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ESTABLISHMENT OF *ENTAMOEBA HISTOLYTICA* FROM LIVER ABSCESS IN MONOXENIC CULTURES WITH HEMOFLAGELLATES*

LI TU WANG, GILBERT JEN, AND JOHN H. CROSS
U.S. Naval Medical Research Unit No. 2, and
National Taiwan University Hospital, Taipei, Taiwan

Abstract. Abscess fluid containing *Entamoeba histolytica* from three Taiwanese with amebic liver abscesses was introduced into monoxenic cultures containing epimastigotes of *Trypanosoma cruzi* or *Trypanosoma conorhini* in a modified Diamond's TP-S monophasic medium. The amebae in the hemoflagellate cultures grew slowly initially, but after serial subcultures they became adapted and multiplied rapidly with maximum growth occurring at 72 hours. Initial growth was better in association with *Trypanosoma cruzi* than with *Trypanosoma conorhini*, but once established the amebae grew equally well in association with either hemoflagellate. The three strains have been designated HT-10, HT-12, and HT-19.

Previous reports on the cultivation of *Entamoeba histolytica* in association with hemoflagellates have indicated that it was first necessary to establish the amebae in cultures containing bacteria.[1-3] Direct establishment of *E. histolytica* in cultures with hemoflagellates, to the best of our knowledge, has not been reported. However, a personal communication from Dr. Louis S. Diamond at the National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, in 1971 indicated he had established a strain of *E. histolytica* directly from a liver abscess in cultures containing *Trypanosoma cruzi* or *Crithidia* sp. The purpose of this report is to detail the isolation and establishment of three additional strains of *E. histolytica* directly from amebic liver abscesses in cultures containing *T. cruzi* or *T. conorhini.*

MATERIALS AND METHODS

Source of organisms. Entamoeba histolytica strains were isolated from patients with proven amebic liver abscesses hospitalized at National Taiwan University Hospital in Taipei. Abscess fluid obtained by needle aspiration was inoculated immediately into culture medium containing the epimastigote stage of the hemoflagellates.

Trypanosoma cruzi, Tulahuen strain, was obtained from Dr. Robert G. Yaeger at Tulane University Medical Center, New Orleans, Louisiana. This strain was maintained in blood agar diphasic medium.

Trypanosoma conorhini was recovered initially from a *Triatoma rubrofasciata* collected in southern Taiwan. Fecal material from this bug was inoculated into a *Macaca cyclopis* (Taiwan monkey) and the parasite was subsequently reisolated from the monkey's blood by culture. Cultures were maintained in a modified monophasic medium described below.

Culture media. Blood agar diphasic medium was prepared according to the method of Tobie, Von Brand, and Mehlman.[4]

The monophasic medium used to culture *T. conorhini* is a modification of the one described by Pan.[5] It was prepared by dissolving 16 g of peptone phosphate broth (Difco) in 500 ml of distilled water and autoclaving at 10 lbs pressure for 10 minutes. After cooling, 30 ml of inactivated horse serum and 30 ml of hemolyzed difibrinated rabbit blood (1 vol blood in 3 vols water) were added. The medium was dispensed in 2 ml aliquots in 16 × 125 mm screw-cap tubes and stored at −20° C. The medium remains stable for at least 3 months at this temperature.

TP-S monophasic medium used for amebatrypanosome culture consisted of 95 ml of Diamond's TP nutrient broth[5] and 5 ml of horse serum dispensed in 12 ml aliquots in 16 × 125 mm screw-cap tubes. This medium was prepared

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TABLE 1

Growth of 3 strains of Entamoeba histolytica *in TP-S medium in association with* Trypanosoma *epimastigotes**

Associate	Ameba		Incubation period (hrs)						
	Strain	Inoculum per tube	24	48	72	96	120	144	168
T. cruzi	HT-10	45,000	2	4	6	4	2	1	1
	HT-12	20,000	2	4	6	4	2	2	1
	HT-19	42,000	3	5	8	5	3	3	1
T. conorhini	HT-10	65,000	2	5	6	5	3	2	1
	HT-12	50,000	2	5	9	7	4	2	2
	HT-19	16,000	2	4	7	5	3	3	2

* Growth is shown as fold increase per tube.

fresh each time it was used for isolation or transfer.

Culture techniques. All hemoflagellate cultures were incubated at 25 ± 2° C in a nearly horizontal position. The organisms were harvested 7 to 9 days after inoculation when used for culture of amebae. Antibiotics were not used in any of the cultures, and cultures were examined periodically for bacterial contamination.

Entamoeba histolytica were isolated by introducing 0.2 to 0.4 ml of material aspirated from liver abscesses directly into TP-S monophasic medium containing approximately five million freshly harvested epimastigotes. Following inoculation the tubes were incubated at 37° C in a nearly horizontal stationary position. Initially the amebae had to be subcultured every 2 days. After the fourth or fifth passage, however, subsequent transfers could be made twice weekly. Cultures were placed in an ice bath for from 3 to 5 minutes before each transfer to dislodge amebae from the walls of the tubes.

Growth of different strains of ameba was determined by simultaneously inoculating known numbers of amebae from pooled cultures into about 28 tubes of TP-S medium containing either *T. cruzi* or *T. conorhini.* The cultures were incubated at 37° C. Every 24 hours two of the culture tubes were used to determine the number of amebae per tube. The culture tubes were chilled before sampling, the sample was diluted with an equal volume of 10% formalin, and the count was performed in duplicate in an A/O Bright-Line Neubauer Hemocytometer.

RESULTS

Three strains of *E. histolytica* isolated directly from liver abscess fluid were successfully established in monoxenic cultures of hemoflagellates in TP-S monophasic medium. Abscess fluid from a number of patients with amebic liver abscess was examined microscopically, but amebae were found in only three specimens. Only abscess fluid known to contain amebae from these three patients was inoculated into hemoflagellate cultures. Strain HT-10 (Human-Taiwan) from a 46-year-old male was isolated in August 1970. Strain HT-12 from a 26-year-old male was isolated in November 1970, and strain HT-19 from a 46-year-old male in February 1971. Strains HT-10 and HT-12 were initially established in association with *T. cruzi*; subsequently they were found to do equally well in cultures containing *T. conorhini* epimastigotes. Strain HT-19 was established initially in *T. cruzi* as well as in *T. conorhini* cultures.

The newly isolated amebae were smaller than those originally found in abscess fluid. They were sluggish and multiplied slowly. After several subcultures over a period of 4 to 6 weeks they appeared larger, were more active, and reproduced rapidly. Amebae of the HT-19 strain when isolated in association with *T. conorhini* initially showed considerably less growth than that observed when they were isolated with *T. cruzi.* Once adapted the HT-19 strain grew equally well in association with either of the hemoflagellate species.

All three strains of amebae have been maintained in continuous culture since their initial isolation. Growth curve studies were done approximately 8 months after each strain was isolated. Peak growths for all three strains occurred at 72 hours, with production of 6 to 9 times as many amebae as in the original inoculum (Table 1). The amebic population decreased

gradually after 72 hours and died out after 168 hours.

Very good growth of *T. cruzi* and *T. conorhini* occurred in the modified monophasic medium. We also have found this monophasic medium useful for isolation of *Leishmania donovani* from human bone marrow and for isolation of a *Trypanosoma* species from the blood of the night monkey, *Aotus trivirgatus*.

DISCUSSION

Although others have established *E. histolytica* directly from liver abscesses into monoxenic bacterial cultures,[2] this is the first published report of establishment of amebae from liver abscesses directly into hemoflagellate monoxenic cultures. Our initial aim in this study was to establish axenic cultures of *E. histolytica* bypassing the ameba-bacterial stage from which most axenic strains have been derived. In earlier experiments sterile liver aspirates were introduced directly into Diamond's TP-S-1 medium,[6] but isolation of *E. histolytica* in this manner failed. We were successful only when sterile pus known to contain *E. histolytica* trophozoites was introduced into the TP-S medium containing *T. cruzi* or *T. conorhini* epimastigotes.

Phillips was the first to establish *E. histolytica* in monoxenic cultures with *T. cruzi*,[1] and in subsequent studies he reported fair growth of amebae in monoxenic cultures containing *Strigomonas fasciculata* but very little growth in cultures containing *T. conorhini*, *T. pipistrelli*, *Leishmania donovani*, *L. tropica*, or *L. brasiliensis*.[7] Subsequently, Pan demonstrated that while ameba yields were highest when *T. cruzi* was used as the associate, *T. lewisi*, *L. donovani*, *L. brasiliensis*, *L. enriettii*, and *Endotrypanum schaudinni* also would support the growth of *E. histolytica*.[5] In the present study the three strains of ameba were found to grow equally well in association with either *T. cruzi* or *T. conorhini*. Although our findings showed that initial growths of the amebae were better in cultures containing *T. cruzi* once the strains of ameba had become adapted the yields were essentially the same in either *T. cruzi* or *T. conorhini* monoxenic cultures.

The medium used for ameba-trypanosome cultures in this study was essentially the same as that used by Diamond[6] in his axenic cultivation of *E. histolytica* except that vitamin mixture 107 was omitted and only 5 ml of horse serum rather than 10 ml per 100 ml medium was used. This monophasic medium is an improvement over that used by Phillips[7] and may explain our success in obtaining good growth of *E. histolytica* in association with *T. conorhini*. In addition, the TP-S medium was undoubtedly a factor in our success in isolation and establishment of the strains of amebae in monoxenic culture.

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