Effect of \textit{Mycoplasma pneumoniae} on Poliovirus Replication

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Results are presented which indicate that different species of mycoplasma may have varying effects on the replication of different viral types.

Recently, Mohadjer and Kaftarians (4) reported that \textit{Mycoplasma hominis} had no effect on the replication of poliovirus type II as judged by infectivity titrations of virus replicated in the presence of \textit{M. hominis}. In contrast to these findings, we found that \textit{Mycoplasma pneumoniae} was capable of inhibiting poliovirus type I RNA synthesis.

\textit{M. pneumoniae} were grown on the surface of 32-oz (ca. 0.946 liter) glass prescription bottles (5) in media containing 70\% PPLO broth (Difco), 20\% agamma horse serum (Grand Island Biological Co.), 10\% fresh yeast extract (Grand Island Biological Co.), 0.5\% dextrose, and 0.004\% phenol red.

\textit{M. pneumoniae} used to inoculate KB cells were prepared as follows. Confluent monolayers of \textit{M. pneumoniae} were rinsed several times with tissue culture maintenance medium (MM; Eagle basal medium with Earle salts) containing glutamine, 2\% calf serum, and sodium bicarbonate (0.001\%, wt/vol) to remove mycoplasma growth medium. \textit{M. pneumoniae} were harvested by scraping the colonies from the glass surface with a rubber policeman into tissue culture MM. \textit{M. pneumoniae} titers were determined by the acid-forming units assay (3).

KB and HeLa cells were passed in Eagle basal medium with Earle salts and glutamine containing 10\% calf serum and sodium bicarbonate (0.001\%, wt/vol) and were periodically monitored for mycoplasma contamination. Plaque-purified poliovirus type 1 (HS/20/63) stocks were prepared in KB cells, and the viral titer was determined by the plaque assay.

KB cell monolayers were inoculated with 16 acid-forming units of \textit{M. pneumoniae} per cell or sham-inoculated with MM and incubated at 33 C for 12 h. Supernatant fluid was then decanted, and the cell monolayers were rinsed with MM. Actinomycin D (10 \textmu g/ml) was then added to the \textit{M. pneumoniae}-infected KB cells, and the sham-inoculated cultures and the cells were incubated an additional 1 h at 37 C. After incubation, the cells were infected with poliovirus (40 plaque-forming units per cell). [5\textsuperscript{-3}H]j uridine was added (8 \mu Ci/ml) at the time of viral inoculation. Viral RNA synthesis was determined by monitoring incorporation of [5\textsuperscript{-3}H]j uridine into acid-insoluble material (3) at various times after viral infection.

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Our results (Fig. 1) indicated that *M. pneumoniae* preinoculation decreased by approximately 47% of the maximum amount of poliovirus ribonucleic acid synthesized.

These results suggest that species of mycoplasma other than *M. hominis* may have differing effects on the replication of other poliovirus types. In addition, studies designed to determine the effect of mycoplasma on viral replication should define the time at which cells were infected with the mycoplasma as well as the mycoplasma input multiplicity, since mycoplasma apparently have varying effects on viral replication at different stages in the mycoplasma multiplication cycle. In the previously described experiments, the inhibitory effect of *M. pneumoniae* on poliovirus ribonucleic acid synthesis was observed when *M. pneumoniae* could not be recovered from the *M. pneumoniae*-infected KB cells (Fig. 2). Although previously described (1, 2), the status of the cell or mycoplasma during this "occult" or "lag" period of mycoplasma replication is not at all understood. The duration of the occult period, however, appears to be related to the input multiplicity of mycoplasma infection.

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**LITERATURE CITED**