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A NEW LIQUID MEDIUM WITHOUT BLOOD AND SERUM FOR CULTURE OF HEMOFLAGELLATES

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Abstract. A liquid medium without blood or serum was developed for cultivation of hemoflagellates. To a basic LIT medium containing liver infusion broth and tryptose, a mixture of RPMI 1640 and Medium 199 was added. This combination permitted high parasite yields useful for biochemical and immunological studies.

Many different media have been used for the cultivation of hemoflagellates. These media can be classified into three major categories: biphasic, semi-solid and liquid media. All principal biphasic and semi-solid media require blood as an important factor for replication of the parasites,[1–3] and most other liquid media require fetal bovine serum (FBS) or blood as an essential factor.[4–10] Disadvantages of these media include the expense of FBS and the fact that blood components complicate immunological and biochemical studies.

In the present paper we report a monophasic medium without blood or serum which is easy to prepare and which supports continuous growth after repeated subcultures yielding large numbers of parasites.

MATERIALS AND METHODS

The composition of the basic medium (LIT) was liver infusion broth (Difco) 2.5 g, tryptose (Difco) 5 g, NaCl 4 g, glucose 2 g, KCl 0.4 g, and Na_2HPO_4 3.15 g, dissolved in 1 liter of water distilled twice. After adjusting the pH to 7.4, the medium was autoclaved, cooled and 25 mg of hemin, dissolved in 1 ml of 1 N NaOH was added.

To this basic medium 1% of a 20× mixture of RPMI 1640 and Medium 199 (Difco) was added just before use. A 1-liter package of Medium 199 was added to a 1-liter package of RPMI 1640 and both were mixed with 100 ml of water to yield a 20× concentrated solution. The pH was adjusted to 7.4 with $NaHCO_3$. This concentrate is referred to as R9.

The parasites were cultured in 15-ml test tubes containing 10 ml of medium or in 72-cm^2 plastic culture flasks containing 25 ml of medium, at 25°C without shaking. Flasks were incubated horizontally.

Parasite growth in the following media was compared: LIT + R9, NNN,[1,2] Warren's medium,[4] LIT + 5% heat-inactivated FBS, LIT without additives and the medium of Berens et al.[10] + 5% FBS. Several combinations of LIT medium and R9 were tested.

Parasites

Leishmania mexicana amazonensis (MHOM/BR/76/LTB 012 Josefa) and *L. donovani chagasi* (MHOM/BR/79 LI 01 Imperatriz) and one strain of *Trypanosoma cruzi* were cultivated. These *Leishmania* were typed by isoenzymes, monoclonal antibodies and DNA buoyant density and were provided by Philip Marsden (University of Brasilia). The strain of *T. cruzi*, isolated from a human patient, was provided by Sonia Andrade (Federal University of Bahia).

Infectivity of parasites after 80, 50 and 10 subcultures for *L. chagasi*, *T. cruzi* and *L. m. amazonensis*, respectively, was tested in hamsters and in BALB/c mice.

SDS-PAGE of different concentrations (1×, 5× and 10×) of the medium was done using the method of Laemmli.[11]

RESULTS

A comparison of the growth of *L. d. chagasi* in different media in 15-ml test tubes is shown in Figure 1. The growth in NNN, Warren's medium, LIT + 5% FBS, Berens et al. medium + 5% FBS, and LIT + 1% R9 was very similar. The stationary phase occurred between days 4

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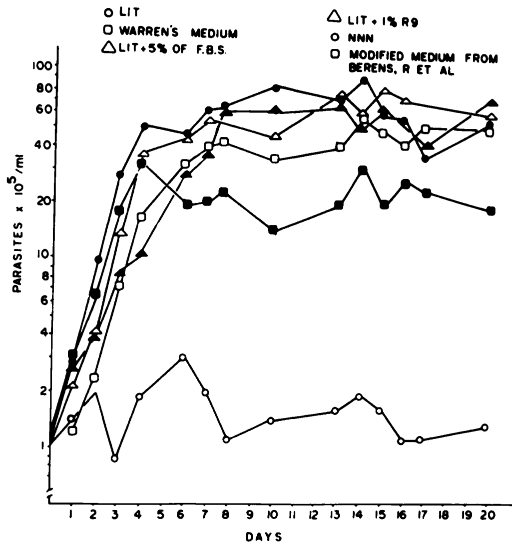

FIGURE 1. Growth curves of *L. d. chagasi* in different media. Cultures were incubated at 25°C in 15-ml test tubes containing 10 ml of medium.

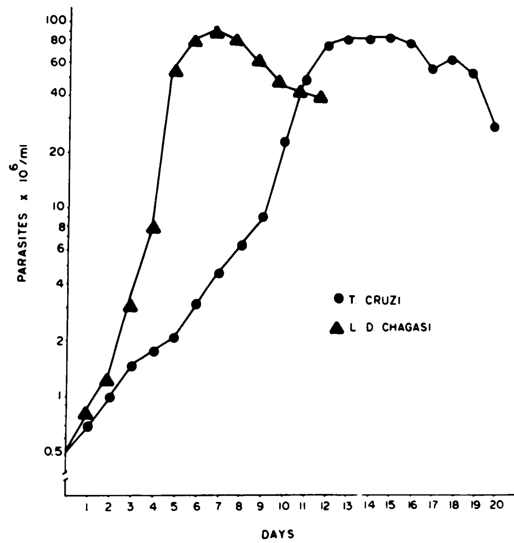

FIGURE 2. Growth curves of *L. d. chagasi* and *T. cruzi* in LIT + 1% R9. Cultures were incubated in a 72-cm² flask containing 25 ml of medium.

and 7. The basic LIT medium did not support proliferation of the parasites.

Figure 2 represents the growth of *L. d. chagasi* and *T. cruzi* in 72-cm² flasks containing 25 ml of medium. Under these conditions a maximum of 9×10^7 *L. d. chagasi* promastigotes per ml was observed and the stationary phase occurred around the 12th day. At the 20th day approximately 80% of the *T. cruzi* were the infective form, trypomastigotes, as determined morphologically. The growth of *L. m. amazonensis* was very similar to that of *L. d. chagasi.*

Infectivity of the three different species of parasites was consistently observed. Hamsters infected with 5×10^7 promastigotes of *L. d. chagasi* at the stationary phase had severe disease after 4 months of infection.

Cutaneous lesions of *L. m. amazonensis* in BALB/c mice infected subcutaneously with 1×10^7 promastigotes occurred after the second week. Blood forms of *T. cruzi* were seen in BALB/c mice infected with 10^5 parasites after the 3rd day of infection, and the maximum number of parasites occurred at the 14th day.

SDS-PAGE of the medium did not show any protein band.

DISCUSSION

The LIT + 1% R9 medium without use of blood or serum promoted good growth of parasites. It is very easy to prepare and is less expensive than most other liquid media. Medium 199 and RPMI 1640, both with FBS, have been used by other authors.[10,12] The absence of blood or serum did not affect infectivity of the parasites. The data show the importance of combining LIT with RPMI 1640 and Medium 199. The combination of 1 ml of R9 to 100 ml of LIT yielded the maximum number of parasites and made the medium less expensive, but the addition of more than 2 ml of R9 also gave a good yield. The hyperosmolarity due to the addition of the concentrated mixtures of RPMI and Medium 199 did not seem to affect the parasites.

The defined medium of Berens and Marr[13] did not give good growth to *L. d. chagasi* or *T. cruzi* and it was not possible to maintain the parasites for more than three serial passages (data not shown).

The advantage of our new medium is the possibility of obtaining parasites free of serum contaminants and suitable for biochemical and immunological studies. Labeling of parasites with radioisotopes such as ^{35}S is easy, since it is possible to transfer parasites to RPMI medium without methionine and then add ^{35}S-methionine. Another important advantage of this medium is for the study of metabolic products of the parasites and purification of these products without contaminants.

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