BOVINE TRYPANOSOMIASIS WITH SPECIAL REFERENCE TO TRYPANOSOMA THEILERI

By

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Thesis presented for the degree of Doctor of Philosophy of the University of Edinburgh in the Faculty of Veterinary Medicine, 1969.
The literature on *Trypanosoma theileri* is reviewed. An argument is presented in support of the collective term 'T. theileri-like' organisms to describe the many miscellaneous observations available, reserving the species name *Trypanosoma theileri* Laveran 1902 specifically for the infections of cattle discovered in South Africa by Théler.

In terms of practical importance, *T. theileri*-like trypanosomes are common and cosmopolitan parasites of cattle but have the appearance of being pathogenic to their mammalian hosts only in rare circumstances, none of which have been repeated experimentally. The possibility exists however that they may complicate the serological diagnosis of salivarian trypanosomes pathogenic to man and domestic animals. The review concludes by suggesting that studies on the natural history of these infections and the handling and storing of *T. theileri*-like trypanosomes in the laboratory are necessary prerequisites to specific serological or other examinations.

The experimental work follows this suggested pattern. An incidence of *T. theileri*-like infections of cattle and other artiodactyls is first described from a total of twenty nine localities in Colombia, England, Nigeria and Scotland, as detected on blood agar media. Observations were made concurrently on: the time of first appearance of organisms on culture, levels of parasitaemia and the age of cattle on first infection. Isolations were stored as stabilates at low temperatures and found to retain infectivity to the same type of media for more than four years. Using stabilates as the principal source of material, the characteristics of growth on blood agar media maintained at 28°C were studied together with the capability of transfer to mammalian cell cultures.
maintained at 37°C. A technique was developed for growing clone populations on blood agar media at 28°C.

An Ayrshire cow known to be infected with *T. theileri* - like trypanosomes was examined intensively by the culture of peripheral blood on blood agar slopes at 28°C. Frequent sampling within a thirty month period showed a marked seasonal variation in the level of parasitaemia. Characteristics of the isolations on culture also showed seasonal variations enabling an hypothesis to be made that organisms possessing the capability of infecting a vector and developing to the metacyclic stage increased in numbers in the peripheral blood of the host in the summer months.

Although the artificial infection of susceptible cattle was achieved by the transfer of whole parasitaemic blood, no success was obtained in attempts to infect cattle with cultures maintained at either 28°C or 37°C. In a search for the vector, dissections of members of the family Tabanidae failed to reveal any trypanosomatid infections.

The results of the experimental work gave support to a working hypothesis of the life cycle of *T. theileri* - like trypanosomes constructed from fragmentary evidence derived from Europe, Africa, Asia and the Americas.
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1. INTRODUCTION

Definition

Trypanosoma theileri has been described as a cosmopolitan parasite of cattle (Herbert, 1964). Both trypomastigote and epimastigote forms (Hoare and Wallace, 1966) may occur in the peripheral blood of infected animals but multiplication is said to occur in the amastigote form in 'fixed' tissues (lymph nodes and brain) (Hoare, 1936).

Kenyon (1926) records the size of the trypanosome in the bloodstream as being 60 - 70 μ long without the flagellum, but adds that smaller forms may be seen of a minimum length of 25 - 30 μ. Hoare (1966) in his classification of mammalian trypanosomes gives a maximum length of 100 μ, but Soulsby (1968) believes that forms of up to 120 μ can occur especially in chronic infections. The posterior end of all bloodstream forms is long and pointed, the nucleus approximately central, and there is a free flagellum with a usually well defined undulating membrane (fig. 1).

Infections are characterized both by very low levels of parasitemia, which make recognition by ordinary microscopy very difficult, and by their lack of pathogenic effect to the host. (Herbert, 1964; Soulsby, 1968).

Historical context

The first recognition of a large trypanosome of cattle was probably made by Lingard in India in 1892 although he did not publish his observations until 1903. Legrain (1896) in France described a trypanosome found on post-mortem in a pericardial haemorrhage of an ox and later, Kossel and Weber recollected in retrospect having seen a single large trypanosome in a heart muscle smear of an ox in Finland about 1900.
2. echaudinn, 1914; Weber, 1909. However, Theiler (1905) was the first to attract major attention to the large trypanosomes of cattle because he believed at that time that it was the cause of an important cattle disease in South Africa known as 'gallziekte' (gall sickness). Although he later identified that disease as bovine anaplasmosis (Theiler, 1910 a) much interest in the trypanosome was aroused, particularly when it was realized that the presence of these organisms in peripheral blood of cattle, difficult to detect on direct microscopy, could be readily detected on culture (Miyajima, 1907; Martini, 1909; Crawley, 1909). In the short period thereafter up to 1914 there are at least seventy published papers giving (a) the failure to establish infection in any small laboratory animal and, (b) the realization that the infection was normally free of pathogenic effect.

The species name Trypanosoma theileri was suggested independently by both Laveran (1902 a) and Bruce (1902) following the receipt of blood slides from Theiler, but Laveran's published description takes precedence. Subsequent authors suggested other species names for other isolations, but early in the story Wenyon (1926) who listed thirteen names which he considered to be synonyms (see section: taxonomy).

Motive for study

Johns (1914) suggested that such a large and easily obtainable
trypanosome would be "very valuable to study the great problem of trypanosomiasis". This view was independently revived by the WHO expert committee on immunology and parasitic diseases (WHO, 1965) who indicated a need for a model trypanosome available for "experimental, immunological and epidemiological study" in temperate climates and recommended that T. theileri be "intensively studied to determine its suitability for the purposes outlined". This recommendation provided the interest and starting point for the present thesis.

Taxonomy

The revised classification of the protozoa proposed by the committee on taxonomy and taxonomic problems of the Society of Protozoologists (Honigberg et al., 1964) placed the genus Trypanosoma as follows:

- Phylum Protozoa Goldfuss, 1818; emend. Siebold, 1845.
- Subphylum Sarcomastigophora Honigberg and Balamuth, 1963.
- Superclass Mastigophora Diesing, 1866.
- Class Zoomastigophora Calkins, 1909.
- Suborder Trypanosomatina Kent, 1880.
- Family Trypanosomatidae Doflein, 1901; emend. Grobben, 1905.
- Genus Trypanosoma Gruby, 1843.

The classification of members of the genus Trypanosoma offered by Wenyon (1926) was based on two main courses of development in the invertebrate host. The first is the development which leads to infection of the biting parts of the invertebrate with the metacyclic stage so that transmission can be termed 'inoculative', and the second is the development which leads to metacyclic forms in the hind gut so that transmission can be termed 'contaminative'. Wenyon therefore divided the genus into
Group A, those trypanosomes which develop in the 'posterior station',
and Group B, those trypanosomes which develop in the 'anterior station'.
Hoare (1949, 1957) considering only the trypanosomes of mammals,
classified them on this basis, giving additional reasons for the
relevance of the grouping. He later proposed the terms Stercoraria and
Salivaria in place of Groups A and B, subdividing both into four subgenera.
T. theileri thus became the type species of the subgenus Neratrypanum
(Hoare, 1964). In Hoare's latest modification (1966) he states that the
hosts of T. theileri are "bovines" and "antelopes" and places in the
same subgenus the following selected species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Author</th>
<th>Hosts</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. theileri</td>
<td>Kinghorn et al., 1913</td>
<td>antelopes</td>
</tr>
<tr>
<td>T. melophagium</td>
<td>Flu, 1908</td>
<td>sheep</td>
</tr>
<tr>
<td>T. mazamae</td>
<td>Massa et al., 1932</td>
<td>deer</td>
</tr>
<tr>
<td>T. cephaloni</td>
<td>Bruce et al., 1915</td>
<td>antelopes</td>
</tr>
<tr>
<td>T. ingens</td>
<td>Bruce et al., 1969 b.</td>
<td>cattle,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>antelopes, chevrotains</td>
</tr>
</tbody>
</table>

Hoare stated in his explanatory notes (1966), in reference to the
Stercoraria, that "fuller studies of such species might necessitate a
re-appraisal of their systematic position". Considering this statement
in relation to his selected species in the subgenus Neratrypanum, posterior
station transmission is proven for T. melophagium (Hoare, 1923), some
circumstantial evidence exists to this effect for T. theileri, but no
evidence exists for any of the others which appear to be allocated solely
on the evidence of their morphology in the hosts affected. The original
descriptions were moreover based on the measurement of very few organisms
(five in the case of T. ingens) although some additional morphological
evidence became available for example: T. ingens in cattle (Hamerton,
T. tracelaphi in an ox (Thomson, 1931), and T. insens, T. tracelaphi, T. cephalophi and T. mazama in artiodactyrs other than domestic oxen (see Table 1).

The unstabilized nature of the situation was further emphasized by Keymer (1967) who described trypanosomes identifiable on morphological grounds as T. theileri, T. cephalophi, T. tracelaphi and T. insens all in the same splenctomized ox in Malawi over a period of six weeks. He, moreover, believed that there was no obvious morphological difference "between any of the recognized species in the subgenus Kinetrpyanum".

Both Hoare (1966) and Keymer (1967) drew attention to a similar difficulty in the stercorarian subgenus Heterosoma and to the solution offered by Davis (1952) to designate as "T. lewisi - like", the trypanosomes of rodents when only morphological data were available. T. crusi - like trypanosomes has similarly become acceptable phraseology within the subgenus Schizotrypanum (e.g. Beane, 1967). Following these precedents, Keymer (1967) suggested that T. cephalophi, T. tracelaphi and T. insens should be referred to as T. theileri - like. Extending this argument, all trypanosomes of the subgenus Kinetrpyanum could be called T. theileri - like excepting those where biological characteristics are known in addition to morphology and host. Trypanosome theileri then refers only to Theiler's original observations in South Africa. A list of T. theileri - like organisms would then absorb not only the list of synonyms offered by Wenyon (1926) but also the selected species of Hoare's 1966 classification with the exception of T. melophagum. A remaining species T. theodori Theodor, 1928 can also be excluded as its vector has been proven. The list of synonyms can then read:

Trypanosome transvaaliense

Trypanosome linsardi

Laveran, 1902 b.

Blanchard, 1904.
Trypanosoma himaevanum
Trypanosoma theileri
Trypanosoma indicum
Trypanosoma auksanarid
Trypanosoma wruobawaiki
Trypanosoma americanum
Trypanosoma franki
Trypanosoma falsavari
Trypanosoma scheineri
Trypanosoma impans
Trypanosoma rutherfordini
Trypanosoma cephaloni
Trypanosoma schonebecki
Trypanosoma tracyaphi
Trypanosoma theileri var.
Somalensis
Trypanosoma theileri var.
Sudanensis
Trypanosoma magarsum

Lingard, 1907.
LMhe, 1906.
Lingard, 1907.
Lingard, 1907.
Wladimiroff and Yakimoff, 1909.
Crawley, 1909.
Frosch, 1909.
Knuth, 1909.
Knuth, 1909.
Bruce et al., 1909 b.
Hadwen, 1912.
Bruce et al., 1915.
Mayer, 1912.
Kinghorn et al., 1913.
Croverie, 1920.
Curasson, 1925.
Mazza et al., 1932.

Selected area of study

The assumption implicit in the WHO recommendation (vide supra) that sufficient is known of the characteristics of Trypanosoma theileri to identify it as a valid and cosmopolitan species is seen to be open to discussion. The review of literature attempted in this thesis will consolidate the information available on Trypanosoma theileri and the T. theileri - like trypanosomes argued and listed above and will discuss the consistencies and inconsistencies of the data. Epidemiological observations will then be described of T. theileri - like isolations made...
in Nigeria (west Africa), Scotland, England and Colombia (South America). Experimental work described mainly relates to isolations obtained from a single Ayrshire cow maintained from 1966 to 1969 at the University of Edinburgh Veterinary Field Station, Easter Bush, Midlothian, Scotland.

Information from the following references has been absorbed into the experimental section as they represent original work relevant to the thesis.


An extension of the work described in this thesis was carried out as part of the programme of the 1966 Edinburgh University Veterinary Expedition to East Africa, and has been recorded by the expedition members: Reid, H.W., Burridge, K.J., Pullan, N.B., Sutherst, R.W., and Wain, E.B.

Nomenclature

The classification of trypanosomes will follow that of Hoare (1966). The new terminology for development stages of trypanosomatid flagellates suggested by Hoare and Wallace (1966) will also be followed. For reference these are:

Old terminology
leishmanial form
leptomonad
trypanosome or trypanomorphic
criithidial
true trypanosome

New terminology
amastigote
promastigote
epistomastigote
epimastigote
choanomastigote
trypomastigote

Laird (1959) believed that two quite distinct genera of the family Trypanosomatidae had been confused under the name Crithidia, and the form usually described as 'criithidial' should be termed 'blastocriithidial'. These are now however designated as epimastigote.

Mammalian hosts will be arranged according to the nomenclature of Morris (1965).
II. REVIEW OF THE LITERATURE:

Trypanosoma theileri - like trypanosomes of mammalian species.

Geographical distribution

Appendix I lists published and some unpublished records of the demonstration of T. theileri - like trypanosomes in mammalian tissues either by direct microscopy or by culture. Although not exhaustive, the list illustrates the cosmopolitan distribution of the organisms ranging from the tropics to the neighbourhood of the arctic circle (Budukaloff, 1908). Small numbers of references for example for Australia and China, and the total lack for New Zealand, are likely to be due only to lack of search. Admittances of failure to demonstrate the organisms using culture techniques successful elsewhere are very few e.g. the failure of Bettencourt and Borges (1912, 1916) to cultivate trypanosomes from approximately 50 head of cattle arriving in Portugal for slaughter from the Azores.

Observations on T. theileri - like trypanosome infections of cattle in Nigeria, Scotland, England and Colombia are later described in section III Experimentation. Only one study of these trypanosomes has been made in Nigeria (Gray and Nixon, 1967) and all other Nigerian references to them have been incidental to observations on salivarian trypanosomes. No previous records appear to exist from Scotland but elsewhere in the British Isles Herbert (1963) made a laboratory study of an isolation made in Eire and several records exist incidental to other investigations. Previous information from Colombia is restricted to occasional demonstration in blood smears (e.g. Virviescas, 1934).

Host range

Records of trypanosomes of the subgenus Megatrypanum are all derived from animals of the mammalian Order Artiodactyla (even-toed ungulates). Megatrypanum are restricted in their host range to members of the Order Artiodactyla.
The two probably valid species, *T. melophagium* and *T. theileri*, infect sheep and goats respectively (family Bovidae, subfamily Caprinae). The majority of records of *T. theileri* -like trypanosomes relate either to the domestic ox (*Bos taurus*) or to the domestic zebu (*Bos indicus*) (family Bovidae, subfamily Bovinae) but as several authors fail to state which of these two species is involved, no distinction will be made in this review. All records of *T. theileri* -like trypanosomes seen in blood smears made from other artiodactyl species are consolidated in Table 1. They relate overwhelmingly to the family Bovidae where infected species represent all of the subfamilies with the exception of Caprinae. This distribution of records may however be misleading for two reasons. Firstly, most records have been incidental to a search in Africa for silent hosts of the salivarian trypanosomes pathogenic to man or his domestic animals. This has resulted in uneven sampling within the order with particular neglect of the 11 species representing the family Cervidae (Table 1). Secondly, since the majority of evidence has come from the examination of blood smears only, many infections have probably been missed. Megatrypanosome parasitaemias of domestic cattle are normally submicroscopic and detected on blood agar culture. Similar levels of parasitaemias may be present in other artiodactyls but only a few culture attempts have been made (Table 2).

Curasson (1925, 1943) claimed to have transmitted *T. theileri* -like trypanosomes of cattle to sheep and goats and to have demonstrated transient infections. Similarly Vrijburg (1912) claimed to have induced a transient infection in a horse and this represents the only successful attempt, amongst many, to infect a representative of another mammalian order (Table 3). Although the approach has been tentative in the majority of cases, the impression is confirmed that trypanosomes of the subgenus *Megatrypanum* are restricted in their host range to members of the Order Artiodactyla.
Methods of detection

1. Blood smears: Many observers have failed to find T. (Nagatrypanum) spp. in the blood of domestic oxen even after exhaustive microscopic examination. They may be seen, however, on rare occasions wherever experiments are carried out involving repeated examination of the blood of oxen for haemoparasites. Among many such examples are Saiawa et al. (1933) in Japan, Atchley (1951) in the U.S.A. and Joyner (1966 - personal communication) in England. Similarly the parasite may be seen on rare occasions during routine blood counts (Benbrook and Stock, 1961). More information comes from countries where diagnostic services routinely examine large numbers of blood smears from cattle. In Uganda, for example (Uganda: Annual Reports Department of Veterinary Services) out of a total of 68,674 blood smears examined for haemoparasites in 22 years, 650 showed one or more T. theileri - like trypanosomes (0.92%). The significance of such a figure will be later discussed in the section on pathogenicity as the level of parasitaemia may be enhanced by concurrent disease.

Information on the relative ease of blood smear diagnosis in other artiodactyls is comparatively very small. Although, for example, Reid et al. (1966) showed that submicroscopic parasitaemias can exist at least in bushbuck (Tragelaphus scriptus), Keymer (1966) found T. (Nagatrypanum) spp. on blood slides of 46 out of 95 duiker (Sylvicapra grimmia) shot in Malawi, Zambia and Rhodesia.

2. Concentration of trypanosomes in peripheral blood: Miyajima (1907), Crawley (1912 b), and Johns (1913, 1914) all variously attempted centrifuging whole blood and examining the leucocyte layer for trypanosomes either on wet or on stained smears. Both Crawley and Johns were successful. Teague and Clark (1916) centrifuged blood after haemolysing with an equal volume of distilled water. The method was later copied by others.
with the finding of trypanosomes on rare occasions (Robinson, 1923; Curasson, 1925; Turner and Murnane, 1930 a, b; Saisawa et al., 1933). Teague and Clark used their method on a calf which showed one trypanosome in every 12 microscopic fields of a stained smear. After concentration, between 1 and 10 trypanosomes were seen in each field (magnification unstated). Martini (1909) and Glaser (1922) also attempted centrifugal methods, the latter seeing one trypanosome on a single occasion. Neither recorded their methods.

3. Culture: T. theileri - like trypanosomes may be cultured both at room temperature and at the temperature of a mammalian host.

a) culture at room temperature: The ease with which T. theileri - like trypanosomes can be cultured from the peripheral blood of cattle after exhaustive blood smear examinations have failed to reveal the organism, has caused some authors to query whether the epimastigote forms seen in culture have any relationship to the trypomastigote forms in peripheral blood (Crawley, 1909; Behn, 1912; Richardson, 1943). In most isolations the relationship has to be assumed, but in a few cases strong circumstantial evidence has been obtained by transferring the flagellates to environments maintained at 37°C and demonstrating a change in morphology (see section (b) following).

Crawley (1912 b) centrifuged peripheral blood from an ox 4 hours after withdrawal and found clumps of flagellates which were probably in active division. Infectivity of the blood to a culture medium was however lost after 4 days when kept on ice and after a shorter period at higher temperatures (see section: storage of strains). Robinson (1923) claimed that this initial growth in blood alone could be maintained to a level of microscopic diagnosis by the simple addition of an isotonic dextrose solution (5.4% in distilled water), and Johns (1914) stated that he was able to cultivate flagellates with greater certainty from
defibrinated blood to which had been added 0.5% dextrose.

The majority of prepared media used have either been variations of 'boil3' or as first employed by Miyajima (1907), Martini (1909) and Crawley (1909, 1912 b), or variations of rabbit blood-agar slopes introduced by Novy and MacNeal (1904) for the cultivation of Trypanosoma brucei. Examples of both types provide efficient isolation media but some authors e.g. Delance (1911), Hanceaux et al. (1911) and Kohl-Yakimoff et al. (1915 a) noticed that subculture was possible on blood agar. Kölliker (1917, 1931) used blood agar plates and demonstrated that the enriching of the agar with nutrient broth enabled indefinite subculture. Herbert (1962, 1965 b) confirmed this and also showed that a minimum of 20% concentration of mammalian blood was necessary.

Trautmann (1922) had already shown that the blood from horse, ox, sheep, goat, rabbit and dog donors could be used but Herbert (1965 b) however showed that many samples of human blood and some samples of ox blood were unsuitable. Julian (1966 - personal communication) found sub-

culture of an isolation in Canada "difficult" unless the medium contained blood from the same ox from which the original isolation was made.

Herbert made a particular study of a continuously subcultured strain of trypanosome which he obtained from Sire and termed Trypanosoma theileri (1965 c). He showed that it "grew well under conditions of aerobiosis, anaerobiosis and reduced oxygen tension. Cultures of T. theileri were depressed in vitro by the presence of toxic products of culture metabolism and of a low pH; they were also depressed when a culture was started with trypanosomes passaged from a declining culture". No growth was obtained on blood free media despite many attempts, although growth became possible when such media were separated from defibrinated blood by a semi-permeable membrane.
Reports of the longevity of cultures at room temperature give wide variations. For bouillon type cultures, Carpano (1913), for example, reported only 20 days of life while at the other extreme Vrijburg (1912) reported 120 days. Herbert (1965 a) ceased counting cultures on blood agar at day 23 as "the organisms had declined in number and were no longer clearly staining due to the protein precipitation that accumulates in the culture vessel as the culture declines". In another reference (1965 b) he stated that for accurate histochemical staining he ceased sampling cultures at day 10 because he found the organisms were too degenerate. Nöller (1917) believed that test tube blood agar cultures died out in about 30 days but recorded having occasionally seen "lively, motile, flagellum bearing forms" on plate cultures at day 47, together with "rounded off vacuolated degenerate forms". Packchanian (1943) found "occasional" cultures still motile at day 120.

The efficiency of culture as a method of detecting infections was questioned by Knuth and Behn (1911). They inoculated a calf with parasitaemic blood but failed to detect infection using a bouillon culture medium although the blood of the inoculated calf was found to be infective to a further calf in which a patent parasitaemia was demonstrated. Vincent in Algeria (1968 - personal communication) has also failed to obtain cultures from an infected calf in which parasitaemia was patent, but he did not state his technique.

*T. theileri* - like trypanosomes have been accidentally cultured from peripheral blood of cattle in Europe; for example by Kerr and Robertson (1945) in Northern Ireland and Pierce in England (1965 - personal communication) during investigations to discover whether trichomoniasis could be a parasitaemic infection. Such accidents are usually
of merely incidental interest, but in Africa they may complicate the diagnosis on culture of important salivarian trypanosomes. Bolton (1965 - personal communication) obtained cultures of \textit{T. theileri} - like flagellates on a diphasic medium which supported \textit{T. congoense} and Gray and Nixon (1967) used for their \textit{T. theileri} investigations a medium originally developed for \textit{T. rhodesiense} and \textit{T. gambiense} (Tobie, von Brand and Nehlsen, 1950). On the other hand Herbert's medium (1961) has not, on the several occasions attempted, supported growth of \textit{T. brucei} derived from parasitaemic mice (McNeillage, 1968 - personal communication).

Novy et al. (1904) obtained flagellates on culture from an ox in the Philippines and assumed that they had cultivated \textit{T. evansi}. Bruce et al. (1909 a) accepted the assumption but Gaiger (1910) pointed out that the flagellates could have been \textit{T. theileri}. The possibility of confusion must still be considered as although the consensus of opinion amongst protozoologists is that \textit{T. evansi} is not infective to blood agar media, the view is not well supported by definitive references (see Balis, 1965) and textbooks may even state that \textit{T. evansi} can be cultured on blood agar media, (e.g. Hutryra et al., 1946; Levine, 1961).

b) Culture at mammalian blood temperatures: There is at least a short period of survival of \textit{T. theileri} - like organisms if parasitaemic ox blood is kept at 37°C. Crawley (1912) and Saisawa et al. (1933) both found that infectivity to culture media maintained at room temperatures was lost within 24 hours but Vrijburg (1912) recorded a 4 day retention of infectivity if the defibrinated parasitaemic blood was kept in flasks and 17 days if kept in test tubes (see section: storage of strains).

Johns (1914) grew his cultures on bouillon between the limits of 27 and 40°C after the addition of 0.5% dextrose but did not indicate the optimum temperature within this range. Several authors have made
isolations at room temperature and then observed the period of survival of the organisms when the temperature was raised to 37°C. Robinson (1922) found no development in broth culture at the higher temperature after 3 days; Glasser (1922) and Herbert (1964) found survivals of 3-5 days and 2-5 days respectively on blood agar slopes, and Trautmann (1922) using blood agar plates described growth at 35°C with change in morphology when the colony from different animals but one organism had been obtained they were readily obtained at 37°C in any of the above combinations of constituents. If Trautmann used the same constituents as Noller (1916) then his plates contained 50% whole blood as compared to the usual 25% in sloped blood agar media.

Ristic and Trager (1958) recorded a successful attempt to isolate T. theileri - like organisms on a medium maintained at 37°C. They used a mixture of lysed ox red blood cells in homologous serum, whole blood from the same animal, and glucose, to which was added whole blood from the same animal. Later, Salted and Soulsby (1965) noted that the trypomastigote form had been reported as a contaminant of tissue cultures. They therefore studied the usefulness of three commercially available media for isolation of T. theileri - like organisms from tissue culture media. Splitter and Soulsby (1967) noted that T. theileri in trypanastigote form had been reported as a contaminant of tissue cultures.
tissue culture fluids as media components for the production of large numbers of the organism for immunological studies. The three tissue culture fluids were also evaluated in combination with veal infusion broth or trypticase soy broth. The media requirements for successful isolation of T. theileri—like trypanosomes appeared to vary with blood samples from different animals but once organisms had been obtained they were readily cultured at 37°C in any of the seven combinations of constituents tried. The authors' observations could however be explained by variations in the level of parasitaemia of the animals sampled (see section: epidemiology). Modifications of media were required for optimum continuous culture (35% veal infusion broth) and for large volume culture (70% veal infusion broth). Failure to passage never occurred if red blood cells were included in the medium or replaced with bovine blood coagulum. Later, Soulod and Soulsby (1968), as a development of this work, modified an initial culture medium over a series of 31 subcultures in a period of one year and succeeded in growing the organisms in a final ambient containing hemin and a defined modification of McCoy 5a tissue culture medium. They claimed therefore that Trypanosoma theileri had become the first mammalian trypanosome to be serially cultivated in a blood and cell free medium at 37°C.

The media of Ristic and Trager, Simpson and Green, and Splitter and Soulsby all had blood as a constituent. However, as the last of these authors had noted, growth of T. theileri—like organisms had been observed as a contaminant of mammalian cell cultures in the total absence of red blood cells. Splitter and Soulsby quoted Lundholm et al. (1959) for contamination of a bovine foetal kidney cell culture, a personal communication from Hare (1965) for blood lymphocytes and Malmquist (1965)
for lymph node cells. Other references can be added e.g. Hulliger (1964 - personal communication) for contamination of a lymphatic tissue monolayer and Cross et al. (1965) for contamination of a buffy coat culture.

The contamination of mammalian cell cultures helped to prompt more specific studies. Thus Lundholm et al. (1959) also observed that trypanosomes could multiply in the absence of cells in tissue culture media containing 10% by volume lamb serum but multiplication appeared more rapid when tissue culture cells were present. Hulliger also made further study and showed that primary isolations could be made routinely on a tissue culture medium containing 20% calf serum or by preparing a monolayer of buffy coat cells from an infected ox. Subcultures could be made from monolayer to monolayer of leucocyte cells but subculture onto medium alone resulted in death of the trypanosomes after a few days. HeLa cells and a hamster kidney cell line were also found capable of supporting continuous growth. Both Whiteside and Woolfe quoted as personal communications by Herbert (1964), have grown T. thalleri - like organisms on tissue culture. Woolfe succeeded in transferring Herbert's continuously subcultured strain at 25°C, which had not grown satisfactorily on haemolysate media (vide supra), on to a tissue culture at 37°C. In these accounts the trypanosomes have been free in the fluid media. Cross et al. (1965) alone described "numerous immature forms" growing within the buffy coat cells which they were cultivating, as well as free swimming forms.

In summary therefore, the most successful media for growth at room temperature are enriched blood agar which allow continuous and indefinite subculture. Growth at 37°C can be achieved in systems requiring either
red blood cells (with Sollod and Sosulby (1968) showing that hemin is probably the most important constituent) or other mammalian cells from fixed tissues. Whereas growth at room temperature is attempting to simulate the environment of the alimentary canal of an insect vector, the two systems allowing growth at 37°C could be construed as simulating two phases of the life cycle in the mammalian host.

4. Serological diagnosis: No method of serological diagnosis has been standardized. The information available is miscellaneous but there has been recent interest because of the possibility of shared antigens interfering with the serological diagnosis of the pathogenic salivarian trypanosomes. Theiler (1903) described agglutination of Trypanosoma theileri in defibrinated blood but there may have been confusion with the rosette appearance of actively multiplying trypanosome colonies. Gray and Nixon (1967) specifically stated that suspensions of T. theileri - like flagellates from cultures were unsuitable for use as antigens in agglutination tests as the organisms were growing in firm clumps when harvested. Nevertheless, Trautmann (1922), summarized by Mesnil (1923), used an agglutination test to show that the sera of infected cattle, as those of the horse, sheep, goat and dog, agglutinated T. theileri - like trypanosomes at dilutions of 1 : 10 to 1 : 80. The diluted sera, if not inactivated, were very trypanocidal. By inference, from Mesnil's summary, Trautmann used culture forms obtained from blood agar plates maintained at room temperature. The antigenic relationship between forms on culture at room temperature and forms in peripheral blood requires to be established, together with their relationship to similar populations of other trypanosome species. This consideration applies both to the work of Robinson (1923) and of Gray and Nixon (1967).
Robinson attempted to make an antigen from room temperature cultures for use in a complement fixation test to detect the pathogenic trypanosomes infections of domestic animals in South Africa. No reactions were observed. Gray and Nixon used an antigen consisting of homogenized blood agar culture forms and failed to show precipitin reactions with sera from cattle infected with T. theileri-like organisms. However, they also found none of the sera from the infected cattle reacted with antigens of Trypanosoma vivax and T. brucei in agglutination and precipitin tests and had no difficulty in infecting cattle which carried infections with T. theileri-like organisms, with T. congolense and T. vivax in laboratory studies. They concluded that infections with T. theileri-like organisms are unlikely to be of practical importance in immunological studies of infections with pathogenic trypanosomes based on agglutination and precipitin reactions. Schilding (1925) also failed to reveal any cross reactions between T. equiperdum and T. theileri-like organisms. He cultured T. theileri-like organisms from 17 out of 26 cattle but none of the cattle sera were positive to a complement fixation test using T. equiperdum as antigen.

Splitter et al. (1967) alone have attempted serological work with T. theileri-like organisms harvested at 37°C (see section: cultural diagnosis) from blood cultures. They found precipitins present "in the sera of 10 of 17 cattle naturally infected with T. theileri, but the indirect haemagglutination test appeared to be of little or no value in detecting T. theileri antibodies, even in cattle whose sera showed precipitin reactions with the T. theileri antigen". The antigen was harvested from culture at about the sixth day when approximately 60-70% of flagellates were in the trypomastigote form (Splitter and Soulsby, 1967). The results therefore keep alive a possibility of precipitation techniques being limited in their application.
21.

to pathogenic trypanosomes if cross-reaction occurs with *T. theileri* -
like organisms. No work however has been done with an antigen prepared
from flagellates grown on tissue culture.

Ouyang (1967), reporting on an indirect fluorescent antibody
test for bovine tsetse transmitted trypanosomiasis, described preliminary
work to provide a baseline for fluorescence. Cattle infected and not
infected with *T. theileri* - like organisms were compared and sera taken
from infected heard were found to be more reactive.

A clear answer is required to the question whether *T. theileri* -
like organisms can interfere with the serological diagnosis of salivarian
trypanosomes.

**Morphology**

1. Morphology in peripheral blood: Wide variations occur in the
descriptions of *T. theileri* - like flagellates under the light microscope.
The alleged polymorphism makes detailed comparisons of the copious and
meticulous measurements recorded by early authors, e.g. Lingard (1907),
unjustified excepting to note the order of size in terms of length and
width and in the relative position of the organelles. Moreover, in
some instances the authors have not clearly stated whether the flagellum
has or has not been included in the length of the organism, and often the
methods used to fix and stain preparations have not been mentioned when
these may significantly alter the size of the flagellates (see Walker,
1963). The thickness of a preparation can also alter apparent width
particularly of a large trypanosome (Keymer, 1966). Laveran (1902 a,b) examined blood slides sent to him by Theiler
and described *Trypanosoma theileri* as a trypanosome varying in length
from 30-65 μ (including flagellum) and in width from 2-8 μ. He further
described a slender and pointed posterior end, a centrally placed nucleus,
a deeply staining kinetoplast at the posterior end, a free flagellum
one quarter of the total length and a finely granular protoplasm. He
claimed however (1902 b) that he identified a second trypanosome species
in further slides sent to him, which he called T. transvaaliense. This
species varied from T. theileri in being 18-50 μ long (including flagellum)
but principally in that the kinetoplast was always near or even attached
to the nucleus (epimastigote form; Hoare and Wallace, 1966). Theiler
himself (1903) disagreed with this view and believed that T. transvaaliense
was merely a development form of T. theileri of greater width, and
comparatively rare. He considered that longitudinal division could occur
in both forms but that in the T. transvaaliense type the kinetoplast
moved adjacent to the nucleus before division commenced. Lühs (1905)
described thin slender forms (average 63 μ x 1.7-2.8 μ excluding
flagellum) and shorter thick forms (average 59 μ x 5 μ). He, like
Theiler, believed that the nucleus and kinetoplast came in close
apposition during division of the trypanosomes, and that these were the
T. transvaaliense of Laveran. He also described longitudinal division,
resulting in daughter trypanosomes of equal size, and transverse division
(see fig. 2) resulting in daughters of unequal size. Both Theiler and
Lühs also described trypomastigote forms with two nuclei, one anterior to
the other. These were later described by Keyser (1966) but apparently
not by any other observer. Their appearance must therefore be considered
rare and problematical in relation to types of division.

Subsequent observers have not challenged the shape and arrangement
of the trypomastigote form as described by Laveran (1902 a) but have
taken as their major items for comment, the range of size, the manner of
division and the occurrence of epimastigote forms. The present review
will be restricted to these phenomena together with a note on observed
motility.
Several authors continued to differentiate between narrow and wide forms e.g. Holmes (1904), Lingard (1907), Rodhain et al. (1912), Johns (1915), Carpano (1915), Dias and Zucarini (1924), Schuets (1930) and Saisana et al. (1935). Behn (1912) and Berger (1913) not only described 'large, narrow' and 'large, wide' trypanosomes but also 'small, slender' as a third distinct morphological type. Johns (1915) alone has described a degree of 'amoeboid' movement in the posterior extremities of the organisms enabling the shape to vary from a long delicate process to a rounded or even knobbed appearance.

Peter (1910) described the range of body length as 30 - 60 μ. He also saw occasional epimastigote forms in peripheral blood as did Schein (1907), Haughton and Youngberg (1920), Glaser (1922), Curasson (1925), and Tendeiro (1949). Carpano (1913, 1924) described a range of body length of 20 - 70 μ and stated that the epimastigote form occurred at the time of division of the organism when the posterior and also rounded off. He later (1932) offered the theory that these were development forms which had been prematurely "washed out" from internal organs. Reichsenow (1940) described a similar range in body length as Carpano, but also described binary division as characteristically resulting in unequal daughter cells (see fig. 2). He stated that within the smaller of these, the kinetoplast was initially adjacent to the nucleus but later moved posteriorly. These short forms continued to multiply and the long forms were in transition to giant forms of "chronic" infections. The idea that the largest forms were seen in "older" parasitaemias had already been put forward by Teague and Clark (1916).

Keymer (1966) plotted a frequency distribution curve of the body length measurements of T. theileri - like trypanosomes from a variety of sources. He found that "the interrupted curve for the grand total of
31. Measurements show a normal length distribution ranging from 10 \( \mu \) to 130 \( \mu \) \( \ldots \) and believed that division occurred mainly in forms under 50 \( \mu \) in length and rarely in the larger forms. Moreover, as already described (see Introduction: taxonomy) the parasites identifiable from Heare’s classification (1966) as \textit{T. theileri}, \textit{T. caproni}, \textit{T. tragelaphi} and \textit{T. ingens} in the same splenectomized ox over a period of six weeks. Touré (1966) also stated that large trypanosomes of cattle observed in Senegal could have a body length of 95 \( \mu \) and gave the opinion that the morphology varied according to the stage of infection in the host.

The variations in size may be related to the variations in recorded motility. Flagella have been described as actively lashng with little or no translatory movement, e.g. Bruce \textit{et al.} (1909 a), Stockman (1910), Johns (1913), Hornby and Bailey (1929) and Hornby \textit{et al.} (1934), while other accounts refer to very rapid translatory movement, e.g. Schein (1907), Carpano (1913) and Croverie (1920). Lühs (1905) described rapid movement but stated that wide forms were slower. Some authors described a capability of the trypanosomes to attach by their posterior ends to leucocytes (Johns, 1913), red blood cells (Lühs, 1905) or to the glass slide (Hornby and Bailey, 1929).

In summary, \textit{T. theileri} - like trypanosomes in peripheral blood show a wide variation in body length of 10-130 \( \mu \), commonly in the trypomastigote form but occasionally epimastigote. The variations probably represent different stages in the life cycle, and the lack of realization in the past of their full extent has assisted the proliferation of species names which may now be ignored.

2. Morphology in tissues other than blood: Smears of tissues containing blood may accidentally reveal \textit{T. theileri} - like trypanosomes
in the trypomastigote form, e.g. Weber (1909) in a smear of heart muscle. Primary cell lines may also be contaminated with such trypanosomes, e.g. Lundhola et al. (1959) who found them in cultures of foetal bovine kidney cells, and Malmquist (1965) who found them in leucocyte cultures (see section: culture).

Souderatschenkoff (1930) found large trypanosomes in ulcerations of the bladder of a cow suffering from haematuria but the principle evidence of T. theileri - like trypanosomes multiplying in tissues other than blood relates to lymph nodes and the brain. Carpano (1932) found T. theileri - like organisms in an hypertrophied bovine prescapular lymph gland. He described: amastigote forms smaller than bovine red blood cells; rounded 'plasmodial' forms exceeding 15 μ in diameter with possibly more than one nucleus but each accompanied by a kinetoplast; promastigote forms of only 5-7 μ with the flagellum either extending externally or twisting internally; epimastigote forms 10-40 μ long including flagellum, identical to those which may be found in peripheral blood or in culture of blood at room temperature, and lastly a few trypomastigote forms 50 - 60 μ long including flagellum which correspond to those found in circulating blood. Gallo (1938) gave a similar description from lymph nodes of cattle which were suffering from haemorrhagic septicaemia (pasteurellosis) but did not see trypomastigote forms. Cruverie (1920) on the other hand stated that the T. theileri - like organism that he studied was never encountered in lymph nodes.

Carpano (1932) and Gallo (1938) also described the amastigote, promastigote, epimastigote and trypomastigote forms in brain lesions. Carpano described the lesion as an area of haemorrhagic softening, and Gallo as a centre of degeneration. Carmichael (1939) found T. theileri - like organisms in the brain of an ox which had died of 'turning sickness'.
He described the brain lesion as a "roughly circular patch of haemorrhagic softening about the size of a walnut with a tendency to necrosis". In smears and sections, however, he found only amastigote and epimastigote forms in large numbers. Barnett (1947) found similar lesions in another animal which had died of 'turning sickness'. In recollection (1966 - personal communication) the brain had several foci of 72 - 200ms. diameter especially in the cerebrum. These consisted of a semi-liquid necrotic centre with haemorrhagic periphery. The organisms present in the lesions were shorter in length than the trypanastigote forms of T. theileri found in peripheral blood.

3. a) Morphology in cultures at room temperature: Many descriptions of T. theileri-like trypanosomes both in liquid and diphasic media. Some variations within the wide range of possibilities described may be entirely attributable to the constituents of the medium used (Carpino, 1958). Adler (1958), for example, believed that the addition of specific sera to the culture medium induced morphological changes in the sternorarian trypanosome T. cruri with no known parallel in the life history of the organism. Nevertheless, comparison of descriptions of primary isolations of T. theileri-like trypanosomes in different media does reveal the common appearance of certain morphological types. These are very clearly described by Crawley (1912) who used a beef bouillon medium (see fig. 3).

Crawley (1912) in addition gave a clear description of the "broad" and "slender" flagellates as solitary organisms, in pairs, or in clusters. Movements of the flagellate in young cultures confirmed, for example, by "bird" and "club" shaped flagellates as solitary organisms, in pairs, or in clusters of a few organisms. By 3-4 days the clusters could be relatively large composed principally of long slender "band" shaped flagellates with a shift in position of the long axis. A few forms showed organisms and others of a 'club' form, the head of the club bearing the flagellum. By 5-6 days there were both large and small clusters with a greater number of free individuals. These, however, were not only of...
the 'band' or 'club' shapes, but also oval and short. Any or all of these forms could be found in division. Crawley was of the opinion that the sequence of development started with the short 'broad' form followed by the 'band' and 'club' shapes. The narrow posterior end of the 'clubs' then reduced to a spike forming finally the oval shape. In old cultures (63 days) Crawley described masses of feebly motile round forms.

Crawley's observations on fixed and stained material gave flagellates at 4-5 day of culture growth a maximum body length of approximately 25 μ. At 10 days the maximum body length approximated 38 μ with the length of the flagellum appearing to increase with the age of the culture. All forms were epimastigote in that the kinetoplast was invariably adjacent to the nucleus, most often anteriorly. Examples of other references giving similar descriptions are Dudukalov and Dudukolova (1910), Swellengrebel (1911 b), Cardamatis and Photinos (1912), Bettencourt and Borges (1916), Glaser (1922), and Iwata et al. (1959).

A terminal flagellar knob or thickening was described by Crawley (1912) and confirmed by many authors in their drawings or photographs if not in their text. Examples are: Martini (1909), Carini (1911), Delance (1911), Manceaux et al. (1911 a,b), Swellengrebel (1911 a,b), Bahn (1912), Bettencourt and Borges (1916), Hartmann and Noller (1918), Leach (1964), and Herbert (1965 a).

Crawley (1909, 1912) in addition, gave a clear description of the movement of the flagella in young cultures confirmed, for example, by Delance (1911) and Robinson (1923). Most movements were confined to vigorous lashings of the flagella and the 'club' forms showed a quick jerking with a shift in position of the long axis. A few forms showed
rapid progressive movement. No author appears to surmise whether these latter minority have any distinctive morphology or are related to the very motile forms seen by Müller (1917) in an old culture (see section diagnosis: culture at room temperature).

Crawley did not however record the trypomastigote forms in culture described by Delanoë (1911), Manceaux et al. (1911 a,b), Herbert (1965 a), and Touré (1968), nor promastigote forms (Herbert, 1965 a) although the latter may be equivalent to his epimastigote forms where the kinetoplast is anterior to the nucleus. In addition his "masses" of round forms in old cultures are usually illustrated or described as individuals e.g. Yakimov and Schokor (1916). The detailed cytological study of Hartmann and Müller (1918) did not mention or illustrate a 'club' form. Their cultures were derived from flagellates infecting Tabanus glaucopus but were said by Trautmann (1922) to be similar to cultures obtained from the peripheral blood of cattle. There appears to be no clear explanation (see section: discussion).

Herbert (1965 a) related changes in morphology on culture to different stages of population growth and associated 'globular' forms (the 'club' and 'oval' forms of Crawley) with the population decline (see fig. 3). This change in morphology was associated with an increasing number and size of cytoplasmic inclusions, and Herbert believed that the changes were comparable to those described as 'degenerative' by Blacklock (1912) for T. rhodesiense and T. gambiense in the cadaver of an animal host (see section: cytochemistry).

The morphology of the alleged Trypanosoma wrublevskii requires mention. The epimastigote form of this trypanosome in peripheral blood described by Wrublewsky (1909) was commented upon by Nesnil (1913).
Nesni suggested that as blood was collected from dead animals and then kept in a pipette for a number of days, the form seen was as if in culture at room temperature. A knobbed flagellum was also described.

Herbert (1965 b) offers the only study of the fine structure of culture forms of a T. theileri - like organism. With the addition of cytochemical techniques he demonstrated mitochondria, lysosomes, ribosomes, endoplasmic reticulum and Golgi apparatus. He also discussed the relationship of these organelles to 'volutin' bodies described in other trypanosomatidae, and phagosomes in particular to the 'probably pinocytic feeding mechanism' (see section: cytochemistry).

3. b). Morphology in culture at mammalian blood temperatures:
Cultures of T. theileri - like flagellates at 37°C results in the formation of trypomastigote forms. Hulliger (1964 - personal communication) observed 'few' epimastigote forms on tissue culture but Splitter and Soulsby (1967) using tissue culture media reinforced with broth and whole blood found 30 - 40% of epimastigote forms at up to six days of culture and 60 - 70% epimastigote forms between the sixth and tenth day.

Cytochemistry:
Herbert (1963, 1965 a) alone has investigated the cytochemistry of a T. theileri - like trypanosome using a strain continually passaged on a blood agar medium (Herbert, 1961). His summary reads, in part:
"when grown in culture at 28°C, T. theileri accumulates no polysaccharides or acid mucopolysaccharide, though it does contain aggregates of lipid, some of which is phospholipid. The cytoplasm contains ribonucleic acid, and occasional large aggregates of this material can also be seen. A number of large cytoplasmic bodies contains polyphosphate. The results of supravitral staining suggests that some, at least, of the cytoplasmic inclusions (or organelles) described are concerned in the uptake and probably digestion and/or detoxification of exogenous materials; and
where this material accumulates, as it does when the organism is in an inequable environment, they die".

Pathogenicity

Theiler (1903) at first claimed that *Trypanosoma theileri* was the cause of "gall sickness" an important cattle disease in South Africa, but later retracted this statement (1910), identifying the disease as anaplasmosis. Other authors of this period and many since have believed *T. theileri* - like organisms to have no pathogenic significance e.g. Martini (1909), Johns (1913), Hornby (1927), Saisawa *et al.* (1933), Ware (1940), Essart (1951) and Gray and Nixon (1963). Nevertheless, acute and sometimes fatal clinical episodes have been described in relation to the presence of *T. theileri* - like organisms. These can be considered firstly in situations where the trypanosome is the only recognized infection, and secondly when the trypanosome occurs as a mixed infection with one or more pathogens.

*T. theileri* - like organisms have been reported in focal lesions in the brain causing acute nervous symptoms (Carpano, 1932; Gallo, 1933; Carmichael 1939; Barnett, 1947). Accounts also exist of *T. theileri* - like organisms being found in large numbers in the peripheral blood of cattle which had died suddenly e.g. Frank (1909) and Kranzle (1912) in Germany, Sassouchin and Matwejef (1922) and Beresine (1930) in Russia, Robinson (1923) in South Africa, and Wyssman (1935) and Bourgeois (1941) in Switzerland. Frosch (1909) believed that Frank had described a new trypanosomal disease and made careful investigation of other cattle in the vicinity of the occurrence. The evidence is discussed in the neighbourhood of the occurrence. Mayer (1909) disbelieved this conclusion but Frank and Frosch (1909) stated that trypanosomiasis must be borne in mind when cases of anthrax were being investigated, a view continued to be held in 1936 by Schmid and independently by Vrijburg.
Other clinical episodes associated with the presence of *T. theileri*-like organisms alone are miscellaneous in character. Valladares (1909) described an acute fatal syndrome in an ox associated with swollen legs and urticarial eruptions on the chest; Sudatschenkoff (1930) saw the trypanosomes in ulcerations of the bladder of a cow suffering from haematuria; Hall (1953) described a Canadian episode where 20 cattle died out of 45 but the reference contains no identification of the trypanosome concerned or the grounds for diagnosis; Levine *et al.* (1956) found *T. theileri*-like trypanosomes in blood smears of a heifer which had suffered from abortion, diarrhoea, and depression for three months; Dikman *et al.* (1957) found the trypanosomes in the stomach contents of an aborted calf; Ristic and Trager (1958) cultivated the trypanosomes from three cows with depressed milk production and a marked eosinophilia; an anonymous contribution to the Communicable Disease Centre Veterinary public health notes (1966) reported parasitaemias associated with a profuse diarrhoea and watery blood in six new born calves, the dams having had high temperatures at the time of parturition, and Lay and Boulay (1967) found a heavy parasitaemia in a calf born at full term in a comatose state. The calf died the same day.

Most reports of *T. theileri*-like trypanosomes in relation to disease have been in association with concurrent infections with agents of recognized pathogenicity. These are listed in Table 4. Only in relation to rinderpest is there any allegation that these trypanosomes can aggravate the course of an infection. The evidence is discussed firstly in terms of general observations from the field, and secondly in relation to circumstance where it is likely that rinderpest virus and *T. theileri*-like trypanosomes were inoculated simultaneously into experimental animals.
From the field, Theiler (1903), Luhs (1905) and Carpano (1913) independently commented that *T. theileri* - like parasitaemias were very common in association with rinderpest; Holmes (1904) believed that such parasitaemias occurred secondarily to an attack of rinderpest; Curasson (1925) detected a parasitaemia in a 'latent' attack of the disease; Kearney (1926) reported that *T. theileri* was the trypanosome most frequently seen in Northern Nigeria in 1925, but added that the cattle examined were also infected with rinderpest, a fact enlarged upon by Glover (1961) who re-examined the records. Lastly, Groveria (1920) stated that the trypanosome appreciably modified the character of the disease.

Most information, however, has come from the 'double inoculation' method of immunising cattle against rinderpest which involved passaging the virus through cattle to obtain immune serum from recovered animals. Following the inoculation of viraemic blood a temperature reaction normally occurred. In some cases after an interval of some days a second temperature rise could be observed associated with a high parasitaemia with *T. theileri* - like trypanosomes. At this second temperature rise death could occur. This phenomenon was reported many times from many countries, e.g. Theiler (1903); Holmes (1904); Durrant and Holmes (1904); Schein (1907); experience of Montgomery, 1912, recalled by Robertson (1956); Haughwout and Youngberg (1920); Carmichael (1926), and Hornby et al. (1934). Other relevant information is provided by: Luhs (1905) who stated that the double inoculation method can be complicated by *T. theileri*; Rao and Ayyer (1931) who said that *T. theileri* parasitaemias occur during virus production, and Uganda (1932) where a cow inoculated with viraemic blood suffered a 'violent' attack of rinderpest associated with a high parasitaemia.

Episodes in Tanzania associated with the 'double inoculation' method of vaccination against rinderpest were investigated more
thoroughly than elsewhere (Hornby and Bailey, 1929; Hornby, 1930; Hornby et al. 1934; Hornby, 1936; Hornby, 1949). Cattle in which these unusual reactions were likely to occur were noted to have originated from one particular district. Believing that these animals were not infected with T. theileri and that concurrent primary infections of both T. theileri and rinderpest virus was a fatal combination, action was taken to avoid this circumstance. Cattle were therefore either given an inoculation of known parasitaemic blood some days before the virus inoculation, or the virus was passed through goats to eliminate the trypanosome, or the viraeemic bovine blood was swabbed onto nasal mucous membrane instead of being injected. As a result the second temperature rise associated with T. theileri - like trypanosomes ceased to occur.

Despite the allegations of pathogenicity, transmission of T. theileri - like trypanosomes alone to presumed susceptible cattle has never provoked more than a febrile reaction (e.g. Behn, 1912, and see section epidemiology: artificial transmission). Moreover these trypanosomes may show high parasitaemias without noticeable associated symptoms (e.g. Ewing and Carnahan, 1967). From the evidence available, therefore, T. theileri - like trypanosomes may be considered non-pathogenic excepting in circumstances of development forms causing mechanical injury in the brain of the host, or in relation to rinderpest virus.

Immunology

The only immunological studies available appear to be the few miscellaneous references relating to serological diagnosis (see relevant section. Gray and Nixon (1967) failed to detect infections in calves born from infected dams and therefore considered prenatal infections unlikely. Since calves, however, could be naturally infected as early as one month of age it seemed also likely that no immunity was acquired from the dams.
Once infected, an animal can remain demonstrably so on culture for periods of at least one year (Vrijburg, 1912), of at least two years (Saisawa et al., 1953; Gray and Nixon, 1967), of at least six years (Robinson, 1923) or for life (Carpano, 1913). Allegations that infected cattle may cease to be parasitaemic after a period of time e.g. Vrijburg (1912), may be based on an incomplete knowledge of natural variations in level of parasitaemia. There is therefore no evidence that a sterile immunity may occur and T. theileri - like trypanosomes appear well adapted to their hosts.

**Epidemiology**

1. **Geographical incidence:** Most of the evidence indicating geographical distribution (see appropriate section and Appendix I) is unsuitable to demonstrate incidence. Not only do the methods of blood examination and culture vary, but the ages of the animals sampled and the season of the year may not be recorded when both may also affect the numbers of animals found infected. Sufficient culture evidence exists, however, to show that in the same geographical area the incidence of infection in adult cattle may vary from 100% to a level not detected on one or more examinations. Reid et al. (1966), for example, used a culture technique to sample four groups of adult cattle in East Africa within a three month period. The numbers of animals found infected were respectively 34 out of 35, 12 out of 30, 5 out of 30, and nil out of 33. The last herd result was later confirmed on a second occasion.

2. **Age incidence:** The highest incidence of infection in a herd of cattle appears to occur in adult animals. Examples are: Knuth (1910), 20/31 adults infected, 1/7 young oxen, 0/3 small calves; Crawley (1912), 20/27 adults, 0/3 calves; Johns (1913), 43/43 adults, 2/7 yearlings, and Glaser (1922), 5/11 cows, 0/14 calves. In addition Reid et al. (1966) found the youngest calf infected in a herd was 2 1/2 months old, thereafter
there was a gradual increase in the incidence with age, 34/35 cattle
over two years of age being infected. Gray and Nixon (1967) found
35/47 cattle infected over one year of age, and 7/20 cattle of less
than one year. Infections have however been found in foetuses (e.g.
Lundholm et al., 1959) or in newborn calves (e.g. Anonymous, 1966;
Lamy and Bouley, 1967) and these are later discussed under methods of
transmission.

3. Levels of parasitaemia: Some indication that levels of parasitaemia
may fluctuate is shown by the examination of blood smears. Folkers
(1965 - personal communication), for example, in Nigeria showed that in
an experiment which required indigenous cattle to be examined daily on
blood smears, T. theileri - like trypanosomes were seen on single
isolated days or, on occasion, on 2-3 successive days. Patent
parasitaemias are also the obvious sign of increasing numbers of
trypanosomes in peripheral blood in relation to many other possible
concurrent infections (see section: pathogenicity and Table 4).

Quantitative information was obtained by Johns (1913) using his
centrifuge technique, finding between two and twenty-one trypanosomes in
each 10 ml. of whole blood. All other such information has been derived
from culture methods. Several authors for example, have determined the
minimum infective dose of blood to culture and thence deduced the
number of trypanosomes in peripheral blood. Miyajima (1907) found that
a platinum loop full of blood was infective to culture; Bahn (1911)
found three drops infective; Vrijburg (1912), two to three drops;
Crawley (1912), 0.34 ml., and Richardson (1930, 1948) obtained positive
cultures from 0.2 ml. blood. These attempts however also revealed that
variations in parasitaemia could occur from day to day (e.g. Saisava et
al., 1933) and raised the possibility that parasitaemias varied season-
ally. Bahn (1910 a), Knuth (1910), Robinson (1923), Saisawa et al. (1933),
Schmid (1938) and Herbert (1964 - personal communication) all made the comment that the trypanosomes were more difficult to demonstrate on culture in winter, but Crawley (1912) recorded the most evidence. In tropical areas, however, seasons may be mainly characterized by rainfall and less by variation of temperature. Gray and Nixon (1967) in Nigeria detected no regular seasonal variation on culture of cattle blood but Curasson (1925) in West Africa and Keymer (1967) in Central Africa both found trypanosomes more easy to find on blood smears in the rainy seasons.

Crawley (1912) found in addition a qualitative change according to the season. Flagellates were detected in culture earlier in spring and summer than in the autumn. His figures for average periods of time before detection were: autumn, 6.04 days, as opposed to 3.86 days in summer and 3.39 days in spring.

4. The effect of chemotherapeutic agents: Trypanocidal drugs which have been, or are being, used to control salivarian trypanosomes have no dramatic effect on T. theileri - like parasitaemias in cattle. Vrijburg (1912) treated two calves with trypan blue but obtained cultures successfully one week later. Hornby et al. (1934) stored blood for one hour following the addition of tartar emetic. Trypanosoma congoense and T. vivax, which had been present, were killed but T. theileri - like trypanosomes present survived. Sergent et al. (1945) believed that T. theileri was eliminated from a calf following treatment with novarsenol (an arsenical not listed in the B. Vet. Codex), but the evidence was only the absence of a patent parasitaemia and the non-infectivity of the blood to another calf. Bolton (1965 - personal communication) noted field reports from Rhodesia of T. theileri - like trypanosomes appearing in blood smears from cattle under treatment with prophylactic trypanocides. Likewise in Nigeria, Folkers (1965 - personal communication) saw T. theileri in blood smears of cattle under an isometamidium (samorin) prophylactic
regime and Ferguson (1967 - personal communication) had a similar experience with a trial of M and B 4427 (sulamin - metamidium complex). Bolton (1965 - personal communication) indicated that diminazine acetate (berenil) may have had a trypanocidal effect on a T. theileri - like trypanosome at 7.0 mg/kg dosage rate but Onyango (1967) found the same treatment level to have an insignificant effect on T. theileri - like infections in six cattle although T. congoense group infections were eliminated in the same animals.

Splitter and Soulsby (1967) examined the effect of antibiotics on culture forms. Penicillin dihydrostreptomycin, oxytetracycline and chloramphenicol failed to have an effect at the concentration used but nystatin (mycostatin = E.R. Squibb) was found to be lethal to the T. theileri - like trypanosomes under examination in culture at levels of 200 units/ml. or higher, and lower concentration resulted in suppression of multiplication.

Natural transmission: The vectors of T. theileri - like trypanosomes are commonly accepted to be horse flies (Diptera: Tabanidae). The opinion is based principally on the work of Nöller (1925) who used three cultures derived from wild Haematopota pluvialis caught near Berlin to attempt infection of four calves. Wallace (1962) critically examined this evidence and concluded "the fact of transmission of Trypanosoma theileri from tabanid flies to cattle appears to be proved", but qualified this by saying "the identity of the insect stage, however, is not so clear".

The evidence for the natural transmission of T. theileri - like trypanosomes is, however, susceptible to wider examination. Firstly, as already argued in the introduction, evidence is not available to declare Trypanosoma theileri a single species of cosmopolitan distribution. Different insects may act as vectors for otherwise apparently similar
trypanosome species, a situation found in isolations of trypanosomes from birds. Baker (1956) found, for example, that T. evum was transmitted in Hertfordshire by a hippoboscid Ornithomyia avicularia, while in Canada, Bennett (1961) considered that hippoboscids were not usually involved in his study location but found consistent development of bird trypanosomes in several species of ornithophilic simuliids.

Secondly, even in a single location, insect host specificity of a trypanosome species should not be assumed. Hanson and McGhee (1963) and Hanson et al. (1968) have emphasized the louse host specificity of insect trypanosomatids (genera Crithidia and Blastocrithidia). A similar capacity may be shown by members of the genus Trypanosoma.

Thirdly, Haematopota pluvialis has a very limited season of activity as an adult fly. This character contrasts markedly with well proven vectors of stercorarian trypanosomes which have long periods of access to their hosts, e.g. kissing bugs (Hemiptera: Reduviidae), fleas (Siphonaptera) and louse flies (Diptera: Hippoboscidae).

Lastly, field observations are lacking on the distribution and availability of possible vectors related to an incidence of T. theileri-like trypanosomes. Gray and Nixon (1967) related seasons of maximum insect activity to the primary infection of young cattle but made no analysis of the haematophagous insects possibly concerned.

The evidence for the natural transmission of T. theileri-like trypanosomes may therefore be summarized as follows:

**Transmission by Arthropods**

1. **Louse flies (Diptera: Hippoboscidae).** Theiler (1903, 1909) considered that he had proven Hippobosca rufipes and H. maculata to be transmitters of *Trypanosoma theileri* (see also Laveran, 1905). His experimental animals however could have been infected prior to the experiment, and, if he was successful, it could only have been through
'mechanical' as opposed to cyclical transmission. Luhs (1905), Knuth (1910) and Kearney (1926) all considered hippoboscids to be likely vectors but van Saceghem (1922) in Rwanda and Nöller (1925) in Germany stated that a high incidence of T. theileri-like infections in cattle were possible in areas where hippoboscids were rare or absent. Baker (1956) considered that the possibility of a hippoboscid transmitting T. theileri was worth re-examination. Kaymer (1966) however examined 132 Echostephus paradorus which had fed on duikers (Sylvicapra grimmia) infected with T. theileri-like trypanosomes and failed to find any trypanosomatid infections.

2. Muscid flies (Diptera: Muscidae). Bohn (1911) thought Stomoxys calcitrans a likely vector and Jegen (1924) demonstrated an incidence of trypanosomatid parasites in this species both of choanomastigote and epimastigote form. Glaser (1922) examined 85 Haematobia servata collected from stables housing infected cattle and found no flagellates. After feeding 10 of the same species on 'vigorou' cultures of T. theileri-like trypanosomes there was a survival of the flagellates in the alimentary canal for up to 48 hours. The same author also examined 92 Musca domestica but only found frequent Haemopoma muscae domesticae infections.

Buxton (1955) considered that there was no evidence of Glossina spp. (tsetse) transmitting members of the subgenus Nogatrypanum but noted that "under natural conditions tsetse flies of several species must from time to time ingest a variety of other trypanosomes", (i.e. other than T. bravi and salivarian trypanosomes) e.g. "T. theileri".

3. Horse flies (Diptera: Tabanidae). Knuth and Rauchbaer (1910) found a trypanosomatid infection of Haematopota pluvialis and suggested that it was the insect stage of T. theileri. Nöller (1916) described flagellate 'resting stages', which he considered to be metacyclic forms of T. theileri, in the hind gut of
Tabanus glaucus. He cultured these forms on blood agar plates at room temperature and believed his cultures were morphologically identical to those obtained from culturing parasitaemic cattle blood. Later (1925) he examined "numerous" Haematopota pluvialis in the neighbourhood of Berlin and found four infected with flagellates. From three of these, each obtained from a different area, he isolated trypanosomatids on blood agar plates, again finding them morphologically similar to cultures of flagellates derived from the peripheral blood of cattle. Plate washings pooled from these three strains were then used to inoculate calves which were assumed to be free of infection (see heading: prenatal transmission). Cultures from plates kept at room temperature appeared to infect a 5 day old calf but failed to infect a 13 day old animal. A further 5 day old calf appeared successfully infected from plates which had been kept at 32°C in order to use cultures containing a greater proportion of trypomastigote forms. A 7-9 month old bullock was unsuccessfully inoculated twice at an interval of one month but a further inoculation after a further interval of a month appeared successful. The successful inoculum was obtained from a plate originating from only one of the Haematopota strains. This culture was 31 days old on the passage used and showed rounded off barley corn shaped flagellates which "abundantly resembled the resting forms of the flagellates in the gut of the gad-fly". These forms either had the kinetoplast at the posterior end or just anterior to the nucleus. Noller had not noticed them in the other inocula used in his experiments but these had been derived from cultures not more than 10 days old in the passages used.

Jegen (1924) found flagellates in 14 out of 60 Haematopota pluvialis but was unable to decide whether they were parasites of the fly alone. He noted a similarity to T. gravi infections in tsetse fly species and vector stages of bovine trypanosomes.
In Java (Djawa) Nieschulz (1931) found flagellates in Tabanus rubidus, T. striatus and T. rufiventris which he considered to be associated with Trypanosoma theileri.

Kraneveld (1931) also suspected tabanids to be the transmitters following the detection on culture of a 'high' incidence of T. theileri-like trypanosomes in the cattle and buffalo populations of Java. On preliminary investigations he found 636 Tabanus striatus out of 3504 dissected to have trypanosomatid infections and likewise 1048 out of 4862 Tabanus rubidus. The flagellates from both tabanid species were cultured on blood agar and Kraneveld stated that their appearance resembled T. theileri-like organisms cultured from the peripheral blood of cattle. Kraneveld found a smaller incidence of flagellates of the same appearance in Tabanus rufiventris, T. bruminennis, T. basalia "indistinguishable from the cultural form of Trypanosoma theileri" and Haematocota javana, but less numbers were examined of these species. However, from data which the author had examined several years previously. He then attempted transmission experiments using large numbers of wild Tabanus striatus which he allowed to feed daily on the shaven skin of two buffalo and an ox. Two out of the three animals used appeared to have been infected by this means, but as care had been taken to prevent exposure to contamination, Kraneveld could only conclude that the flagellates parasitizing this species were not vector stages of bovine trypanosomes.
Kraneveld admitted that he was unable to distinguish between trypanosomatids which were parasites of flies themselves and those which were development forms of *T. theileri* - like trypanosomes. In flies which had been used successfully to transmit trypanosomes he saw in particular:

1. big slow moving epimastigote forms.
2. very mobile small forms.
3. rounded off forms but with flagella.

Types (1) and (2) were in both mid and hind guts while (3) was found with (1) and (2) in the insect faeces.

Packchanian (1957) examined trypanosomatid parasites of *Tabanus atratus* in the U.S.A. and considered that the flagellates seen both in the initial smear of the insect gut and in subsequent cultures were "indistinguishable from the cultural form of *Trypanosoma theileri* isolated from cows" which the author had examined several years previously.

Clarkson (1966 - personal communication) attempted to infect wild populations of *Haematopota pluvialis* with *T. theileri* - like organisms. Even though his experimentation was only exploratory in nature and there was no clear result, he showed that feeding flies on parasiticemic defibrinated blood, kept at 37°C, through membranes was a possible technique.

4. Other Diptera. Mayer (1912) alone has suggested mosquitoes as vectors. Kraneveld (1951) suggested simuliiids in addition to tabanids because of their habit of feeding on eye secretions and also because experimentally he had shown that penetration of mucous membranes by *T. theileri* - like trypanosomes was possible.

5. Ticks (Acaris Ixodidae). O'Farrell (1913) described *Crithidia hyalomae* as strictly a parasitic flagellate of *Hyalomma aegyptium*.
occurring in haemocoelic fluid, salivary glands, ovary, oviducts and ova of the host. Carpano (1932) saw a similar flagellate in one out of twenty ticks of the same species in Libya and offered the theory that it was the vector of *T. theileri*. Reichene (1946) however discounted this, believing that if *T. theileri* and *C. hyalomae* were identical, then the infection was accidental taking the analogy of *T. cruzi* having a period of survival in ticks of several species. Parasites of the genus Blasticrhididae have however been reported in other ticks e.g. *B. christophersi* in Rhipechalus sanguineus and *B. haemophysalisidae* in Haemaphysalis Flava (Wallace, 1966). Whether they represent the vector forms of trypanosomes infecting the hosts of the tick species is unknown.

**Prenatal transmission**

The detection of infections of bovine foetuses or new born calves with *T. theileri*-like trypanosomes has been reported. Bikman et al. (1957) found such flagellates in a foetal stomach, Lundholm et al. (1959) found them contaminating primary monolayers of foetal bovine kidney cells, Anonymous (1966) found patent parasitaemias in six new born calves, and Leary and Bouley (1967) found a heavy parasitaemia in one new born calf.

The preponderance of evidence however indicates that prenatal infection is an abnormal method of transmission, for example, using culture diagnosis, Crawley (1912) failed to detect infection in a day old calf born from a parasitaemic cow; Reid et al. (1966) found five bushbuck (*Tragelaphus scriptus*) foetuses not infected although three of the dams gave positive cultures, and Gray and Nixon (1967) failed to detect infection in fifteen calves of parasitaemic cows examined 1-2 hours after birth. Moreover, Cussick (1966 - personal communication) has never encountered trypanosomes as contaminants of primary bovine
kidney monolayers prepared from 200 calves in Nairobi, Kenya.

6. Artificial transmission: *T. theileri*-like trypanosomes appear to have been successfully transmitted to experimental bovine animals using whole parasitaemic blood; cultures of trypanosomes from peripheral blood maintained at 32°C or 37°C; cultures of trypanosomes obtained either from peripheral blood of cattle, or from intestinal contents of *Tabanus* and maintained at room temperature; using the intestinal content of *Tabanus* flies itself. Comparison of results is often difficult due to incomplete records of experimental methods, but a possible consistent failure has been the inadequate examination of animals for trypanosome infections before the start of experimentation, even without natural transmission; using flies containing trypanosomes from peripheral blood of cattle and 37°C culture media.

The alleged failures to transmit the trypanosomes may have been due to incomplete records of experimental methods, but a possible consistent failure has been the inadequate examination of animals for trypanosome infections before the start of experimentation, although calves seemed mainly to have been chosen to minimize this possibility.

a. Transmission using whole parasitaemic blood: The detection of a patent parasitaemia may have been assisted in some experiments by the concurrent inoculation of a pathogen e.g., rinderpest virus (Schein, 1907; Curasson, 1925) or babsia (Dias and Zuccarini, 1924). Canova (1924) even gave the opinion that the detection of a patent parasitaemia depended on this happening and even then did not reliably occur. Several authors have however recorded no difficulty in infecting either entire or splenectomized cattle with whole parasitaemic blood and detecting infection on blood smears e.g., Theiler (1903), Behn (1912), Bonger (1915), van Saeughein (1922), Levine et al. (1956), Imata et al. (1959), and Hoyte (1966 - personal communication). Others did not succeed in all cases e.g., Behn (1910), Teague and Clark (1918), Hornby et al. (1954) and Keymer (1966), while Holmes (1904) failed with each of four animals.

Keymer's attempts (1966) were of additional interest because he was attempting transmission from a duiker to a calf. He himself however a calf using a broth culture but gave no experimental details.
doubted his one apparently successful result as the calf first showed a large form of 80 μ body length only 4 1/2 days after injection with parasitaemic blood. As he considered these forms represented long standing infections, he felt that the experimental animal may have been previously infected.

The alleged failures to transmit the trypanosome may have been proved successful if culture diagnosis had been used. Vrijburg (1912) only succeeded in demonstrating transmission to calves by these means.

b. transmission using cultures maintained at 32°C and 37°C: Nüller (1925) cultured flagellates from wild Haemotopota pluvialis (see section epidemiology: natural transmission; horse flies) maintaining blood agar plates at 32°C before preparing an inoculum and infecting a calf. Hulliger (1964 - personal communication) infected a calf but with T. theileri - like trypanosomes which had appeared as contaminants on a leucocyte monolayer kept at 37°C. Splitter and Soulsby (1967) used their 'large volume' culture at 37°C (see section diagnosis: culture) to infect a calf whose immunological competence had been temporarily impaired by irradiation from two cobalt 60 sources. Cross et al. (1968) used one of Splitter's media containing trypanosomes to infect one splenectomized calf, and infected another with buffy coat culture fluids which contained trypanosomes.

c. transmission using cultures maintained at room temperatures: Miyajima (1907) detected patent parasitaemias in two calves out of three after inoculating them with broth cultures consisting of motile trypanosomes "in abundance". Martini (1909) successfully infected calves using a broth culture maintained at 29-31°C, but at these temperatures he may have begun to influence the number of trypomastigote forms present. Kohl - Yakimoff et al. (1913 a) also claimed to have infected a calf using a broth culture but gave no experimental details. Nüller
(1925) cultured flagellates from three *Haematopota pluvialis* onto blood agar plates and from growths obtained at room temperature infected one calf out of two at the first attempt, and appeared to infect an older bullock at the third attempt (see section epidemiology: natural transmission; horse flies).

Both Behn (1912) and Vrijburg (1912) failed to infect calves with cultures maintained at room temperature although both authors had been successful using whole parasitaemic blood. Likewise an annual report (India, 1940) recorded that "no infection could be produced by the inoculation of healthy bulls with the crithidial (i.e. epimastigote) forms".

d. transmission using intestinal contents of tabanid flies:
The successful experiments of Kraneveld (1931) using intestinal contents of *Tabanus striatus* have already been described (see section epidemiology: natural transmission; horse flies).

Storage of Strains

Study of *T. theileri* - like trypanosomes most easily commences from isolation of the forms in peripheral blood. The survival of these trypanosomes in whole blood at different temperatures is important, particularly in the field, to determine maximum permissible time lag between collection and inoculation of cultures without loss of infectivity. Following isolation of *T. theileri* - like trypanosomes, investigators have achieved maintenance by continuous subculture at approximately weekly intervals e.g. Crawley (1912) and Noller (1925) even for a period of years (Herbert, 1965 a). There appears to be only one reference to storage at low temperatures (Splitter and Soulsby, 1967).

*T. theileri* - like trypanosomes have been seen to retain motility in wet preparations of peripheral blood for at least one hour by Teague and Clark, (1918), for "some hours" by Schein (1907) and 18-36 hours under
Several authors have commented on the retention of infectivity to culture of peripheral blood following storage at different temperatures. At temperatures approximating $4^\circ C$ blood remained infective to culture for a maximum of 4 days (Crawley, 1912), 5 days (Saisawa et al., 1933), 7 days (Theiler, 1903) or 27 days (Vrijburg, 1912). At room temperatures findings have also been widely divergent e.g. Leach (1964) 6 hours, Johns (1913) 24-30 hours, Crawley (1912) and Saisawa et al. (1933) 3 days, Theiler (1903) 7 days and Vrijburg (1912) over three weeks. At $37^\circ C$ Crawley (1912) and Saisawa et al. (1933) agree on a maximum of 24 hours but Vrijburg (1912) gave 4 days.

Vrijburg (1912) and Splitter and Soulsby (1967) are the two most informative references. Vrijburg reported on loss of infectivity relative to four temperature ranges within the overall range of $4-20^\circ C$ and also on blood kept specifically at $22^\circ C$ and $37^\circ C$ in incubators. Retention of infectivity at $4-7^\circ C$ and $13-15^\circ C$ appeared to be significantly higher than at either of two intermediate ranges of temperature or at $22^\circ C$. Vrijburg also found that if the parasitaemic blood was stored in test tubes instead of flasks, infectivity was retained 3-4 times longer.

Splitter and Soulsby (1967) stored parasitaemic blood at $4^\circ C$ for three days and still infected cultures. In one sample only positive cultures were obtained following storage at $4^\circ C$ for both 6 and 10 days. For storage at lower temperatures, trypanosome cultures were mixed with glycerine, phosphate buffered glucose and saline (pH 7.5), and cooled to $-20^\circ C$ in a commercial deep freeze without regard to speed of cooling. The trypanosomes were then taken rapidly to $-70^\circ C$ in solid CO$_2$ and ethanol. Motility and ability to grow in culture were unaffected by storage at $-20^\circ C$ for 24 hours. Storage at $-20^\circ C$ for 7 and 14 days markedly reduced motility, and cultures of such material were negative. After 30 days storage at $-20^\circ C$, organisms were no longer viable and they failed to grow.
in culture." Cultures which had been quick frozen to -70°C were readily subcultured after at least 50 days storage at this temperature.

Discussion

The literature will be discussed under the following headings:

a. Taxonomy.

b. Detection of infections by serological means.

c. Pathogenicity.

d. Life cycle.

a. Taxonomy: The decision to review the literature on the basis of the term 'T. theileri-like' trypanosomes has been justified. Isolations have approximated to a common pattern, but biological evidence, on which species can be defined, is incomplete. A classification of *Nagatryptanum* infections of artiodactyls in relation to trypanosome infections of only mammalian species removes them from their correct context within the genus *Trypanosoma*. Similarities are evident, for example, between *T. theileri*-like organisms and *Trypanosoma avium* of birds (vide infra). 

b. Detection of infections by serological means: Two main points were made in the relevant section of this review. Firstly, where *T. theileri*-like trypanosomes and pathogenic species may infect the same host, the question of antigenic relationships may be of importance in their differentiation by serological methods. Secondly, the antigenic relationship between the vector and blood stream forms of a single trypanosome species is important in the preparation of antigen. Available information was shown to be miscellaneous in character. A small amount of additional information is available in relation to *Trypanosoma lewisi* and *T. cruzi*. 

simulating
49.

On the first point, Bigalke (1966) used double diffusion precipitin and immunelectrophoretic techniques to show that there were precipitins- ogens common to a number of Trypanosoma spp. The T. rhodesiense strain used to produce the antisera was found to share antigens with other salivarian trypanosomes of the subgenus Trypanozoon and also to the stercorarian T. lewisi.

On the second point, T. cruzi cultures at room temperature simulating the insect vector have been used successfully as antigen for complement fixation tests (Kelser, 1956; Davis, D.J., 1943). Dusanic (1968), however, studying T. lewisi found "a lack of complete immunologic cross-reactivity between culture and blood stream forms" and suggested that "basic antigenic differences may have developed during the continued cultivation of the flagellates and/or by antigenic variations in the original isolates of the parasites". Some clue may have been given by Entner and Gonzales (1966) who found two morphologically distinct forms of T. lewisi associated with several antigenic differences. Entner (1968) further demonstrated that in newly infected and susceptible rats three distinct antigenic stages of T. lewisi occurred.

Parallel situations may exist with T. theileri - like trypanosomes.

c. Pathogenicity: The apparent pathogenicity of T. theileri - like trypanosomes when inoculated, simultaneously with rinderpest virus, into an ox susceptible to both micro-organisms, is the allegation of pathogenic effect most consistently reported.

Although, apparently, the system has never been set up experimentally, Hornby et al. (1934) could anticipate the phenomenon occurring in cattle from one particular district. Interference between two micro-organisms in the same ambient is not uncommon, for example, between an Anaplasma and an Eperythrozoon in cattle (Foote et al., 1957), and between Trypanosoma brucei and ornithosis virus in culture (Biens, 1966),
but in the phenomenon under discussion there is a difference. In the recovery stage of the virus infection a situation appeared to occur which allowed a swarming parasitaemia of *T. theileri* - like trypanosomes and a relapse in the animal's condition. By inference, the normally non-pathogenic trypanosome had acquired pathogenic properties. The question remains whether the relationship is unique or whether the reported anthrax-like episodes are manifestations of the same phenomenon in relation to other infections.

d. Life cycle: The information reviewed can now be discussed in terms of constructing a hypothetical life cycle. The discussion starts with the forms in fixed tissues.

As the parasitaemia in the mammalian host is normally only detectable on culture, and forms in division are seen only exceptionally, multiplication of the trypanosomes in fixed tissues can be argued analogous to other stercorarian trypanosomes (e.g. *T. sagpi*, Davies, 1952; *T. mcerotii* and *T. evotosys*, Molyneux, 1968 b; *T. nabiasi*, Molyneux, 1969 a). Some evidence in support of this is provided by the two available descriptions of *T. theileri* - like organisms in various morphological forms in lymph nodes (Carpino, 1952; Gallo, 1953). The conglomerations reported in brain tissue with associated nervous symptoms could be considered accidental and possibly analogous to the reports of conglomerations of *Theileria* schizonts in the brain (e.g. Carmichael, 1939) or collections of erythrocytes infected with *Babesia* spp. (e.g. Zlotnick, 1955).

Most of the descriptions of dividing forms in the peripheral blood have been with high parasitaemias associated with concurrent infections of rinderpest virus and assumption has to be made that this is either an exaggeration of the same events at sub patent parasitaemias or of the appearance in peripheral blood of stages normally occurring in fixed tissues. Luhrs (1905) believed that division could either be longitudinal,
Giving epimastigote daughters of equal size or transverse giving epimastigote daughters of unequal size. Reichenow (1940) examined slides supplied by Hornby from a similar source. He described unequal division only and theorized that of the two epimastigote daughters, the larger eventually became the giant trypomastigote forms of chronic infections while the smaller were capable of further division. Comparison of the drawings of unequal division made by both Luhs and Reichenow show remarkable similarity (fig. 2). The longitudinal and equal division described by Luhs could well be the further division of the smaller daughter of the initial unequal division and is therefore reconcilable to Reichenow’s theory. From the beginning, the appearance of epimastigote forms in peripheral blood has been well recognized (Laveran, 1902 a; Theiler, 1903). The blood stream forms with two nuclei, one anterior to the other, described by Theiler (1903), Luhs (1905) and Keymer (1966) could represent organisms in which the process of division had been abortive.

If Reichenow’s conclusions are correct, qualitative differences could be expected in blood stream forms, particularly regarding infectivity to an insect vector. Of relevance here may be the widely divergent periods of time for which, firstly, parasitaemic blood has been reported to remain infective to culture, and, secondly, cultures on blood agar have been found to survive. The irregularities seen in transmission experiments from bovine animal to bovine animal using whole parasitaemic blood could also be explained by differences in infectivity or by loss of the capability to divide even before transfer to the new host.

The culture of trypanosomes at room temperature is considered to be analogous to multiplication in the insect vector (Caveny, 1926). Initially division may be a continuation of that described by Reichenow for peripheral blood, as Johns (1913) centrifuged freshly drawn citrated
blood and found forms in unequal division. The larger daughters continued to divide unequally while the smaller daughters divided equally. In older cultures there appears to be an important difference between the morphogenesis described by Crawley (1912) using inocula of peripheral blood and the forms described by Hartmann and Müller (1918) derived from *Tabanus glaucopus*. No club shaped forms were described from the latter cultures and with knowledge of the behaviour of *T. theileri*—like organisms it is difficult to imagine that their non appearance was due to the squash preparations used.

Müller (1925) appears to be the only author who discusses the possibility of metacyclic forms occurring in cultures at room temperature, even though they could reasonably be expected to be trypomastigote in form and such forms have been reported to occur as the cultures age (e.g. Maneaux et al., 1911b). The very active flagellates that Müller (1917) saw on a 47 day old blood agar plate require explanation but he believed that the 'barley-corn' forms in a 51 day old culture (Müller, 1925) were at least morphologically identical to the forms seen lining the hind gut of the tabanids. These he believed to be metacyclic forms and those from which he made his cultures. They could also be the same as the 'rounded off' or oval flagellated forms seen e.g. by Kraneveld (1931) in the hind gut of *Tabanus striatus*, by Patton (1909) in the hind gut of *Tabanus hilarius* and an unidentified *Tabanus* spp., and by Bruce et al. (1911) in *Tabanus secedens*.

Support for this hypothesis is gained by consideration of the metacyclic stage of other trypanosomes whose development occurs in the hind gut of insect vectors and knowledge exists of the appearance of the same forms in culture media e.g. *T. avium* (Baker, 1956, 1966), *T. conorhini* (Deane and Kirchner, 1963), *T. cruzi* (Camargo, 1964), *T. daniilewskyi*.
In all cases the appearance of metacyclic forms in primary culture, at least in recognizable numbers, took longer than the natural development in the insect vector (e.g. days in respect of *T.* avium and weeks in the case of *T.* lewisi). Moreover despite differences of fixing and staining, the metacyclic forms described for *T.* avium, *T.* lewisi and *T.* melophagium in particular have resemblance to the 'barley corn' forms described by Noller.

Wallace (1962) however believed that the 'barley corn' resting stages described by Noller must have been choanomastigote in form representing a trypanosomatid species of the genus *Crithidia*. Certainly, Noller did not eliminate the possibility of mixed infections of trypanosomatid flagellates in the wild tabanids from which he made cultures, neither did he consider the possibility that his cultures were of more than one species. Thomson (1933) for example, clearly figured choanomastigote forms in a tabanid (*T.* africanus) as did Wallace himself in *T.* epistates. Wallace described the possibility that a 'cryptic' infection of *T.* theileri - like trypanosome forms in the hind gut of the vector may have in its turn overwhelmed the choanomastigote forms in the culture. In balance it appears more likely that Wallace was mistaken in his surmise of the nature of Noller's 'resting forms' but resolution of this point will depend on the further work on trypanosomatid infections of tabanids which Wallace himself strongly advocated.

In such work the study of the thickening at the tip of the flagellum, sometimes described in the morphology of *T.* theileri - like trypanosomes in cultures at room temperatures, could possibly assist in the interpretation of the morphogenesis of the flagellates by identifying
stages concerned in the ultimate production of metacyclic forms. The
similar phenomena noted for *Crithidia fasciculata* by Kusel et al. (1967)
and for *Trypanosoma lewisi* by Molneux (1968 b, 1969 b) were interpreted
by the latter author to be an adaptation of the parasite to life in the
insect assisting attachment to the wall of the hind gut. No author has
suggested any significance of these thickenings in relation to *T.
theileri* - like trypanosomes, or whether they are associated with any
other distinctive morphological features or with any particular age of
culture. Few of the available drawings and photographs featuring the
flagellar thickenings are related to the age of cultures but those
available appear to demonstrate their appearance after a few days.
Manceaux et al. (1911) for example figured them from the fourth day of
culture, Crawley (1912) from the fifth day, while Johns (1913) figured
one out of three at 15 hours but all of a group of eight flagellates at
eight days. Some authors have drawn the club shape forms with flagellar
thickenings (e.g. Crawley, 1912; Bettencourt and Borges, 1916) but the
degeneration implicit in the shape was possibly imposed by the medium
rather than representing a stage in an insect vector.

Terminal flagellar thickenings have however been described in
peripheral blood forms of trypanosomes. Mesnil and Brimont (1910)
described such terminal thickenings in blood form of *Trypanosoma lewisi*
of the giant anteater (*Tamandua tridactyla*) likewise Keymer (1966) in
*Trypanosoma brodeni* of elephant shrews (*Elephantulus brachyrhynchos* and
*Petrodromus tetradactylus*) which he considered synonymous with *T.
petro-
dromi* (Bruce et al., 1913 b). Keymer also observed that the characteristic
was easily destroyed on staining. This may account for the poverty of
observations in peripheral blood forms of *T. theileri* - like organisms:
Lilja (1905) referred to the thickened ends of the flagella in "young"
dividing forms and Falshaw and Lingard (1907) reported a fine terminal
The presence of this feature may indicate the peripheral blood forms of the trypanosome infective to the insect vector.

An important obstacle to further work on trypanosomatid infections of tabanids is the present inability to develop laboratory colonies of members of this family. In addition, no studies exist of the habits of tabanids in relation to their hosts and these would obviously affect the sites of deposition of metacyclic forms. Assumption has to be made that this occurs in sites where the host may lick, following the observation by Kraneveld (1951) that an animal was apparently infected by placing intestinal contents of Tabanus striatus in contact with mucous membrane of the mouth. The experience of Hornby et al. (1934) is interesting to recall at this point where peripheral blood forms did not appear to have the same ability to penetrate a mucous membrane (see section: pathogenicity).

Only Carpano (1932) appears to have postulated a series of events in both the mammalian host and the vector. He did this, however, laying emphasis on the forms that he himself had seen in the brain and lymph nodes of cattle and his own observations of trypanosomatid infections in Hyalomma aegyptium. He did not adequately discuss the possibility of other vectors being involved, particularly the evidence in favour of tabanic transmission. Reichenow (1940) discounted both the idea of multiplication in fixed tissues and of tick transmission but Hoare (1936) accepted a conclusion by Carpano that T. theileri behaved similarly to Trypanosoma cruzi in the mammalian host.

Nevertheless the evidence brought together in the present review of the literature does enable a working hypothesis of the nature of the life cycle of T. theileri - like trypanosomes to be constructed, despite the fragmentary and unconfirmed nature of most of the available information.
A diagrammatic representation is given in Fig 4. Multiplication in a 'fixed' tissue, probably lymphoid, results in a terminal unequal division which in exceptional circumstances of high parasitaemia may be also seen in peripheral blood. The smaller daughter divides in the epimastigote form to eventually produce organisms, whether trypomastigote or epimastigote is not clear, which are infective to the insect vectors. The larger of the daughters increase in size, lose their power of multiplication and reach the size credited to T. ingens. The evidence strongly suggests that several species of the family Tabanidae are vectors. Within the alimentary canal of the vector the non infective individuals die out (but may do so quicker than the degeneration seen in culture at room temperature) and the infective forms develop into the metacyclic 'barley corn' like flagellates in the hind gut. A stage on the way may be the highly motile forms such as seen on a 47-day old culture by Noller (1917) and in the midgut of tabanids by Kraneveld (1951). The metacyclics pass out of the vector either in the fluid exuded at the time of feeding or in faecal material. The host licks the area of the bite and the metacyclic forms penetrate the mucous membrane of the mouth. Multiplication of the parasite may initially occur only in the blood of a susceptible uninfected host and a patent parasitaemia may develop. At a later stage multiplication occurs primarily in lymphoid tissue and the life cycle is completed. The parasitaemia is normally subpatent but shows a seasonal variation, the higher parasitaemias probably occurring in the summer months. Once established, the infection may persist for the rest of the life of the host.

In conclusion, the total information concerning the Trypanosoma theileri - like infections of artiodactyls is inadequate to appraise
whether the infections represent a single species or a group of similar trypanosomes. Greater understanding will be acquired by first studying life cycles and developing laboratory methods for the handling and storing of isolations.

The standard diluent used was "solution A" described by Landon et al. (1935), containing a physiologically balanced mixture of sodium, potassium, magnesium and calcium salts, then buffered to approximately the same pH as the blood agar medium used, it is termed in the text "solution ABP 70A". The 70A refers to the phosphate buffer component.

2. Culture from blood or fixed tissues.

As at room temperatures (20°C)

The culture media were blood agar similar to that described by Herbert (1961). Although minor modifications were made in the course of the work, the formula and method of preparation employed in the great majority of experiments was as follows:

1. Blood agar base (Oxoid, London): 35.0 g/litre medium.

2. Dextrose (AnalaR; British Drug Houses, Poole, England): 1.6 g/litre medium.

3. Double distilled water: 800 ml/litre medium.


Constituents (1) and (2) were dissolved in the distilled water and the solution autoclaved at 121°C for 15 minutes. The solution was then cooled to 50°C, the blood mixed to 57°C added, and all maintained at 50°C in a water bath. The medium was then distributed by means of an automatic syringe to screw capped Bïten bottles (Arnold & Horwell Ltd., London) in 2.5 ml quantities. The screw caps were centrally perforated and fitted with white rubber seals. The medium was slopped and cooled at room temperature.

For each batch of slopes prepared, the following checks were made:
III. EXPERIMENTATION

Materials and Methods

1. standard diluent.

The standard diluent used was "solution A" described by Lumsden et al. (1963), containing a physiologically balanced mixture of sodium, potassium, magnesium and calcium salts. When buffered to approximately the same pH as the blood agar medium used, it is termed in the text "solution ABP 7±4". The BP 7±4 refers to the phosphate buffer component.

2. culture from blood or fixed tissues.

a. at room temperatures (28°C)

The culture media were blood agar similar to that described by Herbert (1961). Although minor modifications were made in the course of the work, the formula and method of preparation employed in the great majority of experiments was as follows:

i. blood agar base (Oxoid, London): 32.0 gm/litre medium.

ii. dextrose (Analar; British Drug Houses, Poole, England):

1.6 gm/litre medium.

iii. double distilled water: 800 ml/litre medium.

iv. sheep blood, defibrinated: 200 ml/litre medium (standard donor).

Constituents (i) and (ii) were dissolved in the distilled water and the solution autoclaved at 121°C for 15 minutes. The solution was then cooled to 50°C, the blood warmed to 37°C added, and all maintained at 50°C in a water bath. The medium was then distributed by means of an automatic syringe to screw capped bijou bottles (Arnold R. Horwell Ltd., London) in 2.5 ml quantities. The screw caps were centrally perforated and fitted with white rubber wads. The medium was sloped and cooled at room temperature.

For each batch of slopes prepared, the following checks were made:
the pH was measured following the addition of 3 ml. distilled water to each of two slopes, chosen at random, which had then stood for 1 hour (acceptable range of pH 7.3 - 7.5); all media slopes were allowed to stand at room temperature for four days and any showing contamination were rejected; lastly, a procedure was carried out to check that no flagellates were being cultured from the blood component of the medium itself. For this, on twelve random occasions, twelve slopes were taken from a single batch of medium (120 batches were made averaging 210 slopes per batch), incubated at 28°C, and examined on day 15 for flagellates. No flagellates were found on any occasion.

Stock slopes were prepared at least one week before required, stored at 4°C, and used within 30 days. Antibiotics dissolved in solution ABF 7.4 were added to the cultures more than 2 hours but less than 7 days before they were expected to be used for inoculation. Penicillin and streptomycin were used at final concentrations of 800 and 400 units per ml. of medium respectively.

The described routine was different in the following circumstances:

(i) in Nigeria, universal containers (Arnold R. Horwell Ltd., London) were used and 5ml. of medium was sopped in each.

(ii) in Nigeria, Colombia and in the sampling of herds in Scotland during 1965, defibrinated horse blood was used. (donor horses in Nigeria and Colombia, and Oxoed, SR50 in Scotland).

(iii) in Colombia, Difco blood agar base replaced Oxoed blood agar base No.2 giving an approximate pH of 6.8 instead of 7.4.

The efficiency of isolation of T. theileri - like trypanosomes was checked on single trials following: firstly, the change in size of container from a universal to a bijou, and, secondly, changing from Oxoed blood agar base No.2 to Difco blood agar base. No change of efficiency
was detected in either case.

b. at mammalian blood temperatures (37°C).

Culture at 37°C was attempted on monolayer systems of mammalian cells. An established cell line of bovine kidney (Modin and Darby, 1958), a bovine thyroid cell line on seventh passage (Flowright and Ferris, 1961), and Earle's and Eagle's tissue culture media (Earle, 1943; Eagle, 1955) were obtained from the Department of Veterinary Pathology, University of Edinburgh. The majority of studies were however carried out using bovine leucocyte cultures prepared after the method of Tokuda et al. (1962). The procedure was as follows:

Precautions were taken to obtain a donor animal uninfected with T. theileri-like trypanosomes. A young bullock was selected from Easter Bush farm, Midlothian, Scotland, where the incidence of infection with the trypanosomes was considered to be very low (see results: section 1). As a further check, the peripheral blood of the bullock was cultured for stercorarian trypanosomes on seven consecutive days in the summer. No flagellates were seen.

The bullock was bled aseptically from the jugular vein and the blood inoculated into a universal container to which had been added heparin, streptomycin and penicillin (see section 3; bleeding of animals). The whole blood was then distributed in 0.5 ml. quantities into large Wasserman tubes (12 x 100 mm) with white rubber stoppers. These tubes were then inclined in a rack at about 45° to the horizontal and incubated without rolling at 37°C. After the third day the plasma and free blood cells in each tube were discarded. The leucocytes which had sedimented and adhered to the glass were washed three times with solution A. Whole serum of the same bullock was used as culture medium following inactivation at 56°C for 30 minutes. The inactivated serum was distributed into the tubes in 0.5 ml. quantities and the system was again incubated at 37°C. The
61.

medium was changed every third day. Under conditions of sterility the leucocytes grew into a continuous sheet and supported growths of T. theileri-like trypanosomes for periods up to 70 days. To facilitate staining and microscopic examination, cover slips were added to the culture tubes so that cells would adhere to them.

Slow and careful decanting of old culture medium and equally slow layering of fresh medium did not appear to disturb the relationship between the leucocytes and the adhering flagellates.

3. bleeding of animals, preparation of fixed tissue suspensions, inoculation and examination of cultures.

a. bleeding of animals.

Blood samples from living animals were taken from the jugular vein. In the Nigerian work, all blood samples were taken with aseptic precautions into a 500 ml. conical flask containing glass beads. The blood was defibrinated by shaking the flask until flecks of fibrin could be clearly seen. In the United Kingdom and Colombian work, animals were bled with disposable syringes and needles (Gillette Industries Ltd., London), the blood withdrawn being immediately injected through the wad of a universal bottle containing 100 units heparin, 20,000 units penicillin and 7450 units streptomycin in 0.4 ml. of solution A. The maximum quantity of blood so injected was 20 ml.

All blood samples taken from Cervidae were from animals shot only minutes previously. The preferred site was still from blood vessels of the neck exposed by dissection, but the nature of the shot killing the animal in some cases necessitated exposure of the anterior or posterior venae cavae. The blood was injected, as from living animals, into the universal bottles prepared with heparin, streptomycin and penicillin.

b. preparation of suspensions of fixed tissues.

Experimental animals were killed either with other (laboratory
rodents) or by captive bolt pistol and cutting the carotid artery (cattle). Portions of organs of rodents were removed immediately from the dead animals using aseptic precautions. With cattle, the organs themselves were first removed before samples were taken. The tissue samples, about the size of a pea, were put into tissue grinders with 2 ml. solution A and made into a coarse suspension.

c. inoculation and examination of cultures.

(i) blood agar slopes at room temperature: Bijou bottles containing medium were inoculated through their rubber caps with disposable syringes and needles. One ml. of heparinised peripheral blood was inoculated into each of six bijou bottles, all inoculations being completed within half an hour of bleeding the animal. One ml. of fixed tissue suspensions were inoculated into each of two bijou bottles containing medium, the operation being undertaken with the greatest possible speed, taking only minutes from the death of the animal with some organs, but all inoculations completed within an hour.

Cultures were then incubated at 28°C and wet preparations of the liquid phase were examined microscopically for flagellates on the 5th and 10th days. As a result however of information obtained from the surveys for T. theileri-like trypanosomes (see results: section I) a third day of examination was later added on day 15. A minimum of thirty fields (objective x 20, eyepiece x 10) were examined before negatives were recorded. The situation of at least one flagellate being visible in every field was given a '++' rating; the detection of only a single trypanosome following the examination of all fields was given a '+' rating, and all situations in between a '++' rating.

(ii) leucocyte monolayers at 37°C: Leucocyte monolayers were only inoculated with organisms first grown on blood agar slopes. All transfers of fluids and suspensions were made with disposable syringes
through the rubber wad fitted into the perforated screw caps of containers. The only exception was the final inoculation of the Wasserman tubes containing the monolayers.

About 2 ml. of solution ABP 7.4 was added to the culture on blood agar to wash off any organisms growing up the slope. The augmented liquid phase was then removed and put into a universal container where further solution ABP 7.4 was added to make the required dilution. If the culture inoculum had been peripheral blood the final dilution was required to be at least 1 in 10, otherwise the number of erythrocytes interfered with counts of the flagellates in the final suspension. In addition, before counting or inoculation, the inoculum was drawn into a syringe and expelled through a fine bore needle (25 G x 78") five times to break up clumps of organisms.

The Wasserman tubes were examined by supporting them on the stage of an ordinary light microscope with two parallel glass rods fixed to a cardboard base with plasticine. The magnification used was x 100 (x 10 objective, x 10 eyepiece). Counts of flagellates were possible by firmly swirling the medium in the tubes five times to remove the flagellates from the monolayer. The flagellates were therefore then suspended in the medium.

4. Counting trypanosomes in suspensions, and standard procedure for population studies of the growth of trypanosomes on blood agar slopes.


A minimum of 300 trypanosomes were counted in any one sample using the improved Neubauer haemocytometer. Counting ceased after the square in which this number was exceeded.

b. Standard procedure for population studies of the growth of trypanosomes on blood agar slopes.

Trypanosome populations stored as stabilates at low temperatures
(see section 6) were the only ones used. The contents of a single storage capillary was inoculated into a single bijou containing the medium and incubated at 28°C. On the fifth day, subject to the number of subcultures required, a quantity of solution ABP 7.4 was added to the liquid phase of the culture. The resultant suspension of flagellates was drawn into a syringe and expelled five times through a fine bore needle (25 G x 5/8") to break up clumps of organisms, finally being expelled into a sterile universal container. A prepared number of blood agar slopes each received a 0.1 ml. inoculum of the suspension and were incubated at 28°C. A count was made of the suspension and the number of flagellates per inoculum estimated. From day one onwards, one, two or three cultures were counted as required. Counts were made by adding 2 ml. solution A to the liquid phase of each, again breaking up clumps before placing a drop on the counting chamber of the haemocytometer.

5. the fixing and staining of trypanosomatid flagellates.

a. light microscopy.

(1) thin blood smears, tissue impression smears and cover slips from monolayer tubes: The routine method used came within the recommendations of Curr (1963) for his "improved Giemsa stain R.66" purchased as a ready made up solution.

The slides were fixed in methanol for 2 minutes and placed face down on the bars of a staining tray (see figure 5). Giemsa stain was diluted at the rate of one drop to each ml. of neutral distilled water, and run between the face of the slide and the staining tray by means of a syringe and wide bore needle. After 20 minutes the slides were removed, washed in distilled water and placed on a rack to drain. Cover slips were treated in the same way using a small version of the staining tray, and then fixed to slides for examination using 'Polymount' (Stayne Laboratories Limited, High Wycombe).
This simple procedure gives a clean preparation as sediment is prevented from settling on the slide and evaporation is minimised.

(ii) thick blood smears: A drop of blood was placed in the middle of a slide and spread with the corner of another slide to cover an area approximately the size of a shilling piece. The slide was dried for 24 hours at the temperature of the laboratory by placing face downwards on the staining tray bars. For staining they were turned face upwards. Improved Giemsa stain R.66 was diluted at the rate of 1 part of stain to 50 parts of distilled water buffered to pH 7.2, run onto the slide and left for 1 hour. Distilled water was gently flooded on to the slide to avoid detaching any portion of the thick blood film, the washing process being completed over a sink. The slides were then placed in a rack to drain dry as blotting could result in removal of some of the blood film.

(iii) thin smears of the liquid phase of cultures maintained at 28°C and insect gut contents: Primary cultures of flagellates grown on blood agar were stained as (i) above up to 21 days old. Older cultures, and cultures derived from prepared suspensions of flagellates in solution tended to have a particulate suspension which obscured the organisms if fixed by methanol. The effect was particularly marked with insect gut contents. The method of Lehmann (1954) was therefore used staining with Giemsa after fixing for half a minute in buffered 10% formalin (pH 7.0).

b. electron microscopy, negative staining.

A suspension of organisms was prepared by washing a blood agar slope with solution A and making up a total volume of 10 ml. The suspension was then centrifuged at 250 g for 10 minutes. The supernatant was removed and the organisms fixed for 5 minutes in 2 ml. of a solution made up as follows:

\[ 0.2 \text{ M phosphate buffer pH 7.4} \quad 100 \text{ ml} \]

\[ (19 \text{ ml. KH}_2\text{PO}_4 ; 51 \text{ ml. Na}_2\text{HPO}_4) \]

Taking particular care in the case of glycerol, the mixture was then dispersed into the suspension.
25% glutaraldehyde (Taab Labs., Reading) 12 ml.
CaCl₂ solution (1 x 10⁻³ g/ml.) 4 ml.
Distilled water 84 ml.

The suspension was again centrifuged at 250 g for 10 minutes and the organisms rinsed three times in the phosphate buffer (pH 7.4) using the same centrifuge speed. At this stage wet preparations were made and examined microscopically. The volume of the final suspension was adjusted to give a likelihood of 3-5 organisms per square of the carbon grid. This volume never exceeded 5 ml. Holding a carbon grid in watchmaker's forceps, the suspension was layered on with a Pasteur pipette and allowed to dry in the air. The preparation was then stained in 1% aqueous OsO₄ solution for 2 hours and then rinsed in the phosphate buffer (pH 7.4) for 60 minutes.

6. Low temperature storage.

The method adopted was as used in the Applied Protozoology Research Unit, University of Edinburgh, following the principles laid down by Cunningham et al. (1965). The liquid phase of blood agar slope cultures was the only material stored in the present work.

The liquid phase of the culture to be preserved was drawn into a one ml. syringe and the total volume noted before being discharged into a sterile Wasserman tube. The amount of glycerol or dimethylsulphoxide (D.M.S.) required as cryoprotectant (7.5%) was read off on a prepared table and added to the flagellate suspension. The viscosity of glycerol required that it be drawn into a one ml. syringe before a needle was attached and discharged through a needle having a wide bore (19 G x 2”). The cryoprotectant was then mixed thoroughly with the flagellate suspension using a Pasteur pipette, and taking particular care in the case of glycerol.

Using the same Pasteur pipette, the mixture was then dispensed into sterile capillary tubes (4" x nominal 1.0 mm) (Plowden and Thompson Ltd.,
These were held horizontally by their centres in a rack (Cunningham et al., 1963) leaving both ends of the tubes free. The rack itself was on a stand enabling it to be pivoted along its long axis. While dispensing the mixture the rack was tipped so that the contents of the capillaries approximately filled the middle third. The capillaries were then sealed in a microburner flame starting with the ends from which they were filled. When the side was completed the rack was tipped to check the efficiency of the sealing. If in no case did the contents of a capillary run, the other ends were then sealed.

Sealed capillaries were then transferred to a tube of methanol which had been cooled by placing in a beaker of broken ice. A storage serial number was allocated and written in pencil on a strip of card together with the name of the organism. This label was then placed in the tube which was corked tightly, dried on the outside and put in an insulating jacket made of Onasote (Expanded Rubber Company Ltd., Croydon) with a 25 mm. wall. The insulating jacket was then plugged with non-absorbent neck wool and transferred to the cooling compartment of an insulated cabinet containing solid CO₂. The capillaries were left overnight to cool slowly and transferred to permanent storage the next day.

Transfer of capillaries to permanent storage was carried out under methanol at -79°C. Methanol kept for this purpose was preferably stored in a thermos flask at -20°C as less time was therefore taken to cool it to -79°C ready for use. Solid CO₂ was added to the methanol in a perspex bath until bubbling had almost ceased. A numbered box from the storage compartment of the permanent storage bank was removed and placed in the perspex bath. Pieces of solid CO₂ were laid on top to make sure that it remained cold. The tube of methanol containing capillaries kept in the temporary store overnight was also removed and placed in the methanol bath. The capillaries were then manipulated into the permanent box which was then
replaced full of cold methanol in the permanent bank with maximum speed.
7. the preparation of clone populations on culture.

Cultures of *T. brucei*-like trypansomes were grown from selected stabilates and incubated at 28°C until wet smear examination gave a ++ or +++ rating (see section 2. examination of cultures). One ml. of solution
ABP 7.4 was added to a selected culture by inoculation through the mad of
the screw cap of the bijou bottle. The fluid was withdrawn and expelled
through the needle of the syringe (25 G x 78") five times in order to
break up any colonies or clumps of flagellates. Ten fold serial dilutions
were prepared as far as 2.

The cloning procedure was then carried out entirely in an atmosphere
of at least 90% relative humidity using sterile materials and instruments.
These are displayed in Fig. 6 a. Cover slips (No.1 22 mm x 6 mm) (Chance
Brothers Ltd., London) were placed sideways on to the length of a microscope
slide so that their ends were slightly protruding from the edge of the slide.
They were held in position using capillary attraction exerted by small drops
of solution ABP 7.4.

In the majority of occasions the 2 dilution was found to be the most
suitable and this dilution was the first tried. Using a 1 ml. syringe
and a 26 G x 78" needle whose point was cut with scissors to make a square
end, microdrops of the trypanosome suspension were placed on each of the
prepared cover slips (Fig. 6 b). With practice, the microdrops could be
prepared so that they occupy only part of a microscope field at a total
magnification of x 100 (x 10 eyepiece, x 10 objective). The slide, plus
cover slips plus microdrops, was then placed upside down over a small tray,
containing ABP 7.4, of a convenient size to fit the microscope stage.
This assisted the maintenance of adequate humidity in the environment of
the drops. A flat cover slip box mounted on another slide was found to
be convenient for the microscope used (Fig. 6 c).
The microdrops were then examined by dark ground illumination through the thickness of the slide and cover slips and the number of flagellates in each drop assessed. If all showed more than one flagellate, the suspension was further diluted at the discretion of the operator, or if necessary, another serial dilution tried. A situation could therefore be contrived whereby in every series of microdrops examined, one or more would contain a single flagellate. Allegations of single flagellates, however, required the confirmation of two separate witnesses before being accepted as such. The morphological outline of the single flagellates were recorded and body length estimated using a calibrated eyepiece.

Each cover slip having a single microdrop was then removed by forceps, the operation being facilitated by the small extension over the edge of the slide, and placed onto a blood agar slope (Fig. 6 d) ensuring that the drop was covered by the liquid phase of the medium. If necessary the cover slip was washed down with solution ABP 7.4. Cultures were then examined at 5 day intervals up to the 25th day.

Less than 1% of slopes inoculated were found to be contaminated despite the exposure of materials to the air.


Most of the *Haematopota* spp. caught were attempting to bite man. A calf was also used as a bait animal and two kinds of trap were tried. The simplest was a black screen held between two operators, the other depended on a black plastic sphere of 75 cm. diameter as the attractant (Manitoba fly trap; Thorsteinson et al., 1964). The sphere was suspended in the centre of a tripod the top of which was covered by clear plastic sheeting and surmounted by a no return container.

Students from the Faculties of Science and Veterinary Medicine volunteered to catch biting flies while on summer vacation. They were
supplied with: plastic snap top containers (Arnold R. Harwell, Ltd., London) containing moist blotting paper; stamped addressed and numbered envelopes; a card to enclose with a specimen giving date, place, altitude and vegetation, and a sheet of information for collectors (Appendix 2).

Feeding of flies was accomplished by putting them singly into clear plastic tubes (3" x 1") the bottoms of which had been sawn off and replaced by black nylon netting. The netted ends of the tubes were held against a shaved area of the hide of an ox for twenty minutes or until the fly had fed.

9. dissection of *Haematopota* spp.

Individual flies were killed with ether in a universal container immediately before dissection. Wings and legs were removed, one wing being put into a numbered container to assist later in the final species identification.

The fly was placed dorsal side downwards on a microscope slide with its head near a drop of solution A. The slide was then placed on the stage of a dissecting microscope and the fly held by the thorax. Using curved watchmaker's forceps, the head was slowly drawn off into the drop of solution A. Portions of foregut including salivary glands also came with the head. Traction ceased as soon as the salivary glands were free of the thorax and any connection remaining between head and thorax was cut with micro-scissors. The salivary glands were at once examined at x 100 magnification under a standard light microscope for flagellates and the head was placed with the wing in the numbered container.

The remainder of the fly was pinned by the thorax to an embryo glass filled with paraffin wax. Solution A was placed round the abdomen. Using micro-scissors, and again on the stage of a dissecting microscope, the abdomen was cut laterally on both sides and also at the junction with
the thorax. The ventral portion of the abdomen was then removed.

The abdominal contents were therefore revealed. The alimentary canal was removed starting from the anterior end removing, firstly, two large and brilliantly white air sacs and cutting through masses of tracheae. The contents were entirely removed together with a small portion of anal plate as a point of reference and placed on a microscope slide with solution A. Detailed separation of parts of the alimentary canal could then be made after ovaries and malpighian tubules were identified and removed.

The part of the dissection requiring the greatest care was the demonstration of the rectum. The alimentary canal was then again examined under a cover slip for flagellates.

The flies were identified by the examination of wing venation and antennae.

10. records.

a. stabilate material.

Stabilates were recorded on the record sheets (Kalamasoo Ltd., Birmingham) used as standard with the low temperature storage bank maintained by the Applied Protozoology Research Unit, University of Edinburgh. Stabilates were allocated by serial numbers with the prefix TREU (Trypanosomiasis Research, University of Edinburgh).

b. cultures.

Subcultures made from either primary isolations (recorded in experiment files) or stabilate material were given serial numbers and entered on CCS cards (culture group serials).

c. Haematopota collection and dissection.

The information was consolidated on sheets having columns for:

(i) specimen number

(ii) time and date taken
(iii) collector

(iv) place

(v) altitude

(vi) vegetation

(vii) date of dissection

(viii) site of flagellates if any

(ix) action taken

(x) dissected by

(xi) identification Haematopota spp.

(xii) notes.

Scotland: Seven widely scattered farms were initially chosen and examined within the months of July, August or September, 1965. Three additional farms were examined in later years, Kinloch, Inverness-shire (November, 1966), Trish, Inverness-shire (May, 1968) and Crowsford, Lancashire (July, 1968). Their climate is Northeast European (Andrews, 1963). Data on vegetation, cattle type and annual rainfall is listed in Table 3 and their localisation shown on Fig. 7.

England: A dairy farm at Pinetree, Buckinghamshire was studied in October, 1965. Its climate, again, is Northeast European; annual rainfall is 25.7 in. (65 cm) (Meteorological Office, Buckingham, 9 miles northwest from Pinetree). Advantage was also taken of a visit to Teviotstock, Southwark, in May, 1966 and samples were taken from one animal at each of six widely scattered farms. Teviotstock lies to the immediate Southwest of Perth with an annual rainfall of approximately 45 in. (114 cm) (G.B.A., 1966).

Scotland: Cattle were examined during the months of April to July 1967, on 17 farms in a total of 11 widely scattered localities.
Results


a. Localities studied.

Nigeria: University Farm, Ibadan. Ibadan lies in the lowland rain forest zone of West Africa (Glover, 1961) at approximately 650 feet (200 metres) above sea level. The climate is hot and humid with little seasonal change of temperature; the mean monthly maximum temperature only varies from 93°F (34°C) in February to 80°F (27°C) in August. The mean annual rainfall is 47.5 in. (121.9 cm.) (Records 1953 - 58; Department of Geography, University of Ibadan). The cattle were examined within the months January, February and March, 1965.

Scotland: Seven widely scattered farms were initially chosen and examined within the months of July, August or September, 1965. Three additional farms were examined in later years, Kinloch, Inverness-shire (November, 1966), Traigh, Inverness-shire (May, 1968) and Crossford, Lanarkshire (July, 1968). Their climate is Northwest European (Kendrew, 1963). Data on vegetation, cattle type and annual rainfall is listed in Table 5 and their localities shown on Fig. 7.

England: A dairy farm at Winslow, Buckinghamshire was studied in October, 1965. Its climate, again, is Northwest European; annual rainfall is 25.7 in. (65 cm.) (Meteorological Office, Buckingham, 9 miles northwest from Winslow). Advantage was also taken of a visit to Tavistock, Devonshire, in May, 1966 and samples were taken from one animal at each of six widely scattered farms. Tavistock lies to the immediate Southwest of Dartmoor with an annual rainfall of approximately 45 in. (114 cm.) (B.V.A., 1960).

Colombia: Cattle were examined during the months of April to July 1967, on 17 farms in a total of 11 widely scattered localities.
The Andes mountains enter Colombia in the southwest and split into the Western, Central and Eastern ranges forming between them the Cauca and Magdalena valleys. To the West and North of the Andes lie the Pacific and Atlantic coastal plains while to the East lie the extensive plains (llanos orientales) which account for three fifths of the land area of the country. Because Colombia lies approximately between the equator and $12^\circ$N, these differences in altitude allow dramatic variations of climate and vegetation and make description difficult. A simple understanding of climate can be derived from Davies (1962) who defines areas from sea level to 3000 ft. (914 m.) as the hot country (all year round temperatures $75 - 80^\circ$F (24 - 27°C)), the areas between 3000 and 6000 ft. (914 - 1828 m.) as the temperate zone (average annual temperatures $65 - 70^\circ$F (18 - 21°C)), and 6,5000 - 10,000 ft. (1880 - 3056 m.) as the cold country. Above the cultivatable land up to the snow line at 15,000 ft. (4572 m.) lie treeless pasture lands. Likewise Cole (1965) simplifies the vegetation features of South America by referring to "mountain", "dry", "savannah", and "grass including pampas" areas. The datum in Table 6 therefore includes approximate height above sea level and vegetation as the simplest expression of the variety of characteristics exhibited in the localities where cattle were studied. Fig. 8 shows the geographical distribution of these localities.

b. breeds and types of cattle (following nomenclature of Mason, 1957).

Nigeria: White Fulani type of lyre horned zebu and the Ndama type of small humpless cattle.

Scotland: the dairy cattle were all Ayrshire or Ayrshire crosses and the beef animals were of a variety of crosses.

England: the dairy cattle were Ayrshire at Winslow and South Devon at Tavistock.
all the Colombia: the dairy cattle were predominantly Frisian; the beef cattle from Montaria were Reme-simuna, and those from other areas predominantly sahul.

The only difference between the two series was that both methods of detection were used in Colombia: the dairy cattle were predominantly Frisian; the beef cattle from Montaria were Reme-simuna, and those from other areas predominantly sahul.

In Nigeria, Scotland, and England, culture of peripheral blood on blood agar media was the only method of detection attempted. The detection of epimastigote forms in culture was taken to indicate the presence of trypomastigote forms in peripheral blood. In Colombia, a search was also being made for infections of salivarian trypanosomes and therefore the examination of thick and thin blood films, the inoculation of laboratory rodents and the examination of lymph node biopsy smears were additional techniques used as a routine.

The sampling of the cattle on the University Farm, Ibadan, Nigeria, was taken as the opportunity to establish the days on which cultures should be examined. As a preliminary survey, eight White Fulani cows were sampled and the forty-eight cultures inoculated with their blood were incubated at 25°C and examined daily for 6 days. Because of mould contamination only sixteen of these cultures could be fully satisfactorily examined. Of these sixteen, however, nine became positive, seven of them on day 3 after inoculation or later. A decision was therefore made to examine cultures only from the third day onwards.

In the rest of the work at Ibadan six cultures were inoculated from each animal studied and three of these six cultures were incubated at 25°C and three maintained on a verandah where the temperature fluctuated daily between 29°C and 31°C. Trouble with mould contamination was again experienced but Table 7 gives the data for the twenty-two animals, eleven Fulani and eleven Ndama, for which three cultures were satisfactorily examined in each series daily from day 3 to day 14. It appears that a single examination on day 5 would have discovered nearly
all the positive cultures, 95 per cent in the first and 94 per cent in the second series. The remaining positive cultures would all have been detected on day 10. The only difference between the two series appeared to be that the proportion of cultures becoming positive on day 3 was significantly greater in the second series (28 °C) than in the first (25 °C): $\chi^2$ for the comparison is $7.6; P < 0.01$. A decision was therefore taken that cultures would be routinely examined from that time on days 5 and 10.

**d. observations from Nigeria.**

(i) incidence of infection in animals: In the first eight Fulani cows examined mould contaminants in the cultures (vide supra) interfered with the assessment of numbers of animals infected but, although the rates of contamination were greater on day 3 than on day 10, the difference between the two series was not significant ($P > 0.05$). In the second series infections were detected in five of the six cows examined on day 10 as the first occasion, and two cultures were found positive on day 15. Fifteen further Fulani cows were investigated, of which fourteen yielded positive cultures; similarly, five of six male and thirteen of fifteen female N'dama cattle were found positive. Thus, of a total of thirty-six adult cattle satisfactorily examined, infections were detected in thirty-two (89%).

(ii) degree of parasitaemia in infected animals: The number of cultures found positive out of the 6 inoculated was considered indicative of the number of trypanosomes in the peripheral blood. The incidence of positive cultures is shown in Table 8. Twenty-two of the twenty-eight animals for which six cultures were available infected all six cultures, and the remaining animals infected two to five cultures.

**e. observations from Scotland.**

(i) time of appearance of organisms in primary culture: Guided by the results obtained in Nigeria, cultures from cattle in the seven localities visited during July to September, 1965 were examined twice, once on day 5 and again on day 10. Mould contamination again interfered with results but out of 202 cultures from parasitaemic animals available
139 were positive on the first examination, and a further 9 were positive on the second examination. The results therefore appeared to confirm the Nigerian experience that day 5 revealed the great majority of positive cultures.

However, the results from the two farms visited in 1968 did not fit this pattern. By this time the examination of cultures on three occasions (days 5, 10 and 15) had become routine. At Traigh (visited May, 1968) only one out of seven positive cultures was detected on day 5, the remaining six being detected either on days 10 or 15. At Crossford (visited July, 1968) no positive cultures were detected on day 5 but eight were detected either on days 10 or 15. The results from Kinloch (November, 1966) could not be similarly compared as due to the difficult lines of communication cultures were examined on day 10 as the first occasion, and two cultures were found positive.

(ii) incidence of infection in animals: Table 9 gives the number of cattle found infected at each locality in Scotland. From a total of 231 cattle sampled, 40 gave positive cultures. At least one infected animal was found at each locality but the single animal at Easter Bush had a history of having been imported from West Scotland several years previously.

(iii) degree of parasitaemia in infected animals: The incidence of positive cultures obtained in 1965 is shown in Table 10. Sixteen of the twenty-nine animals for which six cultures were available infected all the six cultures, and the remaining animals infected one to five cultures. In 1968 the cultures were examined on three occasions (days 5, 10 and 15) instead of two (days 5 and 10). Two animals infected all six cultures, one animal two cultures and one animal one culture.

(iv) age at first infection. On five farms (Lephinmore, Hannah, Cochno, Glensaugh and Easter Bush) the animals chosen were
fifteen cows taken at random together with fifteen immature stock animals (calves) which gave positive cultures, one was injected on all representing one progeny of every adult animal sampled. On a sixth farm (Sourhope) the proportion chosen in the same manner was sixteen of the five Tavistock animals which gave positive cultures, three adults and sixteen calves. From these farms there were ninety-one infected all six cultures, one infected four cultures and one, one cases in which an adult female and one of its progeny were both satisfactorily examined. There were only two infected progeny and they were both derived from non-infected dams. None of the twenty infected cows were associated with infected progeny.

The two youngest animals found infected were two years of age.

(i) time of appearance of organisms in primary cultures

Wenslow cultures (October, 1965) were examined twice, once on day 6 and because it was the only one of 15 cows infected to have all again on day 10. No contamination of cultures was seen and of the positive cultures (1/6) detected only on the day 10 examination. The sixty-six cultures available from parasitaemic animals, fourteen were of cultures from the remaining animals were all detected on examination positive on day 6 and thirty three by day 10. The Tavistock cultures (May, 1966) were also examined twice, and of the thirty cultures available from parasitaemic animals ten were positive on day 7 and a further thirteen by day 13. Results from both these localities differed from that of Nigeria or the Scottish farms examined in 1965 in that the majority of positive cultures were revealed on the second examination.

(ii) incidence of infection in animals: Table 11 gives the number of Wenslow animals found infected by years of birth. Out of the twelve animals sampled eleven gave positive cultures. The ages of the Tavistock animals were not recorded but five of the six animals sampled gave positive cultures.

(iii) degree of parasitaemia in infected animals: Of the eleven
Winslow animals which gave positive cultures, one was infected on all six cultures, the remaining animals infected one to five cultures. Of the five Tavistock animals which gave positive cultures, three infected all six cultures, one infected four cultures and one, one culture.

g. observations from Colombia.

(i) time of appearance of organisms in primary culture: Cultures were examined twice, once at day 5 + 1 and once at day 10 + 1. From a total of 418 cultures from parasitaemic animals available, 319 were positive on the first examination and a further 24 in the second examination. Only on one farm were more cultures found on the second examination than the first (Hacienda Cartama day 5 = 3, day 10 = 4). One cow at Villavicencio (Experimental Station, ICA) attracted attention because it was the only adult animal out of 16 found infected to have all its positive cultures (4/6) detected only on the day 10 examination. The 89 cultures from the remaining animals were all detected on examination day 5 with the exception of one from each of two cows.

(ii) incidence of infection in animals: Ages of animals were not recorded excepting calves in one instance. From a total of 171 animals sampled, 71 gave positive cultures. Table 12 gives the breakdown for each locality.

(iii) degree of parasitaemia in infected animals: The incidence of positive cultures obtained is shown in Table 13. Thirty seven of the 64 animals for which six cultures were available infected all the six cultures, and the remaining animals infected one to five cultures.

(iv) age at first infection: At one farm only (Villavicencio, Experimental Station, ICA) young animals were chosen to obtain some idea
of the age of first infection. The first visit had revealed a 100% infection in 16 adult cattle. On the second visit, eleven young stock were chosen whose ages ranged from 2 to 22 months. The youngest animal found infected was four months old (Table 1).

h. discussion.

The decision to examine cultures on days 5 and 10 based on the Nigerian experiences appeared adequate on the majority of occasions. A small number of cultures in Scotland, England and Colombia appeared to grow more slowly with the probability therefore that some positive cultures were being missed. The need for the addition of a further day of examination became apparent by the end of the Colombian survey (July, 1967).

The additional methods of diagnosis attempted in Colombia (thin and thick blood smears, lymph node smears and mouse inoculation) failed to reveal any T. theileri - like trypanosomes.

The results confirmed the presence of T. theileri - like trypanosomes in both temperate and tropical climates. More importantly however, the results from Colombia and Scotland not only revealed a previously unsuspected wide distribution of the infection but also wide variations of incidence between localities in a single country.

The number of cultures found positive out of the six inoculated indicated the variation in quantity of peripheral blood which would infect the blood agar media. This could be taken to infer the quantity of blood which contained a single trypanosome. In Nigeria 2 ml. inocula of peripheral blood were used but in all other localities the inocula were 1 ml. In these latter areas the majority of infected cattle had at least 1 trypanosome per ml. of peripheral blood but this could fall to 1 per 6 ml. of blood. Even lower parasite densities could possibly have been detected if a larger number of cultures had been inoculated. This
is further discussed in section 7.

No evidence of prenatal transmission was obtained from any of the localities studied. The ages of infected and non-infected cattle were known in all of the United Kingdom locations. Only at Winslow however was the infection rate high enough and the scatter of ages suitable to reveal that the incidence of infection increased with age, (Table 11).

2. Attempts to isolate megatrypanosomes from artiodactyls other than domestic cattle, and from a perissodactyl (horses).

a. species of animals and localities.

Animals other than domestic cattle were examined in Scotland and Colombia as and when opportunity arose. The selection of animals was therefore miscellaneous in character and included red deer (Cervus elaphus), roe deer (Capreolus capreolus), reindeer (Rangifer tarandus), feral goats, and domestic sheep and horses. Localities and numbers of animals are listed in Table 15 and the localities are also mapped in figures 7 and 8.

b. results.

The peripheral blood of four out of six sheep examined at the Veterinary Faculty, Bogota, Colombia infected blood agar cultures. The flagellates seen were assumed to be Trypanosoma melophacium. No flagellates were seen in cultures inoculated with the peripheral blood from all the other animals examined.

3. Culture and storage of isolations.

a. retention of infectivity of parasitaemic blood to culture during storage.

The interval between drawing blood from the jugular vein and inoculating culture media was maintained at a maximum of half an hour in all localities of Scotland, England and Colombia where cattle were
In Colombia, experiments were carried out to determine whether the interval could be prolonged without loss of infectivity of parasitic blood to blood agar media. Five experiments were carried out with parasitic blood maintained at laboratory temperatures (diurnal variation 15°-21°). The donor cow was the same animal on all occasions.

Experiment 1: no loss of infectivity was observed in blood inoculated after 1/2 hour, one hour, and then further intervals of one hour to 5 1/2 hours (positive cultures/number inoculated: 6/6, 6/6, 6/6, 4/4, 4/5, 6/6, 6/6).

Experiment 2: no loss of infectivity was observed in blood inoculated on 8 occasions up to 22 hours. (positive cultures/number inoculated: all 6/6 occasion out of four).

Experiment 3: no loss of infectivity was apparent in blood inoculated on 10 occasions up to 48 hrs although a proportion of cultures in this instance were contaminated with fungus (positive cultures/number of those inoculated and available: 6/6, 4/6, 4/5, 4/5, 2/4, 2/4, 3/5, 4/5, 6/6, 3/3).

Experiment 4: the blood was divided into two portions, one using EDTA as a coagulant, the other using heparin. Both samples were examined at the same four approximately equal intervals up to 72 hrs. Some loss of infectivity had possibly occurred in the heparinized sample but was not apparent in the sample with EDTA. (positive cultures/number inoculated and available: EDTA, 6/6, 5/6, 4/5, 3/4; heparin, 6/6, 5/6, 5/6, 1/6).

Experiment 5: blood was inoculated on seven occasions at approximately equal intervals up to 11 1/2 hrs. Infectivity to the culture medium was lost between hours 65 1/2 and 74 1/2. (positive cultures/number inoculated: 6/6, 4/6, 1/6, 0/6, 0/6, 0/6, 0/6).
The results indicated that within this diurnal range of temperature the interval between drawing blood and inoculating culture media could possibly be extended up to 24 hrs. without altering apparent incidence of infection in a herd and that an extension to 48 hrs. would still indicate at least the presence of T. theileri-like trypanosomes.

In Scotland, again using a single donor cow, observations were carried out on parasiticemic blood stored specifically at 4°C, 28°C and 37°C. The results are recorded in Table 16. Blood stored at 4°C took longer to lose its infectivity to the blood agar medium than that stored at 28°C. (still infective on three occasions at 36 hours, and one at 48 hrs., as compared with blood being infective at 72 hrs. on only one occasion out of four). Blood stored at 37°C gave miscellaneous results. On one occasion infectivity was lost between 1/2 and 24 hrs., on another occasion there appeared to have been a multiplication of organisms between the only two observations at 1/2 and 24 hrs., and on a third occasion blood was still infective up to 96 hrs.

b. observations on growth on blood agar slopes at 28°C.

(i) changes in numbers of organisms with age of culture: Two stabilates of field isolations were used of the following history;

TREU 139 - isolated 2.7.65 from a cow at Lephinsmore, Scotland. The stabilate was prepared from a 6 day primary culture.

TREU 144 - isolated 6.7.65 from a cow at the Hannah Dairy Research Institute, Scotland. A primary culture was sub-passaged at day 5 and a stabilate prepared from this second passage at day 3.

Population curves for TREU 139 were prepared on three occasions (materials and methods; section 4). The number of flagellates initially inoculated into each of the culture series and the number of cultures counted daily were as follows:
The organisms counted were those showing movements of their flagella. The results were not uniform. On the first two attempts the population curves had marked fluctuations giving the impression of two peaks of growth. The third population curve showed a comparatively luxuriant growth with a log phase followed by single periods of growth and decline.

A single population curve was prepared from TREU 144 and only one culture counted daily. There was again an appearance of two peaks of growth.

(ii) the infectivity of cultures to other blood agar slopes:

The changes of infectivity of cultures with age was measured twice, once with TREU 139 and once with TREU 144. These were on occasions when population curves were prepared counting only one culture daily.

Following the count, the trypanosome suspension was titrated at ten fold dilutions into series of six blood agar slopes. On both occasions it was seen that the increases or decreases in numbers of organisms was accompanied by an increase or decrease in infectivity, except at the final decline of the culture. In the culture series derived from TREU 139, organisms with active flagella were seen up to the twenty first day but infectivity to other blood agar slopes had been lost by the fifteenth day. Similarly, with TREU 144, organisms with active flagella were seen up to the twentieth day but infectivity to other blood agar slopes had been lost by the fourteenth day.

Where cultures were derived, however, from the inoculation of blood agar slopes with peripheral blood of infected cattle, at least some could remain infective and contain active organisms for much longer
periods. Fifty nine primary cultures found positive in other experiments between 15.8.67 and 7.9.67 were used to demonstrate this behaviour. A few were taken at random at fortnightly intervals and discarded after a single examination. A wet preparation from each culture was examined for motility and 0.1 ml. of the liquid phase was inoculated into each of two blood agar slopes. Observations were made satisfactorily from 42 of the cultures, the rest being found contaminated. In all examinations except one, cultures found to be infective also had active organisms in the liquid phase. One hundred and twenty days was the longest period that any one culture was found to retain infectivity, but this did not represent a limit (Table 17).

(iii) morphology: Thin smears stained with Giemsa were prepared as routine from the liquid phase of primary cultures which had been selected for low temperature storage. A morphology outwith the range of possibility described in the literature was seen only on a single occasion. The slide originated from an isolation made at Easter Bush, Midlothian, Scotland, and stored as TREU 90. An irregularly shaped organism was seen, approximating 50 x 30 μ, which seemed to be divided internally by faintly staining septa into seven compartments. Each compartment appeared to contain a nucleus and a kinetoplast. Close to each kinetoplast originated a flagellum only one of which visibly extended beyond the surface of the organism.

The literature is almost entirely inadequate however in relation to the morphology of the flagellates found surviving on primary cultures for periods of up to at least 120 days. These are discussed in section 7 in relation to the observations from a single Ayrshire cow.

c. observations on growth at 37°C

(1) growth on blood agar slopes, tissue culture media, and bovine tissue cultures: Preliminary experiments were carried out
using a variety of media to obtain an appreciation of the requirements for growth at 37°C.

1. Blood agar slopes: A suspension of flagellates was prepared from a 48 hour culture at 28°C of TREU 63 and 0.1 ml. (approximately 12,000 organisms) was inoculated into each of twenty four blood agar slopes. These were then incubated at 37°C. Flagella were active at hour one but all the cultures were dead by hour 24.

2. Earle's and Eagle's tissue culture media: A suspension of flagellates was prepared from a 5 day culture maintained at 28°C of TREU 63 and 0.1 ml. (approximately 500,000 organisms) was inoculated into a series of bijou bottles containing either 5 ml. of Earle's medium or 5 ml. of Eagle's medium. The bijou bottles were then incubated at 37°C. Infectivity of the cultures to blood agar slopes maintained at 28°C was checked daily. Six blood agar slopes were inoculated from each of two bijou, one taken from both series of media. Infectivity was lost on the seventh day in both series.

3. Bovine kidney cell cultures: A suspension of flagellates was prepared from a 7 day primary culture of peripheral blood of a cow (Cochno, Scotland) maintained at 28°C. A series of Leighton tubes containing a monolayer of kidney cells and Eagle's medium, and a second series with Eagle's medium alone received inocula of 0.1 ml. (approximately 450,000 organisms) and were then incubated at 37°C. One tube of each series was taken daily and examined. The organisms in medium alone remained epimastigote in form and the culture was dead by the third day. In the additional presence of the kidney monolayer, the organisms were, on subjective examination, thinner by the first day, the kinetoplasts were posterior to the nuclei and stages of division were seen in the epimastigote form. The medium was not changed and the organisms had died out by the fifth day. By day 4, however, many organisms were in
trypomastigote form.

In a second attempt, the kidney cells were not growing well and cultures died by the second day in both series of tubes.

1. bovine thyroid cell cultures: A similar comparison was made between growth of organisms on Eagle's medium alone and with the additional presence of a monolayer of bovine thyroid cells. Two comparisons were made using inocula prepared from firstly, an 11 day primary culture from an ox in Uganda (culture having been sent by air) and secondly a 14 day primary culture from a cow (Hannah, Scotland). The results were similar to those obtained with bovine kidney cell cultures.

In the presence of the monolayer, division initially occurred in the epimastigote form, trypomastigote forms appearing at the end of the period of division. In tissue culture medium alone, there was again a relatively short period of survival.

Having established that the presence of bovine cells was important to the growth of the trypanosomes at 37°C, methods of providing a continuous growth of both in association were investigated.

The organisms had been seen to attach themselves by their posterior ends to the bovine cell monolayers. The possibility of changing medium by decanting off the old and adding new appeared to exist. This was therefore compared with inoculating a small quantity of old medium into new tubes containing monolayer systems after having swilled the old medium in the tube to detach the trypanosomes. Both operations were carried out every third day. These series were again compared with the same inoculum into medium alone. The inoculum was prepared from a 15 day primary culture maintained at 28°C from a bullock (Easton Bush, Scotland).

The organisms in medium alone died out by day 2. Where subinoculation was attempted of old medium containing trypanosomes into tubes containing fresh monolayer systems, no trypanosome growth was
apparent on day 2 following the second subinoculation. Where the entire medium was decanted and new medium layered on, a good growth of trypanosomes was still apparent on day 1 after the third change of medium when observations were discontinued.

(ii) leucocyte cell cultures: The decision to use bovine leucocyte cell cultures as the routine system was made on the grounds of simplicity, particularly as inactivated bovine serum provided an adequate medium. (see materials and methods section 2 b). Nine series of leucocyte cultures were prepared at different times for the growth of trypanosomes, 2l. tubes to each series. The origin of materials used is listed in Table 18. The observations made were as follows:

1. period of survival of the leucocyte cell culture: Two series (nos. 3 and 4) appeared free from any contamination and the leucocyte monolayers survived 67 and 58 days respectively. Three series (1, 5 and 9) appeared healthy at the termination of the experiments at 25, 6 and 27 days. The remaining four series (2, 6, 7 and 8) were contaminated, the cytoplasms of the leucocytes became granular and their outline rounded. The observations terminated on days 27, 13, 12 and 21.

2. period of survival of the trypanosome population: Survival of the trypanosomes appeared to depend on the health of the leucocytes. The decline in the number of trypanosomes however occurred quicker than the deterioration of the leucocyte culture, the remnants of which always remained after the trypanosomes could no longer be demonstrated.

3. behaviour of the trypanosomes in relation to the leucocyte monolayer: The trypanosomes inoculated into the leucocyte cultures 4, 5, 6 and 8 were found adhering to the monolayer by their posterior ends as single organisms, occasionally in division, by the first examination at 24 hours. After the same period of time in series 1, 2 and 7, however,
the flagellates were found in active division adhering to the glass wall of the tube away from the monolayer. Not until 72 hours in series 1 and 2 and 48 hours in series 7 were the organisms seen adhering as individuals to the monolayer, again occasionally in division. In series 3 too many red blood cells introduced with the inoculum obscured the view, and in series 9 no observations were attempted.

4. comparison of growth on serum alone with growth on serum plus leucocyte monolayer: The comparison was made in relation to series 3. Growth on serum alone, without renewal, reached maximum numbers at day 3 and then surprisingly took until day 16 for numbers to decline to nil. With the addition of leucocytes to the system, and serum changed every third day, trypanosomes could still be seen in every field of the microscope up to day 67.

5. infectivity to leucocyte cell culture at 37°C of a trypanosome isolation serially passaged on blood agar for four years: Series 8 was inoculated with the isolation from Eire made by Herbert (1961) which had been stored at low temperature as TREU 116. The capability of growth at 37°C was found not to have been lost.

6. infectivity of trypanosomes growing at 37°C to further leucocyte cultures at 37°C: Attempts were made with series 2, 3, 4 and 5. Trypanosomes were swilled off the monolayers and inocula (0.1 ml.) of the media were introduced into new leucocyte cultures. The trypanosomes reattached themselves to the leucocytes in their new environment but no forms in division were seen.

7. infectivity of trypanosomes growing at 37°C to blood agar media maintained at 26°C: Attempts were made with series 2, 3, 4, 5, 6 and 9. Again, trypanosomes were swilled off the monolayer and inocula (0.1 ml.) introduced onto blood agar slopes. Two blood agar slopes were used to every leucocyte culture tube. The inocula were infective in 13 of 25
attempts. Thirteen of these attempts related to the first 17 days of growth of trypanosomes on series 3. The inocula were infective to blood agar on days 1, 2, 6, 7, 11, 15 and 17, but not infective on days 4, 5, 8, 9, 10 and 13.

8. The infectivity of cultures on blood agar slopes maintained at 28°C and established from trypanosome populations growing on leucocyte cultures at 37°C, to further leucocyte cultures also maintained at 37°C. Flagellates on the 5th day of growth on leucocyte cultures infected a blood agar slope. No growth was seen on the blood agar slope on day 10 following inoculation but a "++" growth was seen on a second examination at day 18. This growth was shown to be infective to leucocyte culture series 7 and was observed up to day 7 when the leucocyte culture itself showed deterioration.

9. Morphology: The initial active division of flagellates seen in three series occurred in the epimastigote form. Trypomastigote forms could be identified in all series after the flagellates had adhered to the leucocytes but the position of the kinetoplast in the majority of organisms was difficult or impossible to determine due to the number of cytoplasmic inclusions. A conclusion could not be reached therefore on the period of time required for the morphogenesis. A few organisms however could be distinguished with two nuclei, one anterior to the other, but only one flagellum, similar to those described in the peripheral blood of cattle by Theiler (1903), Lühs (1905) and Keymer (1966).

The flagellates also increased in size with age of culture. Using a measuring eyepiece, the average body length of 100 flagellates in the suspension prepared for the inoculation of leucocyte culture series 3, for example, approximated 15 μ (range 6 - 36 μ). The average body length of 100 flagellates measured at day 5 following growth on leucocyte cultures was 27 μ (range 17 - 56 μ) and at day 9 a few forms had reached
91. The largest form seen on any series was 67 μ body length (series 2, day 10).

No intracellular forms were seen or suspected.

d. storage at low temperature:

A total of 149 stabilates were prepared from the liquid phase of cultures on blood agar slopes. Four stabilates were discarded, two due to the accidental omission of a cryoprotectant and two due to the discovery of bacterial contamination. Storage of 135 of the remainder employed dimethylsulphoxide as the cryoprotectant at the temperature of solid CO₂ (-79°C). The further 10 stabilates were stored with glycerol as cryoprotectant, 6 of these also at -79°C and 4 in liquid N₂ (-196°C).

The cultures stored originated from Canada, Eire, England, Nigeria, Uganda and Scotland. Fifty of the stabilates were clone populations of which 46 were derived from isolations from a single Ayrshire cow under study at the University of Edinburgh Veterinary Field Station (see experimentation section 7).

The infectivity of stabilates to blood agar media was checked at intervals of approximately a year. Two capillaries were taken at random from each stabilate and each inoculated onto a blood agar slope. On the last occasions checked the stabilates ranged in age from 30 to 1686 days. There were no complete failures but some capillaries from 15 stabilates were found to be non infective. At worst this was 3 out of 4 capillaries checked, that is, 1 out of 2 capillaries being found infective at the second attempt. This was not related to the method of storage.

A grouping of stabilates according to the period of time that they had been laid down also failed to show any relationship between the partial failures and their age. Thus:
The observations on culture and storage of isolates were biased towards behaviour rather than morphology. Variations of behaviour were apparent in the following circumstances: the period of time that parasitaemic blood maintained at 37°C retained its infectivity to blood agar cultures; the nature of the population curves derived from capillaries of the same stabilate; longevity of cultures on blood agar, and the differences of behaviour of trypanosome populations grown at 28°C when first placed in leucocyte cultures at 37°C. The evidence suggests therefore that qualitative differences exist between trypanosomes in the same population.

The passage of trypanosomes from culture at 28°C to culture at 37°C was consistently successful, and on the single occasion attempted, a trypanosome population was alternated twice between the two culture systems. This suggests a means by which, if standardised, could be used to compare the behaviour of different trypanosome populations. The ability of the organisms to adhere by their posterior ends to leucocytes in culture is a
probable indication of the means by which they anchor themselves within the body of the mammalian host.

Low temperature storage of the trypanosomes enabled infectivity to be retained for periods of years. The longest period demonstrated was 4 years 225 days (1686 days). There was insufficient information to determine whether the loss of infectivity of some capillaries of a few stabilates was related to the origin of the isolation, the age of the stabilates, the method of storage or human error. No attempt was made to measure loss of infectivity as titration on blood agar slopes was too cumbersome. A mini-titration technique is required possibly involving a system where trypanosome growth can be seen macroscopically.

The attempt was made to locate *T. theileri*-like trypanosomes in tissues other than blood.

The attempt was made to locate *T. theileri*-like trypanosomes in tissues other than blood on a single occasion. A cow known to be infected was found in extremis with a clinical history of kidney dysfunction. The animal was destroyed and impression smears were made of a variety of tissues. Suspensions were made from the same tissues and inoculated into blood agar slopes, two to each tissue.

The tissues selected were:

- Blood
- Adrenal
- Cerebrospinal fluid
- Heart muscle, auricle
- Lymph node, mesenteric
- Lung
- Lymph node, supramammary
- Brain, cerebral cortex
- Haemolymph node
- Brain, choroid plexus
- Bone marrow, rib
- Skeletal muscle
- Liver
- Ascitic fluid
- Spleen
- Kidney, cortex

All cultures were found with some degree of contamination by day 15, the
last day of examination, and no organisms recognizable as a possible stage in the life cycle of a trypanosome were seen.

The post mortem report (see Appendix 3) suggested as a possibility that the trypanosome infection was the cause of a high circulating antibody level fixing onto glomeruli and leading to amyloid changes. The structures in the kidney suspected of being trypanosomes were considered to be distorted kidney tissue cells on re-examination.

5. Attempts at artificial transmission
   a. from cattle to cattle using parasitocemic whole blood.

Two attempts were made using a total of three susceptible animals.

Attempt 1: Two culled animals were received from Easter Bush farm, Midlothian, Scotland, numbered C27 (two years old) and P2 (fifteen months old). Prior infection with T. theileri-like trypanosomes was considered improbable at this location due to the low incidence discovered during the search in Scotland (see results: section 1). Moreover, the animals chosen for the experiment had never been cut to grass and contact with a vector was very unlikely. As an additional check, however, peripheral blood was cultured five times in the week preceding challenge and no flagellates were seen.

The two animals C27 and P2 were transported to the Hannah Dairy Research Institute on 7.5.66 where, without leaving the cattle float, they were inoculated with pooled heparinized blood from two cows known to be infected with T. theileri - like trypanosomes, and returned immediately to Easter Bush. Culture of the inoculum (1 ml. inoculum onto each of 6 blood agar slopes) gave 4 positive cultures by day 5. Animal C27 received a 250 ml. inoculation cleanly into the jugular vein and a further 250 ml. intra-peritoneally. Animal P2 however was considerably excited by the journey, and as a result, some portion of the 250 ml. inoculum intended for the jugular vein was deposited sub-cutaneously. A further 200 ml.
inoculum was given intra-peritoneally. Thick blood smears were then examined daily from both animals and one ml. of peripheral blood was inoculated into each of 6 blood agar slopes, which were then examined on days 5 and 10.

The peripheral blood of P2 was examined daily for 41 consecutive days up to 30.6.66. and then, on culture alone for 7 days in July, 2 days in August, 3 days in September and 5 in October in the same year. No trypanosomes were seen on blood slides and only one culture was found positive and that on the tenth day following challenge.

The peripheral blood of 027 was examined on the same days as P2 but culture continued on a scatter of days in November and December 1966 (4), in 1967 (5), and finally on 16 consecutive days up to 6.5.68. Again, no trypanosomes were seen on blood slides. Single cultures out of the 6 inoculated daily were found positive on day 6, 10 and 33 of the first period of examination, and then on 2 of the 7 days of examination in July. From 7.10.66. until 27.1.67 a total of 12 blood agar slopes were inoculated each with 1 ml. inocula on each day of examination. Single positive cultures were detected on 5 of the 10 days concerned and 3 on a sixth day. On the final 16 consecutive days of examination 2 cultures of 6 inoculated were found positive on the 15th day. This was the only series of examination when cultures were screened on the 15th day as well as the 5th and the 10th.

Attempt 2: A 5 month old calf, numbered T5, which had never been out to grass was also obtained from Easter Bush farm. Peripheral blood was cultured on seven consecutive days up to one hour prior to challenge. The calf was also splenectomized three days before challenge. On 17.5.68. the animal was inoculated at Easter Bush with whole heparinized blood from the same infected cow which was the subject of the special study later described in section 7. Two hundred ml. were inoculated into the jugular vein and a further 200 ml. intra-peritoneally.
Culture of the inoculum on blood agar slopes (one ml. into each of six slopes) failed to give a positive culture.

Examination of thick blood smears was made for the first 21 days following challenge. Peripheral blood was cultured daily for the first 28 days (one ml. inocula onto each of 6 blood agar slopes), then for a further 21 days starting 29.7.66. and a final 7 days starting 10.9.66. Cultures were examined on days 5, 10 and 15.

No trypanosomes were seen in the blood smears. Only ten cultures were found positive out of the 356 inoculated in the course of the experiment. The day of inoculation of cultures after challenge and the day of examination on which cultures were found positive are recorded in Table 19.

b. from cultures maintained at 28°C to cattle.

Two separate attempts were made, one in 1965 in the first year of the programme and one in 1966.

Attempt 1: Three bullocks were obtained from Easter Bush Farm, two aged 14 months and one aged 15 months. They were examined daily from 3.11.65. on thick blood smears and by the culture of peripheral blood (1 ml. inocula onto each of 6 blood agar slopes). Trypanosome suspensions prepared from the liquid phase of blood agar cultures maintained at 28°C were inoculated into bullock 1 after 7 days, bullock 2 after 14 days and bullock 3 at 21 days. Examination of each bullock continued for a further 21 days after challenge.

The inocula were prepared as 10 ml. suspensions of organisms in solution A of which 3 ml. were injected into the experimental animal by each of three routes; intra-venously, intra-peritoneally and subcutaneously. The origin and age of the cultures used were:

Bullock 1: Primary cultures from Winslow, England, subpassaged at day 15 and resulting cultures used at day 5. Each ml. contained approximately
750,000 organisms.

Bullock 2: Three cultures grown from TBEU numbers 193, 154 and 155, one capillary to each culture (Cochno, Glasgow). Liquid phase of the cultures were bulked at day 4. Each ml. contained approximately 1,250,000 organisms.

Bullock 3: Origin of cultures the same as Bullock 2 but the liquid phase was bulked at day 11. Each ml. contained approximately 150,000 organisms.

No trypanosomes were seen on blood smears and no positive cultures were found. Bullocks 2 and 3 were splenectomized in February and March 1966 respectively. Bullock 2 was examined on blood smear and by culture weekly for 13 weeks with no positive results. Bullock 3 was similarly examined weekly for 6 weeks also with no positive results.

Attempt 2: Experimentation carried out after the first attempt gave the information that some primary cultures retained infectivity to other blood agar cultures for periods of time in excess of the 21 days which was the maximum life of most cultures (see results: section 3). The possibility existed therefore that these cultures contained metacyclic forms. The liquid phase of ten such cultures, three of them over 90 days old, was bulked and diluted with solution A to make a 20 ml. inoculation. The experimental animal, a ten week old suckling calf, received 8 ml. of the inoculation intravenously, 6 ml. intraperitoneally and 4 ml. subcutaneously. Each ml. contained approximately 2,500,000 organisms.

The examination of blood smears and the culture of peripheral blood (1 ml. inocula onto each of 6 blood agar slopes) was carried out for the next 19 days and then for a further period of 21 days after an interval of 5 months. The animal was not found infected.

c. from cultures maintained at 28°C to rodents.

Although previous authors had attempted to infect laboratory
rodents (Table 3) none had used animals whose immunological competence had been impaired. The attempt was therefore made to establish infection in rats and mice using the immunosuppressants cortisone (cortisyl - thick Roussel Laboratories Ltd.), azathioprine (imuran - Burroughs Wellcome and Co.Ltd.) and thorium dioxide (British Drug Houses). The dosages used were suggested by Dr. D. James (personal communication). The inocula were the pooled liquid phase of 5 day primary cultures from Fulani cattle. No counts of organisms were made. The rodents were in 4 groups according to age and species, each group again subdivided into four groups of four as follows:—

A. Mice approx. 15 gm.
1. no drug
2. cortisone 2.5 mgm.
3. imuran 0.75 mgm.
4. thorium 0.1 ml.

B. Mice approx. 8 gm.
1. no drug
2. cortisone 2.5 mgm.
3. imuran 0.75 mgm.
4. thorium 0.1 ml.

C. Rats approx. 100 gm.
1. no drug
2. cortisone 2.5 mgm.
3. imuran 0.75 mgm.
4. thorium 0.1 ml.

D. Rats approx. 25 gm.
1. no drug
2. cortisone 2.5 mgm.
3. imuran 0.625 mgm.
4. thorium 0.1 ml.

The inoculum was given intraperitoneally at the rate of 0.1 ml. to the mice and 0.3 ml. to the rats between 09.30 and 10.30 hrs. The immunosuppressants were given first between 16.00 and 16.30 hrs. of the
same day and then repeated at 24 hour intervals for a total of four
days. When the knowledge was available, however, (see results: section
7) there
All the animals were examined daily on both wet smears and thick
stained smears from the 3rd to the 21st day inclusive. The animals
were killed on the 23rd day and a portion of liver and spleen from each
animal was macerated in saline and inoculated into blood agar slopes,
one slope for each organ.
No evidence was found of infection having been established in
the rodents. The organ cultures were heavily contaminated by the third
day and therefore considered of no value.

d. discussion
The only successful transmission experiments attempting to
establish infection in susceptible cattle were those using whole parasit-
asmic blood. Splenectomy of one of the total of three animals inoculated
with parasitaemic blood had no apparent effect as all of the infections
could only be detected on culture. The infrequency of these positive
cultures indicated low levels of parasitaemia. The possibility of such
parasitaemias occurring illustrated the difficulty of selecting susceptible
animals for experimentation and considerably adds to the drawback of
being limited to few animals for reasons of logistics. The results were
in marked contrast to those of Hoyte (1968 - personal communication) who
demonstrated a patent parasitaemia in a calf after the inoculation of
pooled peripheral blood from 8 cattle on the Romney Marsh, England. This
would appear to be further evidence of qualitative differences in the
forms in the peripheral blood of the host.

The experiments using populations grown on blood agar cultures
maintained at 28°C were laborious and time consuming. The experimental
design of the first attempt would have been different if there had been
greater understanding at the time of the possible morphology of the
infective stage and the occasions on which its presence was likely. Even when the knowledge was available, however, (see results; section 7) there was still apparent failure to infect a calf.

6. Natural transmission.

a. transmission by insect vector.

(i) collection of specimens of the family Tabanidae: Collections of tabanids were made in the months of June, July and August of 1966 and 1968, and in the month of July 1967. Catches were made in many locations in the West of Scotland from Sutherland in the north to Galloway in the southwest including islands in the Inner Hebrides. The largest number taken at any location was at Laphinmore, Argyllshire (see Fig. 7) (see adjacent to a herd of cattle known to be infected (Table 9). The numbers of flies caught by species were:

<table>
<thead>
<tr>
<th>Species</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haematopota pluvialis</td>
<td>356</td>
</tr>
<tr>
<td>Haematopota crassicornis</td>
<td>16</td>
</tr>
<tr>
<td>Tabanus sudeticus</td>
<td>2</td>
</tr>
<tr>
<td>Tabanus spp. unidentified</td>
<td>2</td>
</tr>
<tr>
<td>Haematopota spp. unidentified</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>359</td>
</tr>
</tbody>
</table>

Outside of Laphinmore all flies were caught off human clothing or attempting to bite man with the exception of two caught in vehicles. At Laphinmore different methods of collection were tried. In order of success these were (Page, 1966 - personal communication): black screen held between two humans; humans themselves; Manitoba fly trap (see materials and methods), and a calf.

(ii) the examination of tabanids for trypanosomatid infections: A total of 250 flies of these caught were satisfactorily dissected.

Fifty six of these, all of which came from Laphinmore, fed on a parasitaemic cow and were dissected between 2 and 10 days later (average
4 days). No trypanosomatid infections were seen in any fly.

Traces of blood meals were seen in only 18 specimens, apart from those offered a feed. Ovaries containing eggs were seen in only 25 of the total number dissected.

b. prenatal transmission.

Information concerning the age of first infection is recorded in the results section I and the improbability of prenatal transmission discussed.

c. discussion.

Search was made for species of the family Tabanidae in Scotland because of the strong circumstantial evidence from the literature (see review; section, epidemiology) that members of this family are vectors. Enquiries were made at each farm where cattle were sampled (see Table 5 and fig. 7). In answer to the question whether 'clegs' (Haematopota spp.) were seen on the farms, the information given at all western locations and at Easter Bush in the east was that clegs had been numerous in living memory but that the incidence had either markedly decreased or they were no longer seen. Their answers also bore no relationship to the discovered incidence of T. theileri - like trypanosome infections in cattle. At Traigh, clegs were seen annually but trypanosome infections were only found in 2 out of 10 adult cattle. At the Hannah Dairy Research Institute however, clegs had not been seen for 'many years' but trypanosome infections were found in 8 out of 15 adult cattle of which the youngest was 2 years of age. Although this evidence from the Hannah is suggestive of a vector other than a tabenid, populations of Haematopota spp. can exist without the knowledge of even an active and observant farm staff. At Lephinmore, for example, a population was discovered on a small section of stream alongside the cattle pastures at about 800 ft. The farm staff had no working occasion to pass that way during the cleg...
season and only knew of an annual incidence of clegs near the farm
steading at sea level.

The work carried out did not eliminate the possibility of clegs
being the vector in Scotland. A small proportion only of the flies
dissected contained eggs or signs of a meal. This meant that the
majority of flies caught were young females seeking their first feed,
the least likely specimens to be infected with T. theileri - like
trypanosomes. The inconclusive nature of the results was disappointing.

7. The characteristics of infection in a single cow.

a. the provenance of the cow, and its environment.

The animal studied (Elsa IV) was one of the Ayrshire cattle found
infected at the Hannah Dairy Research Institute in July 1965. In 1966,
Elsa was culled from the herd because of a chronic mastitis and the
opportunity was taken to purchase and transport her to the Veterinary
Field Station, Easter Bush, Midlothian.

The cow remained housed for the entire period from September 1966
to April 1969 in one of a range of loose boxes which opened onto a central
enclosed passageway. Although there was no fly proofing, no biting flies
were seen at any time. As in addition the incidence of infection in the
dairy herd at Easter Bush was very low (see results; section 1) the
chances of Elsa being reinfected were considered remote.

b. duration of infection and levels of parasitaemia.

Culture of peripheral blood showed that Elsa remained infected
the entire period that she was under observation, that is, for over a
year at the Hannah Dairy Research Institute where re-infection was a
possibility plus over two and half years at Easter Bush.

Between 26.10.66 and 1.4.69 the peripheral blood of Elsa was
cultured daily for 12 periods of time, 10 of which were of 21 days, one
of 20 and one of 8. (see Table 20). Even though cultures were examined
only twice for the first five periods (days 5 and 10) and three times for
the remainder (days 5, 10 and 15) the evidence showed that the numbers of cultures found positive in the spring and summer, relative to the number available for examination, was far greater than in the autumn and winter (see also fig. 9).

The inoculation of 6 blood agar slopes daily each with a 1 ml. inoculum, and the subsequent observation of the number positive, gave some information on the level of parasitaemia in peripheral blood. In order to obtain more specific information, a titration of peripheral blood was carried out on blood agar slopes daily for two of the periods of examination (19.7.67 - 26.7.67 and 15.8.67 - 4.9.67).

A further six cultures were inoculated with 0.1 ml. inocula and then further series of six at ten fold dilutions. All cultures were examined on days 5 and 10. The results are seen in Table 21. On no occasion did an inoculum of less than 0.1 ml. of peripheral blood infect a culture. An inference could be drawn that the maximum parasitaemia exhibited on any one day approximated one trypanosome/ml. of peripheral blood. A possibility therefore existed that maintenance of the inocula at 1 ml. could be informative in the detection of qualitative differences between peripheral blood forms.

c. characteristics of the isolations on blood agar at 20°C.

(i) day of examination on which cultures were first detected:

The decision to examine cultures on day 5 and day 10 following inoculation was based on the Nigerian observations (see results; section 1). Observations in the other countries visited however indicated that in a few circumstances more information may have been collected if a further day of examination had been added. Confirmation of this was obtained on the fifth period of examination of Elza IV when 30 cultures were found positive on day 5 and a further 30 positive on day 10 (Table 20). Very clearly, the day 10 examination was not merely picking up positive cultures
missed on day 5; some cultures were requiring a longer incubation than others. A third screening was therefore added on day 15. On the next period of examination, the only two positive cultures found were on this additional day.

The further periods of examination were made on this basis and helped to reveal more clearly that the seasonal rhythm of total numbers of cultures was also accompanied by a rhythm of change in proportion of cultures first seen positive on day 5, 10 or 15 (Table 20, and Fig. 9). The rise in numbers of positive cultures in the spring was of those first detected on day 5, but as summer advanced this gave way to larger numbers of cultures detected on days 10 or 15. In the period 29.7.68 - 18.8.68, 22 of the 26 cultures found positive were of this latter group.

(ii) Duration of infectivity of cultures to other blood agar slopes at 28°C: This study was added to the programme of examination of Elsa IV following the observations described in results section 3 b (ii) and Table 17. All primary cultures from Elsa not used in other experiments during 1968 were utilized and examined in the same way excepting that each culture was sampled on 4 occasions approximating days 21, 50, 100 and 150. Any culture which showed contamination at any of these occasions was removed from the experiment. The results can be seen in Table 22.

The total number of cultures available was small, but a comparison can be made between the periods of examination 29.4.68 - 19.5.68 and 29.7.68 - 18.8.68. In the first of these periods 20 cultures out of 21 had lost infectivity by day 100, while in the second the figure was only 9 out of 20.

The period of examination 29.7.68 - 18.8.68 therefore not only had the highest proportion of cultures first found positive on either day 10 or day 15 but also the highest proportion of cultures retaining
infectivity to other media slopes for periods in excess of 100 days. The two characteristics are compared in Table 23 for all the cultures available in 1968. Only 5 out of 34 cultures first detected on day 5 of examination retained infectivity over 100 days while for days 10 and 15 of examination combined the figures were 10 out of 23. These however were all in the period 29.7.68 - 18.8.68, where the ratio was 10 out of 14.

(iii) changes in numbers of organisms with age of culture: Four stabilates were used, all derived from Elsa IV with the following histories:

TREU 444: isolated 7.9.67; primary culture subcultured at 104 days, second culture subcultured at 6 days, third culture low temperature stored on the 6th day.

TREU 429: isolated 3.11.67; primary culture first detected at examination day 15 subcultured at 18 days, second culture low temperature stored on the 6th day.

TREU 497: isolated 12.3.68; primary culture low temperature stored at day 6.

TREU 566: isolated 4.5.68; primary culture low temperature stored at day 5.

The number of organisms inoculated into each of the cultures of the series in which counts were made were:

<table>
<thead>
<tr>
<th>Stabilate</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>TREU 444</td>
<td>1,904,000</td>
</tr>
<tr>
<td>TREU 429</td>
<td>440,000</td>
</tr>
<tr>
<td>TREU 497</td>
<td>560,000</td>
</tr>
<tr>
<td>TREU 566</td>
<td>920,000</td>
</tr>
</tbody>
</table>

For each of the series, two cultures were counted daily. As in section results 3 b (i), the organisms counted were those showing movements of their flagella. The results can be seen in fig. 10.
The series derived from stablate numbers TREU 497 and TREU 566 had similar long lag phases, short growth phases and a decline to death by the 15th day in the former and the 13th day in the latter. TREU 429 similarly showed a single growth phase followed by decline but with an inoculum smaller than the previous two, the growth was relatively luxuriant, dying by the 19th day. TREU 444 again showed a luxuriant growth but with marked fluctuations in numbers, again finally dying on the 19th day.

(iv) observations on the establishment of clone populations on blood agar at 28°C: Clone populations were prepared from nine stablates. A total of 117 blood agar slopes were inoculated with single organisms and 16 were found contaminated with either bacteria or fungi and discarded (13.7%). Of the remaining 101 slopes, cultures were established on 61 (61%) but there were differences in the ease of establishing clone populations relative to the histories of the stablates used (Table 24).

The stablates of isolations first detected positive on examination day 5 (TREU numbers 452, 566, 570 and 572) only had, in total, one success out of 38 attempts. Stablate TREU 429 derived from an isolation first found positive on examination day 15 had 21 successes out of 38 attempts. The stablates from cultures retaining infectivity to other cultures at 28°C for long periods of time (TREU 443 and TREU 444, 104 days) had the greatest success, totalling 19 out of 24 attempts.

(v) morphology: The study of the characteristics of the isolations of trypanosomes in blood agar at 28°C was primarily that of behaviour. No detailed study was made of morphology but observations on both wet and stained preparations showed that differences of behaviour were accompanied by differences in appearance.
Examination of wet preparations: the range of possibility can be demonstrated by describing first, the appearance of cultures on primary isolation from the peripheral blood of infected cattle, and, secondly, comparing the forms seen on population curves prepared from TREU 566 and TREU 444 (see fig. 10).

Flagellates seen in cultures by examination day 5 agreed with the descriptions given by Crawley (1909, 1912) (see review of literature; morphology). Flagellates first seen in culture on either day 10 or day 15 appeared to be smaller and the flagella movements relatively very active. In extreme cases this could be a difference between an awareness to the eye of individual almost stationary flagellates each with a single beating flagellum, and flagella beating at a speed which gave a shimmering effect in the preparation preventing the observation of individual trypanosomes. In the latter circumstances, progressive disturbance of red blood cells could denote that some organisms were capable of translatory movement.

Stabilate TREU 566, was prepared from a primary culture at day 5, while stabilate TREU 444 originated from a primary culture subcultured after 104 days, subcultured again at day 6 and finally stored after a further 6 days of growth. The types of morphology seen on the population curve of TREU 566 followed a similar course to the morphogenesis described by Crawley (1909, 1912) and Herbert (1965 a) but with the addition of two rare but consistently recurring forms. These were, firstly, relatively large elongated organisms with rounded ends from one of which arose several (up to 4) flagella. Secondly, elongate forms, pointed at both ends, each end bearing a flagellum with no break apparent in the continuity of the cytoplasm.

TREU 444 on the other hand showed, particularly from day 3 of growth, a very wide range of size of flagellate. A few of the smallest
were capable of translatory movement. The majority however were still of the 'band' or 'club' shapes. The fall in numbers on day 6 in both cultures counted was due to numbers of organisms whose flagella were not beating and therefore presumed dying or dead. Organisms seen from day 7 and up to the final death of the culture appeared to be smaller and either showed jerky movement in the same spot or were capable of progression. Forms could be seen in both equal and unequal division. The organisms with a flagellum at each end seen with TFEU 566 here also became apparent in small numbers from day 10 of growth and persisted through to the death of the cultures at day 19.

Observations on stained preparations: The subjective observation that flagellates first seen on examination day 5 appeared larger than those first seen on examination day 15 was confirmed on measurement of body length. For example, the average body length of 100 flagellates of TFEU 566 at the time of freezing (day 5) approximated 26 μ (range 15 - 43 μ) measured with a measuring eyepiece. The average body length of 100 flagellates from a population first seen on examination day 15 and later frozen as TFEU 429 was 17 μ (range 10 - 26 μ) (see fig. 11). A similar number of flagellates from TFEU 444 (two subcultures of 6 days duration away from a 104 day old culture) averaged 23 μ (range 13 - 41 μ).

Photographs of the rare but consistently recurring forms noted on wet preparations are shown in figure 11 (multiflagellate form) and figure 12 (form with flagellum at each end).

The shape of the kinetoplast and its position in relation to the nucleus also differed. Flagellates in cultures detected on examination day 5 had rod shaped kinetoplasts with rounded corners situated most often just anterior but distinct from the nuclei. Flagellates from cultures, detected on later days of examination and in 'old' cultures
had more oval or round kinetoplasts which were often situated as if attached to the nuclei and therefore sometimes seen with difficulty. In cultures of all derivations, the flagella with terminal knobs frequently reported in the literature were not seen.

During the experiment studying the duration of infectivity of primary cultures obtained from Else (see (iii) above and Table 22) slides were made from the liquid phase of infective cultures. Slides made in the period 29.7.66 - 18.8.66 contained a proportion of two distinct types of organism which were seen in slides taken at other times of the year only on a single occasion (18.5.66). The first form was epimastigote but with a posterior nucleus. The second form was pear shaped, in the order of 10 μ body length, with the flagellum at the pointed end. There was a rounded nucleus with an adjacent kinetoplast placed either laterally or posteriorly to it. The posterior nuclear forms could be first seen at day 21 (3 out of 20 cultures) and the pear shaped forms at day 50 (4 out of 15 cultures). If the cultures retained infectivity longer than 100 days, both of these forms could still be found amongst a majority of flagellates which were in epimastigote form with nuclei centrally placed.

Examination of negatively stained flagellates by electron microscopy only revealed one additional feature. Most organisms had a faint but definite terminal thickening to their flagella (fig. 12).

d. parallel evidence from other cattle.

(i) duration of infection and levels of parasitaemia: Seven of the cows found infected at the Hamah Dairy Research Institute, Ayr, Scotland on 6.7.65 were examined on a further nine occasions, the last being on 28.6.68 (Table 25). One failed to give positive cultures on any but the first occasion. Three gave positive cultures on the first and last occasions, that is, over a period of three years, and three gave
positive cultures on the first and penultimate occasions, that is, over a period of two years and ten months. One experimental bullock (see results section 5 a.) remained infected for two years before slaughter. Confirmation was therefore obtained that animals could remain infected over a period of years.

Table 25 also offers supporting evidence to illustrate the seasonal variations in levels of parasitaemia in cattle in the herd from which Elsa was purchased. Numbers were small but the larger numbers of cultures were obtained in the summer months and the least in the winter.

Table 26 gives the results of titrations of the peripheral blood of two infected cows to determine a level of parasitaemia. Inferences could be drawn that the number of trypanosomes in the peripheral blood of cow A 992 held at Easter Bush, Scotland, varied from less than one to every 6 ml. blood to one for every 1.2 ml. blood over the eight days of examination. Likewise for cow number 1 held at Bogota, Colombia, the number varied from at least 10 to over 300 per ml. blood. The data is too slight, however, for comment or discussion.

(ii) day of examination on which cultures were first detected:
The observations on T. theileri-like infections of cattle in Nigeria, Scotland, England and Colombia had shown a few occasions when isolations had only been detected on the day 10 examination of cultures. A conclusion was reached (results; section 1) that there was need for the addition of a further routine day of examination.

At the same time that the day 15 examination was added to the specific study of cow Elsa IV (see results section 7 b.), it was also added to the routine examinations of the seven cows at the Hannah Dairy Research Institute (Table 25). Cultures were then detected at examination day 15 in 3 out of the last 4 periods of examination.

(iii) duration of infectivity of cultures to other blood agar
slopes at 28°C. Cultures from the Hannah cattle were included in the duration of infectivity experiments, but too few remained uncontaminated to draw any conclusions.

The cultures obtained from the splenectomized calf following the inoculation of parasitemic blood were few in number (12) but of interest (see results; section 5 a, and Table 19). All four cultures obtained up to day 4 from challenge were infective to other blood agar cultures for long periods of time irrespective of which day of examination they were first detected. Two of them were still infective to other blood agar slopes at day 200, and another two were found infective at day 150 but non-infective at day 200. The remainder of the cultures from day 6 onwards conformed to the trends described with Elsa's infection. None of the 6 cultures first detected on examination day 5 remained infective after day 50. The remaining 2 which were detected on examination day 10 retained infectivity until day 100 in one, while the other was still infective at day 200.

(iv) morphology: The cultures obtained from the infected splenectomized calf (results; section 7 c. (iii)) which retained infectivity to other cultures for one hundred days and over showed the same posterior nuclear and pear shaped forms found in cultures similarly derived from Elsa. The pear shaped forms were seen at the day 50 examination in each case.

e. discussion.

The influence of differences in quality of the blood agar media on their ability to support trypanosome populations was not examined. Although the media batches were prepared in a standard fashion, the blood from the single donor sheep may possibly have varied in quality from week to week or season to season. A possibility that the results
were influenced by differences in the media batches cannot be entirely ignored, and future work would need to take note of this factor.

Another factor relating to media is the change which occurs on age while supporting trypanosome populations. The duration of infectivity of cultures may be a combination of the inherent qualities of the trypanosomes plus changes in the culture media.

Nevertheless, the infection in the cow Elsa showed a pattern of behaviour varying with the season of the year. Parasitaemias detected on blood agar culture were low in the autumn and winter of three consecutive years and relatively high in the intervening spring and summer months. Moreover, the spring isolations were mainly seen on culture after 5 days of incubation while the proportion of cultures detected on days of examination 10 or 15 increased as the summer progressed. The capability of cultures to retain infectivity for periods of 100 days and over was a characteristic most often associated with the same cultures in the summer which were detected at the later days of examination. The changes in numbers of organisms when grown on a blood agar medium (simulating an insect vector) appeared to show differences relative to the derivation of the stabilate. The two stabilates prepared from cultures on which flagellates first appeared on day 5 showed poor growth. The stabilate prepared from a culture on which flagellates first appeared on examination day 15 showed a relatively luxuriant growth. The stabilate prepared from flagellates derived from an 'old' culture showed a similarly luxuriant growth but marked fluctuations occurred suggesting qualitative differences between the organisms comprising the inoculum. The attempts at cloning revealed that the greatest success was possible from 'old' cultures.

An hypothesis can therefore be made that the organisms in the peripheral blood capable of infecting an insect vector and developing
into the metacyclic stage were represented by those cultures requiring the longer incubation. These were also characterized by their long periods of survival, relatively luxuriant growth and the ease by which subcultures could be established from single organisms. The pear-shaped form seen in some cultures could represent the metacyclic stage and therefore be equivalent to the 'barley-corn' forms of Hölter (1925).

The information derived from Elsa was supported by the fragmentary evidence from the other infected cattle examined in Scotland. Additional support is given from the literature (see review of literature; epidemiology) where several authors, notably Crawley (1912), made the comment that T. theileri - like trypanosomes were more difficult to demonstrate on culture in winter than in the summer. Crawley also noted that 'autumn' cultures required on the average a significantly longer period of incubation than isolations made in the 'spring' and 'summer'.

The demonstration that the infections of cattle may last a period of years could be the explanation of the incidence of infection in a herd increasing with age (see results; section 1, epidemiology: review of literature; epidemiology).

The rare but consistently recurring forms noted during the growth and decline of stablate TRYU 566 on blood agar slopes, although not recorded by Crawley (1909, 1912) whose descriptions were taken as the focus of discussion in the review of the literature, have been described by other authors. The multilflagellate forms were noted on several occasions (e.g. Robinson, 1923) but the forms bearing a flagellum at both ends have only rarely been described. From the examination of both texts and illustrations the only references are probably Sergent Ed. and Et. (1911), Hanceaux et al. (1911 b) and Bahn (1912). A similar morphology has however been described for Trypanosoma conorhini.
(Deane and Deane, 1961) and for T. cruzi (Camargo, 1964). Both trypanosomes were being cultivated on liquid media at 26°C. Camargo believed that he was seeing "metacyclic trypanosomes fully formed but still linked in pairs by their posterior tip as if they were undergoing final binary fission". - like trypanosomes were such that study should initially be directed towards understanding life cycles and developing laboratory techniques for handling and storing isolations. The experimental work then described concentrated on the behaviour of isolations rather than on their morphology and provided information in three main directions. Firstly, a previously unsuspected distribution and incidence of T. thalassin - like trypanosomes was demonstrated in Colombia and Scotland and a lesser amount of information was demonstrated for Nigeria and England. Secondly, isolations were successfully stored as stabilesates at -70°C for periods of years. These were used to observe characteristics of growth on blood agar maintained at 37°C, on bovine leucocyte cultures at 37°C and also to develop a cloning technique on blood agar at 25°C. In particular, the ease with which populations growing at 25°C could be transferred to the leucocyte cultures at 37°C suggested that a standardization of this system may enable comparisons of behaviour to be made between trypanosome populations. Thirdly, qualitative and quantitative studies were made of the parasitaemia in a single infection as maintained atmaster Bush, Milltown, Scotland using the character of infectivity of peripheral blood to a blood agar action. The total findings are informative in relation to the life cycle of T. thalassin - like trypanosomes and of interest in their relevance to other trypanosome species.

In the discussion of the literature a working hypothesis of the life cycle was constructed from geographically scattered evidence taken
IV. DISCUSSION:

The experimental observations in relation to the literature.

The concluding paragraph of the discussion following the review of literature gave the opinion that the inadequacies of knowledge concerning *T. theileri* - like trypanosomes were such that study should initially be directed towards understanding life cycles and developing laboratory techniques for handling and storing isolations. The experimental work then described concentrated on the behaviour of isolations rather than on their morphology and provided information in three main directions. Firstly, a previously unsuspected distribution and incidence of *T. theileri* - like trypanosomes was demonstrated in Colombia and Scotland and a lesser amount of information was demonstrated for Nigeria and England. Secondly, isolations were successfully stored as stabilates at -79°C for periods of years. These were used to observe characteristics of growth on blood agar maintained at 28°C, on bovine leucocyte cultures at 37°C and also to develop a cloning technique on blood agar at 28°C. In particular, the ease with which populations growing at 28°C could be transferred to the leucocyte cultures at 37°C suggested that a standardization of this system may enable comparisons of behaviour to be made between trypanosome populations. Thirdly, qualitative and quantitative studies were made of the parasitaemia in a single infected cow maintained at Easter Bush, Midlothian, Scotland using the character of infectivity of peripheral blood to a blood agar medium. The total findings are informative in relation to the life cycle of *T. theileri* - like trypanosomes and of interest in their relevance to other trypanosome species.

In the discussion of the literature a working hypothesis of the life cycle was constructed from geographically scattered evidence taken
from Luhr (1905) in Transcaucasia, Crawley (1912) in the United States of America, Müller (1917) in Germany, Araneveld (1931) in Indonesia, Carpano (1932) in Eritrea and Reichenow (1940) in Tanganyika (fig. 4).

The discovered ability of the trypanosoma populations under study to adhere to the cells of bovine leucocyte cultures at 37°C by their posterior ends may be significant in the understanding of the portion of the life cycle occurring in fixed tissues. If the trypanosomes can anchor themselves to capillary walls in the bovine host and daughter cells from division are shed into the peripheral blood, there is no need to postulate either focal areas of activity or association with a particular tissue. Of greater interest, however, is the support given to the conclusion reached from the literature that peripheral blood forms vary in their infectivity to an insect vector. Moreover the evidence presented from the work in Scotland suggested that the infective forms capable of developing to the metacyclic stage were at their maximum in summer, probably coinciding with the maximum incidence of the vector. The absence of any trypanosomatid parasites in the Haematocota spp. dissected was therefore a disappointment as was the apparent failure to infect a calf from cultures suspected to contain metacyclic forms.

Information on the qualitative differences between peripheral blood forms of other trypanosome species is meagre. A comparison can be made with the salivarian Trypanosoma brucei where the 'short broad' as opposed to the 'long' form is taken to be the one infective to tsetse fly (Glossina spp.) (Wenyon, 1926). Minchin and Woodcock (1912) studied a trypanosome infecting Athene noctua (the little owl) and on morphological evidence surmised a cycle of 'medium slender forms' in the host which in early summer gave way to 'stout spindle forms' which were infective to the vector. Deane and Kloetsel (1969) discussed the poly-
morphism of the blood stream stages of *Trypanosoma cruzi* and thought that they may have the "same significance that it has among species of the *brucei* group, and physiologic differences between the various forms should be expected". Specific study of the forms of trypanosomes present in the peripheral blood of a host which are infective to a vector can therefore be expected to be rewarding. Similarities of behaviour may be revealed not only between stercorarian trypanosomes but between stercorarian and salivarian species.
1. Five main conclusions were reached following the review of the literature:

a) all trypanosomes of the subgenus *Nastropyrum* (Hoare, 1964) should be termed 'T. theileri-like' excepting where biological characteristics are known in addition to morphology and host. The name *Trypanosoma theileri* may then be reserved for the large trypanosome of cattle seen by Theiler in South Africa.

b) a clear answer is required to the question whether *T. theileri*-like organisms can interfere with the serological diagnosis of salivarian trypanosomes.

c) *T. theileri*-like trypanosomes are normally non-pathogenic to their hosts. However, the apparent pathogenicity of the trypanosomes when inoculated, simultaneously with rinderpest virus, into an ox susceptible to both micro-organisms raises the query whether the same phenomenon can occur in relation to other infections.

d) a working hypothesis can be erected of the life cycle of *T. theileri*-like trypanosomes despite the fragmentary and geographically scattered nature of the evidence. The evidence also indicates that more than one vector must exist but they are all likely to be members of the family Tabanidae.

e) the study of the life cycle of these trypanosomes and the development of methods of handling in the laboratory are prerequisites for specific serological or other examinations.

2. The direction of the experimental work was indicated by the deficiencies of the literature, and the following principal conclusions can be made:

a) the results of the search for *T. theileri*-like trypanosomes in Nigeria, Scotland, England and Colombia confirm the presence of the
organisms in both temperate and tropical climates. In addition, the results from Scotland and Colombia reveal not only a previously unsuspected wide distribution of the infection but also wide variations of incidence between localities in each country.

b) qualitative differences in the trypanosomes present in the peripheral blood of a host are suggested by: variations of duration of infectivity of blood maintained at 37°C to blood agar; the lack of uniformity of population curves grown from stabi late 139, an isolation from Lephinmore, Scotland; variations in the longevity of cultures on blood agar, and the differences of behaviour of trypanosome populations grown at 28°C when first introduced into bovine leucocyte cultures at 37°C.

c) three techniques are described which are likely to be informative in any further work to determine qualitative differences between peripheral blood forms. Firstly, the storage of isolations on blood agar media at -79°C using dimethylsulphoxide as the cryoprotectant; secondly, the consistently successful transference of trypanosome populations on blood agar at 28°C to a bovine leucocyte culture at 37°C; thirdly the preparation of clone populations on blood agar at 20°C.

d) the specific study of a T. theileri-like trypansome infection in a single cow demonstrated seasonal variations in level of parasitaemia accompanied by qualitative changes in the isolations grown on a blood agar medium at 28°C. The hypothesis can be made that the form of the trypanosome infective to the insect vector increased in numbers as the summer progressed.

e) the findings from the study of the infection in the single cow, together with the observed capacity for the trypanosomes to adhere by their posterior ends to bovine cells in culture at 37°C, both support and illuminate the hypothetical life cycle erected from the literature.
Grateful acknowledgment is given to Professor W.M.R. Lumaden of the London School of Hygiene and Tropical Medicine for initially suggesting the project and for his continuous encouragement. The work was financed by a research grant from the Ministry of Overseas Development, London.

Access to animals sampled was not only freely given by individual farmers, farm managers and veterinary surgeons but by the following organisations and institutions: University of Ibadan; University of Glasgow; Hill Farming Research Organization, Edinburgh; Nature Conservancy, Edinburgh; National University of Colombia and the Colombian Institute of Agriculture. Some collections of Haematopota spp. were made by students of the Faculties of Science and Veterinary Medicine, University of Edinburgh.

Laboratory facilities were generously provided by the University of Ibadan and the Colombian Institute of Agriculture. Numerous and generous personal communications are acknowledged in the text.

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To these many people, my sincere thanks.
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Published and unpublished records of the demonstration of *T. tenella* -like trypanosomes in bovine tissues either by direct microscopy or by culture.

The following list is not exhaustive particularly as there are many unpublished observations, but the cosmopolitan distribution of *T. tenella* -like trypanosomes is illustrated. The geographical arrangement may also be of use for purposes of reference.

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**APPENDICES**

**Appendices**
- APPENDICES
APPENDIX 1.

Published and unpublished records of the demonstration of *T. theileri* -like trypanosomes in bovine tissues either by direct microscopy or by culture.

The following list is not exhaustive particularly as there are many unpublished observations, but the cosmopolitan distribution of *T. theileri* -like trypanosomes is illustrated. The geographical arrangement may also be of use for purposes of reference.

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Hoare, 1929.
Richardson, 1920, 1948.
Thomson, 1931.
Carmichael, 1939.
Reid et al., 1966, 1958.
Montgomery and Kinghorn, 1908.
Kinghorn and Yorke, 1912.
Kinghorn et al., 1915.
Keymer, 1969.

North and South America

Argentina
Peter, 1910.
Dias and Zuccarini, 1929.

Brasil
Peter, 1910.
Carini, 1911.

Canada
Bowhill, 1909 a, b.
Watson and Hadwen, 1912.
Hall, 1953.

Colombia
Virriessas, 1934.
Wells et al., 1968.

Nicaragua
Gallo, 1938.
Teague and Clark, 1918.
Panama, 1941.
Uruguay
Peter, 1910.

Uruguay and U.S.A.
Crawley, 1909, 1912 a,b.
Johns, 1913, 1914.
Glaser, 1922.
Schoening, 1925.
Packchanian, 1934.
Atchley, 1951.
Simpson and Sanders, 1951.
Dikman et al., 1957.
Levine et al., 1956.
Ristic and Trager, 1958.
Lundholm et al., 1959.
Simpson and Green, 1959.
Ewing and Carmahan, 1967.
Splitter et al., 1967.
Cross et al., 1968.
Sollod and Soulsby, 1968.

1. to investigate the possibility that species of the family Trypanodaeae act as vectors of Trypanosoma theileri, a trypanosome of British cattle.
2. to collect information on the distribution of the British species of Trypanidae.

Identification of Trypanidae
Accurate recognition by collectors is not required. Any fly seen to be biting men or animals should be collected.

There are three genera of Trypanidae present in the British Isles, Gamas, Trypanotaxis and Parnassus. The most familiar is the genus Parnassus, the parasites being venereal in their biting habits. A person may be in the "clag".

Sectors of the family Trypanidae are automatically built flies with a large thorax. There are great differences in size, the largest British species being a member of the genus Loxham with a length of 3 1/2 cm. and a 5 cm. wing span. A characteristic is attached to a common British species, Trypanotaaxis minoris. The wing veination is present to all sectors of the family.

* personal communication.
** personal anonymous communications to Knuth, 1910.
*** personal communications passed on by Hoyte, 1968.

The territories named are now independent and the information available is insufficient to state localities in relation to new territorial boundaries.
APPENDIX 2

TRYPANOSOMIASIS RESEARCH UNIT

DEPARTMENT OF ANIMAL HEALTH

ROYAL (DICK) SCHOOL OF VETERINARY STUDIES, EDINBURGH UNIVERSITY

Survey for Tabanidae, 1966

Information for Collectors

Objectives of the Survey

1. to investigate the possibility that species of the Family Tabanidae act as vectors of Trypanosoma theileri, a trypanosome of British cattle.

2. to collect information on the distribution of the British species of Tabanidae.

Recognition of Tabanids

Accurate recognition by collectors is not required. Any fly seen to be biting man or animals should be collected.

There are three genera of Tabanids present in the British Isles, Chrysops, Haematopota and Tabanus. The most familiar is the genus Haematopota, the females being aggressive in their biting habits. A common name is the "Cleg".

Members of the Family Tabanidae are stoutly built flies with a large head. There are great differences in size, the largest British species being a member of the genus Tabanus with a length of 2 1/2 cm. and a 5 cm. wing span. A photograph is attached of a common British species, Haematopota pluvialis. The wing venation is common to all members of the family.

Methods of Capture

The flies may be caught off man himself or animals, particularly when feeding, or may enter moving vehicles. Traps are being erected on selected farms by the Trypanosomiasis Research Unit. The flies are required alive and should be posted immediately to this Unit in the
envelopes and moist containers provided.

**Records of Capture**

Collectors are requested to fill in the card provided with each container. "Place" should, if possible, be a map reference, or a village and County name. "Vegetation" should follow the nomenclature on the Ordnance Survey 6 miles to 1 inch series, that is:

- Peat Moors, Mosses and Bogs (Cotton grass, Deer grass, Sphagnum)
- Wet Grass Moors (Molinia, Sedges, and Associated Flora)
- Dry Grass Moors (Nardus and Associated Flora)
- Heather Moors
- Arid Grasslands (Fescue - Agrostis with Nardus, Heather, Bracken)
- Sand Dunes, Cliff tops
- Saltings
- Improved land
- Woodland Areas
- Urban Areas

Any difficulty in interpreting vegetation should not prevent despatch of a specimen.

There was little doubt that the subcutaneous oedema, hydrothorax, hydroperitoneum and ascites and the oedema of the abdominal wall were all related to the kidney dysfunction. The cause of this kidney dysfunction was not determined. It may be that the pathological reactions for *Leptospirosis* are significant. On the other hand, it could equally well be reasoned that the *Truncatella thallicta* recovered in this case was the cause of the high circulating antibody fixing on to glomeruli and leading to amyloid change there. Serosa were prepared from the abdominal fluid, popliteal and prescapular lymph nodes, head lymph nodes, bone marrow, lung and kidney and from the liver and spleen and
APPENDIX 3

Copy of the post-mortem report on the cow in which an attempt was made to locate T. theilerii - like trypanosomes in tissues other than blood vessels were found in any of these sections but structures which might be trypanosomes or might be assessed nucleai or blurred.

DEPARTMENT OF VETERINARY PATHOLOGY

Date: 5th May, 1965


Owner: Easter Bush Farm.

Report:

Post-mortem and histological examination of this animal showed that the most significant lesions were in the kidney. There was widespread amyloidosis and a slight interstitial nephritis associated. Throughout the body the arterioles showed slight amyloid change and in the lymphoid tissue throughout the body there was slight reaction and plasma cell formation. There was little doubt that the subcutaneous oedema, hydrothorax, hydropericardium and ascites and the oedema of the abdominal wall were all related to the kidney dysfunction. The cause of this kidney dysfunction was not determined. It may be that the serological reactions for Leptospira pomona are significant. On the other hand, it could equally well be reasoned that the Trypanosoma theileri recovered in this case was the cause of the high circulating antibody fixing on to glomeruli and leading to amyloid change there. Smears were prepared from the abdominal fluid, popliteal and prescapular lymph nodes, head lymph nodes, bone marrow, lung and kidney and from the liver and spleen and
the arteries and veins of these organs. No clear cut trypanosomes were found in any of these sections but structures which might be trypanosomes or might be smeared nuclei or blurred endothelial cells were found in nearly all the smears examined. These were most numerous in the kidney. In the histological sections no clear cut trypanosomes were found but there were some structures which with enthusiasm could be classified as trypanosomes in the kidney. In the smears examined, *Ep. erythrozoon* were found in many of the R.B.Cs. This could be a terminal break-down of a presumed state. Although the small intestine showed slight reaction, the lesion here was not typical of Johne's disease and this is in agreement with the negative complement fixation test, negative faeces examination and doubtful bacteriological examination of the small intestine. Terminally the animal had torn the muscles in the right hind limb and there was an extensive haemorrhagic lesion here with early organisation.

Signed: Kenneth Head

26.11.65.
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* species not recognised by Morris (1965)
** also spelt 'mazamorium' in the same article.
*** Keymer (1966) saw forms resembling *T. theileri*, *inzera*, *cepalophi* and *cephaloophi* commonly and referred to them as 'theileri-like'. See text.
**** Neveu-Lemaire (1943) included these isolations in an appendix to his textbook, additional to those listed elsewhere in this table. He does not however state the source of his information.
***** Morris (1932) and Morris (1940) are probably the same person. The second reference is a personal communication to Curasson (1943) and his name may be miss-spelt as both refer to the Gold Coast.
TABLE 2.

Records of T. theileri-like trypanosomes cultured from the peripheral blood of artiodactyla other than domestic oxen
(Bos taurus and Bos indicus)

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<td></td>
<td>(Bushbuck)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bubalus</td>
<td>T. theileri</td>
<td>Kraneveld (1931)</td>
</tr>
<tr>
<td></td>
<td>bubalis</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Asiatic</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>buffalo)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sylvicapra</td>
<td>'theileri-like'</td>
<td>Bruce et al. (1915)</td>
</tr>
<tr>
<td></td>
<td>grahamiae</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Grey duiker)</td>
<td></td>
<td>Keymer (1966)</td>
</tr>
</tbody>
</table>

Pinnipedia

Rat

Thielser (1903); Novy et al. (1904);

Fowl

Thielser (1903); Novy et al. (1904);

Guinea pig

Thielser (1903); Novy et al. (1904);

Dutton et al. (1906); Lingard (1907);

Schein (1907); Swellengrebel (1911);

Rodham et al. (1912); Dodd (1912);
Some recorded unsuccessful attempts to infect sheep and goats, representatives of other mammalian orders, and birds, using blood or cultures from domestic cattle known to be infected with T. \textit{theileri}-like trypanosomes.

<table>
<thead>
<tr>
<th>CLASS</th>
<th>ORDER</th>
<th>ANIMAL</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mammalia</td>
<td>Artiodactyla</td>
<td>Goat</td>
<td>Theiler (1903); Rodhain et al. (1912); Teague and Clark (1918); van Saezeghem (1922); Dias and Zuccarini (1924); Hornby et al. (1934).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sheep</td>
<td>Theiler (1903); van Saezeghem (1922); Dias and Zuccarini (1924).</td>
</tr>
<tr>
<td></td>
<td>Perissodactyla</td>
<td>Horse</td>
<td>Theiler (1903); Holmes (1904); Lingard (1907); Dias and Zuccarini (1924).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mule</td>
<td>Teague and Clark (1918).</td>
</tr>
<tr>
<td></td>
<td>Rodentia</td>
<td>Mouse</td>
<td>Theiler (1903); Novy et al. (1904); Swellengrebel (1911); Trautmann (1922); Glaser (1922); Robinson (1923); Dias and Zuccarini (1924); Djatkova (1937); Iwata et al. (1959).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rat</td>
<td>Theiler (1903); Novy et al. (1904); Schein (1907); Rodhain (1916); Robinson (1923); Dias and Zuccarini (1924); Djatkova (1937).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Guinea pig</td>
<td>Theiler (1903); Novy et al. (1904); Dutton et al. (1906); Lingard (1907); Schein (1907); Swellengrebel (1911); Rodhain et al. (1912); Dodd (1912).</td>
</tr>
</tbody>
</table>
Table 3 cont...

<table>
<thead>
<tr>
<th>CLASS</th>
<th>ORDER</th>
<th>ANIMAL*</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Teague and Clark (1918); Glasser (1922); Robinson (1923); Dias and Zuccarini (1924); Djatkova (1937); Iwata et al. (1959).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rabbit</td>
<td>Theiler (1903); Holmes (1904); Dutton et al. (1906); Lingard (1907); Schein (1907); Dodd (1912); Glasser (1922); Robinson (1923); Dias and Zuccarini (1924); Djatkova (1937); Nahaian (1939); Iwata et al. (1959).</td>
</tr>
<tr>
<td>Carnivora</td>
<td>Dog</td>
<td></td>
<td>Theiler (1903); Lingard (1907); Schein (1907); Teague and Clark (1918); Dias and Zuccarini (1924); Rao and Ayyar (1931); Djatkova (1937).</td>
</tr>
<tr>
<td>Marsupialia</td>
<td>Opossum</td>
<td></td>
<td>Teague and Clark (1918).</td>
</tr>
<tr>
<td>Primates</td>
<td>Monkey</td>
<td></td>
<td>Lingard (1907); Teague and Clark (1918)</td>
</tr>
<tr>
<td>Aves</td>
<td>Galliformes</td>
<td>Chicken</td>
<td>Teague and Clark (1918)</td>
</tr>
<tr>
<td></td>
<td>Columbiformes</td>
<td>Pigeon</td>
<td>Teague and Clark (1918).</td>
</tr>
</tbody>
</table>

* Authors' descriptions.
Diseases of domestic cattle in which concurrent infections of T. theileri-like trypanosomes have been detected in smears of peripheral blood.

**Bacterial diseases:**

- Anthrax: Lingard (1907).
- Haemorrhagic septicaemia: Gallo (1938).
- Spirochaetosis: Carpano (1924).

**Protozoal diseases:**

- Babesiosis: Theiler (1903); Holmes (1904); Lingard (1907); Falshaw and Lingard (1907); Bowhill (1909 a,b); Heanley (1908); Schmitt (1910, 1911); Stockman (1910); Coles (1913); Carpano (1913, 1924); Robinson (1923); Curasson (1925); Galliard (1925); Maroff et al. (1925); Virviescas (1934); Iwata et al. (1959).
- Theileriosis: Miyajima (1907); Carpano (1913, 1924); Sprinholz-Schmidt (1938); Sergent et al. (1945); Hoyte (1968 - personal communication).
- Trypanosomiasis: Lingard (1907); Carpano (1913); Schweitz (1927, 1928, 1930); Godfrey et al. (1965).

**Virus diseases:**

- Bovine viral diarrhoea: Malmquist (1965).
- Foot and Mouth: Mayer (1912).

- Rinderpest: Theiler (1903, 1907); Durrant and Holmes (1904); Holmes (1904); Lühs (1905); Lingard (1907); Schoen (1907); Carpano (1913, 1924); Crevotte (1920); Haughwout and Youngberg (1920); Curasson (1925); Carmichael (1926); Kearney (1926); Hornby and Bailey (1929); Rao and Ayer (1931); Uganda (1932); Hornby et al. (1934); Robertson (1956).
### Table 4 cont...

**Miscellaneous:**

Anaplasmosis: Theiler (1910); Carpano (1913); Robinson (1923).

<table>
<thead>
<tr>
<th>Locality</th>
<th>County</th>
<th>Vegetation</th>
<th>Cattle type</th>
<th>Annual Rainfall (inches)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lapham, Dalrigh</td>
<td>Argyll</td>
<td>Improved land</td>
<td>Dairy</td>
<td>40.75</td>
</tr>
<tr>
<td>Inverness-shire</td>
<td>Net grass moor</td>
<td>Beef</td>
<td>78.68</td>
<td></td>
</tr>
<tr>
<td>Inverness-shire</td>
<td>Net grass moor</td>
<td>Beef</td>
<td>78.68</td>
<td></td>
</tr>
<tr>
<td>Croagh</td>
<td>Inverness-shire</td>
<td>Improved land</td>
<td>Dairy</td>
<td>not known</td>
</tr>
<tr>
<td>Glensaugh</td>
<td>Kincardine-shire</td>
<td>Heather moor</td>
<td>Beef</td>
<td>36.8</td>
</tr>
<tr>
<td>Crossford</td>
<td>Lanarkshire</td>
<td>Improved moor</td>
<td>Dairy</td>
<td>39.3</td>
</tr>
<tr>
<td>Sourhope</td>
<td>Roxburghshire</td>
<td>Dry grass moor</td>
<td>Beef</td>
<td>36.8</td>
</tr>
<tr>
<td>Easter Bush</td>
<td>Kildonan</td>
<td>Improved land</td>
<td>Dairy</td>
<td>31.18</td>
</tr>
</tbody>
</table>

* Meadow records, Southend, 5 miles from Kochino (1916 - 1950).
** Meteorological office, Skara Brae, 6 miles south-east from Kilmacolm (1916 - 1930).
*** Meteorological office, Skara Brae, 6 miles south-east from Crossford (1916 - 1930).
* Meteorological office, Hamilton, 8 miles north-east from Crossford (1916 - 1930).
‡ Meteorological office, Fairmilehead, 8 miles north from Easter Bush (1916 - 1930).
Data concerning localities visited in Scotland to examine cattle:

vegetation type data from H.M. Ordnance Survey 1:625,000 map

"Vegetation - reconnaissance survey of Scotland".

<table>
<thead>
<tr>
<th>Locality</th>
<th>County</th>
<th>Vegetation</th>
<th>Cattle type</th>
<th>Annual Rainfall (inches)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lephinmore</td>
<td>Argyll</td>
<td>Wet grass moor</td>
<td>Beef</td>
<td>67.7*</td>
</tr>
<tr>
<td>Hannah</td>
<td>Dairy Research Institute</td>
<td>Improved land</td>
<td>Dairy</td>
<td>37.9*</td>
</tr>
<tr>
<td>Cochino</td>
<td>Dunbarton</td>
<td>Improved land</td>
<td>Dairy</td>
<td>40.73*</td>
</tr>
<tr>
<td>Kinloch, Rum</td>
<td>Inverness-shire</td>
<td>Wet grass moor</td>
<td>Dairy</td>
<td>not known</td>
</tr>
<tr>
<td>Kinlocheil</td>
<td>Inverness-shire</td>
<td>Wet grass moor</td>
<td>Beef</td>
<td>78.68***</td>
</tr>
<tr>
<td>Traigh</td>
<td>Inverness-shire</td>
<td>Improved land and wet grass moor</td>
<td>Dairy</td>
<td>not known</td>
</tr>
<tr>
<td>Glens4ugh</td>
<td>Kincardineshire</td>
<td>Heather moor</td>
<td>Beef</td>
<td>34.8</td>
</tr>
<tr>
<td>Crossford</td>
<td>Lanarkshire</td>
<td>Improved moor</td>
<td>Dairy</td>
<td>39.24*</td>
</tr>
<tr>
<td>Sourhope</td>
<td>Roxburghshire</td>
<td>Dry grass moor</td>
<td>Beef</td>
<td>34.6*</td>
</tr>
<tr>
<td>Easter Bush</td>
<td>Midlothian</td>
<td>Improved land</td>
<td>Dairy</td>
<td>31.18†</td>
</tr>
</tbody>
</table>


** Meteorological office, Renfrew Airport, 5 miles from Cochino (1916 - 1950)

*** Meteorological office, Fort William, 6 miles south-east from Kinlocheil (1916 - 1950).

+ Meteorological office, Hamilton, 8 miles north-west from Crossford (1916 - 1950).

† Meteorological office, Fairmilehead, 2 miles north from Easter Bush (1916 - 1950).
Data concerning localities visited in Colombia to examine cattle: vegetation type data after Cole (1965).

<table>
<thead>
<tr>
<th>Locality</th>
<th>Department</th>
<th>Vegetation</th>
<th>Cattle type</th>
<th>Approx. Altitude (metres)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monteria</td>
<td>Cordoba</td>
<td>Forest</td>
<td>Beef</td>
<td>100</td>
</tr>
<tr>
<td>Bogota</td>
<td>Cundinamarca</td>
<td>Mountain</td>
<td>Dairy</td>
<td>2600</td>
</tr>
<tr>
<td>Tibaitata</td>
<td>Cundinamarca</td>
<td>Mountain</td>
<td>Dairy</td>
<td>2600</td>
</tr>
<tr>
<td>Villavicencio</td>
<td>Meta</td>
<td>Savannah</td>
<td>Beef</td>
<td>450</td>
</tr>
<tr>
<td>Puerto Lopez</td>
<td>Meta</td>
<td>Savannah</td>
<td>Beef</td>
<td>450</td>
</tr>
<tr>
<td>Girandot</td>
<td>Cundinamarca</td>
<td>Mountain</td>
<td>Beef</td>
<td>518</td>
</tr>
<tr>
<td>Ibague</td>
<td>Tolima</td>
<td>Mountain</td>
<td>Dairy</td>
<td>1250</td>
</tr>
<tr>
<td>Cali</td>
<td>Valle</td>
<td>Mountain</td>
<td>Dairy</td>
<td>1003</td>
</tr>
<tr>
<td>Palmira</td>
<td>Valle</td>
<td>Mountain</td>
<td>Dairy</td>
<td>1003</td>
</tr>
<tr>
<td>Florida</td>
<td>Valle</td>
<td>Mountain</td>
<td>Dairy</td>
<td>1050</td>
</tr>
<tr>
<td>Marinda</td>
<td>Cauca</td>
<td>Mountain</td>
<td>Dairy</td>
<td>1050</td>
</tr>
</tbody>
</table>
University Farm, Ibadan, Nigeria: results of cultures made from eleven Fulani and eleven Ndama cows.

Numbers of cultures becoming positive, by days after inoculation, expressed as cumulative percentages, in two series maintained, respectively, at 25°C and at 24 - 31°C. Cultures examined from day 3 until positive, or until day 14.

<table>
<thead>
<tr>
<th>Day</th>
<th>25°C</th>
<th>Incubated at 24 - 31°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>38</td>
<td>62</td>
</tr>
<tr>
<td>4</td>
<td>80</td>
<td>89</td>
</tr>
<tr>
<td>5</td>
<td>95</td>
<td>94</td>
</tr>
<tr>
<td>6</td>
<td>97</td>
<td>97</td>
</tr>
<tr>
<td>7</td>
<td>98</td>
<td>97</td>
</tr>
<tr>
<td>8</td>
<td>100</td>
<td>98</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total No. of Cultures positive</td>
<td>65</td>
<td>63</td>
</tr>
<tr>
<td>Total No. of Cultures inoculated</td>
<td>66</td>
<td>66</td>
</tr>
</tbody>
</table>
The number of cultures becoming positive, of six inoculated, on day 5 or a later day, for fourteen Fulani and fourteen N\-dama cattle, at Ibadan, Nigeria (2 ml. inocula).

<table>
<thead>
<tr>
<th>No. positive</th>
<th>Fulani</th>
<th>Ndama</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glassco</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>Kincorth</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Kincorthill</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Tarla</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Glassough</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Crossford</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>Southgate</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>44</td>
</tr>
<tr>
<td>Easter Bush</td>
<td>30</td>
<td></td>
</tr>
</tbody>
</table>

Total

<table>
<thead>
<tr>
<th></th>
<th>Fulani</th>
<th>Ndama</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>231</td>
<td>140</td>
</tr>
</tbody>
</table>
Number of cattle found infected at each locality in Scotland.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Number of cattle examined</th>
<th>Number of cattle examined</th>
<th>Number infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lephinmore</td>
<td>30</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Hannah Dairy Research Institute</td>
<td>30</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>Cochno</td>
<td>30</td>
<td>14</td>
<td>3</td>
</tr>
<tr>
<td>Kinloch</td>
<td>10</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Kinlocheil</td>
<td>16</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>Traigh</td>
<td>10</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Glensaugh</td>
<td>30</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Crossford</td>
<td>11</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Sourhope</td>
<td>32</td>
<td>29</td>
<td>3</td>
</tr>
<tr>
<td>Easter Bush</td>
<td>30</td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

**Totals** 231 40
Number of cultures becoming positive, out of six inoculated, on days 5 or 10, for twenty-nine cattle in Scotland examined between July and September, 1965 (1 ml. inocula).

<table>
<thead>
<tr>
<th>Year of Birth</th>
<th>No. of Animals</th>
<th>No. of positive cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td>1957</td>
<td>29</td>
<td>0</td>
</tr>
<tr>
<td>1958</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>1959</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>1960</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>1961</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>1962</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>1963</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>1964</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>1965</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Totals 16
### TABLE II

Number of animals found infected at Winslow, England, among total animals examined by year of birth.

<table>
<thead>
<tr>
<th>Year of Birth</th>
<th>No. infected</th>
<th>No. examined</th>
</tr>
</thead>
<tbody>
<tr>
<td>1957</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>1958</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>1959</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>1960</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>1961</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>20</td>
</tr>
</tbody>
</table>

*Note: Additional data columns are not visible in the image.*
Number of cattle found infected at each locality in Colombia.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Number of cattle examined</th>
<th>Number infected</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Caribbean lowlands</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Monteria. Experimental Station I.C.A.</td>
<td>29</td>
<td>24</td>
</tr>
<tr>
<td>2. Bogota</td>
<td>37</td>
<td>0</td>
</tr>
<tr>
<td>3. Tibaitata</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td><strong>Eastern Andean range</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Villavicencio. Experimental Station I.C.A.</td>
<td>28</td>
<td>20</td>
</tr>
<tr>
<td>5. Puerto Lopez. Hacienda Pachaquiaro</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Hacienda Lajitas</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td><strong>Eastern Plains</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hacienda Lomitas</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td><strong>Magdalena valley</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Ibague. Las Brisas</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td><strong>Cauca valley</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Cali. El Eden</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>9. Palmira. Experimental Station I.C.A.</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Senor Hernando Reyes</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Santa Rosa</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td><strong>Florida. Senor Buerra</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Senor E. Barney</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Senor B. Barney</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td><strong>Marinda</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td>171</td>
<td>71</td>
</tr>
</tbody>
</table>
TABLE 13.
Number of cultures becoming positive, out of six inoculated, on days 5 or 10, for sixty-four cattle in Colombia examined between April and July, 1967 (1 ml. inocula).

<table>
<thead>
<tr>
<th>No. positive</th>
<th>No. examined</th>
<th>No. of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>64</td>
<td></td>
</tr>
</tbody>
</table>
### TABLE I.

Results from the culture of peripheral blood of eleven young cattle from Villavicencio (Experimental Station I.C.A.) Colombia.

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>No. examined</th>
<th>No. positive on culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>18</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>22</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
Localities and numbers of animals, other than domestic cattle, examined on culture for stercorarian trypanosomes.

<table>
<thead>
<tr>
<th>Country</th>
<th>Locality</th>
<th>Species of animal</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scotland</td>
<td>Kinloch, Rum, Inverness-shire</td>
<td>Red deer</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>(Cervus elaphus)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Penicuik, Midlothian</td>
<td>Roe deer</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>(Capreolus capreolus)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clatteringshores, Kirkcudbrightshire</td>
<td>Feral goats</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Holy Island, Bute</td>
<td>Feral goats</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Aviemore, Inverness-shire</td>
<td>Reindeer</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>(Rangifer tarandus)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colombia</td>
<td>Bogota, Vet. Lab.</td>
<td>Sheep</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Bogota, Vet. Faculty</td>
<td>Sheep</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Arauca</td>
<td>Horses</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Puerto Lopez, Hacienda Saman</td>
<td>Horses</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Palmira, Santa Rosa</td>
<td>Horse</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Date of cultures</th>
<th>Temperature</th>
<th>Scotland</th>
<th>Colombia</th>
</tr>
</thead>
<tbody>
<tr>
<td>1923.7.67</td>
<td>1/6</td>
<td>1/6</td>
<td>1/6</td>
</tr>
<tr>
<td>1926.7.67</td>
<td>1/6</td>
<td>1/6</td>
<td>1/6</td>
</tr>
<tr>
<td>1928.6.67</td>
<td>1/6</td>
<td>1/6</td>
<td>1/6</td>
</tr>
<tr>
<td>1932.3.67</td>
<td>1/6</td>
<td>1/6</td>
<td>1/6</td>
</tr>
<tr>
<td>1934.3.67</td>
<td>1/6</td>
<td>1/6</td>
<td>1/6</td>
</tr>
<tr>
<td>1936.3.67</td>
<td>1/6</td>
<td>1/6</td>
<td>1/6</td>
</tr>
</tbody>
</table>
Duration of infectivity of the peripheral blood of a cow, parasitaemic with *T. theileri*-like trypanosomes, to blood agar medium after having been maintained at 4°C, 20°C, or 37°C for various periods of time:

The numerator is the number of cultures found positive over denominator, number of cultures inoculated.

<table>
<thead>
<tr>
<th>Date of Maintenance</th>
<th>Time interval before inoculation hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleeding temperature</td>
<td>1/2</td>
</tr>
<tr>
<td>19.7.67 28°C</td>
<td>5/6</td>
</tr>
<tr>
<td>26.7.67 4°C</td>
<td>3/6</td>
</tr>
<tr>
<td>28°C</td>
<td>3/6</td>
</tr>
<tr>
<td>15.8.67 4°C</td>
<td>1/6</td>
</tr>
<tr>
<td>28°C</td>
<td>1/6</td>
</tr>
<tr>
<td>37°C</td>
<td>1/6</td>
</tr>
<tr>
<td>21.8.67 4°C</td>
<td>3/6</td>
</tr>
<tr>
<td>28°C</td>
<td>1/6</td>
</tr>
<tr>
<td>37°C</td>
<td>1/6</td>
</tr>
<tr>
<td>7.9.67 37°C</td>
<td>0/6</td>
</tr>
<tr>
<td>28°C</td>
<td>1/6</td>
</tr>
<tr>
<td>37°C</td>
<td>1/6</td>
</tr>
</tbody>
</table>
The duration of infectivity of primary cultures of *T. theileri* - like trypanosomes on a blood agar medium to further slopes of the same medium; primary cultures inoculated between 15.8.67 and 7.9.67, and each examined for infectivity on a single occasion.

<table>
<thead>
<tr>
<th>Ages of cultures when examined (days)</th>
<th>Number examined</th>
<th>Number infective</th>
<th>Number NOT infective</th>
</tr>
</thead>
<tbody>
<tr>
<td>11 - 20</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>21 - 30</td>
<td>5</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>31 - 40</td>
<td>4</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>41 - 50</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>51 - 60</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>61 - 70</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>71 - 80</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>81 - 90</td>
<td>5</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>91 - 100</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>101 - 110</td>
<td>5</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>111 - 120</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>121 - 130</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

*Calculated from the day that preparation started.*
TABLE 18.

Growth of *T. theileri* - like trypanosomes on cultures of bovine leucocytes: origin of materials used.

<table>
<thead>
<tr>
<th>Series Number</th>
<th>Date of experiment</th>
<th>Age of leucocyte cultures</th>
<th>Origin of culture of <em>T. theileri</em> at 28°C</th>
<th>Age of <em>T. theileri</em> culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11.9.67</td>
<td>9</td>
<td>Primary culture, cow, Easter Bush.</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td>13.9.67</td>
<td>8</td>
<td>Primary culture, cow, Easter Bush.</td>
<td>14</td>
</tr>
<tr>
<td>3</td>
<td>20.9.67</td>
<td>3</td>
<td>Primary culture, cow, Easter Bush.</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>2.10.67</td>
<td>10</td>
<td>TREU 114, cow, Ayr.</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>4.10.67</td>
<td>8</td>
<td>TREU 114, cow, Ayr.</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>12.10.67</td>
<td>6</td>
<td>Primary culture, cow, Ayr.</td>
<td>7</td>
</tr>
<tr>
<td>7</td>
<td>19.10.67</td>
<td>4</td>
<td>Primary cultures, from leucocyte monolayer.</td>
<td>18</td>
</tr>
<tr>
<td>8</td>
<td>27.10.67</td>
<td>4</td>
<td>TREU 116, many subcultures away from a cow, Eire.</td>
<td>5</td>
</tr>
<tr>
<td>9</td>
<td>6.1.68</td>
<td>4</td>
<td>TREU 429, second passage from cow, Easter Bush.</td>
<td>5</td>
</tr>
</tbody>
</table>

* calculated from the day that preparation started.*
Transmission of *T. theileri*-like trypanosomes from a cow to a splenectomized calf using parasitaemic blood: results from culturing peripheral blood of the calf at 28°C (one ml. inocula into each of 6 blood agar slopes on each day of examination).

<table>
<thead>
<tr>
<th>Day numbers from challenge on which peripheral blood infected cultures</th>
<th>Number of positive cultures by the day of examination on which they were detected.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 (actual number of days on which cultures were examined)</td>
</tr>
<tr>
<td></td>
<td>10 (first day of examination)</td>
</tr>
<tr>
<td></td>
<td>15</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>21</td>
<td>-</td>
</tr>
<tr>
<td>23</td>
<td>1</td>
</tr>
</tbody>
</table>

No examination on days 26 - 72 inclusive.

| 83                                                                     | 21                                  | 16.3 (13)                           | 1                                   |
| 84                                                                     | 1                                   | -                                   | -                                   |

No examination on days 85 - 137 inclusive.

Last examination, day 145.
The examination, by culture at 28°C, of the peripheral blood of an Ayshire cow infected with Trypanosoma theileri - like organisms; one ml. inocula into each of six blood agar slopes daily during the periods of examination (see also fig. 9).

<table>
<thead>
<tr>
<th>Periods of examination</th>
<th>Number of days</th>
<th>Number of cultures available for examination</th>
<th>Percentage of cultures found positive (actual number in brackets)</th>
<th>Percentage of cultures found positive by days of examination on which they were first detected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>26.10.66-15.11.66</td>
<td>21</td>
<td>126</td>
<td>2.4(3)</td>
<td>2.4(3)</td>
</tr>
<tr>
<td>30.1.67-19.2.67</td>
<td>21</td>
<td>126</td>
<td>4.0(5)</td>
<td>3.2(4)</td>
</tr>
<tr>
<td>7.4.67-27.4.67</td>
<td>21</td>
<td>126</td>
<td>70.6(90)</td>
<td>66.6(85)</td>
</tr>
<tr>
<td>19.7.67-26.7.67</td>
<td>8</td>
<td>48</td>
<td>77.1(37)</td>
<td>66.6(31)</td>
</tr>
<tr>
<td>15.8.67-4.9.67</td>
<td>21</td>
<td>126</td>
<td>47.5(60)</td>
<td>23.7(30)</td>
</tr>
<tr>
<td>20.10.67-8.11.67</td>
<td>20</td>
<td>120</td>
<td>1.7(2)</td>
<td>0</td>
</tr>
<tr>
<td>4.1.68-24.1.68</td>
<td>21</td>
<td>126</td>
<td>10.3(13)</td>
<td>8.8(11)</td>
</tr>
<tr>
<td>7.3.68-27.3.68</td>
<td>21</td>
<td>117</td>
<td>8.5(10)</td>
<td>8.5(10)</td>
</tr>
<tr>
<td>29.4.68-19.5.68</td>
<td>21</td>
<td>126</td>
<td>25.2(33)</td>
<td>21.6(27)</td>
</tr>
<tr>
<td>29.7.68-18.8.68</td>
<td>21</td>
<td>126</td>
<td>22.2(28)</td>
<td>4.8(6)</td>
</tr>
<tr>
<td>7.10.68-27.10.68</td>
<td>21</td>
<td>126</td>
<td>5.5(7)</td>
<td>0</td>
</tr>
<tr>
<td>13.1.69-2.2.69</td>
<td>21</td>
<td>126</td>
<td>3.2(4)</td>
<td>3.2(4)</td>
</tr>
</tbody>
</table>
The titration of the peripheral blood of a cow infected with T. theileri-like trypanosomes on blood agar slopes maintained at 26°C: positive cultures as numerators over the numbers of slopes inoculated as denominators.

<table>
<thead>
<tr>
<th>Date</th>
<th>Inoculum</th>
<th></th>
<th>Date</th>
<th>Inoculum</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.0 ml.</td>
<td>0.1 ml.</td>
<td>1</td>
<td></td>
<td>1.0 ml.</td>
</tr>
<tr>
<td>19.7.67</td>
<td>6/6</td>
<td>1/6</td>
<td>0/6</td>
<td>21.8.67</td>
<td>4/6</td>
</tr>
<tr>
<td>20.7.67</td>
<td>5/6</td>
<td>2/6</td>
<td>0/6</td>
<td>22.8.67</td>
<td>4/6</td>
</tr>
<tr>
<td>21.7.67</td>
<td>6/6</td>
<td>1/6</td>
<td>0/6</td>
<td>23.8.67</td>
<td>3/6</td>
</tr>
<tr>
<td>22.7.67</td>
<td>4/6</td>
<td>2/6</td>
<td>0/6</td>
<td>24.8.67</td>
<td>3/6</td>
</tr>
<tr>
<td>23.7.67</td>
<td>4/6</td>
<td>0/6</td>
<td>-</td>
<td>25.8.67</td>
<td>5/6</td>
</tr>
<tr>
<td>24.7.67</td>
<td>6/6</td>
<td>0/6</td>
<td>-</td>
<td>26.8.67</td>
<td>4/6</td>
</tr>
<tr>
<td>25.7.67</td>
<td>3/6</td>
<td>1/6</td>
<td>0/6</td>
<td>27.8.67</td>
<td>1/6</td>
</tr>
<tr>
<td>26.7.67</td>
<td>3/6</td>
<td>3/6</td>
<td>0/6</td>
<td>28.8.67</td>
<td>6/6</td>
</tr>
<tr>
<td>15.8.67</td>
<td>4/6</td>
<td>2/6</td>
<td>0/6</td>
<td>29.8.67</td>
<td>2/6</td>
</tr>
<tr>
<td>16.8.67</td>
<td>2/6</td>
<td>0/6</td>
<td>-</td>
<td>30.8.67</td>
<td>4/6</td>
</tr>
<tr>
<td>17.8.67</td>
<td>3/6</td>
<td>4/6</td>
<td>0/6</td>
<td>31.8.67</td>
<td>3/6</td>
</tr>
<tr>
<td>18.8.67</td>
<td>2/6</td>
<td>1/6</td>
<td>0/6</td>
<td>1.9.67</td>
<td>5/6</td>
</tr>
<tr>
<td>19.8.67</td>
<td>0/6</td>
<td>1/6</td>
<td>0/6</td>
<td>2.9.67</td>
<td>0/6</td>
</tr>
<tr>
<td>20.8.67</td>
<td>2/6</td>
<td>0/6</td>
<td>-</td>
<td>3.9.67</td>
<td>2/6</td>
</tr>
<tr>
<td>4.9.67</td>
<td>1/6</td>
<td>0/6</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The duration of infectivity of primary cultures of *T. theileri* - like trypanosomes on a blood agar medium maintained at 20°C to further slopes of the same medium; cultures all derived from a single Ayrshire cow, grouped according to the periods in which the cow was examined daily, and each culture checked at approximately day 21, 50, 100 and 150.

<table>
<thead>
<tr>
<th>Periods of examination</th>
<th>No. of cultures available for examination</th>
<th>Day of examination on which cultures were first found positive</th>
<th>No. of cultures dying before day 21</th>
<th>Longest periods of time for which cultures were found to have retained infectivity to other cultures</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-1.68</td>
<td></td>
<td>5 10 15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-1.68</td>
<td>6</td>
<td>6 2 0</td>
<td>15</td>
<td>15 1 0 1</td>
<td></td>
</tr>
<tr>
<td>7-3.68</td>
<td>5</td>
<td>5 0 0</td>
<td>2</td>
<td>2 1 0 0</td>
<td></td>
</tr>
<tr>
<td>27-5.68</td>
<td>21</td>
<td>17 2 2</td>
<td>4</td>
<td>15 1 0 1</td>
<td></td>
</tr>
<tr>
<td>29-4.68</td>
<td>20</td>
<td>6 7 7</td>
<td>0</td>
<td>3 6 4 7</td>
<td></td>
</tr>
<tr>
<td>19-5.68</td>
<td></td>
<td>0 0 4</td>
<td></td>
<td>1 3 0 0</td>
<td></td>
</tr>
<tr>
<td>29-7.68</td>
<td>20</td>
<td>6 7 7</td>
<td>0</td>
<td>3 6 4 7</td>
<td></td>
</tr>
<tr>
<td>19-6.68</td>
<td></td>
<td>0 0 4</td>
<td></td>
<td>1 3 0 0</td>
<td></td>
</tr>
<tr>
<td>7,10.68</td>
<td>4</td>
<td>0 0 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27,10.68</td>
<td></td>
<td>0 0 4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The relationship between the day of examination on which primary cultures of *T. theileri* - like trypanosomes at 28°C were first found positive, to their duration of infectivity to further slopes of the same medium.

<table>
<thead>
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<th>No. of cultures available for examination</th>
<th>Cultures dying before day 21</th>
<th>Longest periods of time for which cultures were found to have retained infectivity to other cultures</th>
<th>Days</th>
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<td>TOTAL</td>
<td>101</td>
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Results of attempts to grow clone populations of *T. theileri* - like trypanosomes on blood agar slopes maintained at 26°C using stablates of isolations, prepared on similar media at the same temperature, from a single infected Ayrshire cow.

<table>
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<th>History of culture prior to freezing; days at each subculture</th>
<th>Stabilate number</th>
<th>Age of culture from stabilate when used</th>
<th>Number of media slopes inoculated with single organisms</th>
<th>Number of clone populations grown</th>
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<td>7-9-67</td>
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<td>104, 6, 6</td>
<td>443</td>
<td>3</td>
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<td>1</td>
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<td>104, 6, 6</td>
<td>444</td>
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<tr>
<td>3-11-67</td>
<td>18, 6</td>
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<tr>
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<td>18, 6</td>
<td>429</td>
<td>4</td>
<td>19</td>
<td>19</td>
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<tr>
<td>3-11-67</td>
<td>18, 6</td>
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<td>6-5-68</td>
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<td>572</td>
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<tr>
<td>8-5-68</td>
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<td>572</td>
<td>4</td>
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**TOTALS**: 101  41
The examination by culture at 28°C of the peripheral blood of a group of seven Ayrshire cattle infected with *Trypanosoma theileri* - like organisms; one ml. inocula into each of six blood agar slopes for each animal on single days of observation.

<table>
<thead>
<tr>
<th>Date of examination</th>
<th>Number of cultures available for examination</th>
<th>Percentage of cultures found positive (actual number in brackets)</th>
<th>Percentage of cultures found positive by days of examination on which they were first detected</th>
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<td></td>
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<tr>
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<td>26.2 (11)</td>
<td>19.0 (8)</td>
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<td>31.0 (13)</td>
<td>28.6 (12)</td>
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<td>22.12.66</td>
<td>42</td>
<td>9.5 (4)</td>
<td>4.8 (2)</td>
</tr>
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<td>30.3.67</td>
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<td>14.3 (6)</td>
<td>11.9 (5)</td>
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<tr>
<td>25.7.67</td>
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<td>21.4 (9)</td>
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<td>19.4.68</td>
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<td>4.8 (2)</td>
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<td>28.6.68</td>
<td>36</td>
<td>25.0 (9)</td>
<td>2.8 (1)</td>
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</table>
The titration of the peripheral blood of two cows infected with *T. theileri* - like trypanosomes on blood agar slopes maintained at 28°C: positive cultures as numerators over the number of slopes inoculated as denominators.

<table>
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<th>Inoculum ml.</th>
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<td>29.4.65</td>
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</tr>
<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>1.5.65</td>
<td>2/6</td>
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<tr>
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<td>27.6.67</td>
<td>6/6</td>
</tr>
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</table>
Fig. 1. Photomicrographs of *T. theileri* - like trypanosomes in the peripheral blood of a splenectomized calf which had received (1968) pooled peripheral blood from cattle on the Romney Marsh, Kent, England (slide by courtesy of Dr. Duncan Hoyte, University of Queensland, Australia).
Fig. 2. Unequal division of T. theileri - like trypanosomes in the peripheral blood. Drawings taken from the illustrations of (a) Lühs (1905), and (b) Reichenow (1940).
Fig. 3. The morphology of *T. theileri*-like trypanosomes on culture at room temperatures. (a) Drawings taken from the illustrations of Crawley (1909); (b) Reproduction of an entire figure from Herbert (1965 a). (1) and (3) epimastigote forms in the lag phase of development. (2) trypomastigote forms. (4) and (5) elongate and dividing forms during logarithmic growth. (6), (7) and (8) globular forms found during population decline. (9) degenerate form.
Fig. 4. A working hypothesis of the life cycle of *T. theileri* - like trypanosomes constructed from evidence provided by Lühs (1905) Transcaucasia, Crawley (1912) U.S.A., Müller (1917) Germany, Kraneveld (1931) Indonesia, Carpano (1932) Eritrea and Reichenow (1940) Tanganyika.
Fig. 5. A tray for the upside-down staining of thin blood smears.
Fig. 6. A technique for cloning stercorarian trypanosomes on a blood agar culture medium maintained at 26°C. (a) the materials required. (b) the preparation of microdrops containing single organisms. (c) microscopic examination of microdrops over an humidity chamber. (d) the inoculation of a culture with a microdrop containing a single organism.
Fig. 7. Map of Scotland showing the distribution of localities where animals were examined for stercorarian trypanosomes.

Fig. 8. Map of Colombia showing the distribution of localities where animals were examined for stercorarian trypanosomes.

Caribbean lowlands.
Eastern Andean range (Cordilleros Oriental).
Veterinary Faculty, National University. Cattle, Sheep.

Eastern plains (Llanos Orientales).
Hacienda Saman. Horses.
Hacienda Lajitas. Cattle.

Magdalena valley.
Hacienda Lomitas. Cattle.

Cauca valley.
Senor Hernando Rejes. Cattle.
Santa Rosa. Cattle, Horse.
Senor E. Barney. Cattle.
Senor E. Barney. Cattle.
Fig. 9. The numbers of cultures found positive in twelve periods of examination of an Ayrshire cow infected with *Trypanosoma theileri* - like organisms, represented as a histogram differentiating the numbers of cultures first detected on day 5, or collectively day 10 and 15 after inoculation (see also Table 20).
Percentage of cultures positive

- 21 cultures positive day 5
- 21 cultures positive day 10 or day 15

Periods of examination

- Negative cultures
- Cultures positive day 5
- Cultures positive day 10 or day 15
Fig. 10. Population curves of *T. theileri* - like trypanosomes on blood agar slopes maintained at 28°C, prepared from four stabilates of cultures derived from a single Ayrshire cow (graphs prepared from the average of counts made of two cultures on any one day).

a. Stabilate TREU 566

<table>
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<td>58</td>
<td>76</td>
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b. Stabilate TREU 497

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### c. Stabilate TREU 429

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### d. Stabilate TREU 444

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Fig. 11. Photomicrographs of *T. theileri* - like trypanosomes growing on a blood agar medium maintained at 28°C; Giemsa staining. (a) single organism 5 day culture. (b) group of organisms 5 day culture showing one with several flagella (c) small group of organisms in a culture first seen positive on examination day 15.
Fig. 12. Electron photomicrographs of *T. theileri* - like trypanosomes from stabilate *M. growing on blood agar maintained at 28°C; negative staining. (a) morphology associated with organisms capable of progressive movement, (b) club shaped form, thickened terminal end of the flagellum faintly visible, (c) form with flagellum at each end.