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Effect of Cycloheximide on the Infective Yield of a Genital Strain of *Chlamydia trachomatis* in McCoy Cells

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The yield of infectious progeny of a genital strain of *Chlamydia trachomatis* in cycloheximide-treated McCoy cell cultures was 11-fold lower than that in untreated monolayers.

Genital strains of *C. trachomatis* produce only a single crop of inclusions in McCoy cell culture (3, 4). The number of progeny inclusion-forming units (IFU) that can be harvested during this single-step growth curve is thus directly related to the number, size, and quality of the inclusions that the input inoculum can establish. Addition of cycloheximide to such cultures increases the inclusion count produced by a given inoculum (8, 12). The purpose of the present experiments was to determine whether cycloheximide treatment of McCoy cultures would also increase the infective yield of *C. trachomatis*. This has been shown to occur in the multicyclic growth of *Chlamydia psittaci* in L-cells (6).

Cover slip cultures of McCoy cells were prepared as previously described (9) in MB752/1 medium (15) supplemented with 10% (vol/vol) fetal calf serum and 1.48 mM glutamine. After a 24-h incubation when the monolayer was confluent, the growth medium was removed and replaced by 1 ml of Earle balanced salt solution (BSS, Flow Laboratories, Irvine, Scotland). Immediately, 0.2 ml of a suitable dilution in BSS of the BK strain of *C. trachomatis* (9) was added and the cultures were centrifuged at 3,500 × *g* for 1 h at 33°C and then incubated for 3 h at 35°C in 5% CO_2 in air. The BSS was removed and replaced by 1 ml of fresh growth medium. Cycloheximide (BDH Chemicals Ltd., Poole, England) was added to half the cultures in a final concentration of 1 μg/ml, which has been found to be optimal in our diagnostic isolation procedures for *C. trachomatis* (8). The cultures were then reincubated as described above. Randomly selected cultures with and without cycloheximide were Giemsa stained after 24, 48, 72, and 96 h for examination and counts of chlamydial inclusions. These procedures and their statistical confidence limits have been described previously (10). The total infective yield from other cultures at the same time intervals was titrated by sonic disintegration of the McCoy cells and inoculation of the suspension into fresh cultures (9, 10). From these titrations, the number of IFU yielded per inclusion at each stage of the primary growth cycle of the BK strain was calculated. All inclusion counts and infective yields were determined on three to six cultures.

In the primary growth cycle, after a 24-h incubation, both cycloheximide-treated and untreated cultures contained only a few very small inclusions containing several large pale-staining reticulate bodies, but only a few elementary bodies. Titration at this stage detected only a few or no progeny IFU. After a 48-h incubation, many large inclusions had appeared; counts (Fig. 1) were similar in both treated and untreated cultures, and all were equally closely packed with deeply staining elementary bodies. The mean inclusion diameter was greater in treated (17.6 μm) than in untreated cultures (11.5 μm). After 72 h the count in untreated cultures had fallen from 2,210 to 1,249, the mean diameter had increased to 14.4 μm, several inclusions were already mature and bursting, and released elementary bodies scattered over the monolayer could be seen. In treated cultures, however, the count had continued to increase to 2,847; fewer inclusions were bursting, but the mean diameter was then 21.6 μm. After 96 h, almost all inclusions in both types of culture had ruptured, and large numbers of released elementary bodies could be seen.

This enhanced size and count of inclusions in cultures treated with cycloheximide was in accord with our previous experience (8) and that of other workers (12).

In contrast, the total infective yield (Fig. 2) from cycloheximide-treated cultures was lower than that from untreated cultures throughout the growth cycle. Between 72 and 96 h, when most of the inclusions were bursting, the yield from untreated cultures remained high (6.7×10^5 IFU per culture), whereas in treated cultures, it fell from 7×10^4 to 5×10^3 IFU per culture.

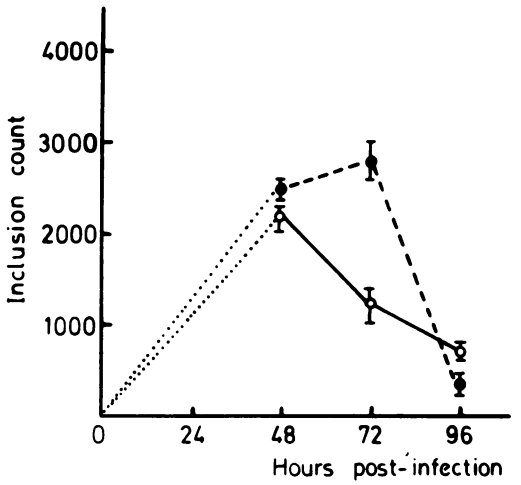

FIG. 1. *Primary growth cycle of C. trachomatis strain BK in fresh MB752/1 medium added to cultures at 3 h postinfection, with* (●) *or without* (○) *cycloheximide. Inclusions were not counted at 0 or 24 h postinfection* (...).

The IFU/inclusion ratio at various stages in the growth cycle (Fig. 3) reached a maximum of 304 in untreated cultures but only 26 in treated cultures.

The effect of varying concentrations of cycloheximide was investigated in McCoy cell cultures which were changed directly to fresh growth medium alone or medium containing cycloheximide in final concentrations of 0.25, 0.5, 1, or 2 μg/ml immediately before infection. After centrifuge-assisted adsorption and incubation for varying periods, inclusion counts and infective yields were determined as described above. In untreated cultures, the peak count was 4,675 inclusions, and the peak yield was 294 IFU per inclusion. Counts of 5,423, 6,597, and 5,889 were found in cultures treated with 0.25, 0.5, or 1 μg of cycloheximide per ml, respectively, but in cultures treated with 2 μg/ml the count fell to only 2,601. Infective yields were low in all treated cultures; only 20, 18, 26, and 13 IFU per inclusion were obtained from those treated with 0.25, 0.5, 1, and 2 μg/ml, respectively.

To determine whether depressed yields might be a nonspecific result of growing *C. trachomatis* in host cells with a greatly reduced replication rate, rather than a specific effect of cycloheximide, cell cultures were infected as above, with or without the addition of hydrocortisone sodium phosphate (Glaxo Laboratories, Greenford, England) in a final concentration of 1 μg/ml, which limits tissue culture cell replication and biochemical activities (2, 14) and increases the inclusion count of a given dilution of the BK strain (2). After a 48-h incubation, the inclusion count in untreated cultures was 4,675, and the yield was 296 IFU per inclusion, and in cortisone-treated cultures, the count was 7,174, and the yield was 250 IFU per inclusion.

Since the inclusions in cycloheximide-treated cultures were consistently greater in number and larger in size than in untreated cultures, contained a dense array of morphologically normal elementary bodies, and burst equally freely as they reached maturity, it is worth considering why such treatment grossly reduces the infective yield. It is possible that many elementary bodies produced in treated cells may be noninfective because of defective development. It is unlikely that cycloheximide could act directly by binding to procaryotic ribosomes, but indirect effects on chlamydial maturation may occur if host cell metabolic processes essential for elementary body synthesis are suppressed by cycloheximide.

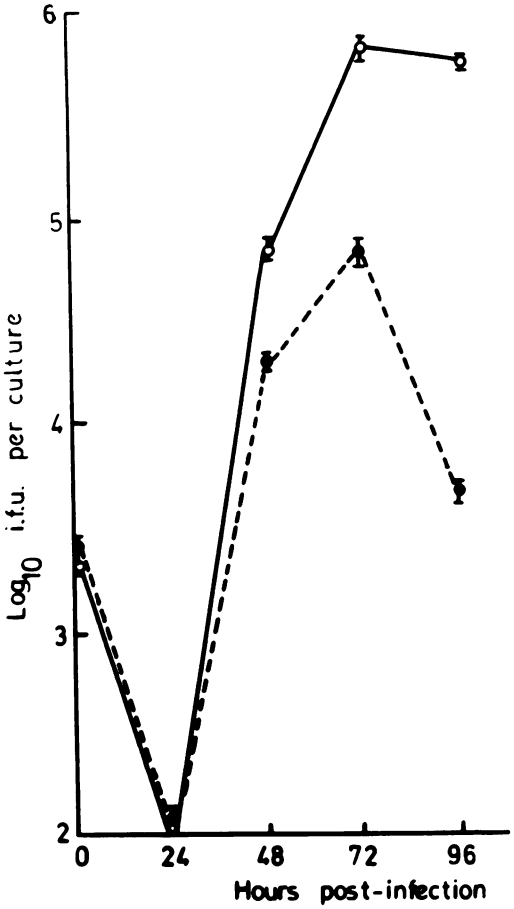

FIG. 2. *Total yields of infective progeny per primary growth culture in fresh MB752/1 medium with* (●) *or without* (○) *cycloheximide.*

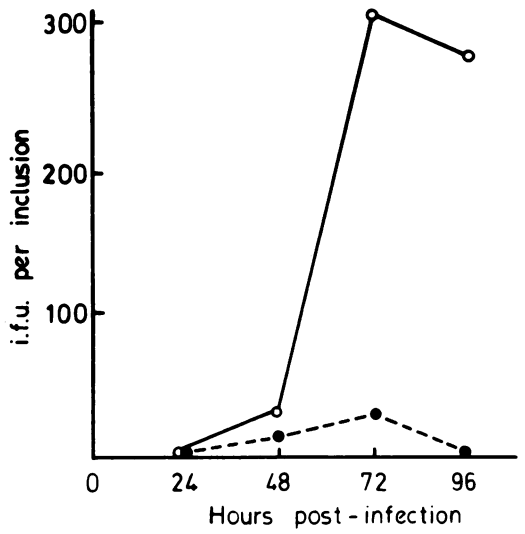

FIG. 3. *Infective yields were expressed as an IFU/inclusion ratio from titration of primary growth harvests from cultures maintained in fresh MB752/1 medium with (●) or without (○) cycloheximide. Inclusions were not counted at 0 or 24 h postinfection.*

For example, host cell enzymes, such as hexokinase, are necessary for chlamydial synthesis (11), and if these were in limiting concentration at the beginning of the growth cycle, it is doubtful if they could be resynthesized in the presence of cycloheximide. Alternatively, the elementary bodies released may be normal and potentially infective but accompanied by late accumulated biproducts of the distorted biochemical activities of cycloheximide-suppressed McCoy cells, which could inhibit chlamydial infectivity in fresh cell cultures used for titration. For example, chlamydial growth in tissue culture can be inhibited by adding interferon or interferon inducers (5, 7), and treatment of virus-infected cultures with cycloheximide increases the amount of interferon that they can produce (13). Further investigation of the means by which cycloheximide affects the yield of *C. trachomatis* in McCoy cells would be of practical value and of academic interest in exploring the apparent differences between *C. psittaci* and *C. trachomatis* in this and in so many other respects (1, 6).

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