Cultivation of *Trypanosoma cruzi* in a Protein-Free Dialysate Medium.* (32203)

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Trypanosoma cruzi has been successfully cultivated in various media including monophasic and diphasic blood agar, semisolid agar, and tissue cultures(1). Most of the media contain blood, serum, peptone, liver fractions and other ill-defined ingredients. The complexity of the medium for the cultivation of *T. cruzi* has hampered immunochemical studies of this organism and has delayed the development of an effective nonallergenic vaccine for Chagas' disease.

In addition, *in vitro* assays for trypanocidal agents have been beclouded by the fact that the proteinaceous material in the assays medium may have inactivated or altered the agent being tested. Since a chemically defined medium is not available for the cultivation of *T. cruzi,* at least a protein-free dialysate medium is essential for certain studies with the trypanosomes.

A dialysate medium for the growth of *T. cruzi* was described(2) and used for the preparations of antigen(3), however, this medium had certain mechanical disadvantages as well as a limited volume for growth of the trypanosomes. This paper deals with a dialysate medium that is simple to prepare, produces abundant growth of *T cruzi* in large volumes, and supports subcultivation.

Materials and method. The composition of the medium consisted of: blood, whole human, 50 ml; liver fraction L (Nutritional Biochemicals Corp.), 1.0 g; NaCl, 0.4 g; Na_3PO_4, 0.5 g; KCl, 0.4 g; distilled water, pH 7.0, 50 ml. Outdated human blood obtained from hospital blood banks was satisfactory as well as blood from horse, cow, sheep, and rabbit. The ingredients were placed in a 250 ml beaker and thoroughly mixed with a magnetic stirring apparatus without the application of heat. The medium was then placed in dialysis bags. Seamless cellulose casings (Visking Corp.) of 5/8" inflated diameter were cut in 6" and 8" strips. After soaking in water, one end of the 6" tube was tied securely in itself, and examined for leaks by filling with water and squeezing. From the open end of the cellulose sac, 25 ml of the medium were introduced with a volumetric pipette. This end was then tied in a knot. To guarantee against leakage or puncture, the sac containing the medium was placed in another sac, 8" long, and knots were secured on both ends of the outer sac.

The sac containing the medium were placed, one each, into 250 ml screw-capped Erlenmeyer flasks. Fifty ml of 0.8% NaCl were added to each flask. This volume completely immersed the dialysis bags containing the medium. The flasks were autoclaved at 15 lb of pressure for 20 minutes. After the flasks had cooled, the screw caps were tightened and the medium was stored at 4°C. The medium was active in supporting growth of *T. cruzi* even after storage for 6 months.

The Brazil strain of *T. cruzi* was obtained from Anita Bayles, Research Department, Parke, Davis and Co., Ann Arbor, Mich. The Three River strain of *T. cruzi* was obtained from A. Packchanian, Dept. of Microbiology, University of Texas Medical Branch, Galveston. In addition, *Trypanosoma ranarum* was studied, which was obtained from D. L. Lehmann, Whitman College, Walla Walla, Wash. These cultures were routinely transferred in a blood-agar disphasic medium(4,5) and in Chang's medium(6).

The inocula consisted of a 10-15 day culture of *T. cruzi* and *T. ranarum* containing approximately 500,000 trypanosomes per ml. One ml of the inoculum was introduced into the medium using a capillary pipette. The inoculated flasks were incubated at 25°C in a Precision BOD incubator. One-tenth ml samples were removed every 2 days for 50 days and examined under the microscope. The cultures were transferred every 15 to 20 days into dialysate medium. These experiments were repeated 15 times. To determine the

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reliability of this medium for the growth of the trypanosomes, 4 different individuals, including the author, maintained subcultures of the trypanosomes in this medium.

Results and discussion. The autoclaved dialysate medium for *T. cruzi* supports growth and subcultivation of the organisms. After 10-14 days of incubation the cultures yielded a 10- to 20-fold increase in population. Microscopic examination revealed counts of the order of 2.0 to 5.0 $\times$ 10^6 trypanosomes per ml. *T. cruzi* and *T. ranarum* have been maintained in this medium by subcultivation every 14 days for over 8 months. Tobie and Rees(2) grew *T. cruzi* in a dialysate medium but other species of trypanosomes could not be carried beyond a few transfers. Zeledon(7) was unable to grow *T. rangeli* in dialysate medium although *T. cruzi* grew well. This is suggestive of divergent nutritional requirements among the mammalian trypanosomes.

Clearly, these investigations indicate that *T. cruzi* may be grown without proteins or other non-dialyzable substances. Furthermore, it is apparent that *T. cruzi* does not require heat-labile substances for growth.

The medium described here has many advantages. It is simple to prepare and may be sterilized in its entirety by autoclaving procedures. The medium is stable and may be stored for many months without a loss of growth-supporting properties. The medium is a protein-free dialysate which promotes excellent growth of the trypanosomes over long periods. Large quantities of the parasite may be harvested with ease.

This medium has definite advantges compared to other media alreary described particularly in the production and testing of the antigens of *T. cruzi.* Currently I am using this medium to determine the trypanocidal activity of several chemical agents. It is hoped that more accurate trypanocidal assays will be possible using this medium.

Summary. A protein-free dialysate medium that can be sterilized by autoclaving procedures is described for the growth and subcultivation of *T. cruzi.*

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Inhibition of the Replication of a Bacterial DNA Virus by Chloroquine and Other 4-Aminoquinoline Drugs. (32204)

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Chloroquine [7-chloro-4(4-diethylamino-1-methylbutylamino)-quinoline] has found extensive use as an antimalarial and on a smaller scale in a variety of other clinical conditions. Although many studies have been published on *in vitro* effects of chloroquine, none has gained wide acceptance as a satisfactory explanation for the action of the drug. Some years ago Irvin and Parker demonstrated that this quinoline antimalarial could interact with nucleic acids, and suggested that this might represent a possible mechanism of drug action(1,2,3). Recent studies on the interaction between chloroquine and DNA indicate that the drug binds more strongly to the purine moieties in DNA, its binding is antagonized by divalent cations, and it binds more strongly to the double helical form of DNA(4). As a consequence of such binding

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