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A new semi-defined medium for *Trypanosoma brucei* sspp.

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Summary

A new, easy to prepare semi-defined medium for the cultivation of trypanosomes of the *T. brucei* complex is introduced. Containing the two commercially available media MEM (Minimum Essential Medium) and Medium 199 TC 45 as well as 10% inactivated foetal calf serum (fcs), the medium supports optimum growth and direct adaptation of bloodstream forms. Growth characteristics, glucose uptake, amino acid utilization and the ultrastructure of trypanosomes grown in this medium are described briefly.

Key words

T. brucei sspp. – culture medium – transformation to culture form – growth requirements.

Introduction

The cultivation of hemoflagellates of the *T. brucei* complex in vitro normally requires blood or blood components. So far, the most suitable media to maintain these trypanosomes and obtain large numbers of cells are the biphasic, blood-containing media of Tobie et al. (1950), Weinman (1960) and the monophasic medium developed by Pittam (1970) and modified by Dixon and Williamson (1970). It is of great importance to have a defined or semi-defined medium available for biochemical, nutritional and immunological studies as well as investigations of the transformation of the bloodstream form to the culture form. The defined medium HX 25 by Cross and Manning (1973) is not ideal for direct adaptation of bloodstream forms and growth of culture forms.

We, therefore, decided to develop a semi-defined medium which is easy to prepare and allows to count the trypanosomes with a cell counter. In addition, it should permit good growth, using even a low inoculum (less than 10^6 cells/ml) as well as direct adaptation of bloodstream forms.

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Material and methods

Trypanosome strains

T. brucei. – STIB 246 was isolated 1971 in the Serengeti National Park from a Hartebeest and cryopreserved after one rat passage. This strain has been man-tested in 1973 at Homa Bay and was not infective when tested with one volunteer (Geigy et al., 1975).

STIB 348 is a clone derived from STIB 246 from a single bloodstream form.

STIB 366 was obtained from Dr. G. A. M. Cross (Molteno Institute, Cambridge) as Molteno S 42/030 and stabilates made after four 3-day passages in rats. This strain was originally isolated in 1966 by Dr. J. R. Baker from a warthog in Uganda.

STIB 101 is a derivative of EATRO 1093 (Geigy et al., 1967) which was isolated in 1966 from a sable antelope. After a cyclic passage (rat-Glossina-mouse) it was cryopreserved as STIB 101 in 1970.

STIB 345 is a derivative of MRC/EATRO 1529 which was isolated from an infected Glossina in Kenya. After 6 mouse passages and 5 short passages in rats it was cryopreserved as STIB 345 in 1973.

T. rhodesiense. – STIB 350 is a clone of STIB 241 which was isolated 1971 in the Serengeti National Park from a lion (Geigy et al., 1975).
This strain was man-tested in 1973 at Homa Bay.

STIB 361 was isolated from a volunteer infected with STIB 350.

T. gambiense. – EATRO 210, isolated originally by Dr. Nelson in 1959, was cryopreserved directly from a *Cercopithecus aethiops* in 1962. We received this strain in 1969 and since then it has been kept at $-70°$ C.

T. congolense. – STIB 68N is a clone of STIB 228, which was originally isolated in 1971 in the Serengeti National Park from a lion (Schläppi and Jenni, 1977).

Culture conditions

Cultures were maintained in 50 ml Falcon Tissue Culture flasks, using 5 ml monophasic medium. They were kept, without shaking, in the dark at 27° C.

For routine maintenance the cultures were transferred twice a week, using 0.3 ml inoculum and 5 ml fresh medium without antibiotics. Flasks were examined every second day directly in an inversion microscope, to check for sterility and general condition of the cultures.

Cell counting

Cell concentrations were determined, using a TOA microcellcounter, CL-661, setting the discriminator at 1.0. To get the 1:500 dilution, a 20 μl sample of culture was sterily taken out of the flask with an autoclaved Eppendorf tip and put in 10 ml of commercially available TOA Cellkit-7.

Electron microscopy

Culture forms were fixed and processed for EM, using the standard proceeding described by Steiger (1973). Ultrathin sections were cut with a diamond knife, using a LKB Ultrotome III and recorded in a Zeiss EM 9.

Glucose determination

Glucose levels in the medium were determined with a Biochemica Test Combination for Glucose, Hexokinase-Methode (Boehringer No. 15931) according to the directions of the manufacturer, using a CE 292 Digital Ultraviolet Spectrophotometer (CECIL Instruments, Cambridge).

Measurements of amino acids

Amino acid analysis of fresh and used medium was done by Dr. G. A. M. Cross, Molteno Institute, Cambridge (for the procedure see Cross et al., 1975).

Measurement of osmolarities

Osmolarities were determined with a osmometer from H. Knauer, Berlin (Germany).

Materials

Minimum Essential Medium (Eagle), for suspension culture, Cat. No. F-14; MEM Amino acids (50×); MEM Nonessential Amino acids (100×); Foetal calf serum (No. 629): GIBCO Bio Cult Ltd. Medium 199 TC 45 (powder): Wellcome Reagents Ltd. HEPES; MOPS: Calbiochem. All other chemicals used were obtained in the purest grade from Merck, Darmstadt (Germany) or Fluka, Buchs (Switzerland).

Results

Development of the medium SDM-77

Initial experiments were done with STIB 366 by testing the available media HX 25 (Cross and Manning, 1973) and HO-MEM (Berens et al., 1976). Both media turned out to be unsuitable to grow large numbers of trypanosomes and for direct adaptation of bloodstream forms. However, a mixture of the two (70% HO-MEM + 20% HX 25 + 10% foetal calf serum) gave a fairly good continuous growth, revealing 2×10^7 cells/ml after 4 days using a starting inoculum of 3×10^6 cells/ml.

We decided to take the simpler HO-MEM as a basis and add components of the more complex HX 25 in order to find the essential substances of the latter which improved growth properties of the basic HO-MEM. A first series of

Table 1. Composition and preparation of the medium SDM-77

Components per liter:		Components per liter:	
8.00 g	Minimum Essential Medium (MEM) (Eagle), for suspension culture: F-14, powder	50 mg	L-Methionine
		650 mg	L-Proline
2.00 g	Medium 199 TC 45, powder	200 mg	L-Threonine
8.00 ml	MEM amino acids (50×)	6 mg	Adenosine
6.00 ml	MEM nonessential amino acids (100×)	6 mg	Guanosine
6.00 ml	Na-pyruvate (100 mM)	10 mg	Glucosamine-HCl
2.50 g	Glucose	0.10 mg	Biotin
9.53 g	HEPES (= 40 mM)	1.00 mg	p-Aminobenzoic acid
4.18 g	MOPS (= 20 mM)		
1.00 g	$NaHCO_3$		

The components are dissolved in 900 ml glass-distilled water, the pH is adjusted to 7.25 with 5N NaOH and the volume is made up to 1 l. Finally SDM-77 is filter sterilized with a Millipore Filter 0.22 μ. 10% inactivated (30 min at 56° C) foetal calf serum and 0.25 ml hemin stock solution */100 ml medium are added before use. The medium is kept frozen in 20 ml batches at −20° C. Osmolarity without fcs: 380 milliosmol.

* 250 mg hemin are dissolved in 50 ml 0.05 N NaOH and made up to 100 ml with glass-distilled H_2O. The pH is adjusted to 8.0 with 1N HCl and autoclaved.

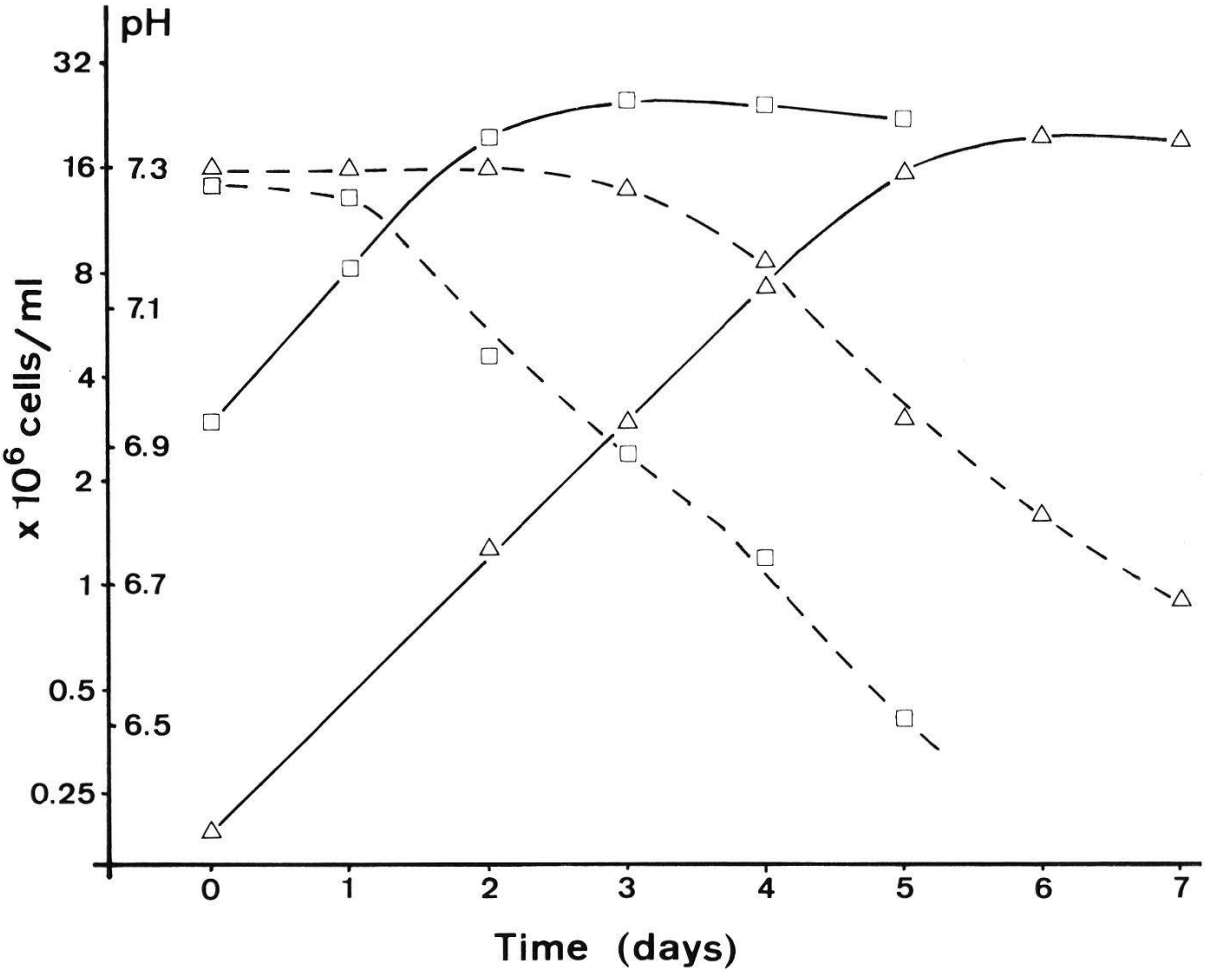

Fig. 1. Growth curve and pH of *T. brucei* STIB 246 in SDM-77 at 27° C. Two experiments are shown, the first using a starting cell density of 2×10^5 cells/ml (△), the second 3×10^6 cells/ml (□). For cell counts, a TOA microcellcounter was used.

experiments indicated that the addition of Medium 199 TC 45 (final conc. 20%) and Proline 600 mg/l gave continuous growth of *T. brucei*. A first amino acid analysis of this medium (HO-MEM with 20% 199 TC 45 and Proline 600 mg/l) showed that the amino acids threonine and methionine were used up completely. We, therefore, added more threonine (200 mg/l) and methionine (50 mg/l) and increased the concentration of buffers to 40 mM HEPES and 20 mM MOPS, in order to keep the pH more stabile. The addition of glucosamine-HCl (10 mg/l), adenosine (6 mg/l) and guanosine (6 mg/l) gave a slightly better growth, whereas all other components of HX 25 (e.g. amino acids, vitamins, salts) did not favour growth. Table 1 shows the composition and preparation of the new semi-defined medium SDM-77.

Growth characteristics

For routine maintenance as well as for growth experiments a culture was normally started with 3×10^6 cells/ml. The cells stayed in log phase for 2–3 days with a doubling time of 16–18 h. They reached stationary phase after 3 days and

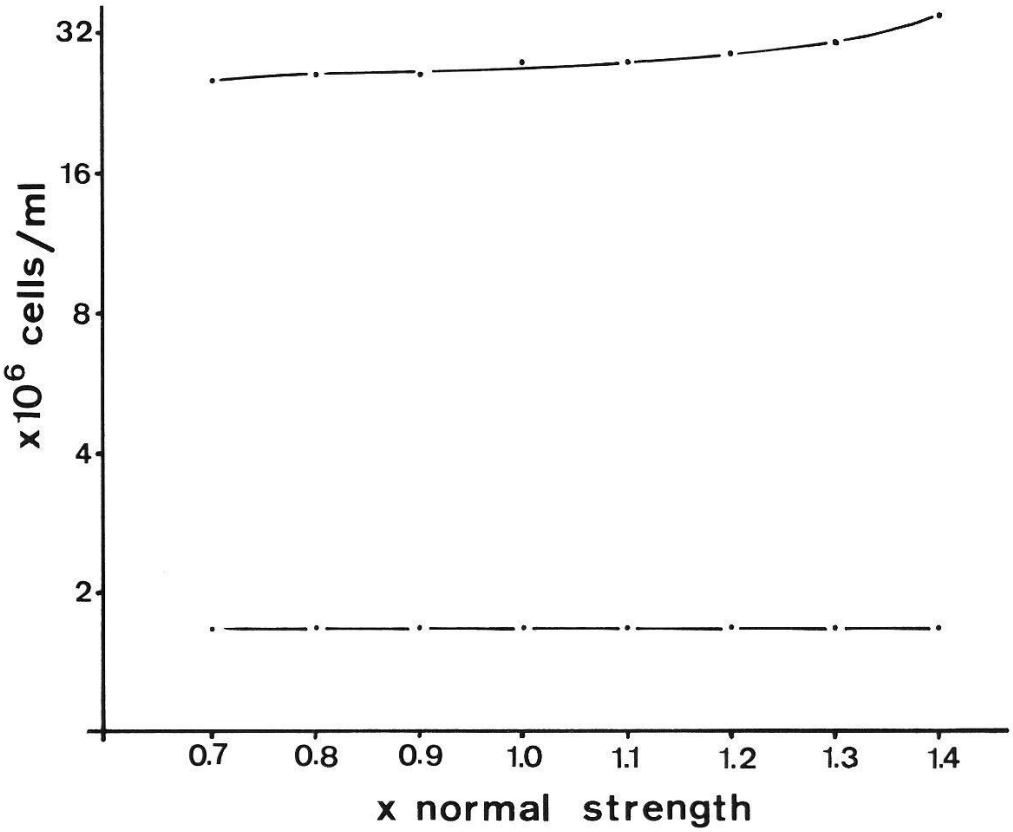

Fig. 2. Cell densities after 5 days at 27° C of different concentrations of SDM-77 (ranging from 0.7× to 1.4× normal strength) inoculated with *T. brucei* STIB 246. The inoculum for all cultures was 1.7×10^6 cells/ml. The osmolarities ranged from 282 milliosmolar for 0.7× to 500 milliosmolar for 1.4× normal strength.

showed a maximum density of $2.5–3.5 \times 10^7$ cells/ml. Log phase could easily be prolonged by using a lower inoculum (Fig. 1). In order to find the lowest possible cell density to start a culture, different inocula of STIB 246 were used in a series of experiments. 10^5 cells/ml turned out to be the lowest starting cell concentration which provided normal growth. In this culture, stationary phase was reached after 7 days revealing a final cell density of 2×10^7 cells/ml. Higher final densities could only be obtained by exchanging the medium. Starting with 10^6 cells/ml a concentration of 10^8 cells/ml was reached with 3 exchanges of the medium at days 4, 5 and 6.

It turned out that the pH is not a critical parameter in that all strains tested grew normally between pH 7.3–6.7. In order to keep the initial pH of 7.3 as long as possible we added 60 mM of buffers (20 mM MOPS, 40 mM HEPES). Depending on the number of cells used as inoculum, the pH dropped below 7.0 after 2–5 days and reached in stationary phase pH 6.5 or less (Fig. 1). Once the pH decreased to a value of 6.0 the cells started to die off. Sterile 1N NaOH may be added in log phase to get the pH back above 7.0 in order to extend the durability of the culture. This pH adjustment had no effect on the final cell density.

In order to find the most favourable osmolarity of our medium, different

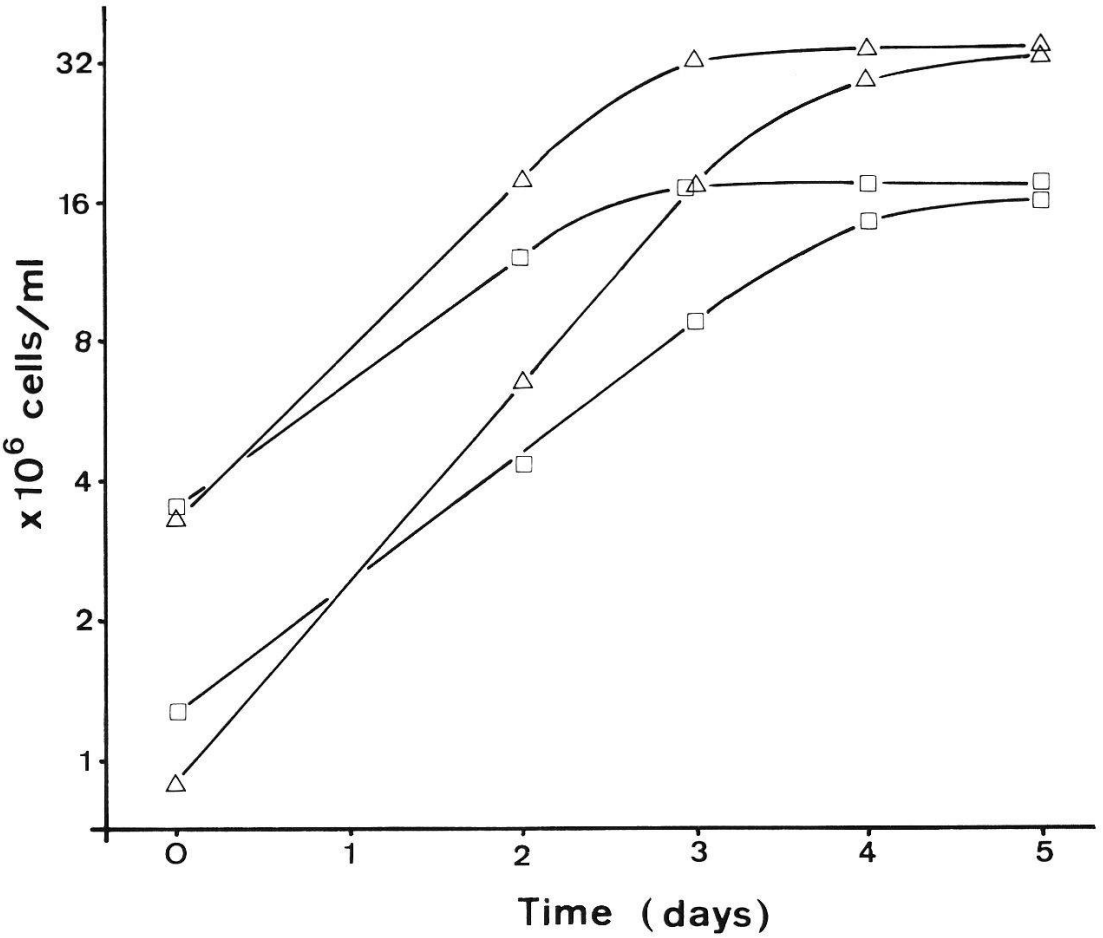

Fig. 3. Growth curves of *T. rhodesiense* STIB 350 and *T. gambiense* STIB 210 in SDM-77 at 27° C. For *T. rhodesiense* (△) two experiments are shown using 9×10^5 cells/ml and 3.4×10^6 cells/ml as inoculum. For *T. gambiense* (□) the inocula were 1.3×10^6 cells/ml and 3.5×10^6 cells/ml. Cell counts were determined with a TOA microcellcounter.

concentrations of SDM-77, ranging from 0.7× to 1.4× normal strength, were inoculated with strain 246. The measured osmolarities of these different concentrations, including 10% inactivated foetal calf serum (fcs), ranged from 282 milliosmolar for 0.7× to 500 milliosmolar for 1.4×. Cell densities after 5 days (using an inoculum of 1.7×10^6 cells/ml) are shown in Fig. 2. With the higher medium concentrations (1.2×, 1.3× and 1.4× normal strength) the cells remained slightly longer in log phase. Starting with 1.7×10^6 cells/ml the densities after 5 days were 2.55×10^7 cells/ml for 0.7×, 2.81×10^7 cells/ml for 1.0× and 3.56×10^7 cells/ml for 1.4×. The pH dropped much less in the higher medium concentrations (pH 6.44 for 0.7×, 6.65 for 1.0× and 6.80 for 1.4×) which contained a proportional higher amount of buffers.

In two other experiments NaCl and DMSO were added to normal strength SDM-77 in order to raise the osmolarity without changing the concentration of its components. Using a range from 335 to 609 milliosmolar, best growth was observed in osmolarities between 335 and 460. However, these two experiments

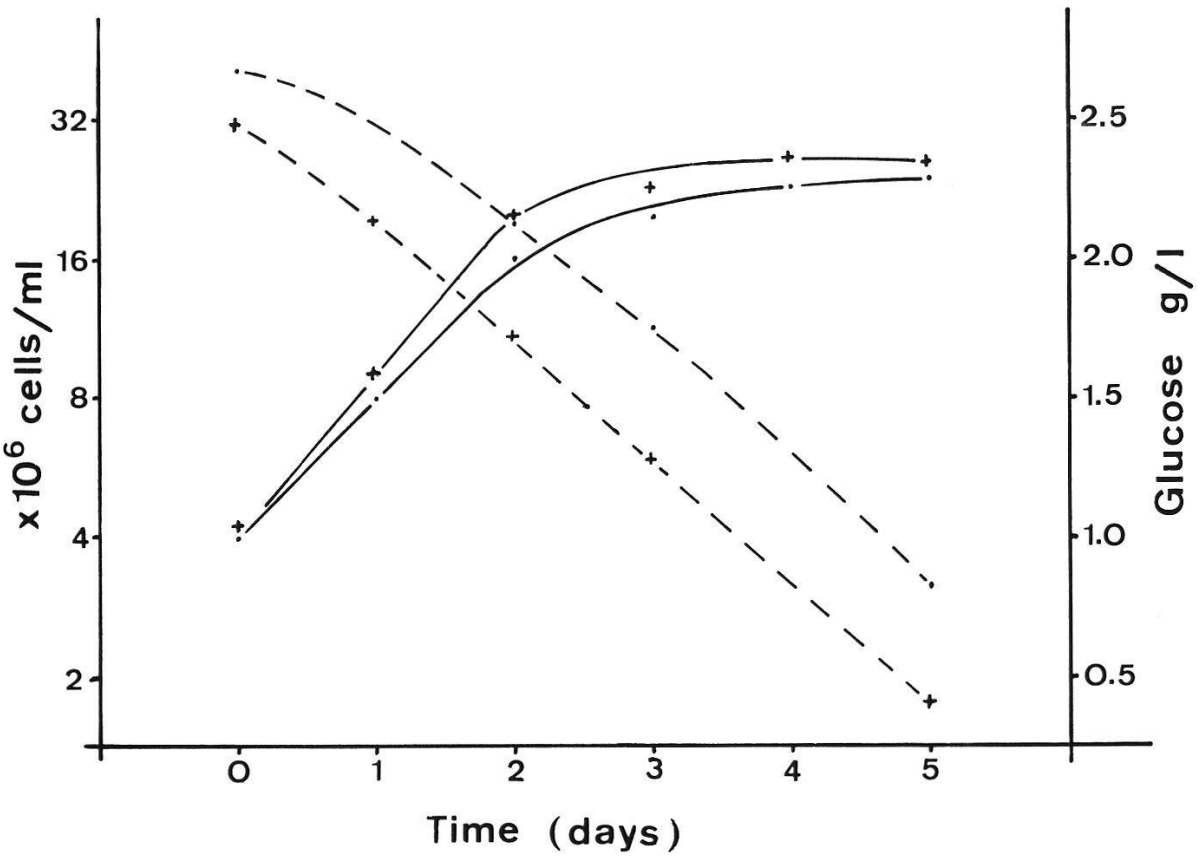

Fig. 4. Growth curve and remaining glucose of *T. brucei* STIB 246 in SDM-77 at 27° C. Two experiments are shown, the first (·) using an initial glucose concentration of 2.68 g/l and the second (+) 2.50 g/l glucose. For both experiments the inoculum was 4×10^6 cells/ml.

have to be considered with care in that the effect on growth might not only be related to the osmolarity but to the effect of NaCl or DMSO resp. added.

T. rhodesiense STIB 350 showed growth properties very similar to those of *T. brucei* STIB 246. The doubling time was 17.0 h and the final cell density after 5 days (3.53×10^7 cells/ml) was slightly higher compared to STIB 246 (Fig. 3).

T. gambiense STIB 210 had a longer doubling time (25.5 h) than STIB 350 and STIB 246. The cell concentration reached after 5 days ($1.5–2.0 \times 10^7$ cells/ml) was distinctly lower for this strain compared to all the others (Fig. 3).

All strains listed in «Material and methods» have been grown in SDM-77 for more than three months.

Glucose consumption

The glucose consumption was determined in several experiments. Two examples are shown in Fig. 4. Glucose was used in log phase as well as in stationary phase. The decrease of the remaining glucose was more or less linear. At the beginning of the stationary phase about half of the initial glucose was left. This indicated that glucose is not a growth limiting factor and, therefore, an initial concentration of 2.5–3.0 g/l seemed to be adequate.

Table 2. Changes in extracellular amino acid concentrations during growth of *T. brucei* STIB 246 in SDM-77

	Initial concentration (mM)	2 days culture (mM)	5 days culture (mM)	△ within 5 days (mM)
Alanine	0.32	1.43	3.30	+2.98
Arginine	0.68	0.49	0.28	−0.40
Aspartic acid	0.18	0.27	0.70	+0.52
Glutamic acid	0.36	0.35	0.29	−0.07
Glycine	0.29	1.53	1.55	+1.26
Histidine	0.26	0.22	0.22	−0.04
Isoleucine	0.47	0.38	0.27	−0.20
Leucine	0.67	0.47	0.23	−0.44
Lysine	0.55	0.46	0.36	−0.19
Methionine	0.45	0.39	0.14*	−0.31
Ornithine	0.10	0.21	0.37	+0.27
Phenylalanine	0.34	0.24	0.10	−0.24
Proline	5.40	4.30	2.75	−2.65
Serine	0.20	0.12	0.12	−0.08
Threonine** + Glutamine	2.66	0.17	0.08	−2.58
(Threonine)***	(1.28)			(−1.20 to −1.28)
Tyrosine	0.26	0.20	0.06	−0.20
Valine	0.60	0.44	0.48	−0.12
Glucose	14.88	11.88	4.44	−10.44
pH	7.35	7.13	6.70	
Cell number	4.4×10^6/ml	1.6×10^7/ml	2.7×10^7/ml	

* In three other experiments the methionine was metabolized completely.

** The programme used for amino acid analysis did not permit threonine, glutamine and asparagine to be resolved. However, asparagine was added initially at a concentration <0.1 mM.

*** According to the amount added the initial concentration of threonine was 1.28 mM. The measured concentration of threonine + glutamine after 5 days was 0.08 mM and represents either threonine or glutamine. Therefore, the amount of threonine metabolized could not be determined exactly, however, it has to be between 1.20 mM and 1.28 mM.

Changes of amino acid levels during growth

Changes in amino acid concentrations in the medium during growth were measured. One out of 6 experiments is presented in Table 2. Amino acid analysis was done for the unused SDM-77 as well as for used media (2 days and 5 days). After 5 days, 4 of the amino acids measured showed increased levels: alanine (+2. 98 mM), glycine (+1.26 mM), aspartic acid (+0.52 mM) and ornithine (+0.27 mM). The low initial ornithine concentration (0.1 mM) derived most probably from the foetal calf serum, because this amino acid was not added separately. Proline and threonine + glutamine (also containing asparagine in an initial concentration of 0.1 mM) were metabolized in a significant amount (−2.65 mM and −2.58 mM resp.). The initial value for threonine

+ glutamine should have been higher according to the amounts of amino acids added. A possible explanation for this might be the instability in solution of glutamine which is included in the powdered media MEM F-14 and 199 TC 45 (components of SDM-77).

Levels in arginine, isoleucine, leucine, lysine, methionine, phenylalanine, tyrosine and valine decreased within 5 days in a range of 0.1 to 0.4 mM, whereas glutamic acid, histidine and serine were metabolized in amounts below 0.1 mM. Methionine was utilized completely in 3 cultures after 6 days.

Adaptation of blood stream forms to SDM-77

Blood from an infected rat was used from the first or second peak parasitaemia showing a high percentage of stumpy forms. 5 ml blood were taken sterily from the heart using approx. 20% of a sterile 3.8% sodium citrate solution. The blood was transferred to a 20 ml universal bottle and 1.7 ml medium and 0.1 ml glucose solution (200 mg/ml) added. After a 4 h incubation period at 27° C another 3.3 ml medium and 0.1 ml glucose solution were added. After another 4 h we added another 5.0 ml medium and 0.2 ml glucose solution. 18 to 22 h after the blood has been taken the blood/medium mixture was centrifuged for 20 min at 50 g, the red blood cells discarded and the supernatant centrifuged for 10 min at 1000 g to concentrate the trypanosomes. The pellet was resuspended in medium in a 50 ml falcon flask to a final cell concentration of approx. 2×10^7 cells/ml. 500 U Penicillin G and 500 μg streptomycin per ml were added. After 24 h and 48 h the medium was exchanged by centrifugation at 1000 g. Morphological changes started after 4 h, and after 18 to 22 h the first fully transformed cells could be seen in division in the blood/medium mixture. There was always a percentage of cells (mainly slender forms) that never transformed but persisted for 2–3 days before they died. The transformed cells started to multiply immediately after their cytodifferentiation. It took about one week after the isolation from the mammalian host to get an established population of dividing culture forms free of red blood cells and dead bloodstream forms.

All the used strains of *T. brucei*, *T. rhodesiense* and *T. gambiense* were adapted to the medium at least two times. The method used is very efficient and enables the isolation and adaptation of parasites from even smaller quantities of blood, such as 1 ml (e.g. from a mouse).

T. congolense STIB 68N could easily be adapted into our medium but not continuously cultured. The bloodstream forms transformed and started to multiply. However, after 2–3 subcultures the cells became swollen, containing large vacuoles, stopped dividing and finally died off.

Ultrastructure

It is not the intention of this paper to describe the ultrastructure of culture forms but rather to demonstrate that trypanosomes grown in SDM-77 exhibit a

normal ultrastructural morphology. Fig. 5 and Fig. 6 show longitudinal to oblique sections of two cells containing kinetoplast, mitochondrion, nucleus and flagellum. Fig. 7 is a cross section with kinetoplast and parts of the mitochondrion which contains a few cristae.

Discussion

The semi-defined medium SDM-77 satisfies the need of having a medium available to grow flagellates of the *T. brucei* complex in large numbers and to adapt bloodsteam forms directly. The basis of SDM-77 are two commercially available media: MEM F-14 (GIBCO Bio Cult Ltd.) and Medium 199 TC 45 (Wellcome Reagents Ltd.). MEM is the main component of semi-defined media for *T. cruzi* (O'Daly, 1975) and *Leishmania* (Berens et al., 1976). Medium 199 is a component of the defined Medium HX 25 for *T. brucei* sspp. (Cross and Manning, 1973).

Our method to adapt bloodstream forms to SDM-77 differs from that of most other workers in that we use a mixture of infected blood and medium to initiate transformation before the blood cells are removed after 18 to 22 h. Due to this procedure or to the properties of our medium, transformed cells start to multiply immediately after transformation. This differs from the results of Cross and Manning (1973) where multiplication was not evident before day 20. All the trypanosome strains used in this study could be adapted and continuously grown in SDM-77. *T. congolense* STIB 68N transformed as well as *T. brucei* but could only be grown for 2–3 subcultures.

The 60 mM buffers added could not hold the initial pH for more than 2–5 days (depending on the inoculum). The cells were able to grow in a pH range of 7.4 to 6.5; only a pH below 6.0 led to the death of a culture.

Different attempts to find the optimum osmolarity of our medium were undertaken either by increasing the concentration of the medium or by adding a small molecule (NaCl and DMSO). By using higher medium concentrations we also increased the concentration of a possible limiting component which might compensate the inhibitory action of a high osmolarity. By adding NaCl or DSMO in order to increase the osmolarity we either raised the levels of Na^+ and Cl^- or introduced a not inert substance. Therefore, it seemed reasonable to keep the medium concentration at normal strength (osmolarity with 10% fcs: 370 milliosmol).

Fig. 5–7. *T. brucei* STIB 101 culture forms grown in SDM-77.

Fig. 5. Longitudinal section containing kinetoplast (K), flagellum (F), Golgi apparatus (G) and nucleus (N). 16500×.

Fig. 6. Longitudinal to oblique section of trypomastigote form showing a large kinetoplast (K), prior to division, and parts of the mitochondrion (M). 14050×.

Fig. 7. Transverse section in higher magnification containing lipid inclusions (L) and the kinetoplast (K) connected with the mitochondrion. 27300×.

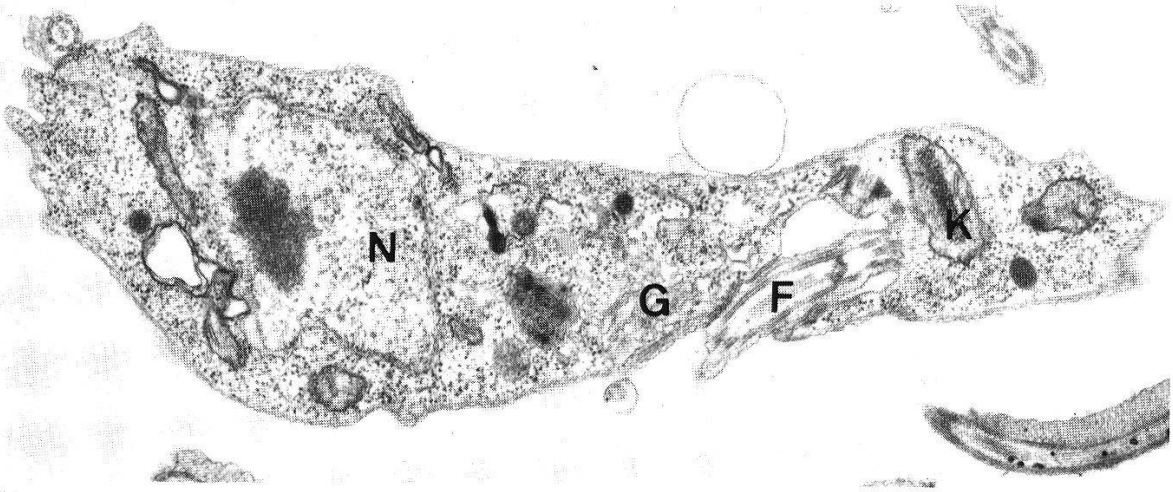

Fig. 5

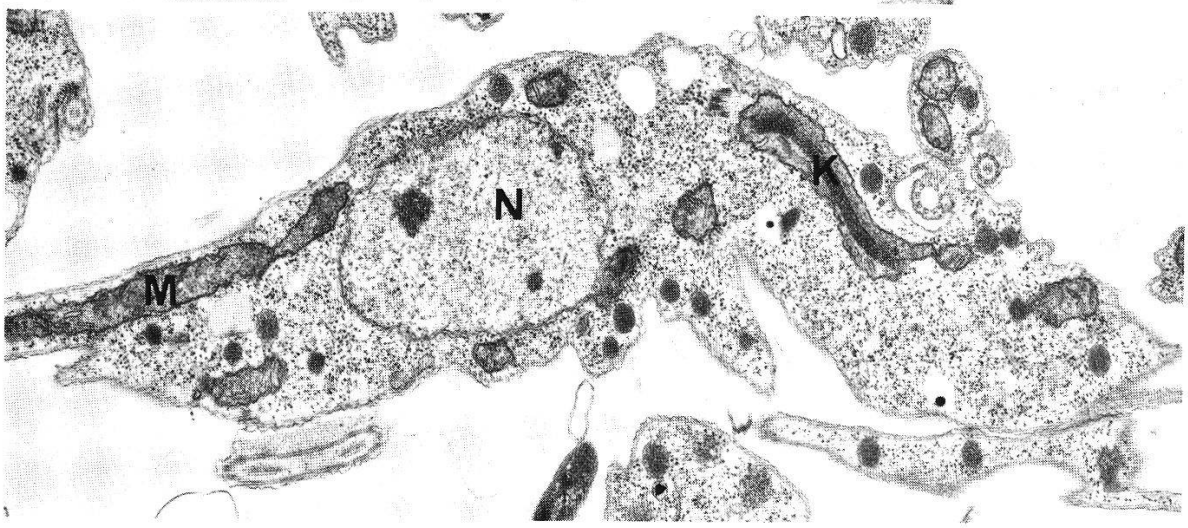

Fig. 6

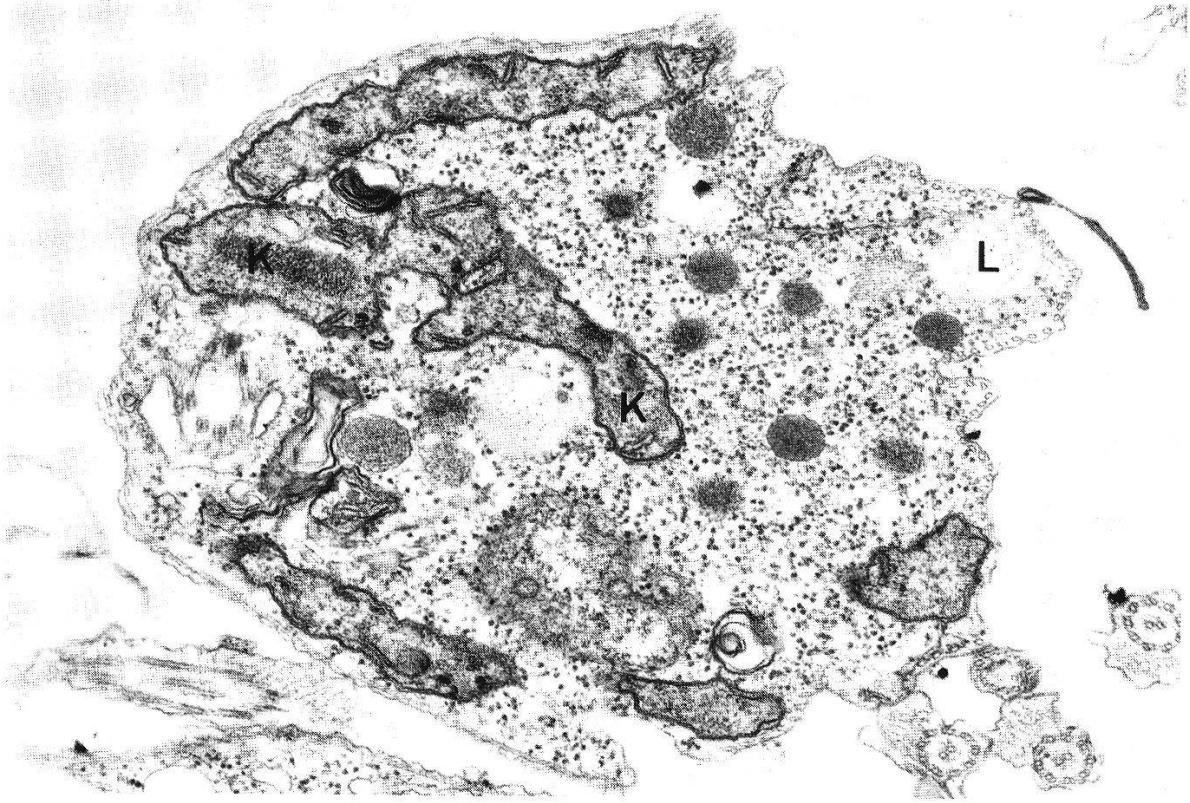

Fig. 7

Glucose was consumed in log phase as well as in stationary phase. The remaining glucose decreased more or less linearly. In some experiments, especially when the inoculum was low (less than 10^6 cells/ml) a delayed utilization of the sugar was noted. Regarding the much lower cell density after inoculation, compared to late log phase, the glucose consumption per cell in early log phase might be as high as in stationary phase or even higher. Our results confirm the findings of Cross et al. (1975) but are in contrast to the findings of other workers. Evans and Brown (1972) stated that *T. brucei* does not utilize glucose before stationary phase is reached. Also for *Leishmania* delayed glucose consumption has been reported: Mukkada et al. (1974) for *L. tropica* and Marr and Berens (1977) for *L. donovani* and *L. braziliensis.*

The results of the amino acid analysis of fresh and used media support the view that proline and threonine play a major role in *T. brucei* metabolism. Srivastava and Bowman (1971) and Bowman et al. (1972) reported a high oxidation rate of proline in culture forms of *T. rhodesiense.* Midgut forms of *T. brucei,* corresponding physiologically to culture forms, show a high proline oxidation, too (Haston, 1975). Although SDM-77 contains 5.4 mM proline the cells never used more than 3.0 mM. In contrast to this threonine + glutamine was utilized almost completely. Cross et al. (1975) demonstrated that threonine could be metabolized completely, even when it was added at higher concentrations, forming acetate and glycine. In our experiments glycine was excreted to an amount corresponding to the threonine metabolized.

The unused medium contained 0.1 mM ornithine. This amino acid was not added to SDM-77 nor was it present in the MEM F-14 or 199 TC 45. The most probable source could be the 10% fcs we added. The used media showed elevated levels of ornithine. *Crithidia fasciculata* metabolizes arginine to proline (G. A. M. Cross, personal communication). The ornithine in our used media would be an intermediate in the conversion of arginine to proline. A step of this pathway could be inhibited by the high proline concentration (even in used media) and result in an excretion of ornithine.

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1 Berens R. L., Brun R., Krassner S. M.: A simple monophasic medium for axenic culture of hemoflagellates. J. Parasit. *62,* 360–365 (1976).

2 Bowman I. B. R., Srivastava H. K., Flynn I. W.: Adaptations in oxidative metabolism during the transformation of *Trypanosoma rhodesiense* from bloodstream into culture form. In: Comparative biochemistry of parasites, p. 329–342. Academic Press, London 1972.

3 Cross G. A. M., Manning J. C.: Cultivation of *Trypanosoma brucei* sspp. in semi-defined and defined media. Parasitology *67,* 315–331 (1973).

4 Cross G. A. M., Klein R. A., Linstead D. J.: Utilization of amino acids by *Trypanosoma brucei* in culture: L-threonine as a precursor for acetate. Parasitology *71*, 311–326 (1975).
5 Dixon H., Williamson J.: The lipid composition of blood and culture forms of *Trypanosoma lewisi* and *Trypanosoma rhodesiense* compared with that of their environment. Comp. biochem. Physiol. *33*, 111–128 (1970).
6 Evans D. A., Brown R. C.: The utilization of glucose and proline by culture forms of *Trypanosoma brucei*. J. Protozool. *19*, 686–690 (1972).
7 Geigy R., Kauffmann M., Beglinger R.: A survey of wild animals as potential reservoirs of trypanosomiasis in the Ulanga District (Tanzania). Acta trop. (Basel) *24*, 97–108 (1967).
8 Geigy R., Jenni L., Kauffmann M., Onyango R. J., Weiss N.: Identification of *T. brucei*-subgroup strains isolated from game. Acta trop. (Basel) *32*, 190–205 (1975).
9 Haston W.: Substrate utilization in midgut forms of *Trypanosoma brucei*. J. Protozool. *22*, 52A (1975).
10 Marr J. J., Berens R. L.: Regulation of aerobic fermentation in protozoans. VI. Comparative biochemistry of pathogenic and non-pathogenic protozoans. Acta trop. (Basel) *34* (1977) (in press).
11 Mukkada A. J., Schaefer F. W., Simon M. W., Neu C.: Delayed *in vitro* utilization of glucose by *Leishmania tropica* promastigotes. J. Protozool. *21*, 393–397 (1974).
12 O'Daly J. A.: A new liquid medium for *Trypanosoma* (Schizotrypanum) *cruzi*. J. Protozool. *22*, 265–270 (1975).
13 Pittam M. D.: Medium for *in vitro* culture of *Trypanosoma rhodesiense* and *T. brucei*. Appendix to Dixon & Williamson (1970) [5].
14 Schläppi B., Jenni L.: Studies on antigenic variation of cyclically transmitted *Trypanosoma congolense*. Acta trop. (Basel) *34*, 43–51 (1977).
15 Srivastava H. K., Bowman I. B. R.: Metabolic transformation of *Trypanosoma rhodesiense* in culture. Nature new Biol. *235*, 152–153 (1972).
16 Steiger R. F.: On the ultrastructure of *Trypanosoma (Trypanozoon) brucei* in the course of its life cycle and some related aspects. Acta trop. (Basel) *30*, 64–168 (1973).
17 Tobie E. J., Brand T. von, Mehlman B.: Cultural and physiological observations on *Trypanosoma rhodesiense* and *Trypanosoma gambiense*. J. Parasitol. *36*, 48–54 (1950).
18 Weinman D.: Cultivation of the african sleeping sickness trypanosomes from the blood and cerebrospinal fluid of patients and suspects. Trans. roy. Soc. trop. Med. Hyg. *54*, 180–190 (1960).