONS MADE BY TWO OBSERVERS

The determination of whether an individual trypanosome in a Giemsa stained bloodsmear is of slender, stumpy or intermediate morphology is subjective. From the literature it would appear that workers have interpreted the standard criteria of Hoare (1972) differently. In studies by Black *et al* (1983a; 1983b; 1985) populations of trypanosomes from pleomorphic lines obtained just after the first peak of parasitaemia were reported to be approximately 80-95% stumpy. In contrast Balber (1972) and McLintock (1990) observed trypanosomes of such populations to be only 30-40% and up to 60% of stumpy form morphology respectively. Therefore, the potential problem of subjective assessment of trypanosome morphologies was addressed. A comparison was made of the percentage prevalences of the three morphological types in several randomly chosen bloodsmears between myself and an experienced observer- C.M.R. Turner. These results are shown in Table 3.1. A comparison of these indicated that I was more likely to classify intermediate forms as stumpy than C.M.R.Turner. My data for the

percentage prevalences of intermediate forms was lower than that obtained by C.M.R. Turner in all 9 slides examined, whilst that obtained for stumpy forms was higher in 7 of the 9 slides. A similar pattern was not observed for slender form data, in this case the differences in prevalence were more evenly distributed in that my data were higher for 4 of the 9 slides and lower in 4 of 5 of the other slides.

3.3.2 A COMPARISON OF GROWTH, REPLICATION AND DIFFERENTIATION RATES BETWEEN CLONED STOCKS

The exponential growth of trypanosome populations in replicate experiments obtained are shown in Figs. 3.1-3.3 for the cloned stocks EATRO 2340, EATRO 1216 and STIB 247 respectively. Growth rates calculated from these are shown in Table 3.2. From a nested analysis of variance of instantaneous rate values (Table 3.3) it was shown that there was a significant difference in PDTs of replicate experiments within a cloned stock and also between stocks. EATRO 2340 had the fastest growth rate (mean PDT = 5.6 hours) and EATRO 1216 the slowest (mean PDT = 9.5 hours), whilst that of STIB 247 was intermediate (mean PDT = 6.1 hours).

Further analyses were conducted to determine whether the overall growth rate concealed differences in the rates of replication and differentiation between the lines. Thus for one experiment from each of the three lines the percentage prevalence of slender, stumpy and intermediate forms at each time point was determined from Giemsa stained bloodsmears. The results obtained are shown in Figs. 3.4(a), 3.5(a) and 3.6(a) for the cloned stocks EATRO 2340, EATRO 1216 and STIB 247 respectively. The patterns of prevalence

of each of the three morphological types were similar for all three cloned stocks of trypanosomes. Stumpy forms predominated over slender and intermediate forms at the earlier time points but declined in prevalence as the infections progressed. In contrast, the prevalence of slender forms was observed to increase as the infections progressed.

The presence of intermediate forms presented a problem in determining replication and differentiation rates because it is not known whether intermediate forms can divide or not. In order to overcome this difficulty these data were analysed twice. In the first analysis it was assumed that all intermediate forms can divide and that they should therefore be grouped with slender forms. In the second analysis it was assumed that intermediate forms cannot divide and that they should therefore be grouped with stumpy forms. If the same general pattern can still be observed under both of these conditions then it can be assumed that the correct classification of intermediate forms is not important with respect to the interpretation of the data.

The numbers of slender, stumpy and intermediate forms/ml of blood increased exponentially with time during the course of an experiment and also when the data for intermediate forms were combined with that of either slender or stumpy forms. The results for the number of slender+ intermediate and stumpy forms/ml of blood for each of the three cloned stocks are shown in Figs. 3.4(b) and 3.4(c), 3.5(b) and 3.5(c) and 3.6(b) and 3.6(c).

The instantaneous values for the rate of increase of slender forms and stumpy forms are shown in Table 3.4. Also

shown are the rate values obtained when the data for intermediate forms were combined with that of slender or stumpy forms. These data show that grouping intermediate forms with slender forms made little difference to rate values of r but when intermediates were combined with stumpy forms some values of d changed considerably. The results of an analysis of variance to determine whether these rates differed between the cloned stocks, and the results of subsequent Scheffés tests to determine where the differences occurred are shown in Table 3.5. There was a significant difference in the rates of increase of slender forms between cloned stocks, and when intermediate forms were combined with slender forms. In both cases the rate of increase of slender forms of EATRO 1216 was significantly lower than that for EATRO 2340 and STIB 247. There was also a significant difference in the rates of production of stumpy forms between the cloned stocks EATRO 1216 and EATRO 2340. The rate of production of stumpy forms of STIB 247 was not significantly different from either EATRO 1216 or EATRO 2340. This difference was also maintained when the data for intermediate forms were combined with that of stumpy forms, in this case EATRO 2340 had a significantly higher rate of production of stumpy forms than either EATRO 1216 or STIB 247.

The finite rates of replication and differentiation/generation were calculated for the cloned stocks (Table 3.6). The results of an analysis of variance of these rates between the cloned stocks and the results of subsequent Scheffés tests are shown in Table 3.7. These data were determined from rate values calculated using

stumpy forms as one category and intermediate forms grouped with slender forms as the other. This combination of data werechosen for several reasons. (1) Grouping intermediates with either slender or stumpy forms did not alter the general pattern observed between rates of r and d between the cloned stocks. (2) There was little difference between rate values derived from the sl+I grouping when compared with the st+I grouping of the data. (3) I had a tendency to classify more trypanosomes in a pleomorphic population as stumpy than one other experienced observer, therefore, those trypanosomes which I classified as intermediate may have been able to divide (Table 3.1).

The pattern of replication rates reflects that previously observed for the overall growth rates (PDTs) between cloned stocks, EATRO 2340 had the fastest replication rate (2.9 hours) and EATRO 1216 the slowest (4.3 hours) whilst that of STIB 247 was intermediate. There was a statistically significant difference in this rate between the cloned stocks: EATRO 1216 had a significantly lower rate than either EATRO 2340 or STIB 247. There was also a significant difference in the finite rates of differentiation/generation between the cloned stocks EATRO 1216 and STIB 247. However the finite rate of differentiation/generation of EATRO 2340 was not significantly different from either of the other two stocks.

3.3.3 THE EFFECT OF RAPID SYRINGE-PASSAGING ON THE GROWTH, REPLICATION AND DIFFERENTIATION RATES OF THE CLONED STOCK STIB 247

The exponential growth of trypanosome populations in replicate experiments obtained for the cloned stock STIB 247

and the lines STIB 247/49 and STIB 247/80 are shown in Figs. 3.3, 3.7 and 3.8 respectively. Growth rates calculated from these are shown in Table 3.2. From a nested analysis of variance of instantaneous rate values (Table 3.3), it was shown that although there was a significant difference in PDTs of replicate experiments within a line, there was no significant difference in PDTs between lines.

The percentage prevalence of slender, stumpy and intermediate forms at each time point during the course of a growth rate experiment was determined from Giemsa stained bloodsmears. The results are shown in Figs. 3.6(a), 3.9(a) and 3.10(a) for the lines STIB 247, STIB 247/49 and STIB 247/80 respectively. As in the previous comparison between cloned stocks, the mean percentage prevalence of slender forms in all three lines increased as the infections progressed, whilst that of stumpy forms decreased.

As in the previous comparison between cloned stocks, the numbers of slender, stumpy and intermediate forms/ml of blood increased linearly with time during the course of a growth rate experiment and also when the data for intermediate forms were combined with that of either slender or stumpy forms. The numbers of slender + intermediate and stumpy forms/ml of blood of each of the three lines are shown in Figs. 3.6(b) and 3.6(c), 3.9(b) and 3.9(c) and 3.10(b) and 3.10(c).

The instantaneous values for the rates of increase of slender forms and stumpy forms are shown in Table 3.4. The rate values obtained when the data for intermediate forms werecombined with that of slender and stumpy forms are also shown in Table 3.4. The results of an analysis of variance

to determine whether these rates differed between the lines and the results of subsequent Scheffés tests to determine where these differences occurred are shown in Table 3.5. There was a significant difference in the rates of increase of slender forms between the lines, the rate value for STIB 247 was significantly higher than that for STIB 247/49 and STIB 247/80. This significantly different result was also maintained when the data for intermediate forms were combined with that of slender forms, in this case the rate of increase of slender forms of STIB 247 and STIB 247/80 were significantly different from each other. However, the rate of increase of slender forms of STIB 247/49 was not significantly different from either STIB 247 or STIB 247/80. There was a significant difference in the rate of increase of stumpy forms between the lines STIB 247/80 and STIB 247/49. However the rate value for STIB 247 was not significantly different from either STIB 247/49 or STIB 247/80. This significantly different result was also maintained when the data for intermediate forms were combined with that of stumpy forms, in this case the rate of increase of stumpy forms of STIB 247/80 was significantly lower than STIB 247 and STIB 247/49.

As in the previous comparison between cloned stocks, combining the data for intermediate forms with that of slender and stumpy forms did not alter the pattern of the results and thus their interpretation. Therefore the slender+intermediate and stumpy form combination of data were used in all subsequent analyses.

The finite rates of replication and differentiation/generation were calculated for the lines

(Table 3.6). The results of an analysis of variance of these rates between the lines and the results of subsequent Scheffés tests are shown in Table 3.7. The replication rates of STIB 247/49 and STIB 247 were similar, 3.4 hours and 3.2 hours respectively whilst that of STIB 247/80 was slower (4.3 hours). There was a significant difference in the replication rate between the lines STIB 247/80 and STIB 247. However the rate of replication of STIB 247/49 was not significantly different from either STIB 247/80 or STIB 247. There was also a significant difference in the rate of differentiation/generation between the lines STIB 247/80 and STIB 247/49. However the differentiation rate of STIB 247 was not significantly different from either of the two other lines.

3.4 DISCUSSION

There were several advantages to the experimental design used in this study. Growth, replication and differentiation rates were determined accurately in inbred mice and were compared between cloned stocks of trypanosomes. All cloned stocks were within 10 mouse passages either of the original field isolate or of transmission through tsetse flies. Therefore the differences in rate values observed between cloned stocks are likely to be representative of those which occured in the field. Previous studies have focused upon comparisons of growth rates between lines of different passage history where trypanosomes of a single genotype are subjected to an artificial selection pressure (Herbert & Parratt, 1979; Barry *et al*, 1979; Inverso & Mansfield, 1983; Diffley, 1987;

Inverso *et al*, 1988) and as is described in this thesis using the cloned stock STIB 247. Although growth rates have been determined in several previous studies, rates of replication and differentiation in pleomorphic populations have not.

To measure replication and differentiation rates, the prevalences of slender and stumpy forms were determined in exponentially growing populations and the unexpected observation was made that stumpy forms were predominant over slender forms at earlier stages in the infection in 4 of 5 experiments. As the infections progressed the percentage prevalence of slender forms increased whilst that of stumpy forms decreased. These results appear to contradict those of previous studies (Balber, 1972; Black *et al*, 1983a, 1983b, 1985; McLintock, 1990). However, in all these studies comparisons of the percentage prevalences of slender and stumpy forms were made from approximately 70 hours p.i., when compared with approximately 20 hours p.i. in the present study.

A comparison of the estimates of growth and replication rates for each cloned stock and line demonstrates that taking account of differentiation rates is important. Replication and growth rates differ by approximately 33-55%. Thus it would appear that measurements of overall growth rates as have been compared in previous studies (reviewed in Herbert & Parratt, 1979; Barry *et al*, 1979; Inverso & Mansfield, 1983; Diffley, 1987; Inverso *et al*, 1988), are not accurate estimates of the rate of slender form replication, except in those studies where monomorphic populations were studied.

Trypanosome growth rates differed significantly between cloned stocks of trypanosomes and there was a direct correlation between rate of growth and the overall parasite load observed in infections with these cloned stocks. EATRO 2340 had the fastest growth rate and the highest overall parasite load whereas EATRO 1216 had the slowest growth rate and the lowest parasite load. A direct correlation was also observed between replication rates and parasite load but there was no apparent pattern of association between differentiation rates and parasite load. These results indicate that quantitative changes in replication rate influence the courses of an infection but that changes in rates of differentiation do not

The results of a second comparison of growth rates between three lines of the cloned stock STIB 247 were not consistent with those of the first. Although these lines differed in their courses of infection there was no significant difference between the growth rates obtained for the lines and no apparent correlation of growth rates with parasite load. An inverse correlation between replication rates and parasite load was observed but this is probably a statistical artifact attributable to variation between experiments and unrelated to passage history (see Table 3.2). No pattern of association between differentiation rates and parasite load was observed.

Differentiation rates were accurately determined for the first time and it was shown that stumpy forms were produced at a constant rate during the exponential phase of trypanosome growth. The rate values obtained for the cloned stocks and lines are indicative of a high rate of

trypanosome differentiation/generation in the range of 1.18-1.30. In other words, approximately 1 in every 4 cell divisions produces a stumpy form.

Estimates of differentiation rates were based upon the assumption that the life expectancy of stumpy form trypanosomes is high. This indeed seems to be the case. Black *et al* (1985) have reported the mean life expectancy of stumpy forms to be approximately 24-36 hours. Moreover, it seems reasonable to assume that the life expectancy of stumpy form trypanosomes will not differ between cloned stocks and lines. The values reported for differentiation rates in Table 3.6, therefore, are unlikely to be affected, relative to one another, by this assumption.

The results of this chapter suggest that the differences in the overall parasite load observed between the cloned stocks compared directly correlate with differences in the rate of trypanosome growth and more specifically with the rate of replication of slender forms. However these conclusions do not appear to hold for the comparison of growth and replication rates between the three lines of the cloned stock STIB 247 for which there is no apparent correlation. There is no evidence from these results to support the notion that quantitative changes in differentiation rates are important in determining the course of an infection as proposed by Black *et al* (1985). Therefore it appears that other factors either combined with or independent of differences in growth rate also regulate the courses of infection observed. The results of this study suggest that these factors are different between field stocks and lines of varying passage history.

Table 3.1

Percentage prevalence of slender, stumpy and intermediate forms in nine slides chosen at random: a comparison of the categorisations of morphological types made by two observers. The results for C.M.R. Turner are shown in the left hand column of each category and can be compared with my own data in the right hand column.

Slide	Prevalence (%) Slender		Intermediate		Stumpy	
1	21	36	63	48	16	16
2	66	43	26	24	9	33
3	64	55	25	10	11	36
4	34	43	42	25	24	33
5	59	54	37	31	5	16
6	60	49	38	13	2	38
7	82	86	17	14	2	1
8	84	96	15	2	1	3
9	90	91	10	8	0	2

Table 3.2 Experimentally determined growth rates of cloned stocks and lines.

Cloned stock/ line	PDT (hours)	Mean PDT (hours)
EATRO 2340	5.5, 5.5, 5.0, 6.2	5.6
EATRO 1216	9.6, 9.4	9.5
STIB 247	5.3, 5.8, 6.5, 6.9	6.1
STIB 247/49	5.5, 5.2	5.4
STIB 247/80	6.3, 6.5	6.4

Table 3.3

Nested analysis of variance of rate values from growth rate experiments. A comparison was made of rate values between experiments within a cloned stock/line and between cloned stocks/lines. Thus two groups of comparisons were made, one between the three cloned stocks and the other between the three lines of STIB 247. F values, degrees of freedom (d.f.) and the level of significance, P, are shown for each comparison.

Cloned stock/ line	Between experiments within a cloned stock/ line	Between cloned stocks/ lines
EATRO 2340 EATRO 1216 STIB 247	8.08 d.f. 7/50 P<0.001	7.32 d.f. 2/7 P=0.019
STIB 247 STIB 247/49 STIB 247/80	6.87 d.f. 5/40 P<0.001	1.58 d.f. 2/5 P=0.295

critical value of F for 7/40 d.f. = 2.25, P=0.05
" " " " " 5/40 " 2.45 "
" " " " " 2/7 " 4.74 "
" " " " " 2/5 " 5.79 "

Table 3.4

Rates of increase of slender forms (r) and stumpy forms (d) assuming that intermediate forms are all grouped with slender forms or all with stumpy forms. sl = slender, st = stumpy, sl+I = slender + intermediate, st+I = stumpy + intermediate.

Cloned stock/ line	sl+I (r)	st (d)	sl (r)	st+I (d)
EATRO 2340	0.155	0.082	0.153	0.132
EATRO 1216	0.099	0.061	0.101	0.067
STIB 247	0.149	0.066	0.163	0.083
STIB 247/49	0.134	0.069	0.140	0.096
STIB 247/80	0.121	0.040	0.126	0.056

Table 3.5

Analysis of variance of rates of increase of slender forms (r) and stumpy forms (d), including and excluding intermediate forms from each category. F values and their level of significance, P, are shown for each of the two comparisons made. One between the three cloned stocks and the other between the three lines of STIB 247. Also shown are the significantly different cloned stocks/lines as determined from Scheffēs test. For those comparisons where two cloned stocks/lines are shown, these were determined to have been significantly different from each other but not from the third cloned stock/line in each group. sl = slender, st = stumpy, sl+I = slender + intermediate, st+I = stumpy + intermediate.

Cloned stock/ line	sl+I (r)	st (d)	sl (r)	st+I (d)
EATRO 2340 EATRO 1216 STIB 247	33.88 P<0.001 EATRO 1216	4.35 P=0.032 EATRO 1216 EATRO 2340	26.16 P<0.001 EATRO 1216	62.45 P<0.001 EATRO 2340
STIB 247 STIB 247/49 STIB 247/80	6.31 P=0.010 STIB 247/80 STIB 247	5.18 P=0.019 STIB 247/80 STIB 247/49	10.62 P=0.001 STIB 247	22.71 P<0.001 STIB 247/80

critical value of F for 2/15 d.f. = 3.68, P=0.05

Table 3.6

Finite rates of replication and rates of differentiation/generation calculated from the slender + intermediate and stumpy combination of data.

Cloned stock/ line	Replication rate, PDT (hrs)	Differentiation rate/generation
EATRO 2340	2.9	1.27
EATRO 1216	4.3	1.30
STIB 247	3.2	1.24
STIB 247/49	3.4	1.26
STIB 247/80	4.3	1.18

Table 3.7

Analysis of variance of replication and differentiation rates. F values and their level of significance, P, are shown for each each of the two comparisons made. One between the three cloned stocks and the other between the three lines of STIB 247. Also shown are the significantly different cloned stocks/ lines as determined from Scheffés test. For those comparisons where two cloned stocks/ lines are shown, these were determined to have been significantly different from each other but not from the third cloned stock/line in each group.

Cloned stock/ line	Replication rate	Differentiation rate
EATRO 2340 EATRO 1216 STIB 247	21.33 P<0.001 EATRO 1216	5.28 P=0.018 EATRO 1216 STIB 247
STIB 247 STIB 247/49 STIB 247/80	6.51 P=0.009 STIB 247/80 STIB 247	4.0 P=0.040 STIB 247/80 STIB 247/49

critical value of F for 2/15 d.f. = 3.68, P=0.05

Fig. 3.1 Growth of trypanosome populations of the cloned stock EATRO 2340 in four separate experiments. n = 6, geometric mean $\pm$ 2SE.

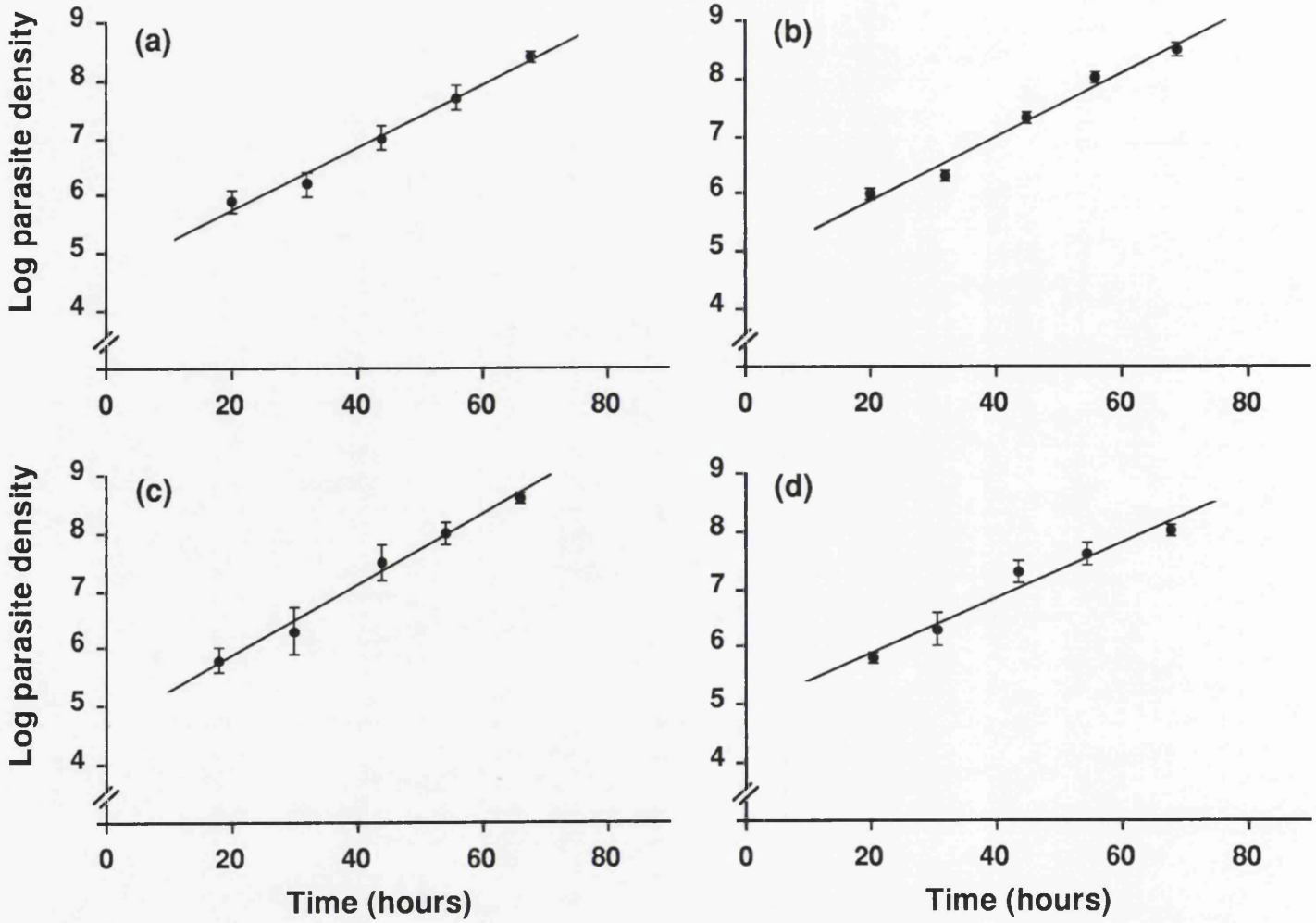

(a)
(b)
(c)
(d)
Log parasite density
Log parasite density
Time (hours)
Time (hours)
9
8
7
6
5
4
0
20
40
60
80

Fig. 3.2 Growth of trypanosome populations of the cloned stock EATRO 1216 in two separate experiments. n = 6, geometric mean ± 2SE.

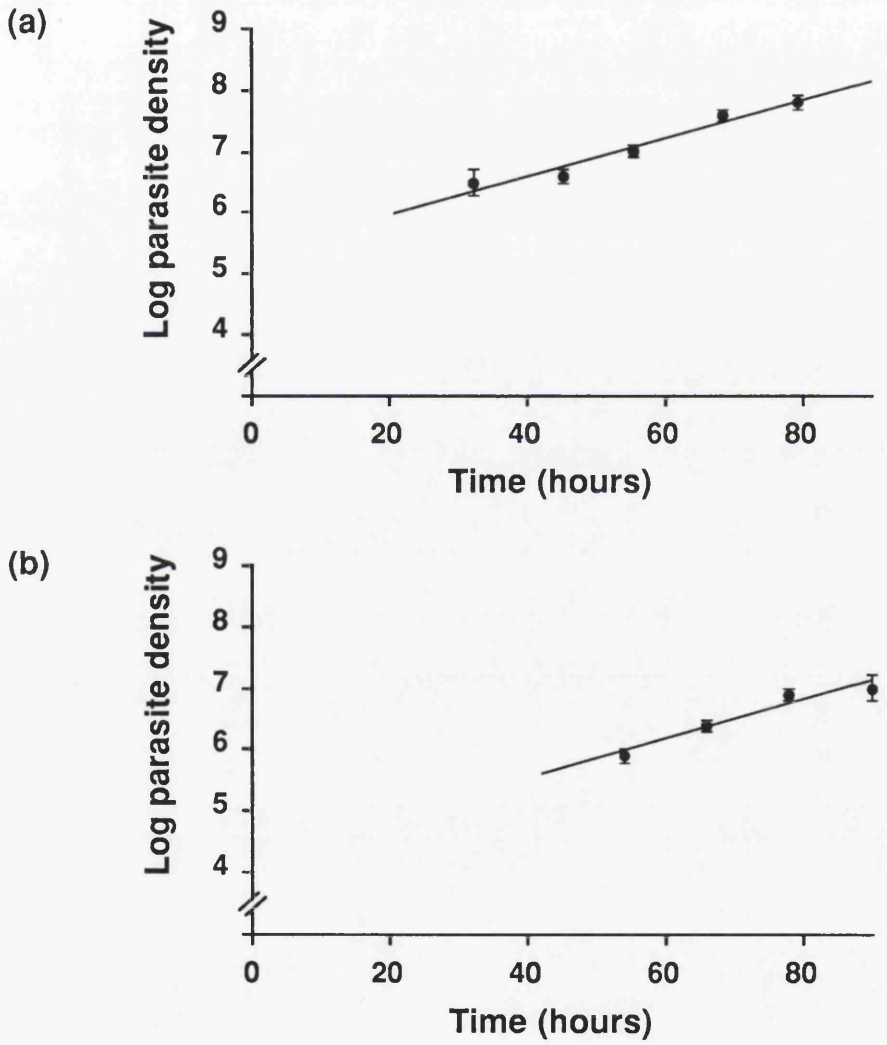
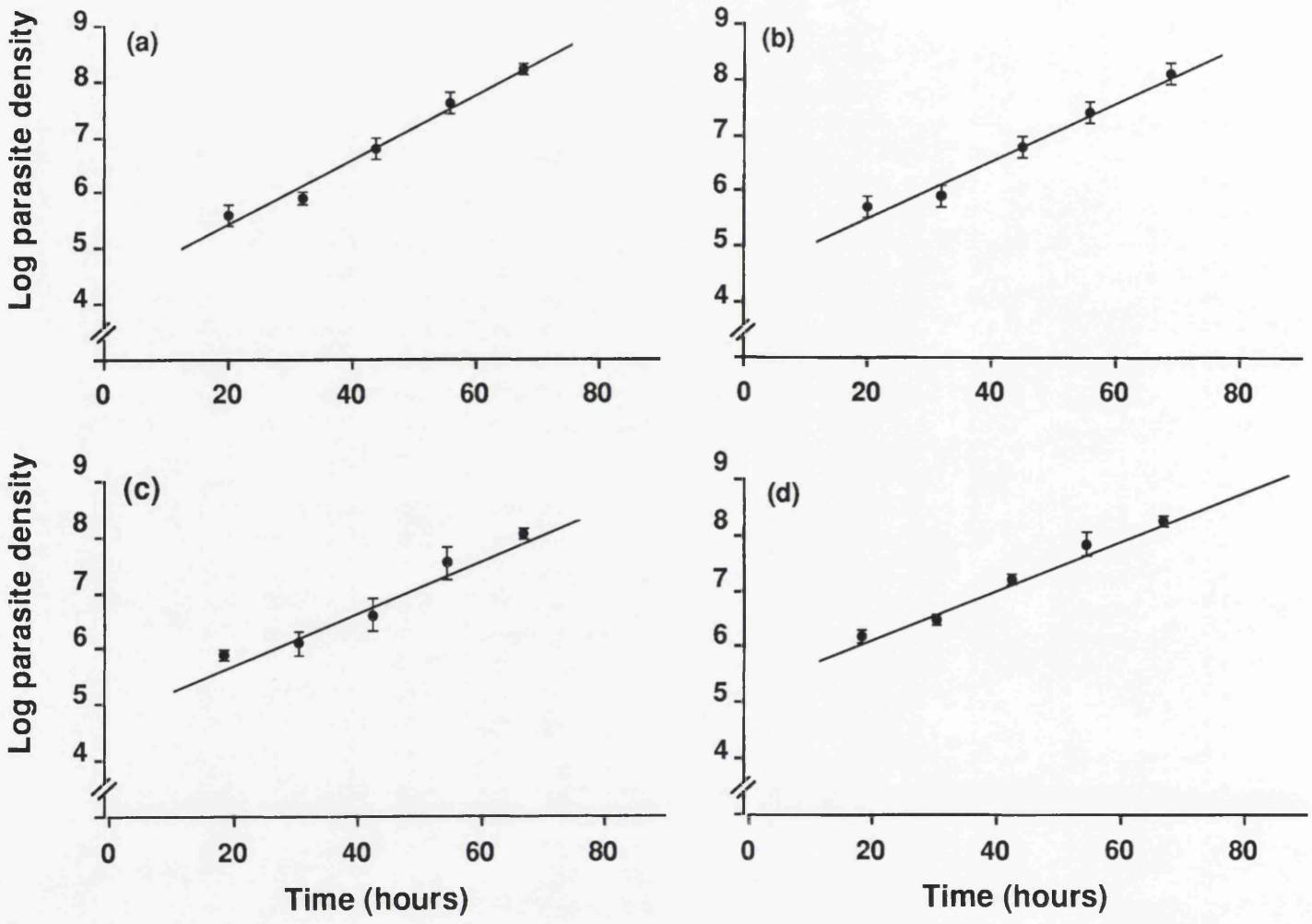


Fig. 3.3 Growth of trypanosome populations of the cloned stock STIB 247 in four separate experiments. n = 6, geometric mean $\pm$ 2SE.


Log parasite density
(a)
(b)
(c)
(d)
9
8
7
6
5
4
0
20
40
60
80
Time (hours)

Figs. 3.4, 3.5 and 3.6

EATRO 2340, EATRO 1216 and STIB 247

(a) The percentage prevalence of slender, stumpy and intermediate forms during the course of a growth rate experiment. n = 6, arithmetic mean ± 2SE. ● = slender, ■ = stumpy and Δ = intermediate.

(b) The rate of replication of slender forms during the course of a growth rate experiment determined from the slender + intermediate combination of data. n = 6, geometric mean ± 2SE, r^2 > 80 %.

(c) The rate of production of stumpy forms during the course of a growth rate experiment. n = 6, geometric mean ± 2SE, r^2 > 80 % for EATRO 1216 and STIB 247, r^2 = 72.9 % for EATRO 2340.

Fig. 3.4 EATRO 2340

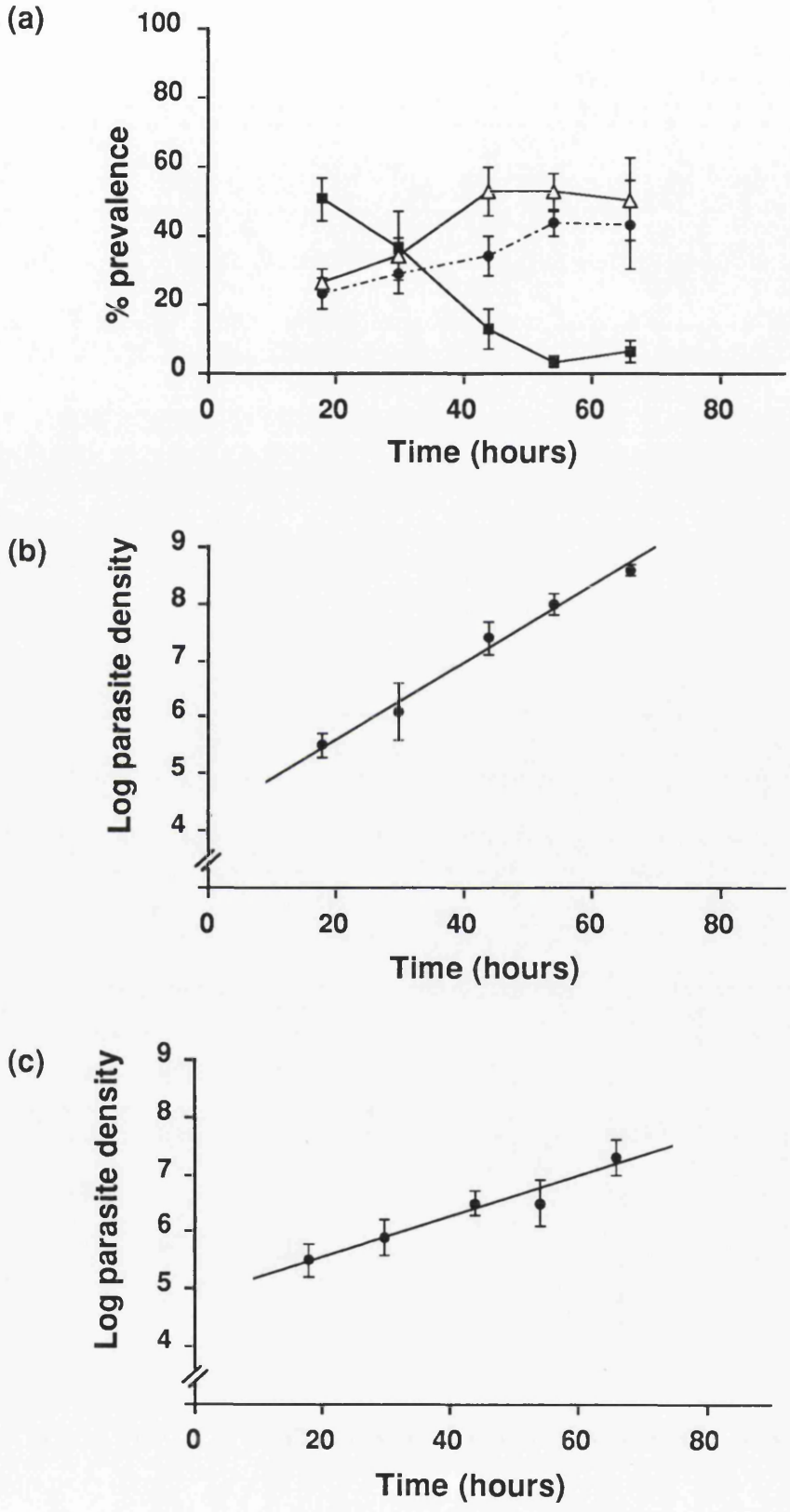


Fig. 3.5 EATRO 1216

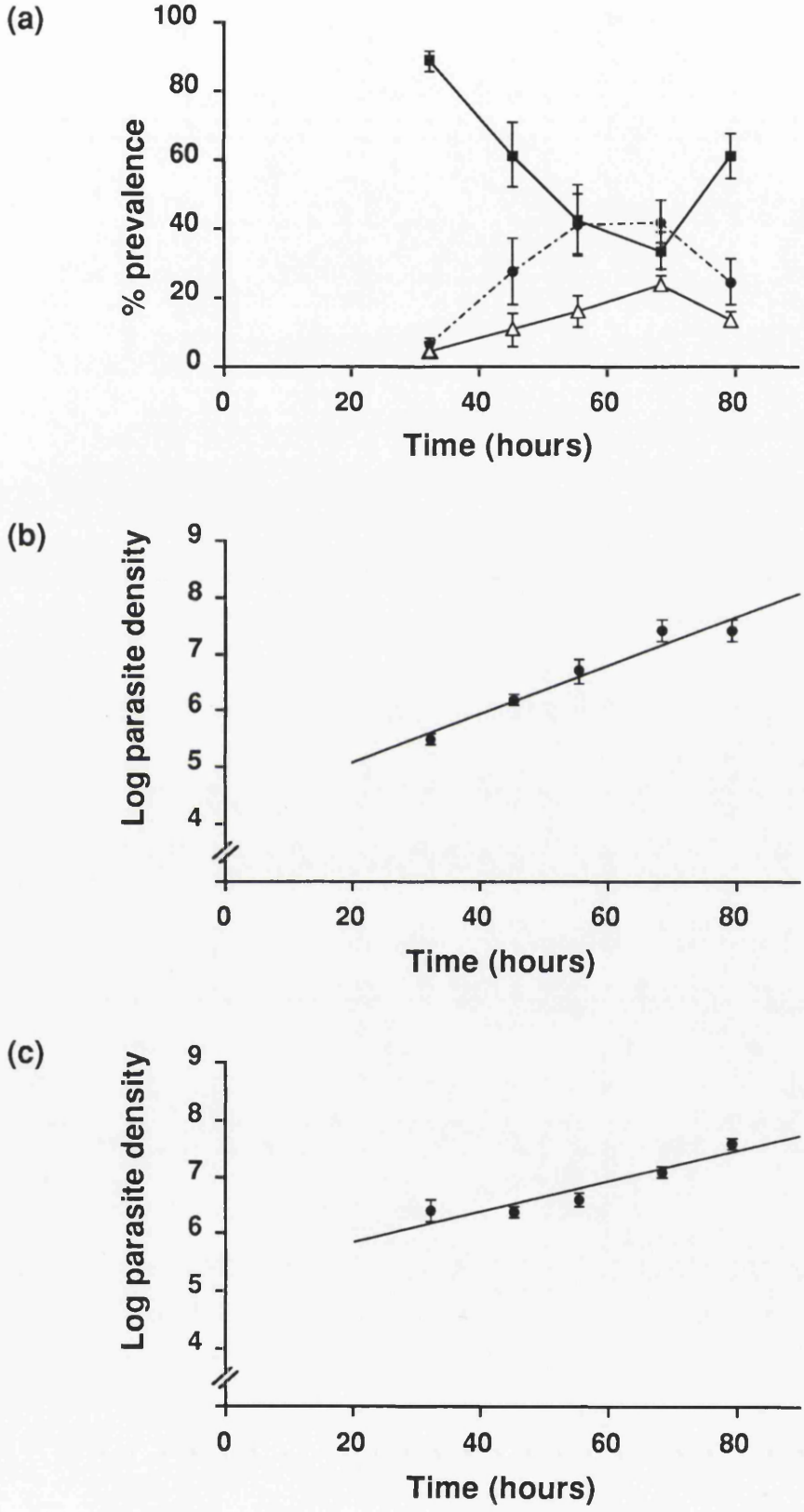


Fig. 3.6 STIB 247

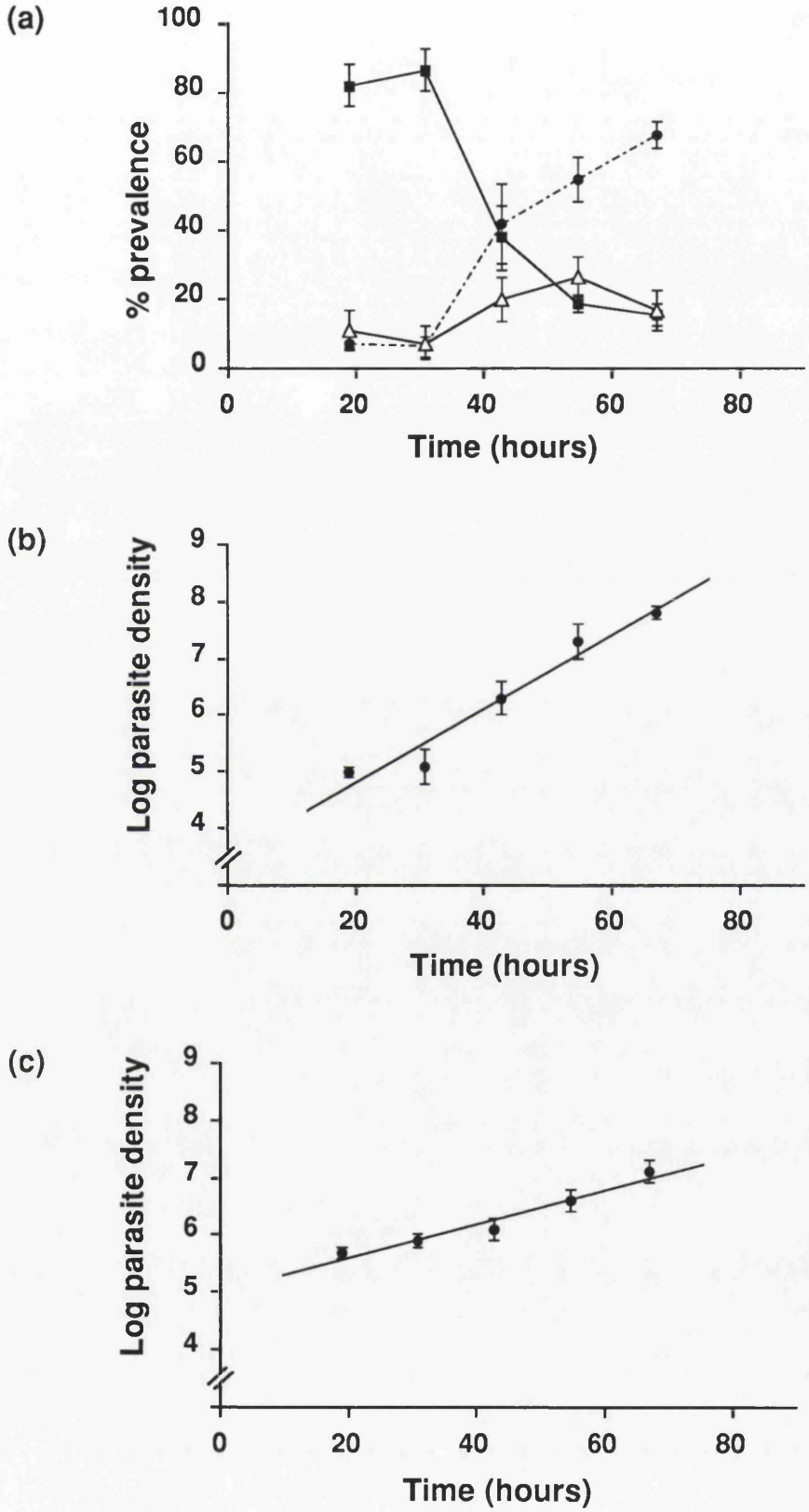


Fig. 3.7 Growth of trypanosome populations of the cloned stock STIB 247/49 in two separate experiments. n = 6, geometric mean ± 2SE.

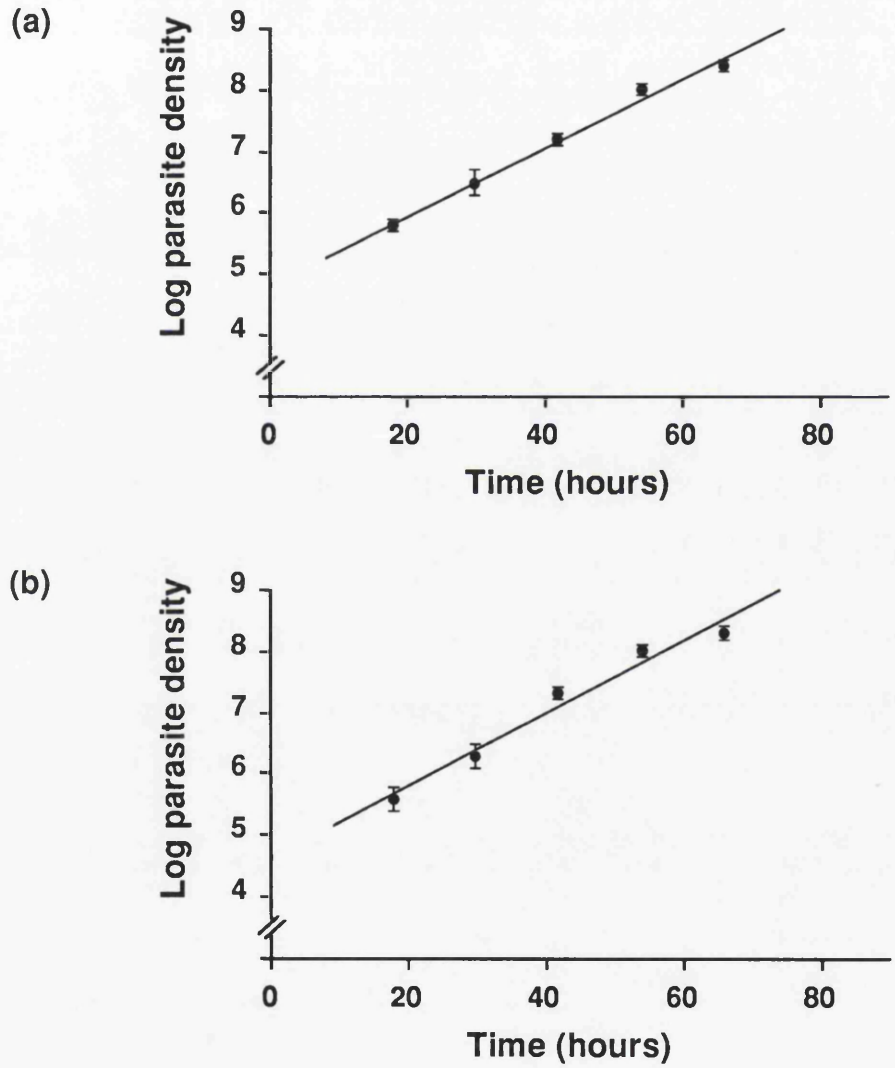
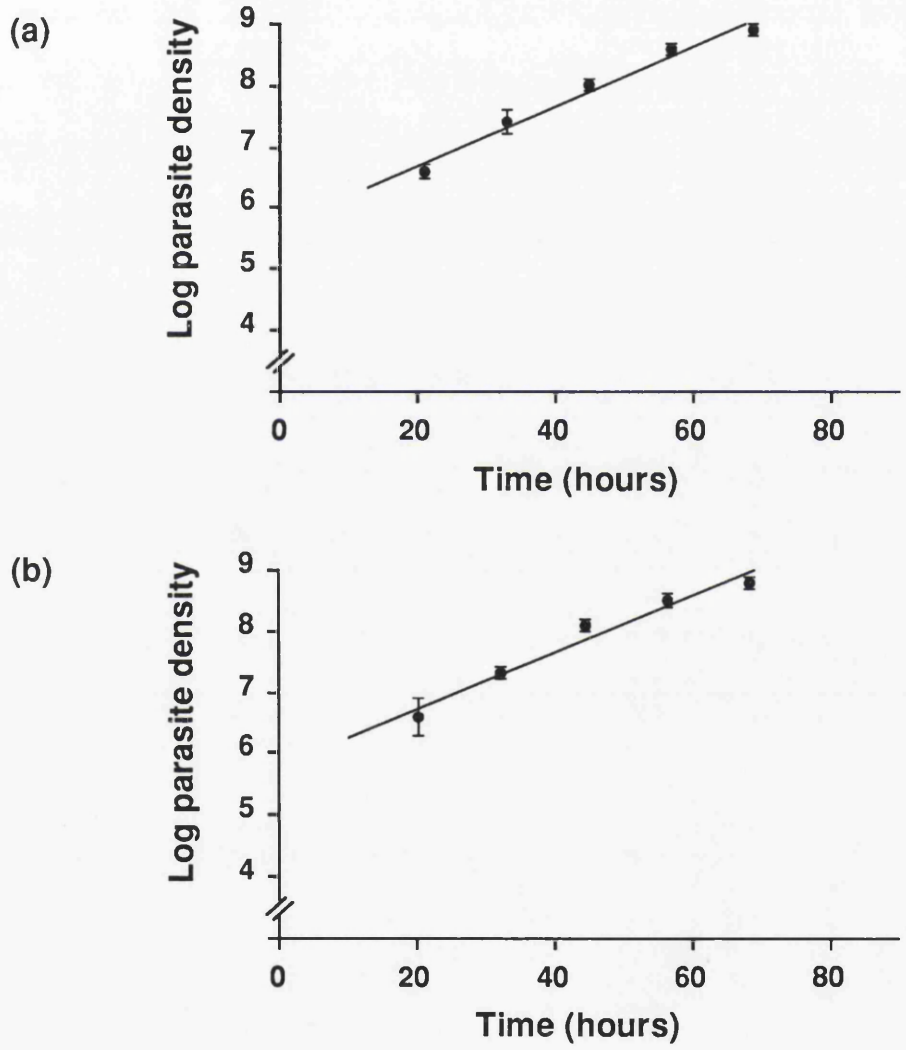


Fig. 3.8 Growth of trypanosome populations of the cloned stock STIB 247/80 in two separate experiments. n = 6, geometric mean ± 2SE.



Figs. 3.9 and 3.10

STIB 247/49 and STIB 247/80

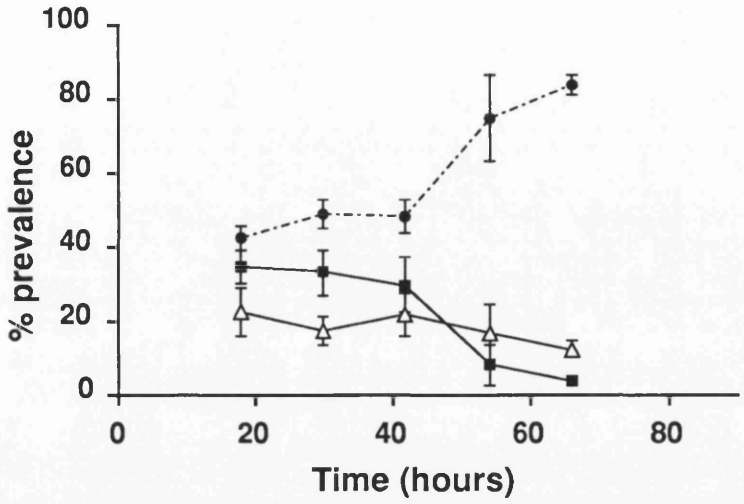
(a) The percentage prevalence of slender, stumpy and intermediate forms during the course of a growth rate experiment. n = 6, arithmetic mean ± 2SE. ● = slender, ■ = stumpy and Δ = intermediate.

(b) The rate of replication of slender forms during the course of a growth rate experiment determined from the slender + intermediate combination of data. n = 6, geometric mean ± 2SE, r^2 > 80 %.

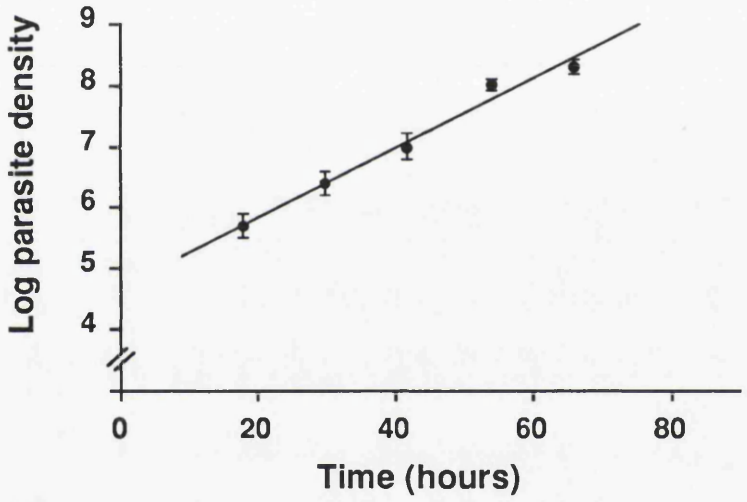
(c) The rate of production of stumpy forms during the course of a growth rate experiment. n = 6, geometric mean ± 2SE, r^2 = 72.2 % for STIB 247/49 and 50.9 % for STIB 247/80.

Fig. 3.9 STIB 247/49

(a)



(b)



(c)

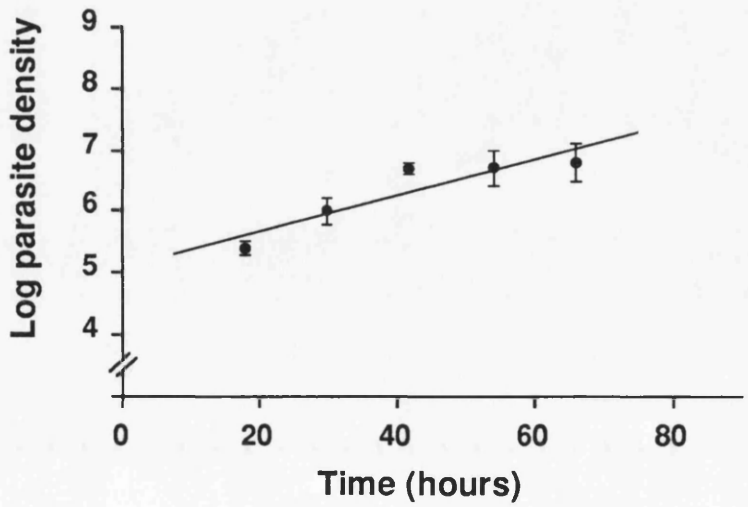
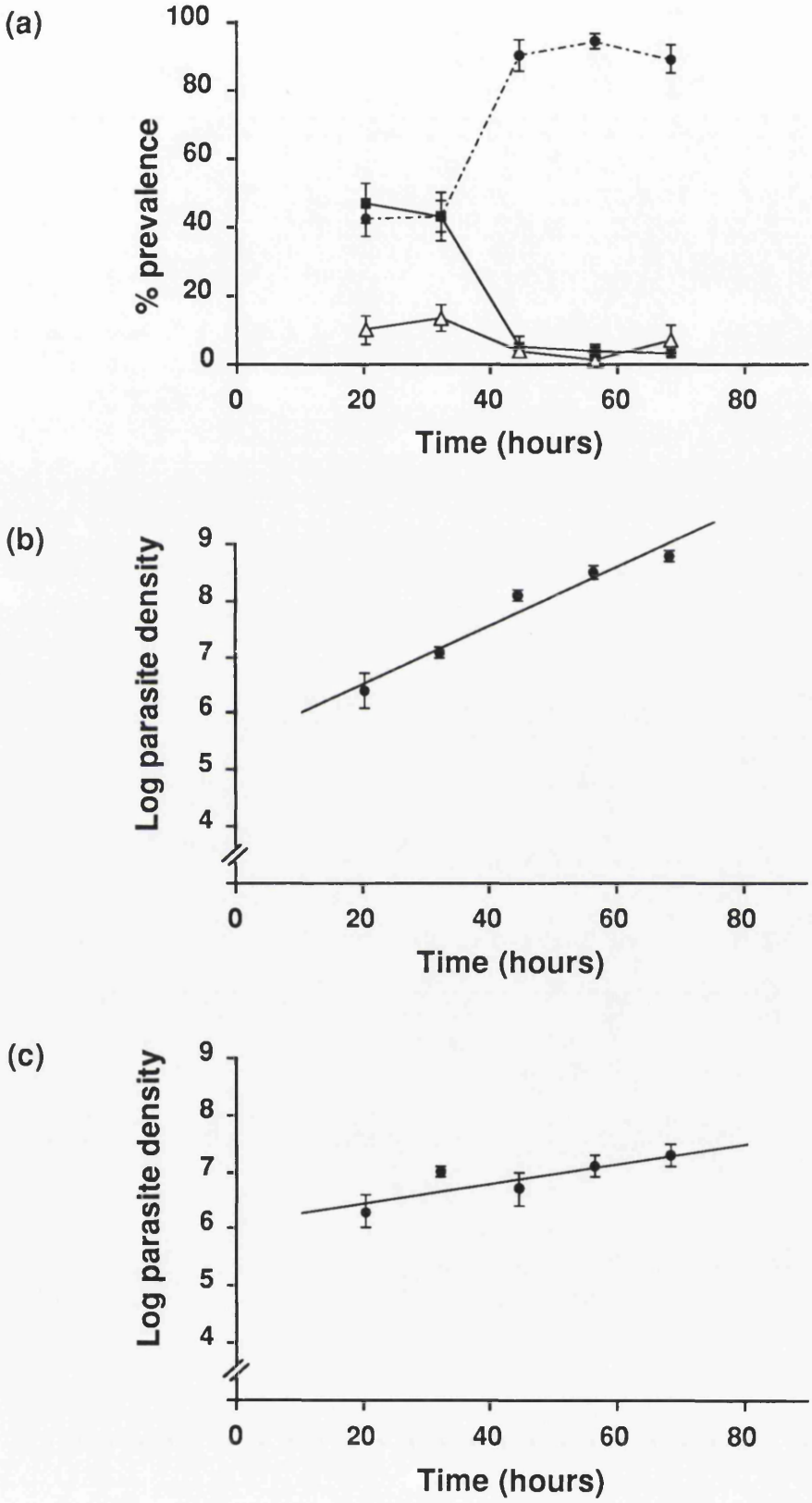


Fig. 3.10 STIB 247/80



CHAPTER 4

THE RELATIONSHIP OF VARIABLE ANTIGEN EXPRESSION AND POPULATION GROWTH RATES IN *Trypanosoma brucei*.

4.1 INTRODUCTION

Variable antigen expression in *Trypanosoma brucei* is hierarchical. When a bloodstream population is analysed at a single point in time VATs differ in their relative abundances. In a longitudinal analysis during the course of an infection these differences in prevalence are observed as a temporal sequence in which VATs are detected in a partially predictable manner. Those VATs at high prevalence in the first wave of an infection are detected early in this sequence, cleared from the circulation by the immune response and other VATs grow and increase in relative abundance thus appearing later (reviewed in Barry & Turner, 1991). It has been previously suggested that this hierarchy may be at least partly caused by trypanosome growth rates being influenced by the VAT expressed. It is this suggestion that forms the subject of this chapter.

A number of previous experimental studies have shown that VAT expression can influence trypanosome growth rates (Van Meirvenne *et al*, 1975; Seed, 1978; Miller & Turner, 1981; Seed *et al*, 1984; Myler *et al*, 1985), but a confounding problem in these studies was that growth rates of VATs were compared between trypanosome lines which often differed in passage history; the way in which they had been handled in the laboratory since original isolation. Since it has been demonstrated that populations of trypanosomes can be manipulated in the laboratory to produce trypanosome lines which vary in their growth rates (Barry *et al*, 1979) the observed differences in growth rates between lines could not be solely attributed to the VAT expressed. The aim of this study was to reinvestigate the problem of VAT-related

growth rates using trypanosome lines that had identical passage histories. Differences in growth rates were determined within each of two groups of VATs each from a cloned isolate of different genotype. All VATs within each group had identical passage history such that any differences in growth rates between VATs could be attributed exclusively to VAT expression and not to passage history.

4.2 MATERIALS AND METHODS

4.2.1 TRYPANOSOMES

Trypanosomes of the GUTAR (Glasgow University *Trypanozoon* Antigen Repertoire) 7 and GUTAR 8 serodemes were used. GUTAR 7 is derived from cloned stock EATRO (East African Trypanosomiasis Research Organisation) 2340 and is 33 mouse passages away from the original isolate. GUTAR 8 is derived from cloned stock STIB (Swiss Tropical Institute Basel) 247L and is 57 mouse passages away from the original isolate (Turner *et al*, 1991). Within GUTAR 7, growth rates were compared between trypanosomes of lines that expressed GUTats 7.3, 7.4 and 7.5 and within GUTAR 8, GUTats 8.2, 8.3 and 8.4 were compared. All VATs within each group have identical passage history (see Appendix).

4.2.2 ANTISERA

VAT specific antisera were prepared in rats and rabbits for use in indirect immunofluorescence. Rabbit antisera against GUTats 7.3, 7.4, 8.2, 8.3 and 8.4 were prepared using a standard procedure (Van Meirvenne *et al*, 1975). Briefly, a rabbit was inoculated with 1 x 10^6 bloodstream trypanosomes expressing a single VAT,

exsanguinated on day 6 p.i. and serum prepared. A rat antiserum against GUTat 7.5 was prepared using the following method. Rats were inoculated i.p. with GUTat 7.5 and when the infections became patent they were cured with an i.p. inoculation of SHAM (salicylhydroxamic acid, Sigma Ltd) at 380 mg/kg body weight with glycerol at 3.8 g/kg body weight (Van der meer *et al*, 1979). The rats received a second inoculation of SHAM/glycerol the following day. Rats were exsanguinated on day 5 post-cure and serum prepared.

VAT specific antisera were titrated against homologous and heterologous VATs to determine optimal working dilutions.

4.2.3 EXPERIMENTAL DESIGN

To determine differences in the relative growth rates of trypanosomes expressing GUTats 7.3, 7.4 and 7.5, equal numbers of trypanosomes (approximately 1 x 10^5 per mouse) of a pair of VATs were inoculated into 6 adult Balb/c female mice. All possible pairwise combinations within this group of VATs were tested in duplicate. Inoculated trypanosomes in infected blood were from exponentially growing populations. They were counted in an improved Neubauer haemocytometer using 0.85% ammonium chloride solution as diluent to determine parasite densities and to ensure that equal numbers of each of a VAT pair were inoculated into mice. Bloodsmears for indirect immunofluorescence were then made from tail blood 90 hours (4 days) later and stored at -70^{0}C in the presence of desiccant until used.

If trypanosomes expressing different VATs grew at the same rate it would have been expected that the two VATs in any combination experiment would be present at equal

prevalence whereas any difference in relative growth rates would lead to dissimilar prevalences.

In experiments with GUTats 8.2, 8.3 and 8.4 an identical procedure was used except that combinations of VATs were tested once.

4.2.4 IMMUNOFLUORESCENCE

Indirect immunofluorescence analysis was performed on acetone fixed bloodsmears using a standard procedure (Van Meirvenne *et al*, 1975). In brief, VAT-specific antisera and species specific fluoroscein isothiocyanate (FITC) conjugated second antibodies (Pasteur Institute Paris, Seralab) were diluted to predetermined optimal titres using phosphate buffered saline (PBS; pH 8.0) as diluent. Propidium Iodide (10^{-5} mg/ml, Sigma Ltd) was used as a counterstain. Reaction zones were painted on each slide using a liquid paint pen and allowed to air dry. A couple of drops of VAT-specific antiserum were added to each well and the slides incubated in a humid chamber for 30 minutes. The slides were then washed in PBS (2 x 10 minute washes) excess PBS drained from the slide and areas of the slide between reaction zones dried with a tissue. A couple of drops of the relevant FITC conjugated second antibody were added to each reaction zone and the slides incubated in a humid chamber for 20 minutes. The slides were then washed in PBS as before and mounted with non-autofluorescing glycerol in PBS, 50% v/v. The prevalence of each VAT in an experiment was determined from counts of 200 trypanosomes/mouse. Statistical analyses of these prevalence data were conducted using Student's t test.

4.2.5 EXPERIMENTAL DETERMINATION OF GROWTH RATES OF GUTats 7.5 AND 8.3

For each VAT, six Balb/c mice were inoculated i.p. with 3×10^6 or 5×10^6 trypanosomes. When the infection became patent, parasite densities were accurately determined at approximately 12 hourly intervals for up to 4 days using an improved Neubauer haemocytometer and 0.85% ammonium chloride as diluent.

The rate of growth of trypanosomes in each experiment, r, was calculated by least squares regression analysis and is expressed as the population doubling time (PDT) where PDT = $\log_e 2/r$.

4.2.6 CALCULATING GROWTH RATES OF VATs WHEN GROWN IN PAIRWISE COMBINATIONS

In those experiments where the PDT of one VAT in a pair had been experimentally determined as described in section 4.2.5 (the "known" VAT), the following method was used to calculate the PDT of the second "unknown" VAT. The ratio of the parasite densities of each VAT, N is equal to the ratio of the percentage prevalence of each VAT, P. i.e.

$$\frac{N_1}{N_2} = \frac{P_1}{P_2}$$

where subscripts 1 and 2 differentiate between the "known" and "unknown" VATs in an experiment. Assuming that the parasite populations are growing exponentially, $N = N_o e^{rt}$ where No is the density of trypanosomes at the time of inoculation, r is the growth rate and t is the duration of the experiment (90 hours).

Therefore: $$\frac{P_1}{P_2} = \frac{e^{r_1 t}}{e^{r_2 t}}$$

Since P_1, P_2, r_1 and t are all known, r_2 can be readily calculated for the "unknown" VAT. For simplicity growth rate values are expressed as PDTs.

4.3 RESULTS

The prevalences of each of the VATs GUTats 7.3, 7.4 and 7.5 when grown in pairwise combinations are shown in Fig. 4.1(a) and a statistical analysis of these data is given in Table 4.1. These results show that the prevalences of the VATs GUTats 7.3 and 7.4 were similar indicating that they had grown at similar rates. The results obtained for the VAT combinations GUTats 7.4 and 7.5, and GUTats 7.3 and 7.5 (experiments 4 and 5) also show that these VATs were present at similar prevalences. However the results of experiments 3 and 6 with these VAT combinations show that the prevalence of each of the VATs in a pairwise combination was significantly different. The results of these two experiments indicate that GUTat 7.4 grew more slowly than GUTat 7.5 which in turn grew more slowly than GUTat 7.3.

The prevalences of each of the VATs GUTats 8.2, 8.3 and 8.4 when grown in pairwise combination experiments are shown in Fig. 4.1(b) and a statistical analyses of these data are given in Table 4.1. These results show that the prevalences of the VATs GUTats 8.2 and 8.4 were similar, indicating that they had grown at similar rates. However the results obtained for both the VAT combinations GUTats 8.2 and 8.3 and GUTats 8.3 and 8.4 (experiments 7 and 8) showed that the prevalences of each VAT in a combination were significantly different. The results of these experiments

indicate that GUTat 8.3 grew more slowly than GUTat 8.2 which in turn grew more slowly than GUTat 8.4.

The results from five of the nine experiments indicated that trypanosomes expressing different VATs grow at similar rates. However the results of the other four experiments (experiments 3, 6, 7 and 8) indicated some effect of VAT upon growth rate and were therefore subjected to more detailed analyses. The rates of growth of GUTats 7.5 and 8.3 were experimentally determined as shown in Fig. 4.2. From these data PDTs of 5.6 hrs and 6.0 hrs were calculated for GUTat 7.5 and PDTs of 6.8 hrs and 6.1 hrs were calculated for GUTat 8.3 (Table 4.2).

Experimentally determined PDT values were used to calculate the PDT of the second VAT in any pairwise combination for each of those experiments that showed a difference between the two VATs using the method described in section 4.2.6. These results are shown in Table 4.2. Two conclusions can be made from the results shown in Table. 4.2. First, that growing VATs in pairwise combinations in order to detect differences in growth rates between VATs was a sensitive assay. Differences in growth rates as low as 1.8% between VATs in a combination produced a statistically significant difference in prevalence between the two VATs. Second, that in all combination experiments the differences in growth rates between VATs were less than 10%.

4.4 DISCUSSION

In this study growth rates were compared between trypanosomes of different VAT but identical passage history.

GUTAR 7 and GUTAR 8 have different genotypes and passage histories, therefore it was only possible to compare growth rates between VATs within each repertoire.

The growth rates of VATs in pairwise combination experiments were compared directly in a sensitive assay capable of detecting differences of less than 2% in PDTs between VATs. If two VATs were present at prevalences of 10% and 90%, such a result would not only be statistically significant but also of biological importance. However if two VATs were present at prevalences of approximately 60% and 40% in an experiment, although such a difference may well be of little biological importance, statistical analysis yields significant results.

These results are inconclusive if based only on the data obtained from statistical analyses of prevalence data (Table 4.1). It was therefore decided to compare PDTs of VATs in those combination experiments that appeared to indicate differences in growth rates between VATs. Experimentally determined PDT values for GUTat 7.5 differed by 7.1% between replicate experiments and for GUTat 8.3 this difference was 11.5%. Such levels of variation between replicate experiments of this type are typical (Chapter 3; Turner, 1990). If trypanosomes of different VAT do not differ in their growth rates, therefore, a similar or lower level of difference between PDTs of the two VATs in pairwise combination experiments would have been expected. As Table 2 shows, PDTs of VATs in all combinations tested differed by less than 10%.

The interpretation of these results is based on the assumption that the growth rate of a population of

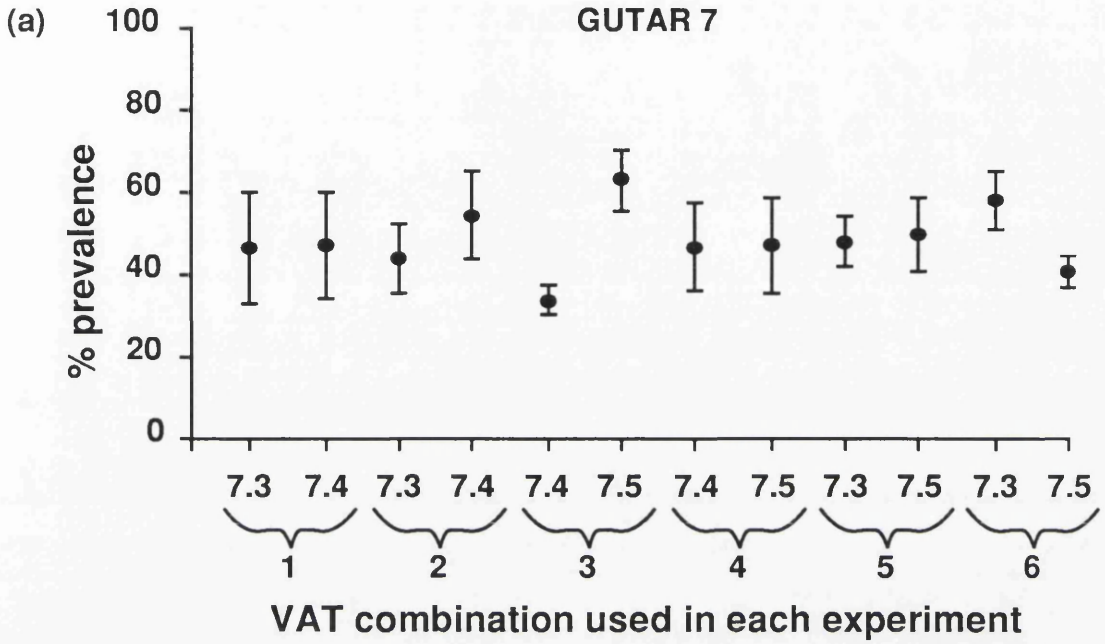
trypanosomes is the same whether the parasites were grown individually or in combination with another VAT. In a recent study McLintock *et al*, (1990) demonstrated that the growth rates of trypanosomes grown in combination with another VAT were slower than if that VAT was present in a single infection. The differences observed however were not significant and do not alter the interpretation of these results.

The relationship between trypanosome growth rate and VAT expression has been extensively studied. Several authors have demonstrated that trypanosome growth rates are determined by the VAT expressed by a population of trypanosomes (Van Meirvenne *et al*, 1975; Seed, 1978; Miller & Turner, 1981; Seed *et al*, 1984; Myler *et al*, 1985). In these studies it was observed that when combinations of VATs were inoculated into mice, one VAT always grew faster and subsequently replaced the slower growing VAT, that is, became the predominant VAT. Thus an order of sequence of VATs was defined based on their respective growth rates, with the fastest growing VAT appearing early in the infection. An alternative approach was used by Barry *et al* (1979) and Myler *et al* (1985). In these studies the authors compared growth rates between trypanosome lines which expressed the same VAT (Barry *et al*, 1979) and those which expressed different VATs (Myler *et al*, 1985). Myler *et al* (1985) showed that trypanosome lines which express different VATs had different growth rates and that these differences correlate with the order of appearance of VATs in a chronic infection, whereas in the study by Barry *et al* (1979) it was shown that lines of identical genotype and VAT had different

growth rates. However, a reservation with the interpretation of the results of these studies was that comparisons of growth rates were made between lines of different passage history. Since growth rates of trypanosome lines have been shown to change on rapid passage (Barry *et al*, 1979), the observed differences in prevalences and growth rates of VATS could not be entirely attributed to the VATs expressed. It was for this reason that the experimental studies presented in this chapter were conducted.

Kosinski (1980) and Agur *et al* (1991) adopted a theoretical approach to the problem of VAT related growth rates using mathematical models to predict the order of appearance of VATs on the basis of differences in growth rates. The results of these studies showed that even when intrinsic growth rates of trypanosomes differed by three to ten fold a partially predictable temporal order of sequence of appearance of VATs could not be generated. In the present study differences of less than 10% were observed between growth rates of VATs. These results would suggest that the regulation of trypanosome growth by the VAT expressed is unlikely to play a significant role in determining the hierarchy of appearance of VATs in an infection.

Fig. 4.1 Prevalences of VATs when grown in pairwise combinations. Results are expressed as $\bar{x} \pm 2SE$, n = 6, except n = 5 for experiments 3 and 6 and n = 4 for experiment 4.



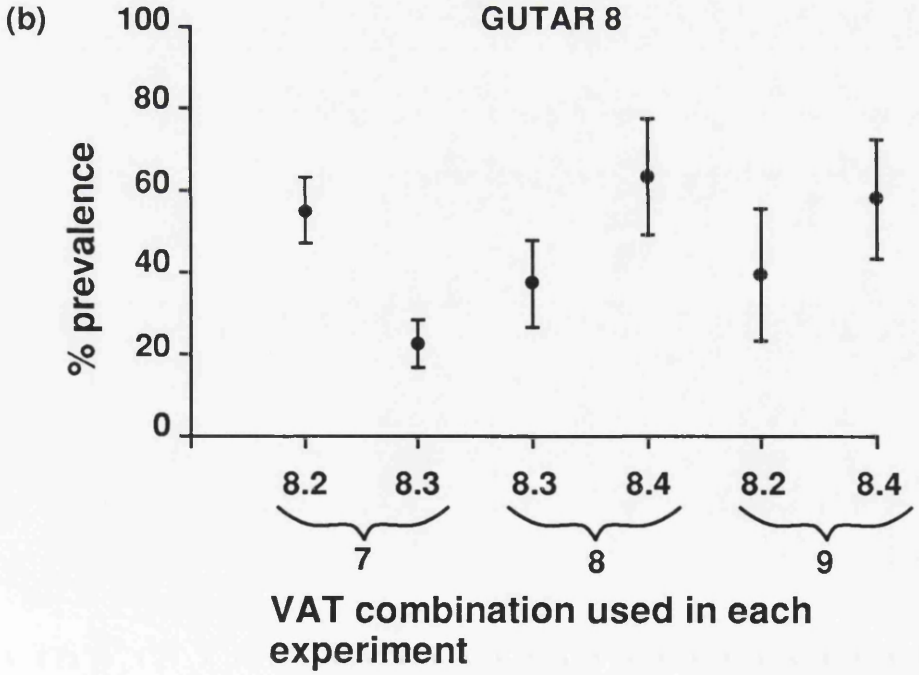


Table 4.1 Statistical analyses of the prevalences of VATs grown in pairwise combinations. * denotes a significant difference between the mean percentage prevalence of each VAT in an experiment.

Experiment	VAT combination		Percent prevalence, $\bar{x} \pm 2SE$		Variance ratio(F)	t	P
	VAT 1	VAT 2	VAT 1	VAT 2			
1	7.3	7.4	46.4±13.8	47.0±13.0	1.1	0.1	P > 0.1
2	7.3	7.4	43.8± 8.2	54.5±10.9	1.8	1.6	P > 0.1
3	7.4	7.5	33.8± 3.3	63.1± 7.4	4.9	7.2	P < 0.001 *
4	7.4	7.5	46.6±10.5	47.1±11.7	1.3	0.1	P > 0.1
5	7.3	7.5	47.8± 6.1	49.6± 9.0	2.2	0.3	P > 0.1
6	7.3	7.5	58.2± 7.1	40.6± 3.9	3.3	4.3	0.001< P <0.002 *
7	8.2	8.3	55.1± 8.2	22.6± 5.6	2.1	6.5	P < 0.001 *
8	8.3	8.4	37.2±10.5	63.0±14.2	1.8	2.9	0.01< P <0.02 *
9	8.2	8.4	39.2±16.2	57.8±14.6	1.2	1.7	P > 0.1

Fig. 4.2 Growth rates of GUTats 7.5 (a,b) and 8.3 (c,d), geometric mean $\pm$ 2SE, n = 6

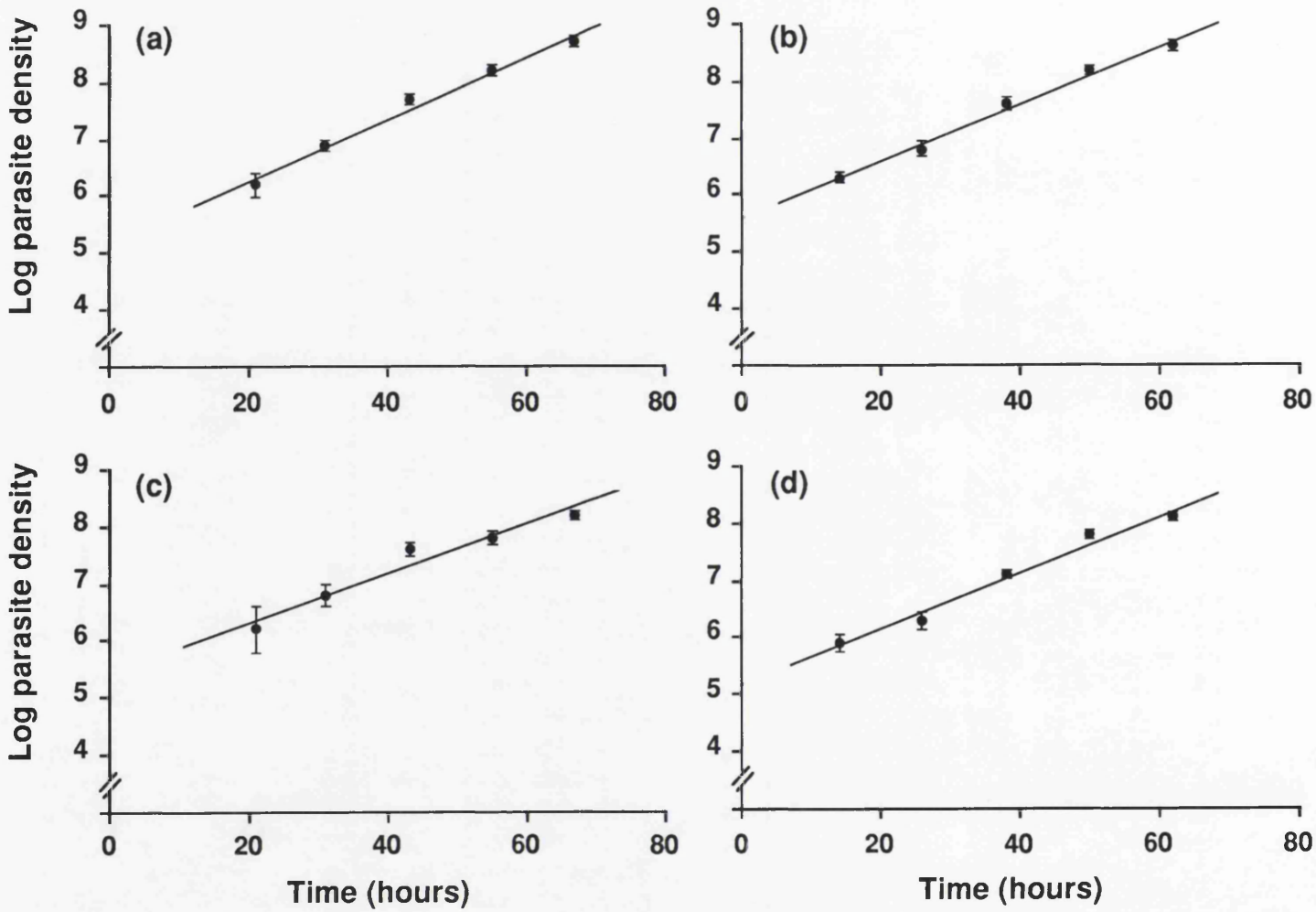

(a)
(b)
(c)
(d)
Log parasite density
Log parasite density
9
8
7
6
5
4
0
20
40
60
80
Time (hours)
Time (hours)

Table 4.2 Detailed analyses of those experiments that appeared to show differences in the rates of growth of VATs when grown in pairwise combinations. For each combination of VATs, two growth rate values were experimentally determined for one of the VATs and subsequent analyses were made with both values of growth rates. The growth rate of the other VAT in in each pair was calculated as described in section 4.2.6.

Experiment	VAT combination		PDT (hours)		Percent difference between VATs
	VAT1	VAT2	VAT1 experimentally determined	VAT2 calculated	
3	7.5	7.4	5.6	6.0	7.1
			6.0	6.4	6.7
6	7.5	7.3	5.6	5.5	1.8
			6.0	5.8	3.3
7	8.3	8.2	6.1	5.6	8.2
			6.8	6.2	8.8
8	8.3	8.4	6.1	5.8	4.9
			6.8	6.4	5.9

CHAPTER 5

GENERAL DISCUSSION

The courses of infection with the economically important species of African trypanosomes have been shown to vary between host species, between individuals within a host species and between strains of a single species of host (see section 1.2). The courses of infection have also been shown to vary between stocks of *T. congolense* (Roelants & Pinder, 1987) and *T. brucei* (Chapter 2). There are four principle trypanosome dependent factors which have been implicated in regulating the courses of infection observed. These are (1) antigenic variation, (2) the rate of trypanosome growth, (3) the rate of differentiation from slender forms to stumpy forms and (4) the spatial distribution of trypanosomes in the host (reviewed in Barry & Turner, 1991). Of the aforementioned factors the importance of antigenic variation in regulating the course of an infection is beyond doubt. Evidence for the importance of the other three factors however, is inconclusive.

The aim of the studies decribed in this thesis was to determine the importance of growth and differentiation in regulating the course of infection. In particular, emphasis was placed upon the use of haemocytometer counts to estimate parasitaemias as opposed to other inaccurate methods often used in previous studies (see Tables 1.1-1.3). Also all stocks were cloned and within 10 mouse passages either of the original isolate, or of transmission through tsetse flies (see Appendix). Any differences between the stocks, therefore are considered to have resulted from selection in the field before isolation rather than after it in the laboratory. Furthermore, age and sex matched inbred mice were used in each of the experiments described.

It is generally accepted that the course of a chronic trypanosome infection is characterised by the occurrence of several cycles of remission and recrudescence of parasitaemia (Fantham & Thompson, 1910, 1911; Ross & Thompson, 1910a, 1910b; reviewed in Herbert & Parratt, 1979). However this pattern of infection was only observed in two of the six cloned stocks examined in Chapter 2. In Chapter 3 it was shown that the observed differences in the courses of infection could be explained by differences in their relative growth rates and more specifically the rates of trypanosome replication.

A considerable proportion of trypanosomiasis research is conducted with trypanosome lines which have been maintained in the laboratory by rapid syringe-passaging in laboratory rodents. Although such lines have been shown to differ in their degree of pleomorphism and homogeneity of VAT expression (Turner, 1990), few formal comparisons have been made of the changes which arise as a result of rapid syringe-passaging of trypanosome lines in laboratory rodents. Barry *et al* (1979) and Inverso *et al* (1988) have studied the effect of rapid syringe-passaging on growth rates with conflicting results. Barry *et al* (1979) showed that PDTs increased with rapid syringe-passaging, whereas the results obtained by Inverso *et al* (1988) showed no change in PDTs. In Chapter 2 it was shown that parasitaemias increased with rapid syringe-passaging and from the results obtained in Chapter 3 it was shown that these differences were not associated with faster growth or replication rates. There was no apparent relationship between the rates of trypanosome differentiation and the

courses of infection observed in either of the two comparisons made. However, estimates of the rates of trypanosome differentiation were based upon the assumption that the life expectancy of stumpy forms is high. As no detailed estimates of stumpy form life expectancy have been made, this assumption was based upon the results of one study by Black *et al* (1985). Therefore it is necessary to accurately determine this parameter to improve confidence in the differentiation rate values.

In this thesis and all previous studies the method of classification of trypanosomes into slender, stumpy and intermediate forms was subjective and therefore of only limited accuracy. Thus for greater accuracy alternative methods of determining functional morphology of trypanosomes should be sought. The development of such methods would enable more reliable estimates of replication and differentiation rates to be made.

In Chapter 3 it was shown that stumpy form trypanosomes predominated over slender and intermediate forms at the begining of the infection but declined in prevalence as the infections progressed during the phase of exponential population growth. In contrast the prevalence of slender forms was observed to increase as the infections progressed. These results are suggestive of a difference in the ability of slender and stumpy form trypanosomes to disperse from the site of inoculation in the peritoneum, through the lymphatics to the bloodstream. The results of this study however, cannot be directly compared with those of previous studies because they have all quantified trypanosome morphology from approximately 70 hours p.i. only

(Balber, 1972; Black *et al*, 1983a, 1983b, 1985; McLintock, 1990). Further studies should be conducted therefore to determine whether differences in the ability of slender forms to enter the bloodstream between cloned stocks and lines could explain the observed differences in the prevalences of the various morphological forms during the initial stages of infection.

During the course of a *T. brucei* infection, VATs are expressed in a partially predictable manner (Gray, 1965a; Capbern *et al*, 1977). Three possible mechanisms by which an ordered sequence of appearance of VATs could be generated have been proposed: (1) VAT related growth rates, (2) the influx of trypanosomes from extravascular sites (expressing novel VATs) into the bloodstream and (3) VAT specific switching rates (reviewed in Turner, 1984; Vickerman, 1989; Barry & Turner, 1991). The first of these proposed mechanisms was reinvestigated in Chapter 4. It was shown that differences in growth rates between VATs when grown in pairwise combinations were less than 10%. It was therefore concluded that the regulation of trypanosome growth by the VAT expressed is unlikely to play a significant role in determining the hierarchy of appearance of VATs in a chronic infection. With respect to the second proposed mechanism, experimental studies by Turner *et al* (1986) have shown that trypanosome populations derived from several extravascular sites were antigenically similar to each other and to the bloodstream populations. Thus it would appear that an hierarchy of appearance of VATs could not be generated by these means. However, experimental studies conducted to investigate the role of VAT specific switching in

determining the hierarchy of appearance of VATs in an infection suggest that the third proposed mechanism is the most likely of the three described. Turner & Barry (1989) obtained switching rate estimates of greater than 2.0-9.3 x 10^{-3} switches/cell/generation in fly-transmitted infections and these are the highest and most accurate estimates to date. More importantly, switching rates were found to be directed to expression of particular VATs and those VATs that were switched to most frequently had the highest prevalences. Therefore it is likely that the switching rate of a trypanosome is of considerably greater importance than its VAT-specific growth rate in determining its prevalence and the timing of its expression in an infection. More recently Turner (unpublished results) obtained switching rate estimates for fly-transmitted trypanosomes which were considerably higher than comparable estimates for lines which had been extensively syringe-passaged in mice. The observed differences in switching rates may explain the differences in the courses of infection between cloned stocks and syringe-passaged lines of different laboratory history. However there is a limitation with the interpretation of the results of these studies in that switching rates were estimated in cloned stocks which had been extensively passaged since isolation before transmission through tsetse flies. Thus in future studies comparisons will need to be made of switching rates between cloned stocks of trypanosomes close to the original isolate.

To conclude, cloned stocks of trypanosomes differ in the courses of infection that they produce in mice, and those stocks which produce high parasitaemias generally have

faster growth and replication rates. Rapid syringe-passaging dramatically alters the courses of infection with cloned stocks of trypanosomes previously shown to produce mild infections in mice. These changes in the courses of infection are not associated with differences in growth and replication rates. Differentiation rates do not appear to regulate the courses of infection observed. A reexamination of the relationship between VAT expression and trypanosome growth rates indicates that the hierarchy of appearance of VATs observed in chronic infections cannot be explained by differences in growth rates alone.

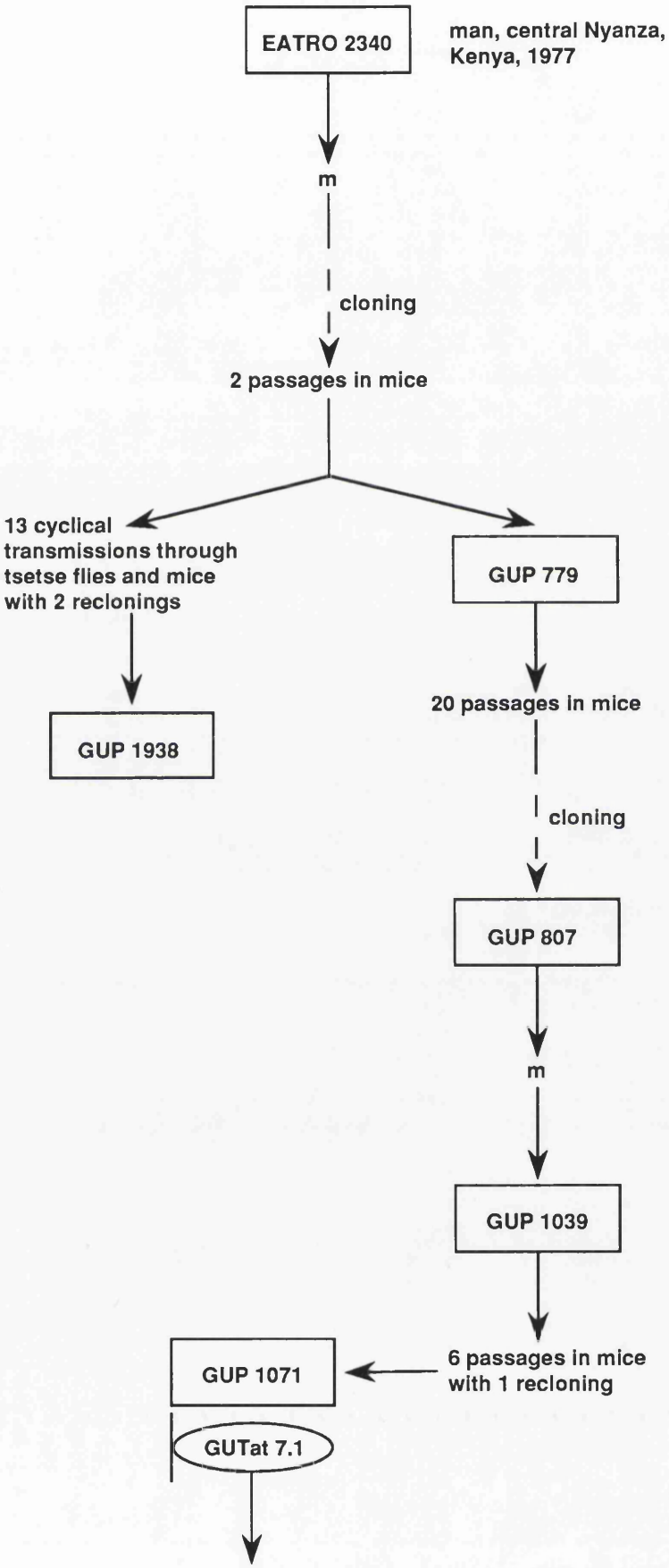
It is hoped that the results of these studies will provide a basis from which further more detailed analyses of those trypanosome dependent factors regulating the courses of infection can be made. For instance, preliminary experiments have been conducted to determine the inheritance of parasitaemia characteristics in F1 hybrid clones derived from the parent stocks STIB 247 and STIB 386 (Turner *et al*, unpublished results). The results of these studies (though preliminary in nature) suggest that the ability to produce a high parasitaemia is inherited as a dominant trait. Also the trypanosome cell cycle which determines replication and differentiation has not been extensively investigated (reviewed in Barry & Turner, 1991). Therefore it would be of interest to determine whether the observed differences in replication rates can be explained by differences in the cell cycle.

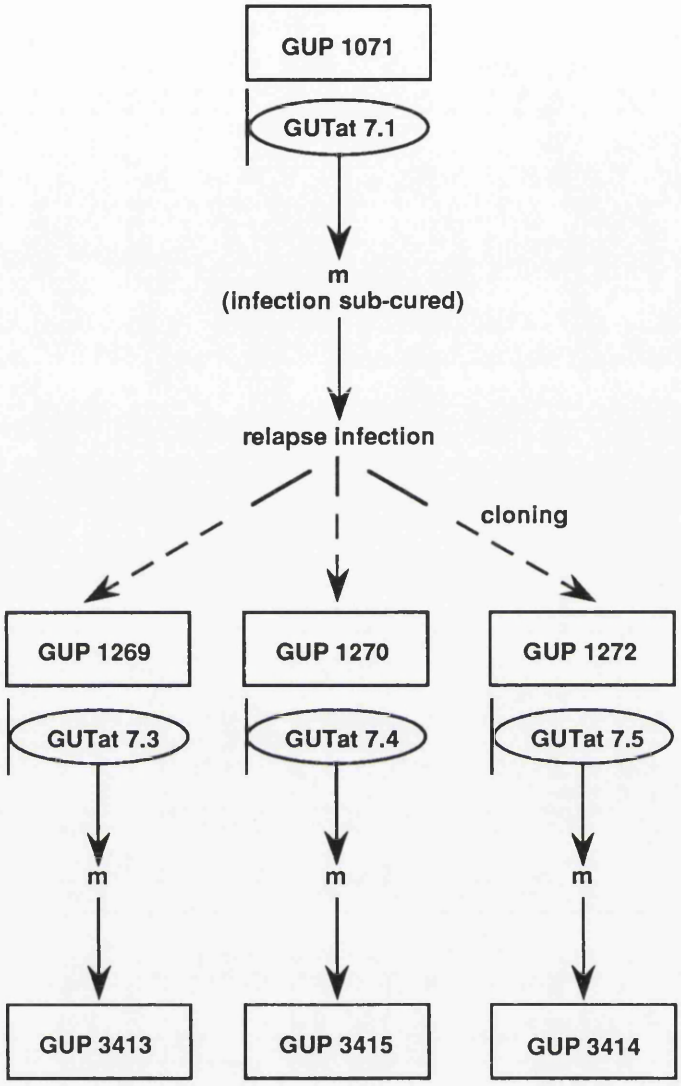
APPENDIX

Pedigree diagrams for each of the cloned stocks, lines and VATs of trypanosomes used in this thesis. These have been presented in a simplified format and are drawn according to the conventions of Lumsden *et al* (1973). A pedigree diagram was not available for the cloned stock TREU 927/4, however, it is known that this stock is within 10 mouse passages of the field isolate (A. Tait, personal communication).

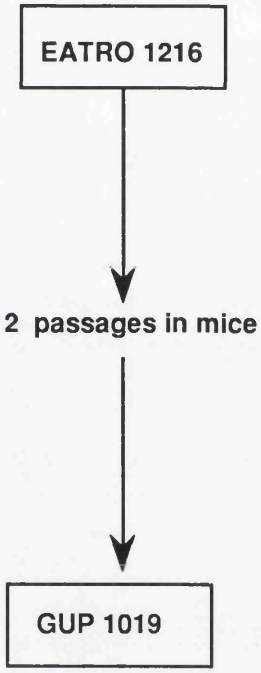
For the comparison of cloned stocks of trypanosomes made in Chapters 2 and 3, the stabilates used were GUPs 1938, 1019, 3280, 3283, 2671 and 2969. The stabilates used for the comparison of lines of STIB 247 in these Chapters were GUPs 3280, 2943 and 3615. For the comparison of growth rates made in Chapter 4, the stabilates used were GUPs 3413, 3415, 3414, 3172, 3173 and 3180. m = mouse.

History of the cloned stock EATRO 2340
(Scott, 1987)

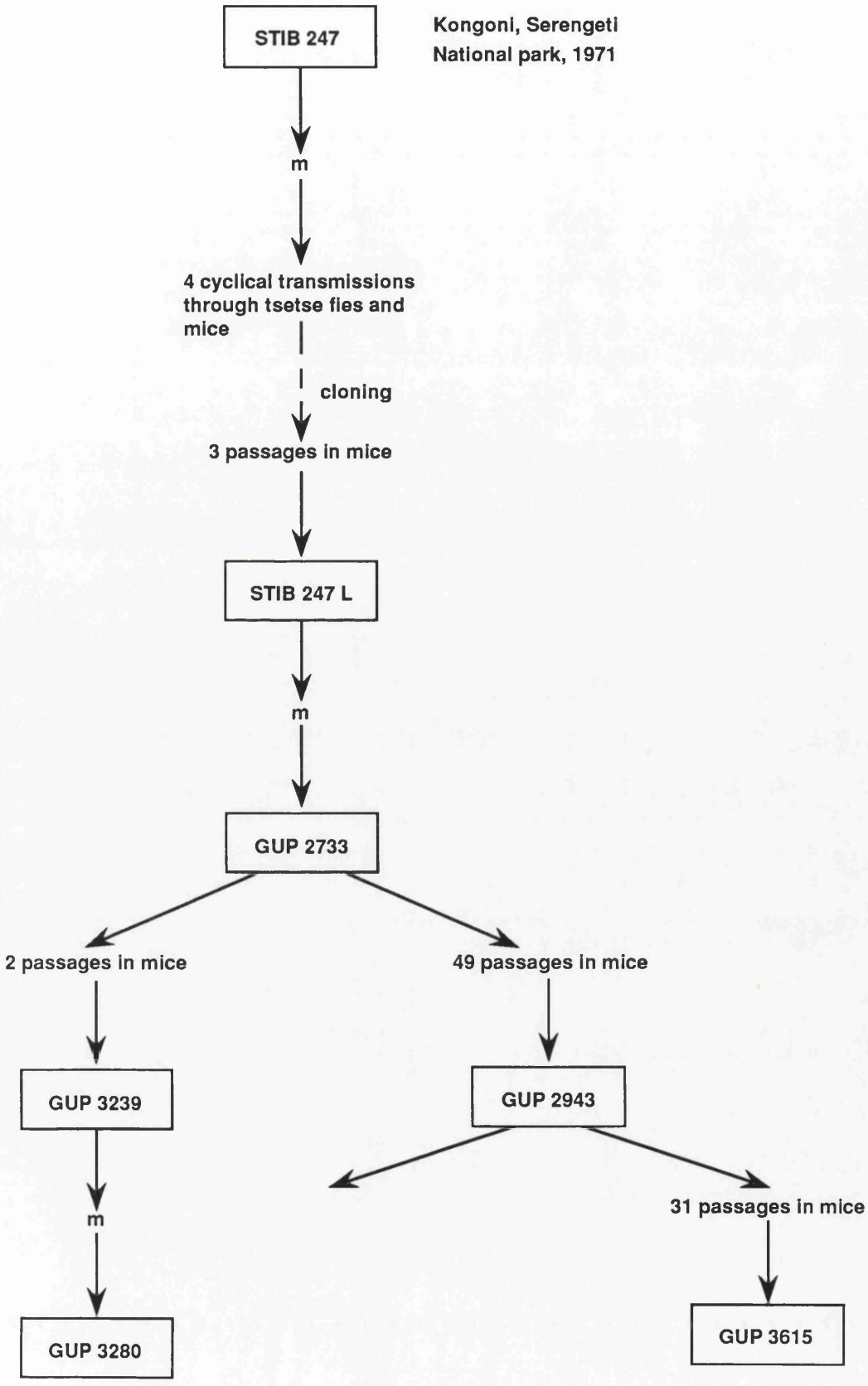


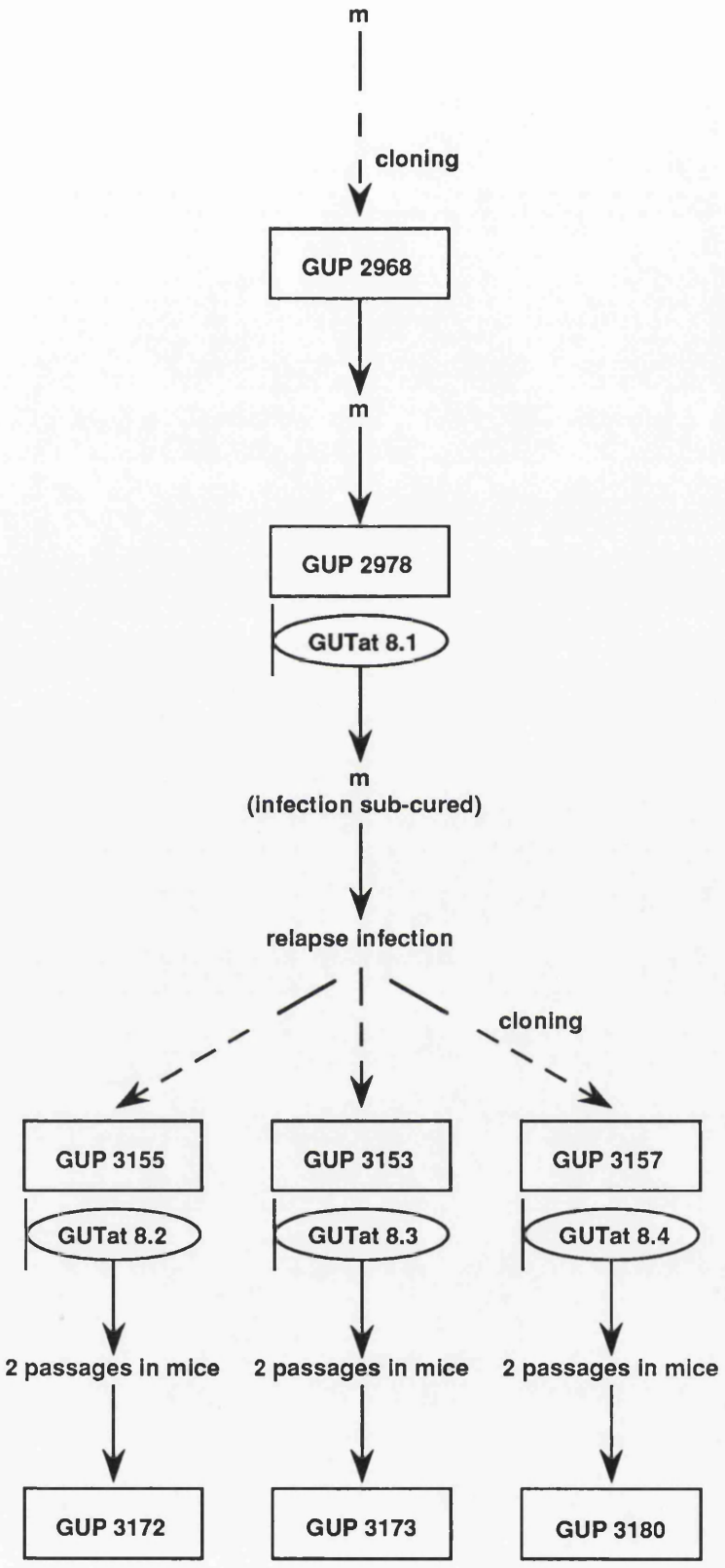

GUP 1071
GUTat 7.1
m
(infection sub-cured)
relapse infection
cloning
GUP 1269
GUP 1270
GUP 1272
GUTat 7.3
GUTat 7.4
GUTat 7.5
m
m
m
GUP 3413
GUP 3415
GUP 3414

History of the cloned stock EATRO 1216

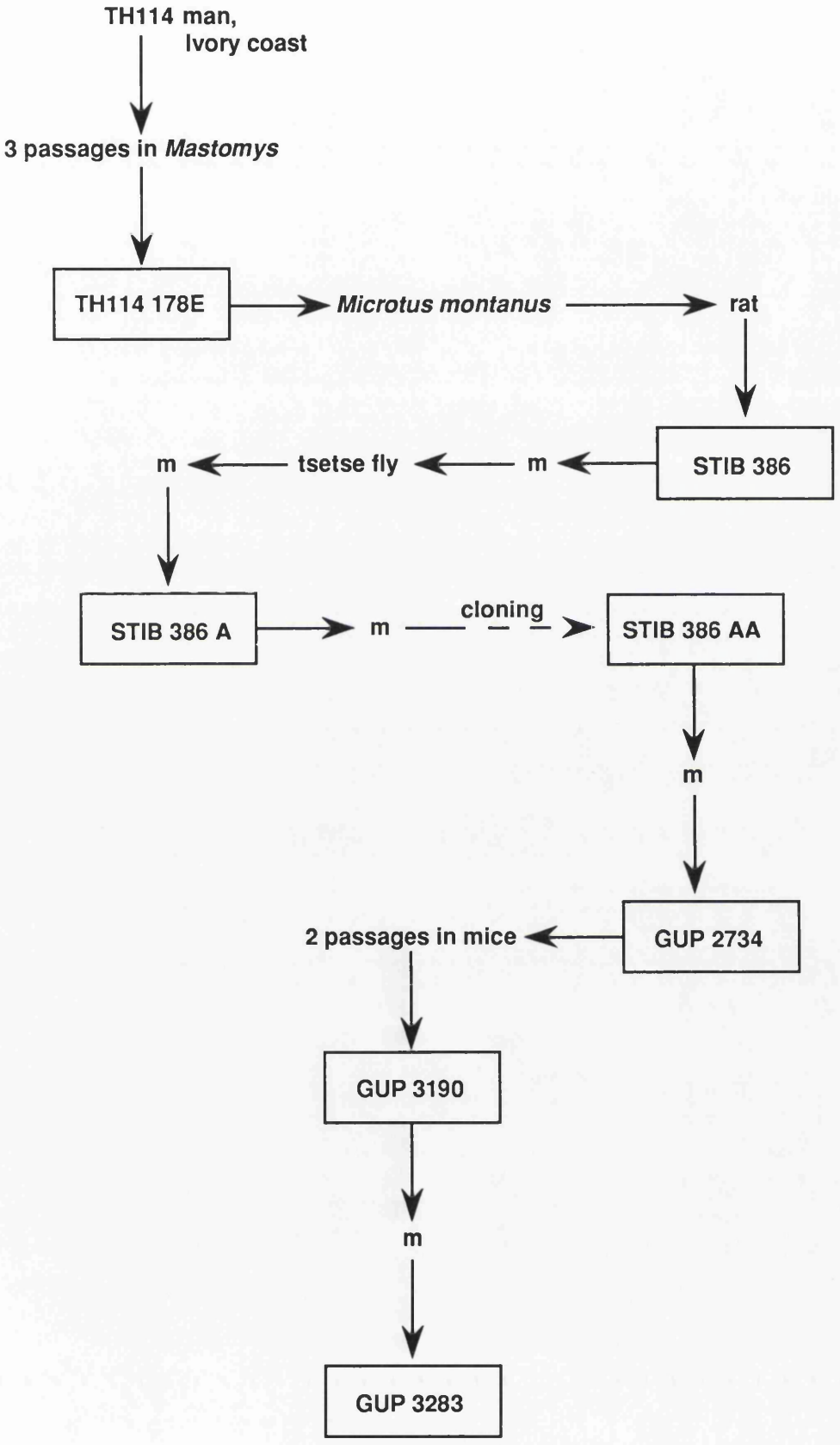


History of the cloned stock STIB 247

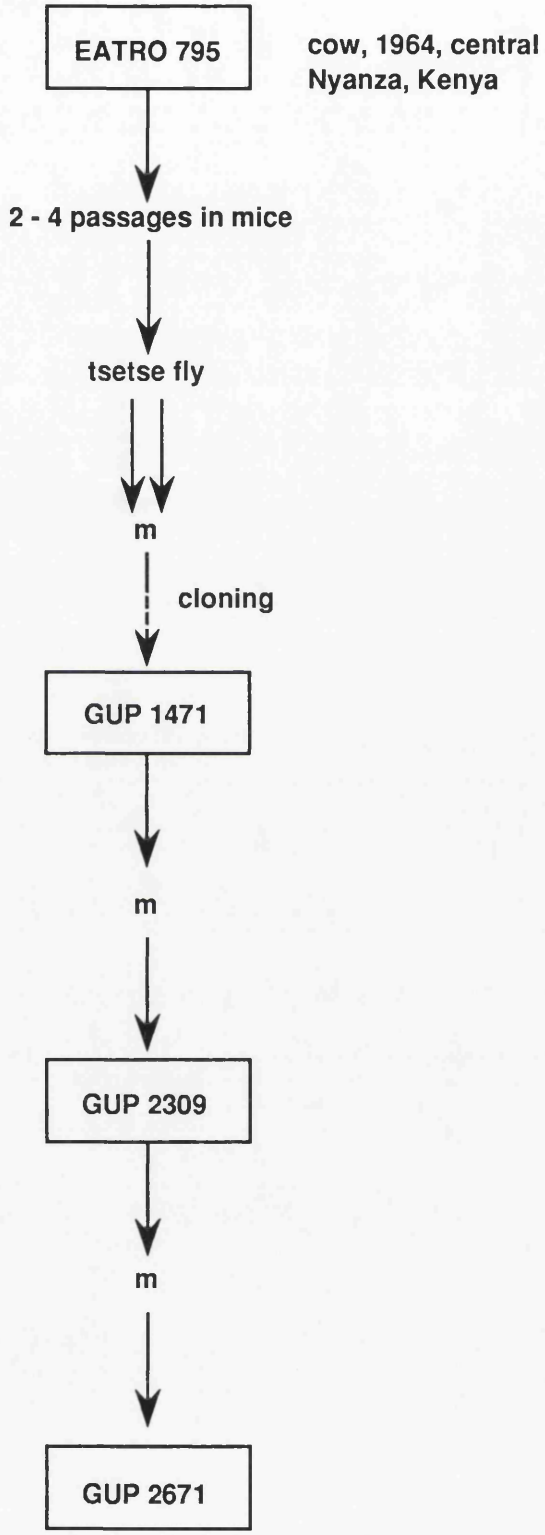



m
cloning
GUP 2968
m
GUP 2978
GUTat 8.1
m
(infection sub-cured)
relapse infection
cloning
GUP 3155
GUP 3153
GUP 3157
GUTat 8.2
GUTat 8.3
GUTat 8.4
2 passages in mice
2 passages in mice
2 passages in mice
GUP 3172
GUP 3173
GUP 3180

History of the cloned stock STIB 386



History of the cloned stock EATRO 795
(Scott, 1987)



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