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# CYTOGENETIC EFFECTS OF MYCOPLASMAL INFECTION OF CELL CULTURES: A REVIEW

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## INTRODUCTION

The presence of mycoplasmas in cell cultures represents a true in vitro infection. This host-parasite relationship is influenced by some of the factors operative in in vivo infection as well as those specific for in vitro cultivation. Mycoplasmal infected cell cultures generally contain two logs more mycoplasmas than cultured cells. Figure 1 shows a scanning electron micrograph of a mycoplasma infected culture. Hamet et al. (1) estimate that 25% of the total protein in infected cultures could be mycoplasmal. It is not surprising that mycoplasmas produce extensive changes in cultures they infect. Since the first report of Robinson et al. (2) in 1956, hundreds of papers have been published on the effects mycoplasmas have on their cell culture hosts. An extensive bibliography of cell culture mycoplasmas, which included effects of infection, has been published (3). The objectives of this report are (a) to provide cytogeneticists and cell biologists an understanding of some basic characteristics of mycoplasmas and (b) review the effects that mycoplasma infection has on the cytogenetics of cells in culture from either published reports or consideration of mycoplasmal characteristics.

The published incidence of infection has ranged from 4 to 92% (4–7). The incidence is influenced by the population of cultures being surveyed, the frequency with which mycoplasmal assays have been performed, the in vitro life span of the cell cultures, and, most importantly, the quality control programs in the laboratories surveyed. Several large scale surveys have been performed. These are summarized in Table 1. The lower incidences of infection reported from our laboratory probably reflect that our assay population includes more primary and early passage material. The majority of cultures tested in our surveys were from laboratories submitting cultures for either cell banking or mycoplasmal assays. Most of these were early passage material that had a lower incidence of infection (6). The incidence in long-term and continuous cultures is probably closer to the figures of Barile et al. (4) and Del Giudice and Hopps (5).

The review of Barile et al. (4) lists 19 different mycoplasma species and strains isolated from cell cultures. However, four species, *Mycoplasma orale*, *M. arginini*, *M. hyorhinis*, and *Acholeplasma laidlawii*, account for the vast majority of isolates. These four organisms account for approximately 85.9% of isolates in our assays (7). *Acholeplasma* is a mycoplasma genus characterized by its nonrequirement of sterols for growth. We have reported a relatively high incidence of *M. salivarium*; however, all but two of the *M. salivarium* isolates came from one laboratory (8). *Mycoplasma salivarium* does not constitute a major isolate in the other large scale surveys (4,5).

*Properties of mycoplasmas.* Mycoplasmas are wall-less prokaryotes, belonging to the Class Mollicutes. Table 2 lists the general characteristics of this class. The four organisms responsible for most cell culture infection differ significantly. *Acholeplasma laidlawii* and *M. hyorhinis* are fermentative organisms. *Mycoplasma orale* and *M. arginini* are not; these organisms convert arginine via the arginine deiminase pathway to citrulline and ornithine, generating ATP (9). Fenske and Kenny (10) studied the role of arginine deiminase in the growth of *M. hominis*. They argue that arginine is but an alternative energy source because increased enzyme levels were not observed until late log phase. Acholeplasmas do not require sterol for growth; mycoplasma species on the other hand cannot synthe-

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size sterols; sterols must be supplied for growth to occur. Therefore, the effects of infection by one mycoplasma species may differ significantly from the effects of infection of the same culture by another mycoplasma species. Infection with *M. orale* will drastically deplete arginine from cell culture media resulting in elevated ornithine (11), producing cytopathology and chromosome aberrations (12). Media arginine is not affected by *A. laidlawii* infection.

*The mycoplasma genome.* The genome size of the *Mycoplasmas* is approximately $0.5 \times 10^9$ daltons. The *Acholeplasmas*, which synthesize sterols, have larger genomes, on the order of $1.0 \times 10^9$ daltons (13). The genome size of the mycoplasma species is among the smallest for a self-replicating prokaryote, approximately one sixth that of *Escherichia coli* (14). The genome sizes of *Mycoplasmas* and *Acholeplasmas* are sufficient to code for a maximum of approxi-

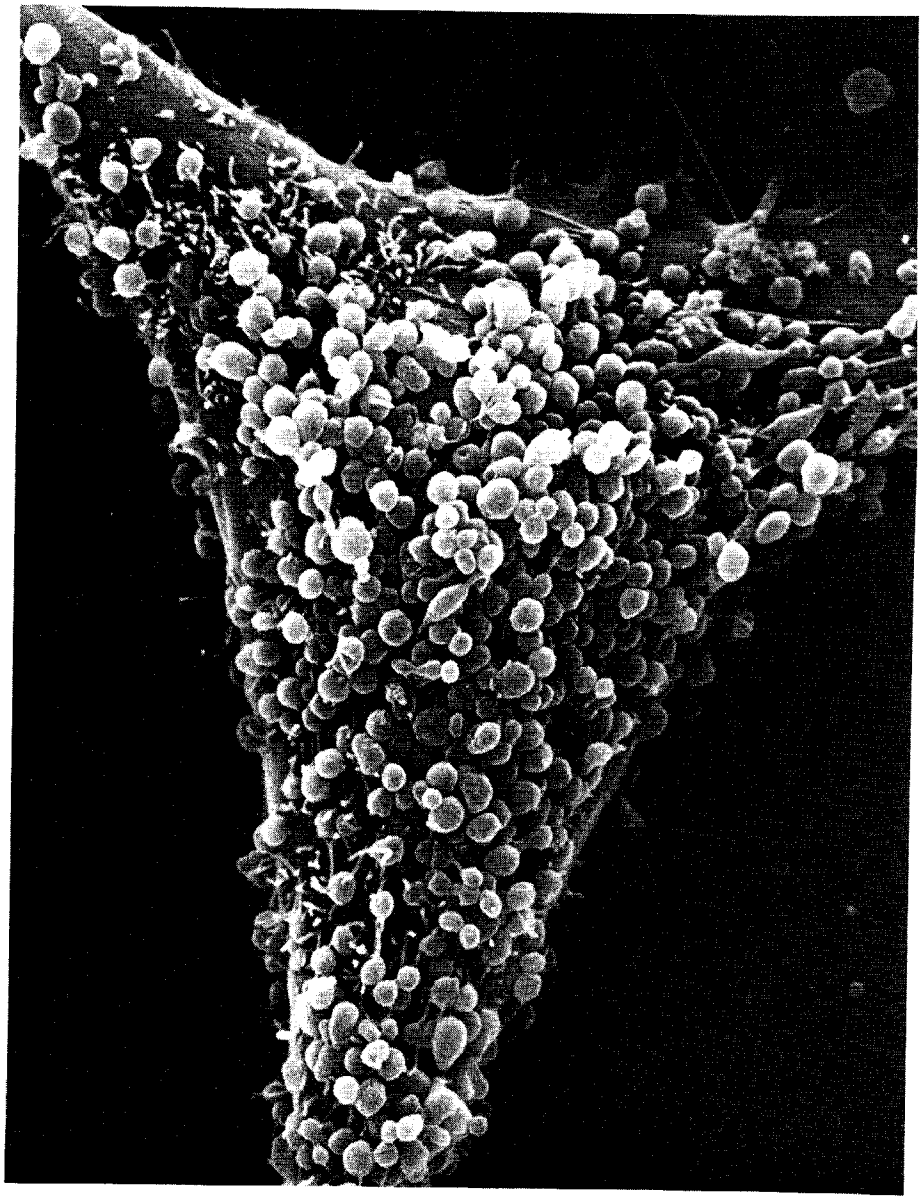

FIG. 1. Mouse tumor A-9 cell line infected with *Mycoplasma hyorhinis*. Photo courtesy of David M. Phillips, Population Council.

TABLE 1

PUBLISHED INCIDENCES OF MYCOPLASMA INFECTION OF CELL CULTURES

| Investigator | Number Infected | Number Assayed | | Reference |
|---|---|---|---|---|
| | | | % | |
| Barile et al. | 2817 | 17,000 | (16.5) | 4 |
| Del Giudice and Hopps | 204 | 2,297 | (8.9) | 5 |
| McGarrity et al. | 373 | 6,432 | (5.8) | 6 |
| McGarrity | 767 | 16,197 | (4.7) | 7 |

mately 625 and 1250 proteins, respectively. This is based on a DNA-to-protein ratio of 20 and an average DNA cistron of $8 \times 10^5$ (*A. laidlawii*: $1 \times 10^9/8 \times 10^5 = 1250$). Mycoplasmas possess a characteristically low guanine + cytosine (G+C) molar ratio, on the order of 25 to 40 moles % (13). These are among the lowest found in prokaryotes. The small genome size and the low G+C content of mycoplasmal DNA restricts the amount of genetic information available to mycoplasmas.

The mycoplasma genome replicates in a manner similar to other prokaryotes, semiconservative and sequentially from several growing points (15). As with other prokaryotes, DNA is synthesized continuously during growth but not during cell division (16). This pause may be due to the requirement for membrane or initiator molecules to be synthesized before DNA synthesis can resume.

Bode and Morowitz (17) showed that the DNA of H39 strain of *M. hominis* is a circular molecule that exists in an unbranched, folded state, similar to *E. coli*, and replicates according to the model of Cairns. More recently, Teplitz (18) showed that *M. hyorhinis* also contained a folded chromosome. Teplitz did not detect RNA or protein associated with the isolated folded chromosome. Obvious replication forks were not seen, although these could still be present. The number of loops varies and the number of loops and the degree of relaxation can result in a wide sedimentation profile in neutral sucrose. Teplitz (18) reports that most of the loops seem to be covalent closed circular DNA.

Razin and Razin (19) demonstrated that mycoplasmal DNA contains methylated bases. These studies included *M. hyorhinis*, *M. orale*, *M. arginini*, and *A. laidlawii*. All contained methylated adenine ($M^6$ methyladenine, $m^6$ Ade); *M. hyorhinis*, the only species tested for methylated cytosine, had 5.8% of the cytosine residues methylated (5-methylcytosine, $m^5$ Cyt). Mycoplasmal DNAs resemble DNA of other prokaryotes in its $m^6$ Ade. Eukaryotic DNA, on the other hand, is almost exclusively methylated in cytosine residues (20). As stressed by Razin and Razin (19), the finding of both $m^6$ Ade and $m^5$ Cyt in *M. hyorhinis* may have important implications on cell culture studies of methylated eukaryotic DNA. They point out that the ratio of eukaryotic DNA to mycoplasmal DNA in an infected cell culture is between 3:1 and 6:1, assuming a multiplicity of mycoplasmas per cell. This means that 15 to 30% of the DNA extracted from an infected cell culture is mycoplasmal DNA!

Ryan and Morowitz (21) demonstrated the presence of a single rRNA cistron in *M. capricolum* str Kid, based on calculations that this organism with a genome size of $6.84 \times 10^8$ has ribosomal DNA to code for one set of 23S plus 16S rRNA and enough DNA complimentary to tRNA to code for 44 different tRNA molecules. Sawada et al. (22) claimed two rRNA cistrons in this organism. Amikam et al. (23), using restriction endonucleases, showed a single rRNA cistron, confirming the work of Ryan and Morowitz (21). In this same study, Amikam et al.

TABLE 2

CHARACTERISTICS OF THE CLASS MOLLICUTES

| | |
|---|---|
| Class: | Mollicutes |
| Order: | Mycoplasmatales |
| Family I: | *Mycoplasmataceae* |
| | 1. Requirement for sterol |
| | 2. Genome size: $5.0 \times 10^8$ daltons |
| | 3. G + C content: 23 to 41% |
| | 4. NADH oxidase activity in cytoplasm |
| Genus I: | *Mycoplasma* (about 66 species current) |
| | 1. Do not hydrolyze urea |
| Genus II: | Ureaplasma (two species with serotypes) |
| | 1. Hydrolyze urea |
| | 2. G + C content: 28% |
| Family II: | *Acholeplasmataceae* |
| | 1. No requirement for sterol |
| | 2. Genome size: $1.0 \times 10^9$ daltons |
| | 3. G + C content: 29 to 35% |
| | 4. NADH oxidase activity in membrane |
| Genus I: | *Acholeplasma* (8 species current) |
| Family III: | *Spiroplasmataceae* |
| | 1. Helical organisms during some phase of growth |
| | 2. Requirement for sterol |
| | 3. Genome size: $1.0 \times 10^9$ daltons |
| | 4. G + C content: 26% |
| | 5. NADH oxidase activity in cytoplasm |
| Genus I: | *Spiroplasma* (3 species current) |

(23) demonstrated two rRNA cistrons in both *M. mycoides subsp. capri* and *A. laidlawii*. These studies also showed a great similarity in rRNA genes in the three mycoplasmas studied and similarities between mycoplasmal and *E. coli* rRNA sequences.

Woese et al. (24) used ribosomal and RNA analysis to conclude that mycoplasmas are phylogenetically related to clostridia. No evidence is available to suggest mycoplasmas represent stable L forms of existing walled organisms. In fact, all available data contradict the presence of genetic information for cell wall products among the Mycoplasmatales. As Razin (25) points out, data on genome sizes of prokaryotes show a gap between $6 \times 10^8$ and $1 \times 10^9$ daltons. Razin, as well as Morowitz and Wallace (26), state that this argues that mycoplasmas did not develop from cell-walled bacteria by deletion of genetic material. Otherwise, genomic sizes would be expected to span all values from the minimal value of $5 \times 10^8$ for mycoplasma to the lowest bacterial value, $1 \times 10^9$. Also, Walker (27), as cited by Razin (25), argues that mycoplasma species with DNA of such extremely low G+C content could not have evolved from walled eubacteria by deletion of nonessential sequences whereas the genome size of mycoplasmas is 25 to 50% of most eubacteria.

Morowitz and Wallace (26) proposed that *Mycoplasma* and *Ureaplasma* species represent the descendants of "protokaryote" organisms that preceded the prokaryote-eukaryote cell split. DNA doubling of the mycoplasma-ureaplasma genome $5 \times 10^8$ led to the $1 \times 10^9$ dalton acholeplasma genome, the intermediate in the evolution from the protokaryotes to the walled prokaryotes. The eukaryotic cell is not longer considered as having developed from the prokaryotic; rather both evolved from a common protokaryote.

Mycoplasmas contain viruses. These have been classified into three groups (28) summarized in Table 3. Group 1 consists of naked bullet-shaped particles containing circular, single-stranded DNA with an approximate $1.5 \times 10^6$ daltons mol wt. Group 2 viruses are spheroidal enveloped particles with circular double-stranded DNA, having a $7.8 \times 10^6$ daltons mol wt. Group 3 viruses are polyhedral particles with short tails and linear double-stranded DNA, mol wt $25.8 \times 10^6$. Most isolates are Group 1 (28) and most have been isolated from *A. laidlawii*, a major isolate from cell cultures. Only some *A. laidlawii* strains have been shown to produce plaques with any of the three virus groups (29). However, other isolates have been made from Mycoplasma and spiroplasma species (30). Viruses of spiroplasmas, helical mycoplasmas, have not been thoroughly classified and characterized. Group 1 and 2 viruses are released from host cells by budding; Group 3 viruses are lytic. Cole (30), Maniloff and Liss (29), and Maniloff et al. (31) have reviewed the ultrastructure and molecular biology of mycoplasma viruses. Phillips (32) has observed viruses

TABLE 3

MYCOPLASMAVIRUSES

| Virion Morphology | | Nucleic Acid | | Progeny Virus Release |
|---|---|---|---|---|
| | | Type | Molecular Weight | |
| Acholeplasma viruses | | | | |
| A1 | Naked bullet-shaped particles | Circular single-stranded DNA | $1.5 \times 10^6$ | Nonlytic |
| A2 | Roughly spherical enveloped particles | Circular double-stranded DNA | $7.8 \times 10^6$ | Nonlytic |
| A3 | Polyhedral particles with short tails | Linear double-stranded DNA | $25.8 \times 10^6$ | Lytic |
| Mycoplasma viruses | | | | |
| M1 | Polyhedron; long contractile tail with base plate and fibers | ? | ? | ? |
| M2 | Polyhedron; medium tail | DNA | ? | ? |
| Spiroplasma viruses | | | | |
| S1 | Rodshaped | Double-stranded DNA | ? | Unknown |
| S2 | Polyhedral with long tails | ? | ? | Lytic (?) |
| S3 | Polyhedral with short tails | Linear double-stranded DNA | 14 $\times 10^6$ | Nonlytic |
| S4 | Isometric sphere | ? | ? | ? |

in mycoplasma-infected cell cultures, although it was not ascertained whether these were mycoplasma viruses.

Liss (33) has reported 0.5 to 2.5 $\mu$g/ml mitomycin C released a Group 1 virus from *A. laidlawii* strain JA2. Similar treatment of another strain (Bju) had no effect. Liss suggested that late log or stationary growth phase organisms must be used to produce virus after mitomycin C treatment, at least in the JA2 strain.

DNA mediated transformation in mycoplasmas has not been reported. However, mycoplasma virus MV-L1 DNA transfects not only *A. laidlawii*, its natural host, but also *M. gallisepticum*, which is resistant to infection by the whole virus (34). Plasmids have been demonstrated in *A. laidlawii*, *M. hominis*, and *M. arthritidis*. Their approximate size was reported to be $20 \times 10^6$ daltons. Maniloff et al. (35) calculated an average of 50 to 100 plasmids per cell.

### *Cytogenetic Effects of Cell Culture Mycoplasmas*

Some general reviews of mycoplasmal infection (MI) of cell cultures have been published. Stanbridge (36) reported on the effects on amino acid, nucleic acid, and other host metabolism. An update of this has been published (13). Barile and Grabowski (37) and Barile (38) published reviews that have focused on changes in cell culture growth, viral titers, and interferon induction. Nichols (39) has also published a short review of some genetic effects of cell culture mycoplasmas. Many workers have shown that the cytogenetic effects of MI on cultured cells can compromise studies in mutagenesis, carcinogenesis, genetics, gene mapping, isoenzyme analysis, and clinical diagnostic procedures significantly.

Fogh and Fogh (40) first reported that mycoplasmas produced chromosomal aberrations in the FL amnion cell line. Mycoplasmal infected cells had a reduced chromosome number, gradually decreasing from 70–76 to 63. A concomitant increase in chromosomal aberrations occurred, which included open breaks and stable and unstable rearrangements. When the infection was cured with antibiotic treatment, the reduced chromosome and stable rearrangements persisted, but unstable aberrations returned to control levels. The organism in this study was not identified.

Paton et al. (41) demonstrated that *M. orale* produced three- to fivefold increases in the number of polyploid cells, chromosomal breakage, and rearrangements in human diploid fibroblasts. Aula and Nichols (12) reported that *M. salivarium* produced a threefold increase (5.6 to 18%) in the number of chromosomal abnormalities 5 d after establishment of human leukocyte cultures. No effects were seen with *M. hominis* Type 2 and *M. fermentans* in these studies. The chromosome damage was caused by mycoplasmal mediated arginine depletion of the media. Addition of 2 m*M* arginine returned the number of cells with breaks in infected cultures to control levels. Freed and Shatz (42) reported that cells deprived of any essential amino acid exhibited mitotic inhibition and chromosome damage. In a later study, we found no increase in chromosomal aberrations in continuous lymphocyte cultures infected with *M. salivarium* (8). The difference between these results and those of Aula and Nichols (12) may be in the age and types of cultures, a 5-d leukocyte culture in the study of Aula and Nichols and a continuous line in our study. The media used in these two studies had different arginine concentrations, 126.4 mg/l in the Aula and Nichols study (12) and 200 mg/l in our study (8). This difference is not thought to be an important factor in the different observed results.

Arginine depletion is not the only mechanism of mycoplasmal induced chromosome damage. Kundsin et al. (43) reported that two freshly isolated human ureaplasmas induced chromosomal abnormalities in human lymphocytes. Ureaplasmas do not possess arginine deiminase. Other mycoplasmas have been shown to induce chromosome abnormalities (44). The organisms included *M. orale*, *M. fermentans*, and *M. hominis*, all arginine utilizers. Antibiotic treatment reduced chromosome damage in these cultures. Stanbridge et al. (44) suggested mycoplasmal inhibition of mammalian DNA synthesis as a possible mechanism for chromosomal abnormalities.

No extensive studies have been published on the effects of MI on chromosome banding or sister chromatid exchange frequency. Fogh and Fogh (45) reported no unusual trypsin banding patterns in FL cells infected with *M. fermentans*. We have examined the effect of MI on sister chromatid exchange (SCE) in human lymphoblastoid cell cultures (Angelosanto et al., unpublished results). No significant increase in SCE was seen in MI. However, mycoplasmas contain uridine phosphorylase, which splits BUdR, rendering it unavailable for incorporation and eventual chromatid differentiation. This necessitated higher levels

of BUdR than usual. Mycoplasmal infection should be considered if relatively high concentrations of BUdR are required to obtain chromatid differentiation in SCE.

Kuzmina (46) reported that mycoplasmas were the presumed cause of Robertsonian fusion in mouse chromosomes that resulted in metacentric and submetacentric chromosome formation rather than the usual mouse chromosomal structure with a terminal centromere. The mycoplasma isolate was not identified.

Schneider et al. (47) reported that a significant increase in chromosome breaks and gaps and aneuploidy were observed in mycoplasma infected amniotic fluid cells. The organism was not identified. One infected culture in this study exhibited a consistent pattern of multiple translocations. The ability of mycoplasmas to induce chromosomal alterations can lead to false positive results in karyotypic analysis of amniotic fluid cells.

*Alterations in nucleic acid metabolism.* Mammalian cells readily incorporate nucleosides into their nucleic acid; free bases, however, are incorporated to only a negligible extent. Plageman and Richeyn (48) showed this is not due to a lack of transport of the free bases but to the lack of phosphoribosyl transferase activity. Mycoplasmas incorporate nucleosides and free bases with equal effectiveness.

McIvor and Kenny (49) reported that eight mycoplasma species representing the major serological groups exhibited significant differences in incorporation of free bases and nucleosides. This study included broth propagated *A. laidlawii*, *M. hyorhinis*, and *M. arginini*. All organisms tested incorporated uracil, adenine, and guanine; none incorporated cytosine. Species frequently isolated from cell cultures did not incorporate thymine independently, but deoxynucleosides enhanced thymine incorporation in *A. laidlawii* and *M. hyorhinis*. These authors reported no nucleoside incorporation in the arginine utilizing species. Significant differences of incorporation of bases and nucleosides occurred in the species tested. Strain differences are also likely. The work of McIvor and Kenny (49) and others (13) clearly

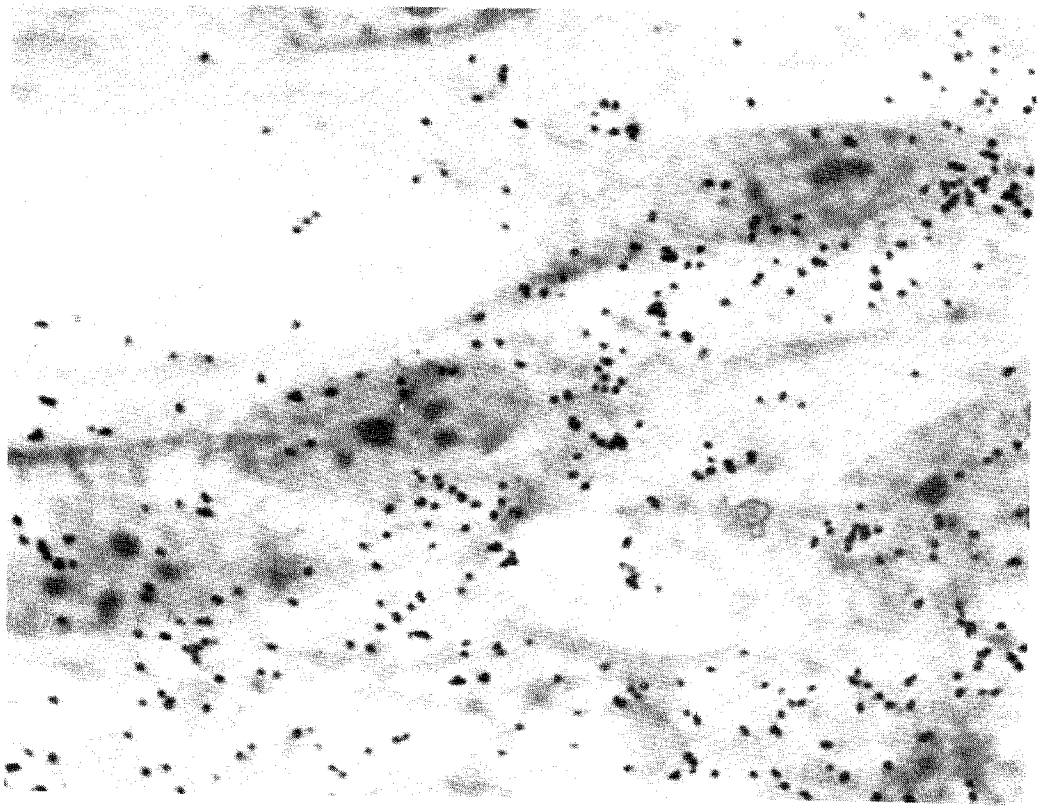

FIG. 2. Autoradiography of human diploid fibroblast IMR-90 infected with *M. orale* with triated thymidine. Note extranuclear labeling due to mycoplasmas.

show that MI can invalidate completely results of studies on mammalian transport and incorporation of nucleosides, free bases, and other studies on nucleic acid metabolism. Figure 2 shows autoradiography of a mycoplasma infected culture.

All mycoplasmas tested lack the orotic acid pathway for pyrimidine synthesis and the enzymatic pathways for de novo synthesis of purine bases (25). For growth to occur, mycoplasmas require at least one purine and one pyrimidine base.

Russell (50) showed that levels of DNase activity were 20-fold higher in mycoplasmal infected BHK-21 cells compared to controls at 20 to 27 h. The same study also demonstrated RNase activity in mycoplasmal extracts and mycoplasmal infected cells. These studies were performed with *M. pulmonis* and *M. fermentans*, not common cell culture isolates. The RNase activities of these organisms, reported by Russell (50) had an optimum pH of between 7 and 8; magnesium ions were not required for activity. Razin et al. (51) found the optimum pH for *A. laidlawii* DNase and RNase was 8.8 and both had an absolute requirement for magnesium ions. Pollack and Hoffmann (52) suggest that *A. laidlawii* strain B has at least two and perhaps as many as five nucleases. The DNases from *A. laidlawii* B., *A. equifetale*, *M. arthriditis*, and *M. pneumoniae* had an optimum pH range of 6.5 to 9.0. The two discernible DNase peaks of *A. laidlawii* with sedimentation coefficients of 3.1S and 4.3S displayed endonuclease activity with two substrate specificities.

Pollack and Hoffmann (52) suggest that Mollicutes nucleases present in inocula degrade medium DNA or oligonucleotides to more assimilable precursors necessary for growth. Nucleases released from the mycoplasmas during growth or present in media serum may also alter nucleic acids in the medium to usable forms.

Randall et al. (53) demonstrated mycoplasmal DNase activity in infected HeLa cells. These workers measured the release of host acid-soluble oligonucleotides into the supernatant medium, demonstrating the lability of host cell DNA in mycoplasma infected cultures. The organism was not identified. As mentioned, Stanbridge et al. (44) hypothesize that mycoplasmal interference of host cell DNA synthesis can induce chromosomal damage. Interference could be through competition for the acid soluble pool of nucleotide DNA precursors or by degradation of host cell DNA by mycoplasmal nucleases.

Mills et al. (54) published the first description of DNA polymerase from Mycoplasmatales using *M. orale* and *M. hyorhinis*. The purified enzyme from each species had an estimated 130,000 daltons mol wt as determined by gel filtration, a sedimentation coefficient of 5.65, and a sp act of >50,000 U/mg protein. These workers did not find evidence for more than a single DNA polymerase in these two mycoplasma species. Also noteworthy was the lack of any demonstrable exonuclease activity, including $3' \rightarrow 5'$ exonuclease activity. These characteristics are similar to those observed in a HeLa cell infected with mycoplasmas (55). Huppert et al. (56) showed that cell culture mycoplasmas were cosedimented with tumor virus preparations and the unidentified mycoplasma exhibited an endonucleolytic DNase activity (56).

Hellung-Larsen and Frederiksen (57) studied the incorporation of labeled precursors into RNA components of *M. hyorhinis* infected cell cultures. With [$^{32}$P]orthophosphate or [$^{3}$H]adenosine labels, incorporation is primarily in host RNA. [$^{3}$H]methyl groups are incorporated only into mycoplasma tRNA, 23S and 16S rRNA. 5-[$^{3}$H]uracil was incorporated into mycoplasmal tRNA, 4.7S RNA, a mycoplasmal low molecular weight RNA component M, and 23S and 16S rRNA. With 8-[$^{3}$H]guanosine or 5-[$^{3}$H]uridine, some incorporation was observed into host 28S and 18S rRNA, but it was significantly less than into mycoplasmal 23S and 16S rRNA. On the basis of these studies, the authors concluded that MI may prevent completely the identification of low molecular weight components.

*Mycoplasmal enzymes.* Mycoplasmas also possess nucleoside phosphorylases that cleave nucleosides to their free bases by the general reaction:

$$\text{Nucleoside} + PO_4 \rightleftharpoons \text{free base} + \text{ribose} - P$$

In fact, Levine (58) has utilized mycoplasmal phosphorylases as an indirect assay for MI. This technique monitors the conversion of [$^{14}$C]uridine into [$^{14}$C]uracil as an indicator of uridine phosphorylase activity. Negligible conversion occurs in mycoplasma-free cultures. Conversion rates of 40 to 90% are observed routinely in mycoplasmal infected cultures. Other methods of detection have involved measurement of incorporation or enzymatic conversion of radiolabeled thymidine or adenosine, or orthophosphates (59,60). Hatanaka et al. (61) detected adenosine phosphorylase activity in 13 of 16 species of Mycoplasmas, Acholeplasmas, and Spiroplasmas

(61). These authors reported no detectable activity in *M. pneumoniae*, *M. lipofilum*, and *M. sp.* Serogroup 70-159. This last organism has been detected only in cell cultures (5).

Hamet et al. (1) studied mycoplasmal enzymes involved in nucleotide degradation and base salvage pathways of purine and pyrimidine metabolism. Organisms studied were: *M. hominis*, *M. buccale*, *M. orale*, *M. salivarium*, and *M. arginini*. Enzymatic activities detected in mycoplasmas included: 5-nucleotidase, adenosine phosphorylase, inosine phosphorylase, adenine phosphoribosyltransferase (APRT), hypoxanthine phosphoribosyltransferase (HPRT), 5-pyrimidine nucleotidase, uridine phosphorylase, thymidine phosphorylase, cytidine deaminase, and uracil phosphoribosyltransferase. These authors did not detect uridine phosphorylase in *M. buccale*, the only mycoplasma species reported to lack it. However, we obtained this strain from these workers. It contained uridine phosphorylase when assayed by the method described by Levine (58).

We have exploited mycoplasmal phosphorylases as the basis for another indirect detection procedure (62). A purine analogue, 6-methylpurine deoxyriboside (6MPDR) has no apparent effect when added to mammalian cells in culture. In the presence of mycoplasmal adenosine phosphorylase, two potent antimetabolites, 6-methylpurine and 6-methylpurine riboside are produced. Extensive cytopathology occurs in mycoplasmal-infected cell cultures 3 to 4 d after addition of 6MPDR. The metabolic pathway is shown in Fig. 3. This method detected adenosine phosphorylase activity in all mycoplasmas tested, including *M.* sp. Serogroups 70-159 and two spiroplasma species.

*DNA repair.* Light and dark repair has been demonstrated in *A. laidlawii* (63). Das et al. (63) showed that sensitivity to ultraviolet irradiation and photoreversal was dependent on growth

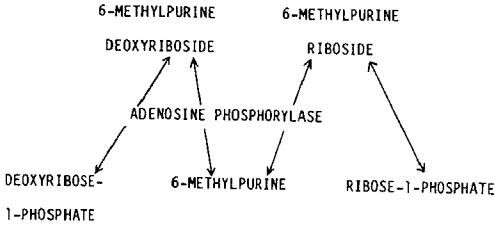


FIG. 3. Metabolism of 6-methylpurine deoxyriboside by adenosine phosphorylase.

phase of the culture. These workers stated that earlier reports of a lack of dark repair in *A. laidlawii* was due to a loss in viability of cells held in buffer, both in the light and in the dark. Some failures to demonstrate repair activity should be reexamined in light of this finding.

Smith and Hanawalt (64) studied DNA repair of broth propagated *A. laidlawii* after ultraviolet irradiation. This mode of replication consisted of small, single strand regions in parental DNA strands; the newly synthesized DNA was capable of normal semiconservative replication. Smith and Hanawalt stated that incorporation of nucleotides into parental strands might occur directly from resynthesis in excision repair or indirectly through recombination between normally replicated DNA molecules. This sister chromatid exchange would also yield the observed result, i.e. the presence of recently replicated DNA in parental DNA strands. Ultraviolet irradiation induces SCE in mammalian cells as shown by Kato (65). BUdR, used in the Smith and Hanawalt (64) study at a concentration of 7.4 μg/ml, also induces SCE in mammalian cells. The effect of this concentration of BUdR on *A. laidlawii* is difficult to determine. Uridine phosphorylase activity of *A. laidlawii* will cleave BUdR, rendering it unavailable for incorporation and reducing BudR concentration in the system. On the other hand, Smith and Hanawalt (64) reported that some repair replication occurred in the unirradiated culture in BUdR medium.

Mills et al. (54) showed that the principal DNA polymerase activity from *M. orale* and *M. hyorhinis* lacked exo- and endonuclease activities and could not excise thymine dimers. These findings would suggest a lack of dark repair. Ghosh et al. (67) detected neither dark nor photoreactivation repair in ultraviolet irradiated *M. gallisepticum*. Aoki et al. (68) demonstrated dark but not light repair in *M. buccale*. The dark repair system was inhibited by caffeine. *Mycoplasma buccale* is an infrequent isolate from cell cultures (4).

Results of these studies on broth propagated mycoplasmas suggest that MI, at least by some mycoplasma species, can compromise DNA repair studies in mammalian cell cultures. The effect of MI can be direct, i.e. through its own DNA repair, or indirect through incorporation of radiolabeled precursors or inhibition of mammalian DNA synthesis, among other possibilities.

Mycoplasmal infection can interfere with DNA repair studies in other ways. Gruneisen et al. (69)

reported that in four different *M. hyorhinis*-infected cell cultures, concentrations of $10^{-2}$ *M* to $10^{-5}$ *M* hydroxyurea failed to block incorporation of [$^3$H]thymidine during S phase growth. This was shown by autoradiography and pulse cytophotometry. Apparently, mycoplasmas have a lower sensitivity to hydroxyurea, similar to that observed in other prokaryotes. Rosenkranz et al. (70) showed that bacteria require two orders of magnitude greater concentration of hydroxyurea for inhibition of DNA synthesis than mammalian cells. Cell cultures whose DNA syntheses are not inhibited by the normal concentrations of hydroxyurea ($10^{-2}$ to $10^{-5}$ *M*) should be assayed for mycoplasmas.

A significant amount of work in mammalian cell genetics involves the use of enzymes and isoenzymes as markers for hybridization, gene mapping, genetic diseases, and metabolic pathways. Cell culture mycoplasmas can contribute isoenzymes that can confuse research and diagnostic procedures. Standbridge et al. (71) demonstrated that *M. hyorhinis* and *A. laidlawii* contained significant levels of endogenous hypoxanthine phosphoribosyltransferase (HPRT) activity. This activity was detected in a human cell line, D98/AH-2, that lacks measurable HPRT activity (71). Van Diggelen et al. (72) demonstrated HPRT activity in *A. laidlawii*, *M. hyorhinis*, *M. orale*, *M. arginini*, and *M. salivarium*. These workers showed that significant strain differences occur. *Mycoplasma hyorhinis* strain GDL formed 27 nmol IMP/h per milligram, whereas a wild-type strain had significantly more activity, 362 nmol IMP/h per milligram protein. The study of van Diggelen et al. (72) also showed that mycoplasmal HPRTs are more susceptible to 6-thioguanine than the mammalian isoenzyme, have a higher substrate binding constant for hypoxanthine and 5 phosphoribosyl-1-pyrophosphate (PRPP), and are more heat labile than the mammalian isoenzyme, as summarized in Table 4. Mycoplasmal infected mammalian cell lines that have HPRT activity died in hypoxanthine and aminopterin (HAT) medium, presumably due to mycoplasmal utilization of thymidine (72,73). Electrophoretic migration of mycoplasmal and mammalian HPRTs is shown in Fig. 4.

Clive et al. (74) demonstrated that MI prevented the selection of mutant cells in the thymidine kinase locus (TK +/—) assays. This system can select for forward and reverse mutations. For forward mutant selection, the cells are exposed to mutagens, then grown in BrdU. Mutated cells that have lost the TK+ gene do not incorporate BrdU and are resistant. The nonmutated cells incorporated BrdU and are killed under the conditions of the experiment. With MI, BrdU is not incorporated; all cells act like mutants. In a variation of this assay, TK—/— cells can be treated with a mutagen and then selection made for backward mutation to TK+/— by growing the cells in a selective medium of HAT. De novo cellular synthesis of purines and pyrimidines is prevented by aminopterin. Only cells that can incorporate the exogenous pyrimidine as well as purines can survive. Mycoplasmal infection prevents the incorporation of TK even when a cell has back mutated to TK+/— by cleaving the deoxyriboside to the base. Mycoplasmal infection prevents this and the selection process. In another report, Stanbridge and Reff (13) described the phenotypic conversion of TK—

TABLE 4

SPECIFIC ACTIVITY, HEAT SENSITIVITY, AND INHIBITION BY 6-THIOGUANINE OF ENDOGENOUS HPRT ISOZYMES IN FIVE COMMON MYCOPLASMA SPECIES[a]

| Species | Specific Activity | Heat Inactivation | Inhibition by 6-Thioguanine |
|---|---|---|---|
| | *nmol IMP formed/ hr/mg protein* | *% activity remaining after 2 min at 70° C* | *% inhibition at equimolar concentration* |
| *A. laidlawii* MG | 176 | 19.0 | 92.7 |
| *M. arginini* VA | 86 | 0.7 | 99.6 |
| *M. hyorhinis* (GDL) | 27 | 3.1 | 97.5 |
| *M. hyorhinis* (Einstein) | 362 | 5.6 | 98.2 |
| *M. orale* JS | 75 | 0.4 | 99.6 |
| *M. salivarium* VV | 68 | 0.3 | 99.4 |
| Mouse L929 | 187 | 91.0 | 56.0 |
| Mouse A9 | <2 | — | — |

[a] From Van Diggelen et al. Endogenous HPRT activity in mycoplasmas isolated from cell cultures. *In Vitro* 14: 734–739; 1978. Reprinted with permission.

to TK+ after infection by an unnamed mycoplasma, but data and study methods were not presented. This is presumably by a strain of mycoplasma supplying the thymidine kinase to the cell similar to the phenotypic conversion.

Bradlaw et al. (75) reported that *M. arginini* infection of rat hepatoma cell line H-4-II-E actually increased the responsiveness of these cells to aryl hydrocarbon hydroxylase (AHH) induction by 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (TCDD). Elimination of the infection by antibiotic treatment rendered the cells less reponsive to AHH induction by TCDD. Almost double the TCDD dose was required to achieve half maximal enzyme induction.

O'Brien et al. (76) reported on expression of other enzymes in 22 species of *Mycoplasma* and *Acholeplasma* and related how these could potentially interfere with studies on mammalian cell biology. In this comprehensive study, mycoplasmas were analyzed for 21 isoenzymes routinely used to characterize mammalian cells. Most mycoplasmas had significant activity of nine of the isoenzyme systems: triose phosphate isomerase, glucose phosphate isomerase, dipeptidase, glyceraldehyde-3-phosphate

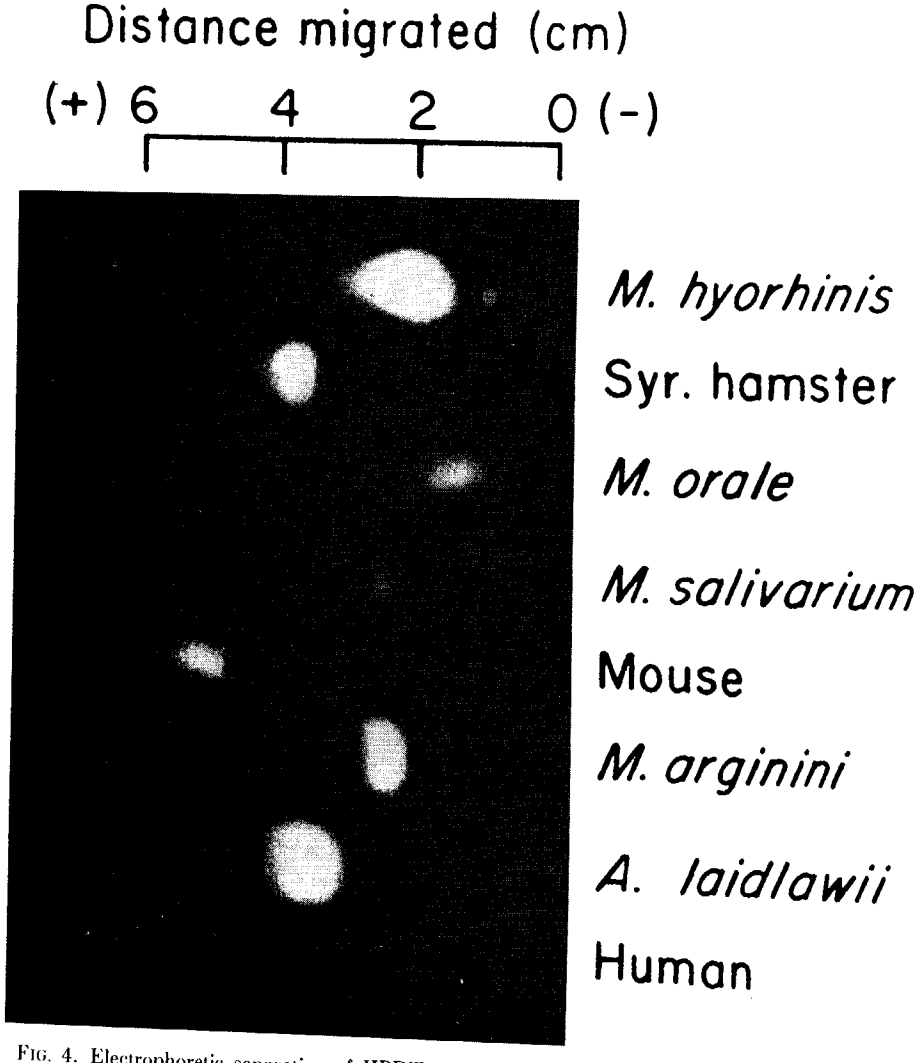


FIG. 4. Electrophoretic separation of HPRT isozymes from five mycoplasma species and three selected mammalian species. Cell-free lysates were run in the cellulose acetate gel (Cellogel) at pH 7.0. Syrian hamster: BHK 21/C1 13; Mouse: L929; Human: HeLa. (Reprinted from *In Vitro* 14: 734-739; 1978.)

dehydrogenase, adenylate kinase, inorganic pyrophosphatase, acid phosphate esterase, and nucleoside phosphorylase. In addition, acholeplasmas, but not mycoplasmas, contained superoxide dismutase, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. The appearance of the latter two enzymes suggests that the pentose shunt is functional in acholeplasmas, but not mycoplasmas. The expression of mammalian isoenzymes was not apparently altered by mycoplasmas. These workers suggest that triose phosphate isomerase, glucose phosphate isomerase, and nucleoside phosphorylase were the best candidates to use as a screen for MI. However, they caution that such a screen can be influenced significantly by the level of activity in the infecting mycoplasma and the coincidental mobilities of homologous enzymes produced by the host cells and the infecting mycoplasmas.

In other enzyme systems, Clark et al. (77) reported that mycoplasmas have higher relative activities of pyruvate dehydrogenase (PDH) and pyruvate dehydrogenase complex (PDHC) relative to human fibroblasts. Constantopoulos et al. (78) measured several oxidative enzymes in fermentative and nonfermentative mycoplasmas. They found high activities of PDH and PDHC in *A. laidlawii*, *M. hyorhinis*, and *M. pneumoniae*, all fermentors. Activity of $\alpha$-ketoglutarate was minimal in all five mycoplasmas tested. *Acholeplasma laidlawii* had lipoamide dehydrogenase activity comparable to human fibroblasts, and all five mycoplasmas examined contained lactate dehydrogenase and NAD-H-DCPIP oxidoreductose activities. Increased levels of PDH were detected in a human cell line deficient in PDH activity after MI (in press).

*Potential effects on cytogenetic/mutagenic assays.* Mycoplasmas can influence in vitro cytogenetic/mutagenic assays by virtue of their large concentrations and metabolic turnover. The potential general effects of MI on cell cultures, i.e. cytopathology and reduced growth rates, can reduce significantly the sensitivity of these assays and affect the qualitative results. Mycoplasmas can also act as a metabolic sink for cell culture supplements or mutagenic chemicals, reducing the effective concentration of these agents available to the target cells, as was illustrated in Table 4, which showed that mycoplasmal HPRT had a greater affinity for 6-thioguanine than the mammalian isozyme. Therefore, a mycoplasma infected HPRT positive cell line in a mutagenic assay could lead to survival of the cells due to mycoplasmal metabolism of 6-TG. Such interference would also be apparent in selection of mutants in HAT medium; HPRT + cells containing mycoplasma die in HAT medium (72,73), probably due to mycoplasma depletion of thymidine from the medium. Mycoplasmal infection can lead to false negatives in HAT selection for cell hybrids.

Mycoplasmas may present themselves in metaphase spreads with Giemsa, Hoechst 33258, or other fluorochrome staining. Use of a DNA binding fluorochrome, such as Hoechst 33258, can show that structures observed in Giemsa preparations contain DNA. Use of Hoechst 33258, first introduced as a cytogenetic reagent, was shown to be effective in detection of MI by Chen (79). DNA fluorescence has been shown to be a highly efficient method of detection when performed under controlled conditions in an appropriate indicator cell line (5,6). Figure 5 shows a mycoplasma infected cell culture stained with Hoechst 33258.

Other mycoplasmal mediated effects on cytogenetic/mutagenic assays can occur. Mycoplasmas can alter mitogenic responses of freshly drawn blood lymphocytes. Different mycoplasmal species have been shown to stimulate lymphocytes. Different mycoplasmal species have been shown to stimulate mitogenesis in the absence of phytohemagglutinin; other species inhibited the action of phytohemagglutinin. This effect of mycoplasmas seems to be related to utilization of arginine; arginine utilizing mycoplasmas inhibit mitogenesis, nonarginine utilizers stimulate mitogenesis (80–82). The mechanism of the mitogenesis is unknown. It has been shown to be nonspecific and polyclonal (82,83). Copperman and Morton (84) demonstrated that PHA stimulation of lymphocyte cultures could be inhibited by heat killed as well as viable mycoplasmas.

As described above, another potential effect in SCE by MI is inhibition of chromatid differentiation. This is due to mycoplasmal uridine phosphorylase cleavage of BUdR rendering this analogue unavailable for cell incorporation and chromatid differentiation. This would result in large concentrations of BUdR being used to overcome the effects of MI to achieve chromatid differentiation.

Mycoplasmal infection can also influence transformation assays. This can be via effects on growth rates or by effects on transforming viruses.

McPherson and Russell (85) showed that mycoplasma infected BHK21/13 cells mimicked transformation by growth in soft agar. Mycoplasmal infected cells had altered morphologies, containing fibroblastic/epithelioid morphologies, and grew in soft agar. Clonal efficiencies ranged from 0.5 to 5%. Cells picked from these agar colonies retained their altered epithelioid and fibroblast morphology when grown on glass. Infections with *M. fermentans* (PG18) and *M. hominis* (PG21 and PG27) yielded the highest levels of growth in soft agar. When the mycoplasmas were eliminated from the cell cultures by antibiotic treatments, plating efficiencies were 10 to 150 times higher than controls, indicating that, once induced, the presence of mycoplasmas were not necessary for continued observation of the changes. Under these conditions, microcolonies of mycoplasmas can develop around developing colonies of the BHK cells, rendering the BHK cells more susceptible to effects of the mycoplasmas. The authors suggest that the manifestation of a permanent change in the genetic apparatus induced by the mycoplasmas was analogous to the irreversible chromosomal changes reported by the Foghs (40).

Mycoplasmal infection has also been shown to mimic auxotrophy in cultured cells. The aliphatic diamine putrescine is of demonstrated importance in cell replication (86). Luk et al. (87) showed that a block of endogenous putrescine synthesis impairs mammalian cell growth in vitro. We have shown that putrescine stimulated the growth of a murine lymphoblastoid line, R.I.I. infected with *M. orale* (88). The mechanism was shown to be putrescine inhibition of the mycoplasma arginine deiminase pathway, which inhibited mycoplasma growth. Without putrescine, *M. orale* grew to high titers and prevented growth of R.I.I.

### *In Vivo Correlates*

The above data are of potential importance to a variety of in vitro systems. Are the various characteristics expressed by cell culture mycoplasmas related to in vivo pathogenesis? If so, cell and organ culture systems would be of value as model systems. Mycoplasmas, as a rule, do not elaborate many of the extracellular factors or enzyme systems that bacteria use to enhance invasiveness or pathogenesis (25,89).

*Chromosome damage.* A variety of viral diseases have been shown to cause chromosomal

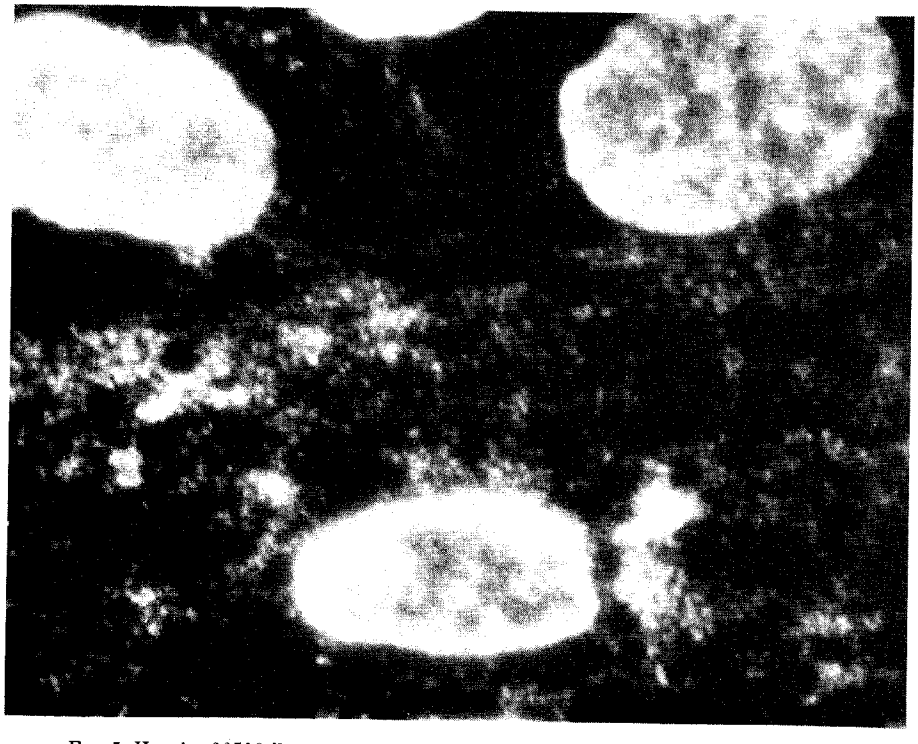

FIG. 5. Hoechst 33528 fluorescent stain of HeLa cell culture infected with *M. hyorhinis*.

aberrations in peripheral leukocytes. These have been reviewed by Nichols (39). No data exist to demonstrate chromosomal aberrations caused by clinical mycoplasmal disease. Mycoplasmas are not generally detected in the blood stream during clinical disease. In fact, Birke et al. (91) speculate that natural killer cells may prevent serious clinical manifestations of mycoplasmal infections, including blood invasion. These authors showed that mycoplasma infected cell cultures, as well as tumor cells and virus infected cells, are targets of human NK cells and induce interferon (IFN). Beck et al. (92) reported similar findings in a murine system. Both studies showed that mycoplasma infected, but not mycoplasma free cell cultures induced interferon. There were some minor differences between the studies in human and murine systems. No human cell line studied resisted NK lysis despite inducing high titers of IFN. In the murine system Beck et al. (92) reported the L929 cell was resistant. A heat and acid stable IFN-$\alpha$ was produced in human cells; an acid labile IFN-$\gamma$ was produced in murine cells.

The incidence of blood carriage of mycoplasma increases with immunosuppression, due to either disease or therapy (93). A concomitant increase in chromosomal aberrations has not been reported. We are unsure if a study to measure chromosome aberrations in mycoplasma infection has been performed. However, such an association might be difficult to prove inasmuch as many therapeutic regimens used in these patients cause aberrations.

In vivo, mycoplasmas are generally found on epithelial cell surfaces. Chromosomal aberrations would not be expected in such microenvironments. Epithelial tissues exhibit histiotypic gradients in vivo, cells at the basal surface exhibiting a relatively high incidence of mitosis and a low level of differentiation. The cells at the luminal surface are highly differentiated but undergo few, if any, mitoses. Under these conditions, it is not surprising that chromosomal aberrations are not observed.

*Abortions, infertility, and stillbirths.* Taylor-Robinson and McCormack (94) have reviewed the role of genital mycoplasmas in human disease. Cassell et al. (95) have reviewed the involvement of mycoplasmas in diseases of laboratory animals. In their review, Taylor-Robinson and McCormack (94) state that *Ureaplasma urealyticum* is a suggestive cause of chorioamniotis and low birth weight. Some data also show a correlation between involuntary fertility, spontaneous abortion, and stillbirth with *U. urealyticum*. Fowlkes et al. (96) have demonstrated lower sperm motility with *U. urealyticum*. Spermatozoa adsorb to colonies and suspensions of certain mycoplasmas. Taylor-Robinson and McCormack (94) do not believe this to play a significant role in clinical disease due to the relatively short exposure times and the small numbers of mycoplasmas per spermatozoa. It may have a greater effect on banked sperm; this has been reported in bovine sperm (97).

Published reports have shown that mycoplasmas, and especially ureaplasmas, have been isolated in greater frequency from fetal membranes of fetuses aborted spontaneously than from therapeutically aborted fetuses (98). Antibiotic treatment of women having a history of spontaneous abortions resulted in successful pregnancies (99,100). However, antibiotic trials have often been uncontrolled, the role of other microorganisms has not been studied, and small survey populations render interpretation of results difficult. The role of *U. urealyticum* is further complicated by the presence of serotypes. Black and Kroggsgaard-Jensen (101) originally proposed eight serotypes. Certain serotypes may be more virulent than others. Quinn et al. (102) have reported that women with pregnancy losses had elevated mean titers to Serotypes 4 and 8. Infants of mothers with pregnancy losses had elevated titers to Serotypes 6 and 8. Serotyping is not performed routinely because of cross reactions at low dilutions and the necessity of large volumes of specific antisera and antigen, both significant problems with *U. urealyticum*. Quinn et al. (103) developed an immunoperoxidase assay that circumvented many of these difficulties. More recently, Robertson and Stemke (104) have expanded the serotyping scheme for *U. urealyticum* to 14 types. If ureaplasmas are associated with various diseases, some but perhaps not all serotypes may be involved. It is hoped that the availability of improved assays and reagents will clarify the role, if any, these organisms play in such diseased states.

The association between *U. urealyticum* infection and low birth weight is strong but not definitive for many of the reasons listed for spontaneous abortions. An association has been noted between low birth weight and *M. pulmonis* in rodents (95).

In laboratory animal studies, rodents frequently acquire *M. pulmonis* via aerosol the first weeks of life (105); the organism can also be acquired in utero (106). Transplacental trans-

mission of mycoplasmas has been demonstrated in other animals (107). The effects of respiratory and genital *M. pulmonis* infections on research involving rodents has been reviewed (95). Latent and acute mycoplasmal infection of laboratory rodents can influence experimental results significantly. In fact, some of the effects cited by Cossell et al. (96) are reminiscent of the tales of cell culture mycoplasmas.

*Sex ratio organism.* A fascinating study in parasitism exists in the spiroplasma-induced sex ratio (SR) trait in certain neotropical strains of drosophila. Spiroplasmas are helical mycoplasmas that have been shown to cause disease in plants, insects and, under certain laboratory conditions, suckling rodents. Not all isolates are associated with diseases. A text on plant and insect mycoplasmas has reviewed the field (108).

The sex ratio trait was first reported to be caused by a small treponema-like spirochete in the hemolymph of female *Drosophila willistoni* (109). The sex ratio has been detected in four closely related neotropical species of Drosophila: *D. equinoxialis*, *D. nebulosa*, *D. paulistorum*, and *D. willistoni*, and results in the total elimination of males from the progeny of infected females. Williamson and Paulson (110) and Williamson et al. (111) have reviewed the SR trait and the sex ratio organism (SRO).

The SROs, now shown to be spiroplasmas, can be transferred through injection of infected hemolymph to noninfected drosophila species females. Within days, the newly infected females begin to produce progeny with a SR distorted in favor of females. The SRO's are transovarially transmitted; female progeny contain SRO's in their hemolymph and evidence total or partial elimination of male offspring. Williamson and Paulson (110) reported a total of 1501 female progeny of *D. pseudoobscura* and 0 male over eight broods.

Sex ratio organisms grow to unusually high titers in the hemolymph of infected females, on the order of 1.0 to $2.0 \times 10^6/\mu l$ hemolymph. Interestingly, no apparent pathology is observed in females. Despite these large concentrations, the SROs cannot be cultivated in vitro. Media used to propagate other spiroplasmas are unsuccessful in cultivation of SROs (111). This has been a significant obstacle to investigators.

The mechanism of male killing by SROs is not understood. Studies of various special strains of *D. melanogaster* infected by injection have shown that the presence of a Y chromosome is not involved in male lethality. Only embryos with two X chromosomes are able to survive an established SR infection; XXY females survive, but XO males die (110). The existence of a specific male lethal factor has been suggested (112). A maternally inherited nonmale killing spiroplasma has been found in *D. hydei* natural populations near Ito, Japan (113). This organism cannot be propagated in laboratory media.

Despite the shortcomings associated with the failure to propagate SROs outside drosophila hosts, these organisms present an intriguing study model in infectious disease, gene action, sex differentiation, and natural selection. The process by which this infection persists in nature without eliminating the population is unknown.

## CONCLUSIONS

Mycoplasmal infection represents the venereal disease of cell cultures! It does not happen to "nice" laboratories. It often results from contacts with "questionable" sources, and too often it is not the subject of frank, open discussion. The true incidence of infection may be higher than published figures. If ignored, the infection will not go away but will continue to fester, spreading to other cultures and other laboratories to generate more meaningless and confusing data. Such confusion and wasted effort are bad enough. Too frequently, however, other investigators in other laboratories must spend time to clarify confusing results generated with infected cultures.

Effective and relatively simple preventive detection and control measures are available. No laboratory need be susceptible to chronic, repeated disasters of cell culture mycoplasmas. An effective disease control program should include assay of cell cultures for mycoplasmas. Detection methods have been reviewed recently (7). Investigators can select effective procedures that are appropriate for their facilities. For optimum effectiveness, detection procedures must be combined with efforts to prevent introduction of mycoplasmas and to control their spread if they do gain entrance. Methods of prevention and control consist, in general, of adherence to classical microbiological procedures. In practice, these are quite simple and highly effective (114,115). Strict adherence to these preventive and control measures would greatly reduce and perhaps eliminate the need for future reviews.

## REFERENCES

1. Hamet, M.; Bonissol, C.; Cartier, C. Enzymatic activities on purine and pyrimidine metabolism in

nine mycoplasma species contaminating cell cultures. Clin. Chim. Acta 103: 15-22; 1980.

2. Robinson, L. B.; Wichelhausen, R. K.; Roizman, B. Contamination of human cell cultures by pleuropneumonia organisms. Science 124: 1147-1148; 1956.
3. McGarrity, G. J.; Meredith, D.; Gruber, D.; McCall, M. Cell culture mycoplasmas: a bibliography. McGarrity, G. J.; Murphy, D. G.; Nichols, W. W. eds. Mycoplasma infection of cell cultures. New York: Plenum Press; 1978: 243-334.
4. Barile, M. F.; Hopps, H. E.; Grabowski, M. W. Incidence and sources of mycoplasma contamination: a brief review. McGarrity, G. J.; Murphy, D. G.; Nichols, W. W. eds. Mycoplasma infection of cell cultures. New York: Plenum Press; 1978: 35-45.
5. Del Giudice, R. A.; Hopps, H. E. Microbiological methods and fluorescent microscopy for the direct demonstration of mycoplasma infection of cell cultures. McGarrity, G. J.; Murphy, D. G.; Nichols, W. W. eds. Mycoplasma infection of cell cultures. New York: Plenum Press; 1978: 57-69.
6. McGarrity, G. J.; Sarama, J.; Vanaman, V. Factors influencing microbiological detection of mycoplasmas in cell cultures. In Vitro 15: 73-81; 1979.
7. McGarrity, G. J. Detection of mycoplasmal infection of cell cultures. Maramorosch, K. ed. Advances in cell cultures. New York: Academic Press; 1982: 99-131.
8. McGarrity, G. J.; Phillips, D. M.; Vaidya, A. B. Mycoplasmal infection of lymphocyte cell cultures: infection with *M. salivarium*. In Vitro 16: 346-356; 1980.
9. Schimke, R. T. Studies on the metabolism of arginine by mycoplasmas. Ann. NY Acad. Sci. 143: 573-577; 1967.
10. Fenske, J. D.; Kenny, G. E. Role of arginine deiminase in growth of *Mycoplasma hominis*. J. Bacteriol. 126: 501-510; 1976.
11. Stanbridge, E. J.; Hayflick, L.; Perkins, F. T. Modification of amino acid concentrations induced by mycoplasmas in cell culture medium. Nature 232: 242-244; 1971.
12. Aula, P.; Nichols, W. W. The cytogenetic effects of mycoplasma in human leukocyte cultures. J. Cell. Physiol. 70: 281-290; 1967.
13. Stanbridge, E. J.; Reff, M. E. The molecular biology of mycoplasmas. Barile, M. F.; Razin, S. eds. The mycoplasmas. Vol. 1. New York: Academic Press; 1979: 157-185.
14. Razin, S. Mycoplasmas: the smallest pathogenic prokaryotes. Isr. J. Med. Sci. 17: 510-515; 1981.
15. Smith, D. W. DNA replication in *Mycoplasma laidlawii* B. Biochim. Biophys. Acta 179: 408-421; 1969.
16. Quinlan, D. C.; Maniloff, J. Deoxyribonucleic acid synthesis in synchronously growing *Mycoplasma gallisepticum*. J. Bacteriol. 115: 117-120; 1973.
17. Bode, H. R.; Morowitz, H. J. Size and structure of the *Mycoplasma hominis* H39 chromosome. J. Mol. Biol. 23: 191-199; 1967.
18. Teplitz, M. Isolation of folded chromosomes from *Mycoplasma hyorhinis*. Nucleic Acids Res. 4: 1505-1512; 1977.
19. Razin, A.; Razin, S. Methylated bases in mycoplasma DNA. Nucleic Acids Res. 8: 1383-1390; 1980.
20. Razin, A. Methylated bases in the mycoplasmas. Denhardt, D. T.; Dressler, D.; Ray, D. S. eds. The single stranded DNA phages. Cold Spring Harbor Monograph Series. 1978: 165-175.
21. Ryan, J. L.; Morowitz, H. J. Partial purification of native rRNA and tRNA cistrons from *Mycoplasma sp.* (Kid). Proc. Natl. Acad. Sci. USA 63: 1282-1289; 1969.
22. Sawada, S.; Osawa, S.; Kobayashi, H.; Hori, H.; Muto, A. The number of ribosomal RNA genes in *Mycoplasma capricolum*. Mol. Gen. Genet. 182: 502-504; 1981.
23. Amikam, D.; Razin, S.; Glaser, G. Ribosomal genes in mycoplasma. Nucleic Acids Res. 10: 4215-4222; 1982.
24. Woese, C. R.; Maniloff, J.; Zablen, L. B. Phylogenetic analysis of mycoplasmas. Proc. Soc. Natl. Acad. Sci. USA 77: 494-498; 1980.
25. Razin, S. The mycoplasmas. Microbiol. Rev. 42: 414-470; 1978.
26. Morowitz, H. J.; Wallace, D. C. Genome size and life cycle of the mycoplasma. Ann. NY Acad. Sci. 225: 62-73; 1973.
27. Walker, R. T. Mycoplasma tRNAs. Proceedings of the international conference on the synthesis, structure and chemistry of the tRNAs and their components. Poznan, Poland; 1976: 291-295.
28. Maniloff, J.; Das, J.; Putzrath, R. M.; Nowak, J. A. Mycoplasma and spiroplasma viruses: molecular biology. Barile, M. F.; Razin, S. eds. The mycoplasmas. Vol. 1. New York: Academic Press; 1979: 411-430.
29. Maniloff, J.; Liss, A. Comparative structure, chemistry & evolution of mycoplasma viruses. Kurstak, E.; Maramorosch, K. eds. Virus, evolution and cancer. New York: Academic Press; 1974: 584-604.
30. Cole, R. M. Mycoplasma and spiroplasma viruses: ultrastructure. Barile, M. F.; Razin, S. eds. The mycoplasmas. Vol. 1. New York: Academic Press; 1979: 385-410.
31. Maniloff, J.; Das, J.; Nowak, J. A. Single stranded DNA mycoplasma virus. Denhardt, D. T.; Dressler, D.; Ray, D. S. eds. The single stranded DNA phages. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory; 1978: 177-184.
32. Phillips, D. M. Detection of mycoplasma contamination of cell cultures by electron microscopy. McGarrity, G. J.; Murphy, D. G.; Nichols, W. W. eds. Mycoplasma infection of cell cultures. New York: Plenum Press; 1978: 105-118.
33. Liss, A. Release of a group 1 mycoplasma virus from *Acholeplasma laidlawii* after treatment with mitomycin C. J. Virol. 40: 285-288; 1981.

34. Liss, A.; Maniloff, J. Transfection mediated by *Mycoplasmatales* viral DNA. Proc. Natl. Acad. Sci. USA 3423–3427; 1972.

35. Maniloff, J.; Das, J.; Christensen, J. R. Viruses of mycoplasmas and spiroplasmas. Adv. Virus Res. 21: 343–380; 1977.

36. Stanbridge, E. Mycoplasmas and cell cultures. Bacteriol. Rev. 35: 206–227; 1971.

37. Barile, M. F.; Grabowski, M. W. Mycoplasma-cell culture-virus interactions: a brief review. McGarrity, G. J.; Murphy, D. G.; Nichols, W. W. eds. Mycoplasma infection of cell cultures. New York: Plenum Press; 1978: 135–150.

38. Barile, M. F. Mycoplasma-tissue-cell interactions. Tully, J. G.; Whitcomb, R. F. eds. The mycoplasmas. Vol. 2. New York: Academic Press; 1979: 425–474.

39. Nichols, W. W. Genetic effects of mycoplasma. McGarrity, G. J.; Murphy, D. G.; Nichols, W. W. eds. Mycoplasma infection of cell cultures. New York: Plenum Press; 1978: 151–157.

40. Fogh, J.; Fogh, H. Chromosome changes in PPLO-infected FL human amnion cells. Proc. Soc. Exp. Biol. Med. 119: 233–238; 1965.

41. Paton, G. R.; Jacobs, J. P.; Perkins, F. T. Chromosome changes in human diploid-cell cultures infected with mycoplasma. Nature 207: 43–45; 1965.

42. Freed, J. J.; Schatz, S. A. Chromosome aberrations in cultured cells deprived of single essential amino acids. Exp. Cell Res. 55: 393–409; 1969.

43. Kundsin, R. B.; Ampola, M.; Streeter, S.; Neurath, P. Chromosomal aberrations induced by T strain mycoplasmas. J. Med. Genet. 8: 181–187; 1971.

44. Stanbridge, E.; Onen, M.; Perkins, F. T.; Hayflick, L. Karyological and morphological characteristics of human diploid cell strain WI-38 infected with mycoplasmas. Exp. Cell Res. 57: 397–410; 1969.

45. Fogh, J.; Fogh, H. Chromosome changes in cell culture induced by mycoplasma infection. Ann. NY Acad. Sci. 225: 311–329; 1973.

46. Kuzmina, S. W. Action of mycoplasmas on the chromosomal apparatus of mouse fibroblasts in tissue culture. Sov. Genet. 8: 126–127; 1972.

47. Schneider, E. L.; Stanbridge, E. J.; Epstein, C. J.; Golbus, M.; Abbo-Halbasch, G.; Rodgers, G. Mycoplasma contamination of cultured amniotic fluid cells: potential hazard to prenatal chromosomal diagnosis. Science 184: 477–480; 1974.

48. Plagemann, P. G.; Richey, D. P. Transport of nucleosides, nucleic acid bases, choline and glucose by animal cells in culture. Biochem. Biophys. Acta 344: 263–305; 1974.

49. McIvor, R. S.; Kenny, G. E. Differences in incorporation of nucleic acid bases and nucleosides by various mycoplasma and acholeplasma species. J. Bacteriol. 135: 483–489; 1978.

50. Russell, W. C. Alterations in the nucleic acid metabolism of tissue culture (hamster fibroblast) cells infected by mycoplasmas. Nature 212: 1537–1540; 1966.

51. Razin, S.; Knysynski, A.; Lifshitz, Y. Nucleases of mycoplasma. J. Gen. Microbiol. 36: 451–459; 1964.

52. Pollack, J. D.; Hoffmann, P. J. Properties of the nucleases of mollicutes. J. Bacteriol. 152: 538–541; 1982.

53. Randall, C. C.; Gafford, L. G.; Gentry, G. A.; Lawson, L. A. Lability of host-cell DNA in growing cell cultures due to mycoplasma. Science 149: 1098–1099; 1965.

54. Mills, L. B.; Stanbridge, E. J.; Sedwick, W. D.; Korn, D. Purification and partial characterization of the principal deoxyribonucleic acid polymerase from *Mycoplasmatales*. J. Bacteriol. 132: 641–649; 1977.

55. Bolden, A.; Noy, G. P.; Weissback, A. DNA polymerase of mitochondria is an γ polymerase. J. Biol. Chem. 252: 3351–3356; 1977.

56. Huppert, J.; Delain, E.; Fossar, N. Endonucleolytic DNase in oncorna viruses: role of mycoplasma. Virology 57: 217–226; 1974.

57. Hellung-Larsen, P.; Frederiksen, S. Influence of mycoplasma infection on the incorporation of different precursors into RNA components of tissue culture cells. Exp. Cell Res. 99: 295–300; 1976.

58. Levine, E. M. A simplified method for the detection of mycoplasma. Methods Cell Biol. 8: 229–248; 1974.

59. Markov, G. G.; Bradvarova, I.; Mintcheva, A.; Petrov, P.; Shiskov, N.; Tsanev, R. G. Mycoplasma contamination of cell cultures: interference with $^{32}$P-labelling pattern of RNA. Exp. Cell Res. 57: 374–384; 1969.

60. Perez, A. G.; Kim, J. H.; Gelbard, A. S.; Djordjevic, B. Altered incorporation of nucleic acid precursors by mycoplasma-infected mammalian cells in culture. Exp. Cell Res. 70: 301–310; 1972.

61. Hatanaka, M.; Del Giudice, R.; Long, C. Adenine formation from adenosine by mycoplasmas: adenosine phosphorylase activity. Proc. Natl. Acad. Sci. USA 72: 1401–1405; 1975.

62. McGarrity, G. J.; Carson, D. A. Adenosine phosphorylase mediated nucleoside toxicity: application towards the detection of mycoplasma infection in mammalian cell cultures. Exp. Cell Res. 139: 199–205; 1982.

63. Das, J.; Maniloff, J.; Chaudkuri, U.; Ghosh, A. Repair of DNA damage in mycoplasmas. Hanawalt, P. C.; Friedberg, E. C.; Fox, C. F. eds. DNA repair mechanisms. New York: Academic Press; 1978: 277–281.

64. Smith, D. W.; Hanawalt, P. C. Repair replication of DNA in ultraviolet irradiated *Mycoplasma laidlawii B*. J. Mol. Biol. 46: 57–72; 1969.

65. Kato, H. Spontaneous and induced sister chromatid exchanges as revealed by the BUdR-labelling method. Int. Rev. Cytol. 49: 55–97; 1977.

66. Perry, P.; Evans, H. J. Cytological detection of mutagen-carcinogen exposure by sister chromatid exchange. Nature 258: 121–125; 1975.

67. Ghosh, A.; Das, J.; Maniloff, J. Lack of repair of ultraviolet light damage in *Mycoplasma gallisepticum*. J. Mol. Biol. 116: 337-344; 1977.
68. Aoki, S.; Ito, D.; Watanabe, T. UV survival of human mycoplasmas: evidence of dark reactivation in *Mycoplasma buccale*. Microbiol. Immunol. 23: 147-158; 1979.
69. Gruneisen, A.; Rajewsky, M. F.; Remmer, I.; Uschkoreit, J. Inhibition of $^3$H-thymidine incorporation by hydroxyurea: atypical response of mycoplasma infected cells in culture. Exp. Cell Res. 90: 365-373; 1975.
70. Rosenkranz, H. S.; Carr, H. S.; Pollack, R. D. Studies with hydroxyurea: VI effects of hydroxyurea on the metabolism strains of *Escherichia coli*. Biochim. Biophys. Acta 149: 228-245; 1967.
71. Stanbridge, E. J.; Tischfield, J. A.; Schneider, E. L. Appearance of hypoxanthine guanine phosphoribosyltransferase activity as a consequence of mycoplasma contamination. Nature 256: 329-331; 1975.
72. Van Diggelen, O. P.; McGarrity, G. J.; Shin, S. Endogenous HPRT activity in mycoplasmas isolated from cell cultures. In Vitro 14: 734-739; 1978.
73. Shin, S.; Van Diggelen, O. P. Phenotypic alterations in mammalian cell lines after mycoplasma infection. McGarrity, G. J.; Murphy, D. G.; Nichols, W. W. eds. Mycoplasma infection of cell cultures. New York: Plenum Press; 1978: 191-212.
74. Clive, D.; Flamm, W. G.; Patterson, J. B. Specific-locus mutational assay systems for mouse lymphoma cells. Hollaender, A. ed. Chemical mutagens. Vol. 3. New York: Plenum Press; 1973: 79-103.
75. Bradlaw, J. A.; Casterline, J. L., Jr.; Reynaldo, E.; Scott, W. Influence of *Mycoplasma arginini* on the induction of aryl hydrocarbon hydroxylase by TCDD in rat hepatoma cell cultures. Food Chem. Toxicol. 20: 599-602; 1982.
76. O'Brien, S. J.; Simonson, J. M.; Grabowski, M. W.; Barile, M. F. Analysis of multiple isoenzyme expression among twenty-two species of *Mycoplasma* and *Acholeplasma*. J. Bacteriol. 146: 222-232; 1981.
77. Clark, A. F.; Farrell, D. F.; Burke, W.; Scott, C. R. The effect of mycoplasma contamination on the in vitro assay of pyruvate dehydrogenase activity in cultured fibroblasts. Clin. Chim. Acta 82: 119-124; 1978.
78. Constantopoulos, G.; McGarrity, G. J.; Barranger, J. A. Oxidative metabolism of mycoplasmas. Fed. Proc. 40: 1794; 1981.
79. Chen, T. R. In situ detection of mycoplasma contamination in cell cultures by fluorescent Hoechst 33258 stain. Exp. Cell Res. 104: 255-262; 1977.
80. Barile, M. F.; Levinthal, B. G. Possible mechanism for mycoplasma inhibition of lymphocyte transformation induced by phytohaemagglutinin. Nature 219: 751-752; 1968.
81. McGarrity, G. J. Effects of mycoplasmas on lymphocyte cell cultures. McGarrity, G. J.; Murphy, D. G.; Nichols, W. W. eds. Mycoplasma infection of cell cultures. New York: Plenum Press; 1978: 167-181.
82. Naot, Y.; Tully, J. G.; Ginsburg, H. Lymphocyte activation by various mycoplasma strains and species. Infect. Immun. 18: 310-317; 1977.
83. Biberfeld, G.; Gronowicz, E. *Mycoplasma pneumoniae* is a polyclonal B cell activator. Nature 261: 238-239; 1976.
84. Copperman, R.; Morton, H. Reversible inhibition of mitosis in lymphocyte cultures by non viable mycoplasmas. Proc. Soc. Exp. Biol. Med. 123: 790-795; 1966.
85. McPherson, I.; Russell, W. Transformation in hamster cells mediated by mycoplasmas. Nature 210: 1343-1345; 1966.
86. Jänne, J.; Pösö, H.; Raina, A. Polyamines in rapid growth and cancer. Biochim. Biophys. Acta 473: 241-293; 1978.
87. Luk, G. D.; Goodwin, G.; Marton, L. J.; Baylin, S. B. Polyamines are necessary for the survival of human small cell lung carcinomas in culture. Proc. Natl. Acad. Sci. U.S.A. 78: 2355-2358; 1981.
88. Kamatani, N.; Willis, E. H.; McGarrity, G. J.; Carson, D. A. Putrescine dependent growth of mycoplasma infected mammalian cells. J. Cell Physiol. 114: 16-20; 1983.
89. Tully, J. G. Biology of the mycoplasmas. McGarrity, G. J.; Murphy, D. G.; Nichols, W. W. eds. Mycoplasma infection of cell cultures. New York: Plenum Press; 1978: 1-33.
90. Nichols, W. W. Studies on the role of viruses in somatic mutation. Hereditas 55: 1-27; 1966.
91. Birke, C.; Peter, H. H.; Langenberg, U.; Müller-Hermes, W. J. P.; Peters, J. H.; Heitman, J.; Leibold, W.; Dallügge, H.; Krapf, E.; Kirchner, H. Mycoplasma contamination in human tumor cell lines: effect on interferon induction and susceptibility to natural killing. J. Immunol. 127: 94-98; 1981.
92. Beck, J.; Engles, H.; Brunner, H.; Kirchner, H. Interferon production in cocultures between mouse spleen cells and tumor cells: possible role of mycoplasmas in interferon production. J. Immunol. Methods 38: 63-73; 1980.
93. Murphy, W. H.; Bullis, C.; Cabick, L.; Heyn, R. Isolation of mycoplasma from leukemic and nonleukemic patients. J. Natl. Cancer Inst. 45: 243-251; 1970.
94. Taylor-Robinson, D.; McCormack, W. M. The genital mycoplasmas. N. Engl. J. Med. 302: 1003-1010, 1063-1067; 1980.
95. Cassell, G. H.; Lindsey, J. R.; Davis, J. K. Respiratory and genital mycoplasmosis of laboratory rodents: implications for biomedical research. Isr. J. Med. Sci. 17: 548-554; 1981.
96. Fowlkes, D. M.; MacLeod, J.; O'Leary, W. M. T mycoplasmas and human infertility: correlation of infection with alterations in seminal parameters. Fertil. Steril. 26: 1212-1218; 1975.
97. Jurmanova, K.; Sterbova, J. Correlation between impaired spermatozoan motility and mycoplasma findings in bull semen. Vet. Rec. 100: 157-158; 1979.

98. Kundsin, R. B.; Driscoll, S. G. The role of mycoplasmas in human reproductive failure. Ann. NY Acad. Sci. 174: 794-797; 1970.
99. Driscoll, S. G.; Kundsin, R. B.; Horne, H. W., Jr.; Scott, J. M. Infections and first trimester losses: possible role of mycoplasmas. Fertil. Steril. 20: 1017-1019; 1969.
100. Quinn, P. A.; Shewchuk, A. B.; Shuber, J.; Lie, K. I.; Ryan, E.; Chipman, M. L.; Nocilla, D. M. Efficacy of antibiotic therapy in preventing pregnancy loss among couples colonized with genital mycoplasmas. Am. J. Obstet. Gynecol. 145: 239-244; 1983.
101. Black, F. T.; Kroggsgaard-Jensen, A. Application of indirect immunofluorescence, indirect haemagglutination and polyacrylamide gel electrophoresis to human T-mycoplasmas. Acta Pathol. Microbiol. Scand. [B] 82: 345-353; 1972.
102. Quinn, P. A.; Shewchuk, A. B.; Shuber, J.; Lie, K. I.; Ryan, E.; Shen, M.; Chipman, M. L. Serologic evidence of *Ureaplasma urealyticum* infection in women with spontaneous pregnancy loss. Am. J. Obstet. Gynecol. 145: 245-250; 1983.
103. Quinn, P. A.; Arshoff, L .U.; Li, H. C. S. Serotyping of *Ureaplasma urealyticum* by immunoperoxidase. J. Clin. Microbiol. 13: 670-676; 1981.
104. Robertson, J. A.; Stemke, G. W. Expanded serotyping scheme for *Ureaplasma urealyticum* strains isolated from humans. J. Clin. Microbiol. 15: 873-878; 1982.
105. Lemcke, R. Association of PPLO infection and antibody response in rats and mice. J. Hyg. (Lond.) 59: 401-412; 1961.
106. Stone, S. S.; Masiga, G.; Read, W. C. S. *Mycoplasma mycoides* transplacental transfer in cattle. Res. Vet. Sci. 10: 368-372; 1969.
107. Tram, C.; Guillon, J. C.; Chowroulinkov, I. Isolement de mycoplasmes de foetus de rats obtenus par cesarienne aseptique. C.R. Soc. Biol. 164: 2470-2471; 1970.
108. Whitcomb, R. F.; Tully, J. G. The mycoplasmas III: plant and insect mycoplasmas. N.Y.: Academic Press; 1979.
109. Poulson, D. F.; Sakaguchi, B. Nature of "sex ratio" agent in *Drosophila*. Science 133: 1489-1490; 1961.
110. Williamson, D. L.; Poulson, D. F. Sex ratio organisms (spiroplasmas) of *Drosophila*. Whitcomb, R. F.; Tully, J. G. eds. The mycoplasmas: plant and insect mycoplasmas. Vol. 3. New York: Academic Press; 1979: 175-208.
111. Williamson, D. L.; Steiner, Th.; McGarrity, G. J. Sex ratio organisms: can they be cultivated? Yale J. Med. In press.
112. Oishi, K. Spirochete-mediated abnormal sex-ratio (SR) condition in *Drosophila:* a second virus associated with spirochetes and its use in the study of the SR condition. J. Genet. Res. 19: 45-56; 1971.
113. Ota, T.; Kawabe, M.; Oishi, K.; Poulson, D. F. Non-male killing spiroplasmas in *Drosophila hydei*. J. Hered. 70: 211-213; 1979.
114. McGarrity, G. J. Spread and control of mycoplasmal infection of cell cultures. In Vitro 12: 643-648; 1976.
115. McGarrity, G. J.; Steiner, Th.; Gamon, L. Prevention, detection and control of mycoplasmal infection of cell cultures. Kilbey, B. J.; Legator, M.; Nichols, W.; Ramel, C. eds. Handbook of mutagenicity test procedures. New York: Elsevier. 1984: 161-173.

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