JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 1984, p. 217–222
0095-1137/84/080217-06$02.00/0

Vol. 20, No. 2

Enhancement of *Chlamydia trachomatis* Infectious Progeny by Cultivation in HeLa 229 Cells Treated with DEAE-Dextran and Cycloheximide

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Received 2 April 1984/Accepted 16 May 1984

The effects of DEAE-dextran and cycloheximide on the infection of HeLa 229 cells with *Chlamydia trachomatis* serotype G were studied in terms of the number of cells infected and the yield of infectious progeny per infected cell. Pretreatment of the host cells with DEAE-dextran resulted in an increase in the number of infected cells but had no significant effect on the yield of infectious progeny per infected cell (burst size). In contrast, the addition of cycloheximide to the medium of infected cells had no significant effect on the number of infected cells but greatly enhanced the burst size. The burst size was calculated to be close to 500. The enhanced burst size was also observed in cells treated with DEAE-dextran and cycloheximide. In addition, there was an increase in the number of cells infected and an augmentation of the infectious progeny yield. Under the conditions of combined treatment, the yield of *C. trachomatis* serotype G cultivated in HeLa 229 cells was found to be approximately threefold higher than the yield of the organisms cultivated in McCoy cells. The results suggest that HeLa 229 cells treated with DEAE-dextran and cycloheximide offer a most suitable system for the high-yield cultivation of *C. trachomatis* organisms and possibly also for the diagnosis of infection with these organisms.

Members of the species *Chlamydia trachomatis* are gram-negative, spherical bacteria capable of multiplication only inside susceptible mammalian host cells (3).

Currently, 15 serotypes of *C. trachomatis* strains are recognized. Three (L1, L2, and L3) are the lymphogranuloma venereum (LGV) strains. Twelve (A, B, Ba, C, D, E, F, G, H, I, J, and K) are the trachoma-inclusion conjunctivitis (TRIC) strains (11). In tissue culture, LGV strains readily infect their host cells and give rise to a high yield of infectious progeny (17). Compared with the LGV organisms, the TRIC organisms are rather poor infective strains that require some sort of assistance to optimize their ability to infect host cells. Even with assistance, the TRIC strains give rise to relatively low yields of infectious progeny (17).

This investigation was initiated with the immediate objective of searching for conditions that enhance the infectious progeny yield of TRIC organisms and with the long-term objective of doing physiological and biochemical studies that require the availability of the infectious organisms in large quantities.

MATERIALS AND METHODS

***C. trachomatis* strains.** *C. trachomatis* G/UW-57/CX was used throughout this study. Some preliminary studies were also carried out with the TRIC strains: H/UW-4/Cx, I/UW-12/Ur, and B/TW-5/OT. All strains were from the culture collection of H. D. Caldwell at the Rocky Mountain Laboratories, Hamilton, Mont.

Cell culture. HeLa 229 and McCoy cells were used for cell cultures. The cells were from the culture collection of H. D. Caldwell and were grown as monolayers in tissue culture flasks or roller bottles in Eagle minimal essential medium supplemented with 10% fetal bovine serum (MEM-10). The medium consisted of the following in a final volume of 1,000 ml: 100 ml of 10× Hanks balanced salt solution (HBSS) (GIBCO Laboratories, Grand Island, N.Y.), 20 ml of 50× basal medium Eagle amino acids (GIBCO), 100 ml of heat-inactivated fetal bovine serum (Hyclone Sterile Systems, Inc., Logan, Utah), 10 ml of 100× BME vitamins (GIBCO), 10 ml of 100× L-glutamine (GIBCO), 10 ml of gentamicin (1 mg/ml) (Schering Corp., Bloomfield, N.J.), and 10 ml of 7.5% $NaHCO_3$; finally, a volume of sterile 0.2 N NaOH was added to adjust the pH of the medium to approximately neutral.

Large-scale cultivation of chlamydiae. Plastic tissue culture roller bottles (850 cm^2; Corning Glass Works, Corning, N.Y.) containing 300 ml of MEM-10 were seeded with 10^8 host cells and incubated at 37°C with rotation at a speed of 1.5 rpm. After 24 h, the medium was removed, and unless otherwise indicated, the monolayers were treated for 30 min at 37°C with DEAE-dextran (DEAE-D) solution (Sigma Chemical Co., St. Louis, Mo.) and with rotation as described above. The DEAE-D (molecular weight, 500,000) was prepared in HBSS at a concentration of 45 μg/ml, and 20 ml of the solution was used per roller bottle. At the end of the treatment, the DEAE-D was removed, and the monolayers were washed with 20 ml of HBSS per bottle. The infective dose of chlamydia suspended in 10 ml of sucrose-phosphate-glutamate (SPG) (18) was then added to each roller bottle, and incubation at 37°C with rotation was continued for 2 h. At the end of the incubation, the inoculum was removed, and the infected monolayers were washed with 20 ml of HBSS per bottle. The monolayers were then fed with 300 ml of MEM-10 per roller bottle. Unless otherwise indicated, the medium contained cycloheximide (Sig-

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ma) at a concentration of 1 μg per ml of medium. With this lot of cycloheximide, the concentration of 1 μg/ml gave the highest number of inclusions in a titration assay. The roller bottles were incubated at 37°C with rotation. At 72 h postinfection, the medium was removed, and the infected cells were removed from the plastic with glass beads and suspended in 20 ml of HBSS per roller bottle. To release the chlamydiae from their host cells, the suspended cells were subjected to sonic disruption for two 30-s intervals at 100 W. The sonically disrupted cells were centrifuged for 10 min at 1,200 × *g* to sediment unbroken cells and cell debris. The supernatant fluid from this step was centrifuged for 30 min at 30,000 × *g* to sediment the chlamydiae. Pelleted chlamydiae from each roller bottle were suspended in 15 ml of SPG and subjected to sonication as described above, and the sonic extracts were overlaid on 18 ml of 30% Renografin (E. R. Squibb & Sons, Princeton, N.J.) and centrifuged for 1 h at 18,000 rpm in an SW28 type rotor and in a Beckman L8-70 ultracentrifuge. The pellet from this step was further suspended in 15 ml of SPG, overlaid on 18 ml of 30% sucrose, and centrifuged for 1 h as described above. The partially purified pelleted chlamydiae were suspended in SPG, dispensed in portions, and stored frozen at −70°C. Unless otherwise indicated, the chlamydiae used in this study were purified through the sucrose gradient step.

Titration of infectious chlamydiae. The number of inclusion-forming units (IFU) in chlamydial preparations was assayed as described by Kuo et al. (18), with some modification, and was quantitated as described by Furness et al. (9). Briefly, 2 × 10^5 HeLa 229 cells in 1 ml of MEM-10 were grown as monolayers on glass cover slips (12 mm diameter) in dram shell vials. The vials were fitted with stainless steel caps and were incubated for 24 h at 37°C in a 5% CO_2 incubator. After removal of the medium, the monolayers were incubated with 1 ml of DEAE-D solution per vial. Incubation was for 30 min at 37°C in a 5% CO_2 incubator, after which the DEAE-D was removed and the monolayers were washed with HBSS. Each vial was then inoculated with 0.1 ml of serially diluted chlamydia preparation. The vials were plugged with silicone rubber stoppers and centrifuged at 500 × *g* for 60 min at 35°C. The inocula were removed, and the infected monolayers were washed with HBSS. Each vial then received 1 ml of MEM-10 medium containing 1 μg of cycloheximide. The vials were covered with stainless steel caps and incubated at 37°C in a 5% CO_2 incubator. After incubation for 48 to 72 h, the medium was removed, and the cover slips were fixed with 1 ml of methyl alcohol for 20 min at room temperature. The fixed cover slips were washed with phosphate-buffered saline, and the chlamydial inclusions were stained by using an indirect fluorescent-antibody staining method.

Indirect fluorescent-antibody staining. The fixed and washed cover slips were incubated with 0.2 ml of an appropriately diluted rabbit anti-*Chlamydia* serum. The antiserum was specific for the *C. trachomatis* 155,000-dalton, species-specific antigen isolated from the LGV strain L2/434/BU (5). The incubation was for 1 h at 37°C in a 5% CO_2 incubator. The serum was then removed, and the cover slips were washed three times with phosphate-buffered saline. A 0.2-ml volume of an appropriately diluted fluorescein-labeled goat antirabbit immunoglobulin specific for heavy and light chains (Cappel Laboratories, Cochranville, Pa.) was then added to each of the cover slips, and incubation was continued for 45 min at 37°C. The stained cover slips were washed three times with phosphate-buffered saline and mounted on slides, and the fluorescent inclusions were counted in a fluorescent microscope at a magnification of ×400.

RESULTS

Effect of DEAE-D and cycloheximide on the size and number of *C. trachomatis* serotype G inclusions formed in HeLa 229 cells. The infection of HeLa 229 cells with *C. trachomatis* serotype G in the absence of DEAE-D or cycloheximide treatment resulted in the production of relatively small inclusions (Fig. 1A). In contrast, inclusion size was greatly enhanced by each of the following culture conditions: (i) pretreatment with DEAE-D (Fig. 1B), (ii) incubation with cycloheximide (Fig. 1C), or (iii) a combination of DEAE-D and cycloheximide treatment (Fig. 1D).

Pretreatment of HeLa 229 cells with DEAE-D resulted in a threefold increase in the number of inclusions formed (Table 1). These findings are in agreement with the earlier work of Kuo et al. (18). Cycloheximide treatment did not significant-

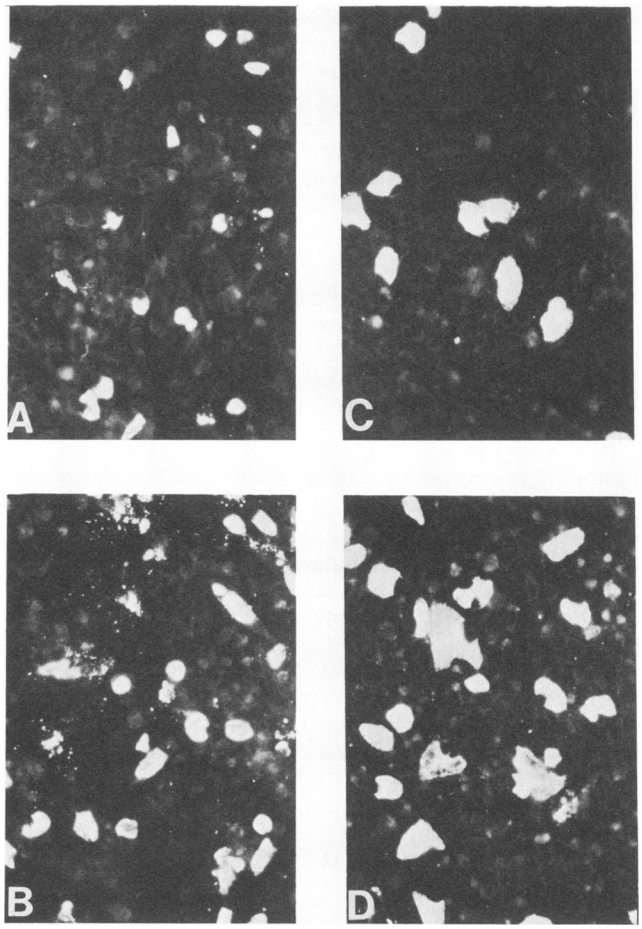

FIG. 1. Effects of DEAE-D and cycloheximide on the size of *C. trachomatis* serotype G inclusions in HeLa 229 cells. The cells were grown as monolayers on cover slips. Volumes of 0.1 ml of the chlamydiae, diluted to 10^{-4} in SPG, were centrifuged onto the monolayers. The chlamydial preparation was purified through the 30% sucrose gradient step as described in the text. The titration assay, fluorescent staining, and treatments with DEAE-D and cycloheximide are also described in the text. The fluorescent inclusions were photographed at a magnification of ×200 with B1W Tri-X pan (Kodak) film, ASA 400, 30-s exposures. (A) No treatment. (B) Treated with DEAE-D. (C) Treated with cycloheximide. (D) Treated with DEAE-D and cycloheximide.

TABLE 1. Effect of DEAE-D and cycloheximide on the number of *C. trachomatis* serotype G inclusions formed in HeLa 229 cells[a]

Cell treatment	No. of inclusions[b]	Treated/untreated ratio
None	108	1.0
DEAE-D	285	2.6
Cycloheximide	108	1.0
DEAE-D + cycloheximide	216	2.0

[a] HeLa 229 cells were infected as described in the legend to Fig. 1.
[b] The number of inclusions represents the total count of 15 microscopic fields.

ly affect the number of inclusions formed. The combination of DEAE-D and cycloheximide resulted in an increase in the number of inclusions formed, but the increase was less than that observed with DEAE-D treatment alone.

Effect of DEAE-D and cycloheximide on the yield of infectious progeny. Since treatment with DEAE-D and cycloheximide affected both the size and the number of inclusions formed, it was of interest to investigate the effect of these treatments on the yield of infectious progeny obtained at the end of the development cycle. DEAE-D treatment increased the yield by approximately threefold (Table 2). The increase in the yield correlated well with the increase in the number of inclusions formed (Table 1). Treatment with cycloheximide resulted in a striking increase in the yield of the infectious progeny. The yield was increased even further when cycloheximide treatment was used in combination with DEAE-D treatment. Under these conditions, the yield was approximately 70 times higher than that obtained from the untreated cells and approximately 23 times higher than that obtained from DEAE-D–treated cells. Preliminary data (not shown) indicated that the DEAE-D plus cycloheximide treatments of HeLa 229 cells caused a marked increase in the infectious progeny yield of other TRIC organisms as well (serotypes I, H, and B).

In the experiments described above, monolayers of ca. 2×10^8 cells grown in roller bottles were infected with ca. 2×10^8 IFU. This was a multiplicity of infection of one, and according to Poisson distribution, approximately 63% of the cells should have been infected. (For details of Poisson distribution, see reference 7a). It should be noted, however, that the titer of the inocula was determined on monolayers grown on cover slips, and the infection was accomplished by centrifugation. The monolayers grown in roller bottles were, on the other hand, infected by rotation with the inocula as described above. Visual examination by phase microscopy of infected cells in roller bottles that had been treated with DEAE-D and cycloheximide indicated that at least 60% of the cells were infected. Thus, it appears that under these treatment conditions, the efficiency of infection in the roller bottles system was at least as efficient as that of the cover slip system. If the multiplicity of infection was at least one, then the number of infected cells per roller bottle was at least 1.26×10^8. At most, the number of infected cells was 2×10^8. Dividing the total number of IFU released from the infected cells by the number of infected cells gave the average burst size. The apparent average burst size of *C. trachomatis* serotype G was calculated to be close to 500 (Table 2). As the number of infected cells varied under the various treatment conditions (Table 1), the calculated burst sizes from the DEAE-D–treated cells and from the cycloheximide-treated cells were corrected to adjust for these variations. The calculation of the adjusted burst size was based on the following assumptions: (i) the number of infected cells per roller bottle under conditions of DEAE-D and cycloheximide treatment was 1.26×10^8, (ii) the relative infection observed with the cover slips (Table 1) also reflected the relative infection obtained in the roller bottles. Accordingly, the number of infected cells under conditions of no treatment or cycloheximide treatment was 0.63×10^8, and the number of infected cells under conditions of DEAE-D treatment was 1.66×10^8.

The yield of infectious progeny from DEAE-D–cycloheximide-treated HeLa cells was highest when the chlamydiae were harvested 72 h postinfection. The yield declined to ca. 50% when the chlamydiae were harvested at 48 h postinfection and declined to ca. 76% when the chlamydiae were harvested 86 h postinfection (data not shown). Similar experiments to determine the optimum harvest time of the untreated cells or of the cells treated with DEAE-D alone or cycloheximide alone were not carried out. Thus, some of the differences observed in infectious progeny yield may have been due to the choice of time of harvest.

Effect of DEAE-D and cycloheximide on the size and number of *C. trachomatis* inclusions formed in McCoy cells. Because McCoy cells have been used most widely for the cultivation of TRIC agents, it was of interest to study the effect of the combined DEAE-D and cycloheximide treatments on the size and number of inclusions formed in these cells. In the absence of any treatment, the inclusions formed were numerous but tiny (Fig. 2 and Table 3). Treatment with DEAE-D did not appear to significantly affect the size or the number of inclusions formed. Cycloheximide treatment resulted in a decrease in the number of inclusions formed but a marked enhancement of the size of the inclusions. The combined DEAE-D and cycloheximide treatment resulted in an enhancement of the size of inclusions with little reduction in the number of inclusions. Several investigators have reported on the cycloheximide enhancement of the size of TRIC inclusions in McCoy cells (4, 19, 20). These investigators have also reported an increase in the number of inclusions detected by iodine staining in the cycloheximide-treated cells. The numerous tiny inclusions stained by fluorescent antibody and observed in this study in untreated cells may have been devoid of glycogen and thus would have escaped detection in the studies involving the iodine-staining method.

Comparison of infectious progeny yields of HeLa 229 cells and McCoy cells. Since the highest number of large inclusions formed in McCoy cells was observed when the cells

TABLE 2. Effect of DEAE-D and cycloheximide on the infectious progeny yield of *C. trachomatis* serotype G cultivated in HeLa 229 cells[a]

Cell treatment	Infectious progeny yield (IFU $\times 10^8$)	Treated/untreated ratio	Calculated burst size	Adjusted burst size[b]
None	8.6	1	6.8	13.6
DEAE-D	26.7	3	21.2	16.0
Cycloheximide	354.0	40	280.9	561.8
DEAE-D + cycloheximide	623.0	70	494.4	494.4

[a] HeLa 229 cells were grown as monolayers in roller bottles in MEM-10 medium. The monolayers were infected at a multiplicity of infection of one and by using the chlamydial preparation described in the legend to Fig. 1. At 72 h postinfection, the infected cells were removed from the roller bottles and disrupted by sonication. The sonically disrupted cells were diluted in SPG, and the infectious progeny were titrated on HeLa 229 cells grown on cover slips. The titration was carried out as described in the text.
[b] The adjustment was made to account for the differences in the number of infected cells with the various cell treatments.

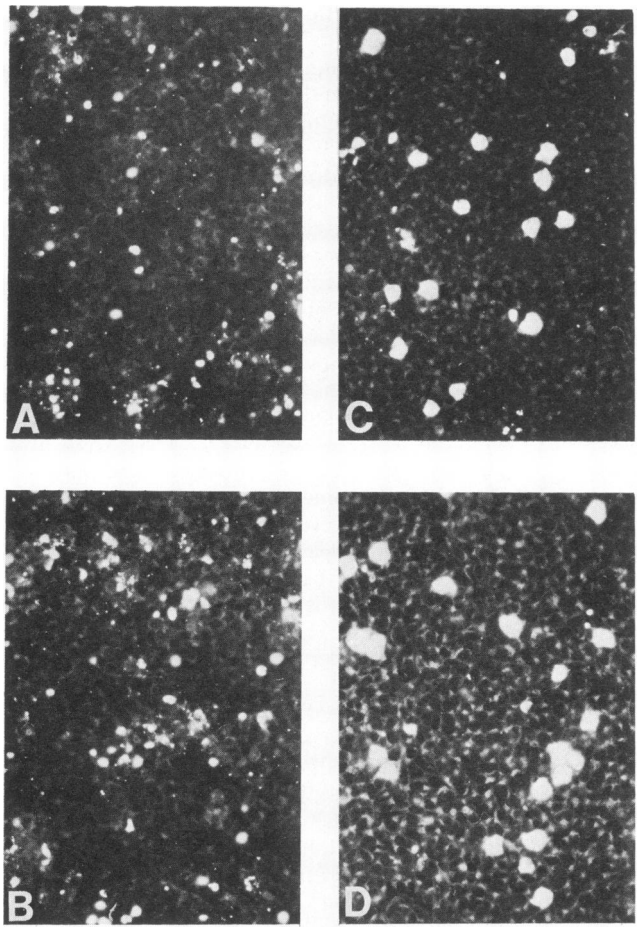

FIG. 2. Effects of DEAE-D and cycloheximide on the size of *C. trachomatis* serotype G inclusions in McCoy cells. The cells were grown on cover slips and infected with the same chlamydial preparation and under the same conditions as described in the legend to Fig. 1. (A) No treatment. (B) Treated with DEAE-D. (C) Treated with cycloheximide. (D) Treated with DEAE-D and cycloheximide.

were treated with DEAE-D and cycloheximide, it was of interest to determine the yield of the infectious progeny under these conditions and to compare it with that obtained from HeLa 229 cells. The yield of infectious progeny obtained from HeLa 229 cells was approximately three times higher than that obtained from McCoy cells (Table 4).

The *C. trachomatis* strain used in these experiments was passed extensively in HeLa cells. The history of the preparation described in Tables 1 to 3 was H16/E10/H22/McCoy 1/H3. The question arose whether the strain had adapted to infect and grow in HeLa 229 cells. Adaptation may affect the number of cells infected or the yield of infectious progeny or both. Chlamydiae harvested from HeLa 229 cells (H16/E10/H22) did not give appreciably increased IFU counts when titers were determined on HeLa 229 as compared with McCoy cells (Table 5). Similarly, chlamydiae harvested from McCoy cells (H16/E10/H22/McCoy 1) did not give increased IFU counts when titrated on McCoy cells as compared with HeLa 229 cells. At present, however, it cannot be ruled out that adaptation affected the multiplication of the chlamydiae within the host cells and the yield of the infectious progeny.

The inclusions formed in HeLa 229 cells were larger than the inclusions formed in McCoy cells (Fig. 1D and 2D). The HeLa cell being larger than the McCoy cell evidently supported the development of a larger inclusion.

Compared with the burst size of 500 obtained from infected HeLa 229 cells grown in roller bottles (Table 2), the burst size from the cells grown in 150 T-flasks was only 275 (Table 4). The roller bottle system and conditions might have supported a better infection and development of the infectious progeny.

TABLE 3. Effect of DEAE-D and cycloheximide on the number of *C. trachomatis* serotype G inclusions formed in McCoy cells[a]

Cell treatment	No. of inclusions[b]	Treated/untreated ratio
None	498	1.00
DEAE-D	441	0.88
Cycloheximide	211	0.42
DEAE-D + cycloheximide	369	0.74

[a] McCoy cells were infected as described in the legend to Fig. 1 and by using the same chlamydial preparation.
[b] The number of inclusions represents the total count of 15 fields.

TABLE 4. Comparison of *C. trachomatis* serotype G infectious progeny yields obtained from HeLa 229 cells and McCoy cells

Cell treatment	Infectious progeny yield (IFU × 10^7)		Avg burst size	
	HeLa	McCoy	HeLa	McCoy
None	2.3	0.8	5.9	2.0
DEAE-D	3.8	2.7	9.7	6.7
Cycloheximide	54.0	22.2	135.2	55.7
DEAE-D + cycloheximide	109.9	45.4	274.9	113.6

[a] HeLa 229 and McCoy cells were grown as monolayers in tissue culture flasks (150 cm^2) containing 50 ml of MEM-10 medium. The monolayers of ca. 10^7 cells of each were incubated for 2 h at 37°C with an infective inocula containing ca. 5×10^6 IFU. For incubation, the flasks were placed on a Bellco rocking platform and allowed to rock gently.

DISCUSSION

Compared with *Chlamydia psittaci* and *C. trachomatis* LGV strains, the *C. trachomatis* TRIC organisms have long been recognized as rather inefficient strains with respect to their infectivity and growth in tissue culture systems. The inability to obtain high yields of infectious progeny of these strains has limited studies on their biochemical and antigenic properties. Many studies have been conducted in a search for conditions that enhance the infectivity of these organisms. Numerous cell lines have been tested for susceptibility to infection (7, 21). The two cell lines that have emerged as the most widely used are the mouse fibroblast McCoy cell line and the human cervical carcinoma HeLa 229 cell line. In

TABLE 5. Effect of the source of *C. trachomatis* G on the number of inclusions formed in HeLa 229 cells and McCoy cells[a]

Expt	Cell line used for titration	Avg no. of IFU per field in chlamydia from:	
		HeLa	McCoy
1	HeLa	70	23
	McCoy	54	16
2	HeLa	14	19
	McCoy	15	20

[a] HeLa 229 and McCoy cells were grown on cover slips and infected under conditions of DEAE-D plus cycloheximide treatments as described in the text.

both cell lines, it has been shown that infectivity is enhanced when the chlamydiae are centrifuged onto the host cell monolayer (10, 15). Both cell lines were used in this study. For the purpose of titration of the infectious inocula and of the infectious progeny yield, the infection was always assisted by centrifugation.

Kuo and co-workers (15, 17, 18) have shown that pretreatment of HeLa 229 cells with DEAE-D enhances the infectivity of TRIC organisms. We found a threefold increase in the number of inclusions formed in DEAE-D–treated HeLa 229 cells. These observations are in close agreement with those of Kuo et al. The yield of infectious progeny from the DEAE-D–treated cells was also increased by approximately threefold; however, the burst size was not affected. The increase in yield appears to be a reflection of the increase in the number of inclusions formed. These findings suggest that DEAE-D treatment affects the *C. trachomatis* serotype G adsorption to host cells or phagocytosis by the host cells, but has no effect on the intracellular development of the infectious progeny. DEAE-D is a positively charged cation and is believed to enhance infectivity of TRIC organisms by suppressing the replusive negative surface charges of the chlamydiae and their host cells (14, 16).

Cycloheximide treatment has been used extensively for the cultivation of TRIC organisms in McCoy cells (4, 19, 20). However, the effect of this antibiotic on the propagation of TRIC organisms in HeLa 229 has not been investigated. As shown in this study, cycloheximide had no effect on the number of inclusions formed but caused a marked enhancement of the size of the inclusions and a striking increase in the yield of infectious progeny. Since cycloheximide was added to the cells after the chlamydiae had been allowed to adsorb and after the removal of unadsorbed organisms by washing, cycloheximide was not expected to affect adsorption. In addition, because the number of inclusions formed was not affected, one would conclude that cycloheximide had no effect on phagocytosis. The enhancement of inclusion size, coupled with the increase in infectious progeny yield, suggests that cycloheximide promoted the intracellular multiplication of the chlamydiae or the development of their infectious progeny or both. Cycloheximide is known to inhibit macromolecular synthesis of mammalian cells (8) but not that of their chlamydial parasites (1). In a study on *C. psittaci* infection of McCoy cells, Hatch (12) has shown that depletion of amino acids from the culture medium results in the production of dormant infection (i.e., an infection with poor infectious progeny yield). More recently, Allan and Pearce (2), working with the same host-parasite system, have shown that the omission of certain amino acids from the culture medium of cycloheximide-treated cells results in a significant reduction in the yield of infectious progeny, yet the number of inclusions formed is not affected. Thus, the inclusions formed are dormant or unproductive.

In this study, a comparison between DEAE-D–treated HeLa 229 cells and the cycloheximide-treated cells in terms of the size of inclusions, the number of inclusions formed, and the yield of infectious progeny suggested that the inclusions of the DEAE-D–treated cells were unproductive. The low yield obtained from the DEAE-D–treated, infected HeLa 229 cells was probably due to competition of the host cells with amino acids needed for the maturation of the chlamydial bodies into the infectious elementary bodies. Electron microscopy studies are needed to distinguish whether the poorly productive inclusions of DEAE-D–treated cells consist mainly of reticulate bodies or uninfectious elementary bodies.

The highest yield of infectious progeny was obtained from HeLa 229 cells that were treated with both DEAE-D and cycloheximide. It appears that DEAE-D and cycloheximide treatments act in concert, with the former treatment resulting in an increase in the number of inclusions formed and the latter treatment allowing the maturation of the formed inclusions to the productive stage.

From the data reported herein, the burst size of *C. trachomatis* serotype G grown in HeLa 229 cells and under conditions of DEAE-D and cycloheximide treatment was calculated to be close to 500. Data on chlamydial burst size are scarce. In one study, Collier (6) reported a burst size of 35 to 60 for TRIC organisms cultivated in McCoy cells. Karayiannis et al. (13) reported burst sizes of 304 and 26 for *C. trachomatis* BK strain grown in untreated McCoy cells and cycloheximide-treated cells, respectively. Karayiannis et al. concluded that the infectious progeny yield from cycloheximide-treated McCoy cells was lower than that from untreated cells. In contrast, the data shown in Table 4 indicate that the response of the McCoy cells to DEAE-D and cycloheximide treatments paralleled that of the HeLa 229 cells, with the highest infectious progeny yield obtained under conditions of the combined treatment. The differing results may be due to differences in the chlamydial strains used or to differences in the conditions of infection. Recently, Allan and Pearce (2) reported a yield per infected cell (burst size) of 1,075 for *C. psittaci* cultivated in cycloheximide-treated McCoy cells. Clearly, different strains of chlamydiae, even if grown in the same host cell and under the same environmental conditions, may have different burst sizes. Furthermore, the same chlamydiae growing in different host cells but under the same environmental conditions may exhibit different burst sizes. The higher yield of *C. trachomatis* serotype G infectious progeny in the HeLa 229 cells compared with the McCoy cells suggests a larger burst size in the former cells. In summary, it appears that HeLa 229 cells treated with DEAE-D and cycloheximide offer a most suitable host cell system for large-scale, high-yield cultivation of TRIC organisms and possibly also for diagnostic purposes.

ACKNOWLEDGMENTS

This work was supported in part by Public Health Service Biomedical Research support grant 2 S07 RR05 771-05 to Eastern Virginia Medical School.

S.F.S. thanks John Swanson, Chief of the Laboratory of Microbial Structure and Function, Rocky Mountain Laboratories, Hamilton, Mont., for space and supplies in support of a major portion of this work. We thank Susan Smaus and Karen Milliken for secretarial assistance.

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