the bottom of an empty well, the plate covered immediately and the drop examined in an inverted phase contrast microscope. If neither of two observers could detect more than one trypanosome, 100 μl of the following cloning medium was added to the well:

- 6.0 ml SDM-79 (with haemin and 10% inactivated FBS), 3–4 days conditioned by STIB 366 procyclic culture forms
- 6.0 ml SDM-79 (with haemin)
- 2.0 ml double distilled water
- 1.3 ml inactivated FBS
- 0.4 ml penicillin/streptomycin (100×)
- Filter sterilized with Millipore 0.22 μm
- Tonicity: 290 milliosmolar.

The well was covered with Parafilm and the plate was sealed by a lid. After 7 days, 50 μl medium was carefully removed from the top of the well and 100 μl fresh cloning medium was added. After about 2 weeks or whenever the well showed a sufficient cell density, the whole well was transferred into a T-25 tissue culture flask with 3.0 ml medium. In one experiment on day 19 when the cell density reached 6×10^7/ml, the first subculture could be made.

Besides its excellent properties for the trypanosomes of the *T. brucei* subgroup, SDM-79 proved to be a very good culture medium for promastigotes of *Leishmania* species. *Leishmania donovani. L. tropica* and *L. braziliensis* were grown with two weekly subcultures for over one year in this medium revealing growth characteristics similar to *T. brucei.*

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Cultivation and in vitro cloning of procyclic culture forms of *Trypanosoma brucei* in a semi-defined medium*

Short communication

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Attempts to cultivate the vector forms of *Trypanosoma brucei* in defined media have made good progress in the last few years. Good growth of most *T. brucei* stocks which were tested could be achieved in semi-defined media (supplemented with 5–10% foetal bovine serum): RE I (Steiger and Steiger, 1976) and modifications of this medium (R. Steiger, personal communication); SDM-77 (Brun and Jenni, 1977); SM (Cunningham, 1977). Fully defined media (e.g. HX 25, Cross and Manning, 1973) are still unsuitable for most *T. brucei* stocks.

The medium SDM-77 has been used extensively in our laboratory and has been improved several times, resulting in the modification SDM-79 (Tab. 1). The main alterations are the following: lower concentration of glucose; higher concentrations of Na-pyruvate, Na-bicarbonate, adenosine and guanosine; addition of folic acid and a group of amino acids which are known to be present in tsetse fly haemolymph (Cunningham and Slater, 1974).

Several strains and stocks of *T. brucei* and *T. b. rhodesiense* were grown for several months in T-25 tissue culture flasks containing 5 ml of medium at 27° C, with two weekly subcultures. The trypanosome material was the same as used in an earlier investigation (Brun and Jenni, 1977). Additionally, the monomorphic derivatives of the *T. brucei* stocks of S427 and LUMP 227 were also grown.

The growth characteristics of *T. brucei* stock STIB 366 (a derivative of S42/030) is given in Fig. 1. In SDM-79 the maximum cell density after 4 days was about twice that in SDM-77. The stationary phase in SDM-79 (with 5% or 10% inactivated FBS) is less than 24 h; the high cell density after 4 days very rapidly makes the medium unsuitable for the growth and survival of the trypanosomes. With a lower serum concentration of 2.5% the trypanosomes remain longer in the stationary phase but the final cell density is lower than with higher serum concentrations.

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Table 1. Composition and preparation of the medium SDM-79

Components per liter:

7.0 g	MEM, F-14, powder (GIBCO Bio Cult)
2.0 g	Medium 199 TC 45, powder (Wellcome)
8.0 ml	MEM amino acids (50×), w/o L-glutamine (GIBCO Bio Cult)
6.0 ml	MEM nonessential amino acids (100×) (GIBCO Bio Cult)
1.0 g	glucose
8.0 g	HEPES (= 33.6 mM)
5.0 g	MOPS (= 24.0 mM)
2.0 g	$NaHCO_3$
100 mg	Na-pyruvate
200 mg	L-alanine
100 mg	L-arginine
300 mg	L-glutamine
70 mg	L-methionine
80 mg	L-phenylalanine
600 mg	L-proline
60 mg	L-serine
160 mg	L-taurine
350 mg	L-threonine
100 mg	L-tyrosine
10 mg	adenosine
10 mg	guanosine
50 mg	glucosamine-HCl
4 mg	folic acid
2 mg	p-aminobenzoic acid
0.2 mg	biotin

The pH is adjusted with 2N NaOH to 7.3 and the medium filter sterilized with a Millipore filter 0.22 μm. Bovine haemin stock solution (2.5 mg/ml in 0.05 N NaOH) is autoclaved and added at a final concentration of 7.5 mg/l. Before use the medium is supplemented with 2.5 to 10% inactivated foetal bovine serum (GIBCO Bio Cult).
Tonicity of complete medium: 330 milliosmolar.

Two different trypanosome sources can be used to initiate a culture: 1. bloodstream forms from an infected mammalian host, 2. midgut forms from an infected *Glossina*. Bloodstream forms are the common source for all pleomorphic strains. Midgut forms from infected tsetse flies are often the only possibility to initiate a culture of monomorphic strains which hardly transform to culture forms in vitro.

To adapt bloodstream forms to culture conditions a parasitaemia with mainly intermediate and stumpy forms is needed (Brown et al., 1973; Ghiotto et al., in press). Citrated heart blood from a mouse or a rat was mixed with an equal volume of medium. After centrifugation for 7 min at 1000 *g* the supernatant was discarded and the pellet (trypanosomes and blood cells) resuspended in SDM-79 to a trypanosome concentration of about 5×10^6/ml. The medium was supplemented with additional glucose (3 mg/ml) and penicillin/

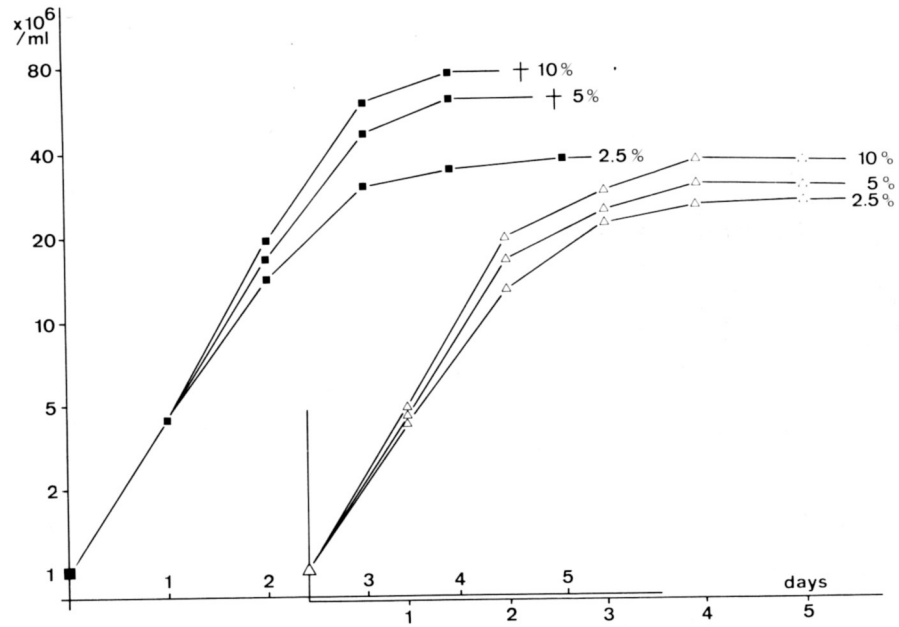

Fig. 1. Growth curves of established procyclic culture forms of *Trypanosoma brucei* STIB 366 at 27° C in SDM-79 (——■——) and SDM-77 (——△——) supplemented with 2.5%, 5% and 10% inactivated foetal bovine serum. † = death of the culture.

streptomycin (250 U/ml, 250 μg/ml respectively). After 24 h the medium was exchanged by centrifugation. After 48 h the blood cells were removed by slow centrifugation for 20 min at 50 *g*. The trypanosomes were pelleted from the supernatant (7 min at 1000 *g*) and resuspended in SDM-79 without additional glucose and with lower concentrations of antibiotics.

Whenever bloodstream forms cannot be adapted to in vitro conditions, midgut forms from infected tsetse flies are an excellent source for the initiation of a culture. An infected *Glossina* was killed and dissected under sterile conditions. The midgut was removed, opened and the gut forms transferred into SDM-79 with 500 U/ml penicillin and 500 μg/ml streptomycin for the first 48 h.

To find out the lowest number of trypanosomes required for subcultivation in SDM-79, different inocula ranging from 10^6/ml to 10^3/ml were checked. For *T. brucei* STIB 366 an inoculum of 10^3/ml was sufficient to start a new subculture. After 14 days the cell density in this culture reached 6×10^7/ml. Based on this finding, we tried to clone STIB 366 in vitro. For that purpose a good culture in logarithmic growth phase was diluted to approx. 10^4 trypanosomes per ml. All the peripheral wells of a Micro Test II tissue culture plate (Falcon) were filled with sterile distilled water to provide a humid atmosphere during the period of observation. With a drawn out capillary tube a tiny drop was placed at