IN VITRO
Vol. 8, No. 2, 1972

GROWTH OF *CHLAMYDIA PSITTACI* STRAIN MENINGOPNEUMONITIS IN MOUSE L CELLS CULTIVATED IN A DEFINED MEDIUM IN SPINNER CULTURES

SUSAN J. MORRISON AND H. M. JENKIN

The Hormel Institute, University of Minnesota, Austin, Minnesota 55912

SUMMARY

L cells were grown in spinner cultures in a defined medium consisting of Waymouth medium MB752/1 (19) supplemented with 2 mg of fatty acid-free bovine serum albumin (BSA) per ml and 5 μg of oleate per ml (WO_5 medium). Growth in WO_5 medium was comparable to spinner L cell growth in two serum-containing media. The optimal concentration of oleate in the WO medium was 5 to 10 μg per ml. The use of 20 to 80 μg of oleate per ml of medium resulted in lower peak populations and earlier declines in viable cell counts. Cell death occurred rapidly in WO_{100} medium. Cell growth in WO medium containing 5 to 80 μg of oleate per ml was well above the level of growth observed when no oleate was present in the medium. Since the total lipid and fatty acid compositions of the BSA used in this study have been characterized by the authors, the WO medium may be considered a defined medium. L cells have been continuously maintained in spinner cultures in WO_5 medium for over 50 passages with no major variation in the growth pattern. A 1000-fold increase in *Chlamydia psittaci* strain meningopneumonitis, with a peak titer of 9.3×10^7 plaque-forming units per ml, was observed when the chlamydial agents were grown in spinner L cells in WO_5 medium.

Key words: defined medium; L cells; spinner culture; *Chlamydia psittaci* strain meningopneumonitis; Waymouth oleate medium.

INTRODUCTION

Historically, it has been difficult to grow mammalian cells and obligate intracellular microorganisms in well defined media, particularly in suspension cultures. There is a limited number of reports on the cultivation of mammalian cells in semi-defined or defined media using spinner cultures (1, 2) or shaker cultures (3–11).

Chlamydial agents have been grown in mouse L cells cultivated in spinner cultures by using serum-containing media (12, 13). Growth of chlamydiae occurred in monkey kidney cells (LLC-MK2; 14) cultivated in monolayers in a defined medium composed of Waymouth medium MB 752/1 (19) supplemented with fatty acid-poor bovine serum albumin (2 mg per ml) and sodium oleate (10 to 20 μg per ml), with initiation of cell growth by a 1% serum medium (15, 16). However, this system was not convenient for use in suspension culture, because the cells often formed large clumps. The cultivation of chlamydial agents in mammalian cells grown in serum-free defined medium in suspension cultures has not been reported.

A defined medium system with the advantages of suspension cultures was desired for studying lipid metabolism of normal and chlamydiae-infected cells. Therefore, we investigated the cultivation of L cells in spinner cultures in a defined, supplemented Waymouth medium and the growth of a chlamydial agent in this cell system.

MATERIALS AND METHODS

Cells. Mouse L cells (17, 18) clone 5b (12) were obtained from J. W. Moulder (Department of Microbiology, University of Chicago).

Growth media. Waymouth medium (19; imMEDIAte Dry Tissue Culture Medium MB 752/1, Schwarz/Mann, Orangeburg, N. Y.), pH 7.2, was supplemented with 2 mg of fatty acid-

free bovine serum albumin (BSA; Pentex, Miles Laboratories, Inc., Kankakee, Ill.) per ml and various concentrations of oleic acid. The oleic acid (Lipids Preparation Laboratory, The Hormel Institute, Austin, Minn.) was added as the sodium salt prepared by the method previously described (20). Henceforth, this Waymouth-BSA-oleate medium will be referred to as WO medium with a subscript number designating the number of micrograms of oleic acid per milliliter of medium. Cells were also grown in Waymouth medium containing 10% heat-inactivated fetal calf serum (FCS_{10}), pH 7.2, and in Medium 199 (21; Instant Tissue Culture Powder Medium, Grand Island Biological Company, Grand Island, N. Y.), plus heat-inactivated FCS_{10}, pH 7.2. Vancomycin hydrochloride (Eli Lilly & Co., Indianapolis, Ind.) and streptomycin sulfate were each used at concentrations of 100 μg per ml of medium. All media were sterilized by membrane filtration (0.45-μ porosity; Millipore Corporation, Bedford, Mass.)

Cell cultivation. The L cells were propagated in WO_5 medium in spinner cultures in a 5% CO_2, 95% air incubator at 37°C. Cells were grown in 250-ml centrifuge bottles equipped with sampling ports and suspended magnetic stirring bars, rotated by a magnetic stirrer. Every 3 to 5 days the L cell populations were determined and the cultures were centrifuged (200 × *g*, 10 min). The supernatant fluid was decanted, and the cells were resuspended in fresh medium with a starting population of approximately 5×10^5 cells per ml.

Sterility of the cultures and media was ascertained by using blood agar slants, trypticase soy broth, and thioglycollate broth. The cell culture and meningopneumonitis (MN) agent were determined to be free of mycoplasmas by T. F. Smith, Microbiology Section, Mayo Clinic, Rochester, Minn.

L cell growth curves. The growth of L cells in Waymouth-FCS_{10} medium, Medium 199-FCS_{10}, unsupplemented Waymouth medium, and in Waymouth-BSA medium containing concentrations of oleic acid ranging from 0 to 160 μg per ml of medium was compared, by using the cultivation procedures described above. Two to six spinner cultures were used to determine each growth curve. Duplicate 1.5-ml samples were withdrawn from each bottle at 12- or 24-hr intervals, and duplicate counts of each cell sample were made microscopically with the aid of a hemacytometer (15). Viable cells were determined by the trypan blue exclusion technique (22).

Meningopneumonitis agent. Chlamydia psittaci strain MN (23), used to infect spinner L cells, was serially propagated in L cell monolayers grown in WO_5 medium. The stock inoculum of MN was prepared in L cells as described by Jenkin (24). MN in its 3rd to 7th L cell passage was pooled and used as inoculum for the experiment.

Growth of meningopneumonitis agent in defined medium L cell spinner cultures. L cells were grown in spinner cultures in WO_5 medium for 80 hr at 37°C. The cell suspension was centrifuged (200 × *g*, 10 min), the supernatant medium was discarded, and the cells were resuspended in 30 ml of WO_5 medium. A cell count was made and 1.8×10^5 cells were distributed to each spinner bottle. L cells were infected using the procedure of Tribby (12). The MN agent [6.8×10^5 plaque-forming units (PFU)] was added to each bottle, and the total volume was brought to 20 ml with WO_5 medium. The cells and agent were kept in suspension during the 2-hr absorption period which was carried out at 37°C in a 5% CO_2, 95% air atmosphere. At the end of the absorption period, the cell suspension was centrifuged (200 × *g*, 10 min, 4°C), and the supernatant medium containing unabsorbed MN was decanted. The infected L cell culture was resuspended in 120 ml of WO_5 medium. A starting cell population of approximately 1.5×10^6 cells per ml was used in an attempt to establish a nonmultiplying cell population (25). Duplicate infected and uninfected L cell spinner cultures were used.

Triplicate Leighton tubes containing cover slips and 0.5 ml of WO_5 medium were inoculated with 0.5 ml of cell suspension for each spinner culture and incubated at 37°C for 24 hr. The L cells were stained with May-Grünwald-Giemsa stain and examined to determine the percentage of infected L cells (13). Greater than 90% of the L cells contained one or more inclusions.

At 6-hr intervals, from 0 to 96 hr, duplicate 1.5-ml samples were withdrawn from each spinner bottle and centrifuged (200 × *g*, 10 min, 4°C) to sediment the cells. One 0.5-ml aliquot of supernatant medium from each sample was