The Interaction between *Mycoplasma hominis* and Poliovirus in Cell Culture

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**SUMMARY**

Eagle’s culture medium containing 2% heat-inactivated calf serum is inadequate for the growth of *Mycoplasma hominis*. However, when the medium was conditioned by remaining in contact with the monolayers for several days, or when it was supplemented with 10% mycoplasma medium, it supported the growth of this organism.

The presence of *Mycoplasma hominis* in MK and MS cell cultures had no effect on the replication of poliovirus type II, as judged by the titration of the infectivity of the virus produced. However, the presence of poliovirus in primary cultures of monkey kidney cells enhanced the growth of mycoplasma in such a way that the delay in the logarithmic phase of growth of this organism was overcome.

**INTRODUCTION**

Since the first isolation of mycoplasma from cell cultures by Robinson, Wichelhausen & Roizman (1956), these organisms have been isolated from a variety of cell cultures and it is apparent that contamination is encountered mainly in continuous cell lines, whereas cultures of primary cells are rarely contaminated (Carski & Shepard, 1961; Herderschee, Ruys & Rhijn, 1963; Kenny & Pollock, 1963).

There are several reports on the effect of mycoplasma on virus growth. Some of these fail to detect any effect of mycoplasma on virus replication in cell cultures (Herderschee *et al.* 1963) and others indicate that mycoplasma either enhance (Singer, Barile & Kirschstein, 1969; Singer, Kirschstein & Barile, 1969; Hargreaves & Leach, 1970) or suppress (Gori & Lee, 1964; Afshar, 1967) virus yield. On the other hand, the results obtained by Hayflick & Moorhead (1961) showed that cell lines which are continuously propagated differ in many respects from primary cultures, quite apart from their heteroploid character. All of these findings raise the important question of the extent to which contamination with mycoplasma may interfere in different ways with the multiplication of viruses in primary or continuous cultures of cells.

In this communication we compare results on the effects of *Mycoplasma hominis* and poliovirus upon each other in cultures of primary monkey kidney and continuous monkey kidney cells.
METHODS

Cell cultures. Primary monkey (African green) kidney cell cultures were prepared by Flow Laboratories Ltd., Irvine, Scotland. The cells had been plated in Melnick A medium and fed with Melnick B medium after 3 days. Tube cultures at 10 to 12 days old were received by this laboratory and incubated for 1 to 2 days before use in experiments.

Cell of a continuous line of monkey (African green) kidney cells were kindly provided by Dr Mirshamsy, Razi Institute, Iran. The cell monolayers were prepared in 2 oz Roux bottles using the usual trypsinization method. Tests for the presence of endogenous mycoplasma in the culture were negative. For production of monolayers for experimental use, cells were plated in stationary, inclined screw-capped tubes and incubated at 36 °C for 2 to 3 days. After formation of the monolayers and before the inoculation of mycoplasma, the tubes were tested again for mycoplasma to confirm the absence of contamination during processing.

Mycoplasma. Mycoplasma hominis type I (PG-2I), kindly provided by Dr Chanock of N.I.H., received four passages in our laboratory before being used in experiments.

Virus. The Lansing strain of poliovirus (type II) was kindly provided by Dr R. Nategh, Teheran University, School of Public Health, Section of Virology. A stock of the virus was prepared in cultures of continuous monkey kidney cells (MS). The infectivity of the supernatant fluid was $10^8$ TCD$_{50}$ per ml.

Media

For cell cultures. Cells were plated in Eagle's medium (Eagle, 1955) containing 10% heat-inactivated calf serum and 50 to 100 units/ml of penicillin. The medium was adjusted to pH 7.4 with 1.2 M-sodium bicarbonate. The cells were then maintained in the same medium but with 2% heat-inactivated calf serum.

For mycoplasma. The organism was grown in the medium described by Taylor-Robinson et al. (1963) and Manchee & Taylor-Robinson (1968). The liquid medium consisted of 70 ml of Difco PPLO broth without Crystal Violet (Difco Laboratories, Detroit, Michigan), 10 ml of 25% aqueous extract of active dried yeast (Distillers Co., Ltd., Scotland), 2 ml of a 2.5% aqueous solution of thallium acetate and penicillin G (1000 units/ml). In addition, in order to detect the growth of Mycoplasma hominis, which is an arginine-utilizing mycoplasma, 0.1% (w/v) arginine and 0.002% (w/v) phenol red were added and the medium adjusted to pH 7.2. The solid medium was Difco PPLO-agar supplemented with yeast extract, unheated horse serum, thallium acetate and penicillin in the same concentrations as above for the liquid medium.

Titration of mycoplasma. Samples were diluted serially tenfold in mycoplasma medium and growth was detected by a change in the colour of the medium from yellow (pH 7.2) to pink (pH 7.8 or above). The highest dilution which produced a colour change after continued incubation at 36 °C was taken as the end-point of the titration and was considered to contain one colour changing unit (c.c.u.)/ml. At the same time, the number of colony-forming units (c.f.u.) of the organism was determined by inoculation of 0.1 ml of the above dilutions on to agar medium and counting the colonies formed after incubation for 2 to 3 days at 36 °C.

In the first series of experiments, titration of mycoplasma was done by estimating both the number of c.f.u./ml and c.c.u./ml. Since the preliminary results obtained by these two
methods showed little difference, the titrations of the organism were later performed by determining only the c.c.u./ml.

**Titration of virus infectivity.** The infectivity of virus samples was determined by the tube titration method (Merchant, Kahn & Murphy, 1964). Virus-infected cultures were examined by microscope daily and were scored according to the progression of cytopathic changes: none = 0; significant morphological changes of less than 25 % of the cells = trace; definite morphological change of 25 % of the cells = 1; 25 to 50 % cellular degeneration = 2; 50 to 75 % cellular degeneration = 3; and complete cellular destruction = 4. The highest dilution of a virus sample producing infection in 50 % of the cultures was calculated by the method of Reed & Muench (1938) and expressed as TCD₅₀/ml.

**Inoculation of cell monolayers with mycoplasma and virus.** Cell monolayers in tubes were inoculated simultaneously with 100 c.c.u. of mycoplasma in Eagle's medium and 80 TCD₅₀ of virus, and incubated at 36 °C. Two tubes were taken out every day and titrated for growth of *Mycoplasma hominis* and infectivity of poliovirus.

**Conditioning of the cell culture medium.** Eagle's basal medium (BM) containing 2 % heat-inactivated calf serum and 50 to 100 units/ml of penicillin was left in contact with the monolayers of primary and continuous monkey kidney cells for 3 to 6 days at 36 °C. Each day a sample of the supernatant fluid was taken and the remaining medium adjusted to pH 7.0 to 7.2 by 0.1 M-sodium bicarbonate, before inoculation with 100 c.c.u./ml of *Mycoplasma hominis* and incubation at 36 °C. These samples were then titrated each day for growth of mycoplasma. Each experiment was repeated three times and the mean calculated.

**RESULTS**

**Growth of Mycoplasma hominis in cell culture medium**

Eagle's medium containing 2 % heat-inactivated calf serum is inadequate for the multiplication of *Mycoplasma hominis*, but it can maintain some viable counts for one to two days at 36 °C. However, when this medium is supplemented with 10 % mycoplasma medium, or is conditioned by contact with the cell monolayers for a time, a good growth of mycoplasma in this medium is obtained (Fig. 1, 2). Eagle's medium conditioned by remaining in contact with primary monkey kidney cells (Fig. 2) showed the same optimum growth of the organism as was obtained in the presence of the cells (Fig. 3). However, the growth of the organism in Eagle's medium conditioned by contact with the continuous line of monkey kidney cells was less (10⁴ c.c.u./ml) than that in the presence of the cells (10⁷ c.c.u./ml).

**Growth of Mycoplasma hominis in cell cultures**

The infection by *Mycoplasma hominis* showed no observable c.p.e. (Table 1) in cultures of either primary or continuous monkey kidney cells. The infected cells showed only a higher degree of granularity when compared with the controls. An increase in acidity was also noted in the supernatant fluids of cultures infected with *M. hominis*.

**Effect of Mycoplasma hominis on poliovirus infection**

The susceptibility of cell cultures to the lethal action of the virus is shown in Table 1. The c.p.e. of the virus in either cell culture system infected with *Mycoplasma hominis* did not differ from that in cells not infected by this organism.

The biosynthesis of poliovirus was investigated by titration of the virus yield from the *Mycoplasma hominis*-infected and the corresponding control cultures at 1, 2, 3 or 4 days...
Fig. 1. Growth of *Mycoplasma hominis* in Eagle's medium containing 10% PPLO broth.
- - - - - - , c.c.u./ml; △-△, c.f.u./ml.

Fig. 2. Growth of *Mycoplasma hominis* in Eagle's medium conditioned by remaining in contact with monolayers of primary monkey kidney cells (MK). Δ-Δ, Eagle's medium conditioned for 1 day; ○-○, Eagle's medium conditioned for 3 days; , Eagle's medium conditioned for 6 days.
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Fig. 3. Growth of Mycoplasma hominis in cultures of primary and continuous line monkey kidney cells. Δ—Δ, cultures of continuous line of monkey kidney cells; ▲—▲, cultures of primary line of monkey kidney cells.

Table 1. Cytopathic effect of poliovirus in cultures of primary or continuous monkey kidney cells infected by Mycoplasma hominis

<table>
<thead>
<tr>
<th>Cell culture</th>
<th>Days after infection</th>
<th>Cell cultures infected with:</th>
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<tr>
<td></td>
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<td>virus or mycoplasma</td>
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<td>plus uninfected control</td>
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<tr>
<td>Primary monkey kidney (MK)</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
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<td></td>
<td>2</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Continuous monkey kidney (MS)</td>
<td>1 Trace</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3 Trace*</td>
<td>4</td>
<td>Trace*</td>
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Degree of cytopathic change: o, no detectable change; trace, slight but significant morphological changes; 1, definite morphological changes in 25% of cells; 2, 25 to 50% cellular degeneration; 3, 50 to 75% cellular degeneration; 4, complete cellular destruction.

* Some slight changes are due to the medium remaining unchanged during the experiment.

after infection. The harvested materials were titrated also for mycoplasma growth daily for 8 to 9 days.

The titrations of virus yields showed that the presence of Mycoplasma hominis in cultures of primary (MK) or continuous (MS) monkey kidney cells had no effect on the biosynthesis of poliovirus.

Effect of virus infection on the growth of mycoplasma

The growth of Mycoplasma hominis in cultures of primary monkey kidney cells (MK) was determined as shown in Fig. 4. The logarithmic phase of growth of M. hominis started...
4 days after incubation and the maximum infectivity was obtained after incubation for 7 days. The decline of infectivity was gradual and a level of $10^{4.5}$ c.c.u./ml remained after incubation for 9 days. On the other hand, the presence of poliovirus enhanced the growth of mycoplasma so that this delay in the logarithmic phase was overcome. However, there was a rapid decline in the number of viable mycoplasma (Fig. 4).

The growth of *Mycoplasma hominis* in cultures of continuous monkey kidney cells in the presence and absence of virus showed no delay in either case in the logarithmic phase of growth of mycoplasma. This started a day after incubation and the maximum infectivity was obtained after incubation for 2 to 3 days. However, the viability of mycoplasma again declined rapidly in the presence of virus, as in the tests with cultures of primary monkey kidney cells.

**DISCUSSION**

Our results demonstrate that *Mycoplasma hominis* cannot grow in Eagle's medium. Carski & Shepard (1961) found that even cell culture medium which has been in contact with HeLa cells for 3 days did not support the growth of this organism. However, our results and those of Mazzoli & Taylor-Robinson (1971) show that medium conditioned by exposure to cell monolayers supports the growth of *M. hominis*. On the other hand, Reed (1972a) has reported similar results with *M. hyorhinis* in tracheal organ culture. She found that the presence of tissue was necessary in order to obtain optimal growth of the organism, as we have shown for the continuous line of monkey kidney cells. We also found that *M. hominis* would grow optimally when Eagle's medium was supplemented with 10% mycoplasma medium.

The presence of poliovirus in cell cultures has an effect on mycoplasma growth. The growth of *Mycoplasma hominis* following infection of cells with or without poliovirus is different for primary monkey kidney cells (MK) and the continuous line of monkey kidney cells (MS). A marked delay in the logarithmic phase of mycoplasma growth was observed...
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only in cultures of MK cells. Primary monkey kidney cells, but not continuous monkey kidney cells, may have inhibitory factors which are neutralized either by prolonged incubation or through the liberation during cell lysis of intracellular nutrients and other components. This is in accordance with the results of Reed (1972a), who found that heating the medium and tissues was the best way of increasing the growth of mycoplasma. The enhancing effect of virus infection upon mycoplasma growth has been reported by Nakamura & Sakamoto (1969) using M. orale in cell cultures infected with Japanese encephalitis virus and by Reed (1972b) using M. hyorhinis in tracheal organ cultures infected with rhinovirus and parainfluenza virus. Our results are similar to those of Reed (1972b), who reported that the degree to which virus infection enhanced the growth of mycoplasma was related to the extent to which the virus destroyed the epithelium.

The presence of Mycoplasma hominis type I, an arginine-utilizing mycoplasma, has no effect on the biosynthesis of poliovirus type II, either in cultures of primary or continuous monkey kidney cells. Similar results were reported by Herderschee et al. (1963) using human embryonic lung fibroblasts and by Hargreaves & Leach (1970) using three sublines of HeLa cells. All of these studies indicate that for poliovirus, an arginine-dependent and intracytoplasmic RNA virus, the depletion of available arginine by growth of contaminating mycoplasma does not result in a decrease of virus yield, as has been reported for adenoviruses (Rouse, Bonifas & Schlesinger, 1963) and herpesviruses (Hargreaves & Leach, 1970), which are intra-nuclear DNA viruses.

These findings raised the speculation that the requirement for arginine may constitute a common feature of all intra-nuclear DNA viruses (Goldblum, Ravid & Becker, 1968). On the other hand, Singer et al. (1970), using hamster embryo fibroblasts, chick embryo fibroblasts and a continuous line of human amnion cells, reported that the growth of vaccinia virus, a DNA virus which replicates in the cellular cytoplasm, is also dependent on arginine and thus is inhibited by an arginine-utilizing mycoplasma. On the contrary, the results obtained by Hargreaves & Leach (1970) using vaccinia in HeLa cells showed that not only is the yield of vaccinia virus not decreased by an arginine-utilizing mycoplasma, but the yield of infective virus is even raised by such contamination.

There are also several reports indicating that, unlike poliovirus, the replication of some of the RNA viruses, such as measles (Romano & Brancato, 1970) and influenza (Hargreaves & Leach, 1970), is inhibited when the cell culture is depleted of available arginine through mycoplasma contamination. Since both measles and influenza viruses have been reported to replicate in the nucleoli of cells (Wilner, 1969), we consider that the site of virus replication in the cell may be important in defining the effect of an arginine-utilizing mycoplasma. Also, the specificities and the differences in the properties of the cells and, in particular, their intracellular amino acid pools (Mohadjer & Gabliks, 1966), may be responsible for this inhibition. More experiments are needed to evaluate these interactions.

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