APPLIED MICROBIOLOGY
Vol. 12, No. 4, p. 337-342 July, 1964

Aerosols as a Source of Widespread *Mycoplasma* Contamination of Tissue Cultures[1]

ROBERT C. O'CONNELL[2], RUTH G. WITTLER, AND JOHN E. FABER

Department of Microbiology, University of Maryland, College Park, Maryland; Veterans Administration Central Laboratory for Clinical Pathology and Research; and Department of Bacteriology, Walter Reed Army Institute of Research, Washington, D.C.

Received for publication 2 March 1964

ABSTRACT

O'CONNELL, ROBERT C. (University of Maryland, College Park), RUTH G. WITTLER, AND JOHN E. FABER. Aerosols as a source of widespread *Mycoplasma* contamination of tissue cultures. Appl. Microbiol. **12**:337-342. 1964.—*Mycoplasma* isolates were cultured from 15 antibiotic-free cell cultures obtained from a single laboratory. Complement-fixation tests showed that these isolates were antigenically related to each other but were unrelated to *M. hominis* type 1, *M. hominis* type 2, *M. arthritidis*, *M. laidlawii* type B, *Mycoplasma* sp. H.Ep. #2 (Barile), or *M. salivarium*. Examination of serum used to feed the infected cell lines revealed no *Mycoplasma*. Infection resulting from cross-contamination by a single *Mycoplasma* strain from one cell culture to another was investigated. Although the organisms were not found in the air over the work area, aerosols containing these contaminants were produced in tissue culture bottles during the trypsinization of cell monolayers. The minimal infectious dose of *Mycoplasma* for tissue cultures was measured, and it was determined that one organism was capable of initiating an infection in a tissue culture. The pattern of contamination and the small dose required for infection indicated that *Mycoplasma* contamination was spread from one tissue culture to another via aerosols. It was demonstrated that *Mycoplasma* can be transferred from one cell culture to another through the use of a common burette for dispensing medium.

The reports of Pollock, Kenny, and Syverton (1960) and Barile, Malizia, and Riggs (1962) indicated that more than half of the tissue cell lines in some laboratories are contaminated with *Mycoplasma* spp. Unlike bacterial contamination, which is easily recognized and eliminated, *Mycoplasma* contamination is often difficult to detect and to eliminate. *Mycoplasma* may be present in tissue culture supernatant fluids at concentrations of 10^5 to 10^7 organisms per ml without causing noticeable turbidity of the medium or gross cytopathology of the cells.

Kenny (1961) demonstrated that *Mycoplasma* infections can cause tissue cultures to grow more slowly and to a lower final population than noninfected cultures by depleting the tissue culture medium of arginine. Arginine depletion in infected cell lines also inhibits plaque formation by certain viruses (Rous and Bonifas, 1962). (Throughout this report reference to infection or contamination of tissue cultures will be understood to mean that due to *Mycoplasma* unless otherwise stated.) Nelson (1960) and Powelson (1961) reported that certain animal strains of *Mycoplasma* produce cytopathogenic effects (CPE) in experimentally infected tissue cultures, and O'Connell (1963) observed that *Mycoplasma* strains isolated from various tissue cell cultures destroy African Green monkey kidney cells.

Little is known about the effect of *Mycoplasma* on the metabolism of tissue cells or on the virus-host cell relationship. McCarty et al. (1964) suggested that *Mycoplasma* supplies infected cells with carbamyl phosphate, a substrate for the synthesis of pyrimidines. Brownstein and Graham (1961) reported that virus yields from infected cells are low compared with yields from noninfected cells and that infected cells are unusually fragile when inoculated with virus. We have also observed (*unpublished data*) that one *Mycoplasma* strain enhances CPE from a latent simian virus when it is added to African Green monkey kidney cells. Thus, the desirability of using known *Mycoplasma*-free tissue cultures for metabolic and virological studies is obvious.

The task of maintaining noninfected tissue cultures presents a very real problem, since the source of *Mycoplasma* contamination of tissue cultures remains unknown. It is frequently suggested that the occurrence of *Mycoplasma* in tissue cultures may result from the use of antibiotics which convert bacterial contaminants to stable L forms indistinguishable from *Mycoplasma*.

We observed that tissue cultures carried without antibiotics became infected, while in the same laboratory tissue cultures containing antibiotics remained uninfected. This pattern of contamination would not be expected if the infection originated from conversion of bacterial contaminants to stable L forms in the presence of antibiotics. An investigation into the source of *Mycoplasma* in these antibiotic-free tissue cultures was undertaken and is here described.

MATERIALS AND METHODS

Origin of tissue cultures. Four antibiotic-free continuous cell lines (Chang liver, H.Ep. #2, Henle intestine, WISH)

[1] This paper is part of a dissertation presented to the Graduate School of the University of Maryland in partial fulfillment of the requirements for the Ph.D. degree.

[2] Present address: Flow Laboratories Inc., Rockville, Md.

and 11 antibiotic-free human embryonic cell strains from a single laboratory were examined culturally for the presence of *Mycoplasma*. Cell strains were designated by the number of the embryo from which the tissue culture was made followed by a letter indicating the tissue of origin (L, lung; S, skin; K, kidney; E, whole embryo).

Infected monolayer cultures of embryonic lung, Henle intestine, and Chang liver cells in 32-oz prescription bottles were obtained from the tissue-culture laboratory under study. Tubes and plaque bottles of African Green monkey kidney were obtained from Flow Laboratories Inc., Rockville, Md.

Media. Heart Infusion Broth and Heart Infusion Agar (Difco) were enriched with 20% unheated horse serum and 10% yeast extract (HIB-HoS-YE and HIA-HoS-YE, respectively) for isolation and maintenance of *Mycoplasma* spp. Yeast extract was prepared by the method of Chanock, Hayflick, and Barile (1962), modified to provide for sterilization by filtration.

Incubation conditions. All cultures were incubated at 37 C. Agar cultures of *Mycoplasma* were incubated in an atmosphere of 5% CO_2 and 95% H_2. All other cultures were incubated aerobically.

Isolation of Mycoplasma. A 1-ml amount of serum or tissue culture supernatant fluid to be tested was inoculated into a tube containing 10 ml of HIB-HoS-YE. After incubation for 48 hr, a drop of this medium was inoculated onto HIA-HoS-YE. Plates were examined at 100× magnification for *Mycoplasma* colonies after incubation for 4 to 7 days.

Mycoplasma strains. Mycoplasma isolates from tissue cultures were designated by the name or number of the tissue culture from which they were isolated. Strains obtained from the American Type Culture Collection (ATCC), Washington, D.C., were: *M. arthritidis* strain H 606 (ATCC 13988); *M. hominis* type 1, strain 4387 (ATCC 14027); *M. hominis* type 2, strain Campo (ATCC 14152); *M. laidlawii* type B (ATCC 14192); and *M. salivarium* strain Buccal 1 (ATCC 14277). *Mycoplasma* strain H.EP. #2 isolated from tissue culture (Barile et al., 1962) was kindly supplied by M. F. Barile. *Mycoplasma* strain HeLa was isolated prior to this study from a HeLa cell culture in this laboratory.

Preparation of antigens and antisera for complement-fixation test. Mycoplasma strains were grown in 250-ml Erlenmeyer flasks containing 100 ml of medium. Initially HIB-HoS-YE was used, but subsequently 1.5% PPLO Serum Fraction (Difco) was substituted for horse serum (HIB-SF-YE). A 2-ml amount of a 48-hr broth culture (HIB-HoS-YE) was used as inoculum for each flask. After incubation for 4 days, cultures were centrifuged at 35,000 × *g* for 25 min. The precipitate was washed once in Heart Infusion Broth, recentrifuged, suspended in 0.85% saline, and stored at −20 C. The saline suspension represented a 20× concentration of the broth culture.

Antigens prepared from four strains (Chang liver, 31-K, 24-E, 37-E) were used to prepare antisera. White New Zealand rabbits weighing 5 to 7 lb were used for production of antisera. Each rabbit was inoculated intravenously with 1 ml of antigen every 4 to 6 days until a total of 10 ml had been given. The animals were bled 10 days after the last injection.

Each antiserum was tested by complement-fixation test (O'Connell, Wittler, and Faber, 1961) against its homologous antigen and against all of the other isolates, as well as *M. arthritidis*, *M. hominis* type 1, *M. hominis* type 2, *M. laidlawii* type B, H.Ep. #2 (Barile), and *M. salivarium*. Fifteen antigens, including those used to produce antisera, were grown in HIB-HoS-YE. All other antigens were grown in HIB-SF-YE. Two batches of *Mycoplasma* 31-K were produced, one in each medium.

Tissue cultures. Eagle basal medium containing 10% calf serum (BME-CS) or 2% horse serum (BME-HoS) was used. Cultures of McCoy synovial cells in screw-capped tubes and in 200-ml milk dilution bottles were fed twice weekly with BME-CS by complete medium replacement. On the seventh day, the cultures were split. The old medium was discarded, and trypsin (2.5% in 0.85% NaCl diluted 1:10 in Hanks' balanced salt solution) was added to the monolayer. The trypsin remained in contact with the cells for 1 min, and was then discarded. Several additional minutes were allowed for the residual trypsin to loosen the cells from the glass. BME-CS (10 ml) was added to the bottle, and the cells were suspended by aspiration with a pipette; 3 to 5% of the resulting cell suspension was left in the bottle, and a like amount was used to inoculate each new bottle. A 1-ml amount of a 1:20 dilution of this suspension was used to inoculate each screw-capped tube. Tube cultures of African Green monkey kidney cells were maintained on BME-HoS.

Infected 32-oz bottles of Chang liver cells were trypsinized as described above. Fresh medium was then added from a burette equipped with a dispensing bell. Immediately after the addition of medium to the infected Chang liver bottle, six plaque bottles of African Green monkey kidney cells were fed from the same burette. Negative and positive controls consisted, respectively, of unopened monkey kidney bottles and of unfed bottles inoculated with the supernatant fluid from the infected Chang liver cells. After incubation for 1 week, the monkey kidney bottles were observed and cultured for *Mycoplasma*.

Aerosol sampling. Plates of HIA-HoS-YE were opened and placed next to the pour-off bucket during the feeding of bottles known to be infected with *Mycoplasma*. In subsequent experiments, an Andersen air sampler (Andersen, 1958) was substituted for the open petri dishes. During the feeding operation in the latter experiments, 20 to 25 ft^3 of air were sampled.

The air within freshly trypsinized bottles of infected tissue cultures was also examined with the Andersen air sampler. Bottles (32-oz) of infected cells were trypsinized,

and the cells were suspended by aspiration. Bottles were sampled immediately and at intervals up to 6 min after aspiration. To examine the air within bottles of tissue cultures, it was necessary to modify the Andersen air sampler. A 3-in. (7.6-cm) Büchner funnel was inverted and sealed over the air intake of the sampler. The spout of the funnel was connected by a 40-cm plastic tube to a 30-cm glass tube which was inserted into the tissue culture bottle 5 to 8 cm from the bottom. The sampler was operated for 30 sec, examining approximately 8.5 liters of air. Plates of HIA-HoS-YE used in the sampler were incubated 5 to 7 days and examined for the presence of *Mycoplasma* colonies.

Enumeration of Mycoplasma. The concentration of *Mycoplasma* in broth cultures and in supernatant fluids from infected tissue cultures was determined simultaneously by plate count and by titration. Serial decimal dilutions (10^{-1} to 10^{-8}) of the broth culture or supernatant fluid were made in BME-CS. Triplicate 0.05-ml samples of each dilution were plated on HIA-HoS-YE, and colonies were counted after 5 to 7 days of incubation. Four systems were used to titrate the *Mycoplasma*. Samples of each dilution were inoculated into each of the specified number of tubes or bottles as follows: (i) African Green monkey kidney cells (0.1 ml, 10 tubes per dilution), (ii) HIB-HoS-YE (0.1 ml, 10 tubes per dilution), (iii) McCoy synovial cells (0.1 ml, 5 bottles per dilution), and (iv) McCoy synovial cells (0.5 ml, 10 tubes per dilution). Monkey kidney cells were inoculated when the growth medium was replaced with maintenance medium. Synovial cells were inoculated at the time the cells were planted. The occurrence of *Mycoplasma* in each bottle was determined by the method described above (*Isolation of Mycoplasma*) or by the CPE on monkey kidney cells (Table 2, experiments 3 and 4).

TABLE 1. *Complement-fixation results comparing Mycoplasma from tissue cultures with Mycoplasma from several sources*[a]

Mycoplasma antigens	Anti-*Mycoplasma* serum			
	Chang liver	31-K	24-E	37-E
Isolates from tissue cultures				
Chang liver[b]	2,048	1,024	1,024	2,048
WISH[c]	2,048	1,024	2,048	2,048
H.Ep. #2[c]	2,048	2,048	2,048	2,048
Henle[c]	2,048	2,048	2,048	2,048
1-S[c]	2,048	1,024	1,024	2,048
2-L[b]	2,048	1,024	1,024	2,048
4-L[c]	2,048	1,024	1,024	2,048
22-L[c]	2,048	1,024	2,048	2,048
24-E[b]	1,024	1,024	2,048	2,048
31-S[c]	2,048	1,024	2,048	2,048
31-K[b]	1,024	2,048	1,024	1,024
31-K[c]	1,024	2,048	1,024	1,024
32-K[c]	1,024	1,024	1,024	2,048
37-S[b]	2,048	1,024	1,024	2,048
37-L[c]	2,048	1,024	2,048	2,048
37-E[b]	2,048	1,024	1,024	2,048
Stock culture strains				
M. arthritidis[c]	8	8	8	64
M. hominis type 1[b]	128	64	128	256
M. hominis type 2[c]	16	16	64	64
M. laidlawii type B[c]	16	16	64	64
H.Ep. #2 (Barile)[c]	16	32	64	64
M. salivarium[b]	256	256	256	256

[a] Results are expressed as complement-fixation titers. The numbers represent the reciprocal of the highest dilution of serum giving a 2+ reaction (50% hemolysis).

[b] Antigen grown in HIB-HoS-YE

[c] Antigen grown in HIB-SF-YE.

An infectious unit of *Mycoplasma* was defined as the number of organisms necessary to initiate an infection in a tissue culture. The median number of infectious units of *Mycoplasma* per tube or bottle in that dilution which represented the end point of the titration was calculated from the equation $m = 1n\ p_0$, where m is the median number of units per tube and $1n\ p_6$ is the natural logarithm of the fraction of sterile tubes (not infected) over total number of tubes at that dilution. The concentration of infectious units of *Mycoplasma* in the original sample was obtained by multiplying the median number of units per tube by the dilution. For each sample, the number of infectious units per milliliter as determined by tissue culture titration was compared with the number of colony-forming units per milliliter as determined by plate counts. Since the colony-forming unit of *Mycoplasma* has been demonstrated to be a single organism (Wittler, Conference on the Molecular Biology of the Pleuropneumonia-Like Organisms, Storrs, Conn., 1962; Weibull and Lundin, 1962), it was possible to express the infectious unit of *Mycoplasma* for tissue culture in terms of individual organisms.

RESULTS

Mycoplasma strains were isolated from each of the 15 antibiotic-free tissue cultures examined. These strains were all closely related serologically, producing titers of 1,024 or 2,048 with the four antisera employed (Table 1). *M. arthritidis*, *M. hominis* type 1, *M. hominis* type 2, *M. laidlawii* type B, H.Ep. #2 (Barile), and *M. salivarium* produced titers of 8 to 256, and were not closely related to the tissue culture isolates. The serological similarity of the *Mycoplasma* strains isolated from the tissue cultures suggested that they were originating from a common source within the laboratory.

One of the continuous cell lines (Chang liver) was contaminated with *Mycoplasma* when received by the laboratory. Early cultural examination had shown that the other three cell lines were uncontaminated at that time. Information was not available concerning the original status of the 11 cell strains; 6 months after the original cultural examination, all of these tissue cultures were contaminated.

Examination of each lot of serum which had been used

TABLE 2. *Population of Mycoplasma calculated from plate count and titration experiments**

Expt	Inoculum	Plate count method	Titration			
			Synovial cells		Monkey cells	HIB-HoS-YE
			Bottles	Tubes	Tubes	Tubes
1	*Mycoplasma* 24-E†	1.7×10^8	NT	NT	NT	1.1×10^8
2	*Mycoplasma* 24-E†	3.9×10^5	NT	NT	NT	1.4×10^5
3	Henle intestine‡	3.6×10^6	1.6×10^7	NT	1.3×10^7	3.6×10^5
4	Henle intestine‡	2.0×10^7	NT	2.4×10^6	NT	3.2×10^7
5	Embryonic lung‡	5.5×10^5	1.4×10^5	NT	NT	NT
6	*Mycoplasma* HeLa†	2.4×10^8	NT	NT	7.0×10^7	NT
7	*M. hominis* type 1†	1.2×10^8	NT	NT	1.6×10^8	NT

* Results are expressed as organisms per milliliter. NT = not titrated.
† Broth culture (HIB-HoS-YE) of *Mycoplasma*.
‡ Supernatant fluid from infected tissue culture.

to feed the cells during this 6-month period revealed no *Mycoplasma*. Likewise, tissue cultures were maintained in another laboratory for 1 year on media containing the same lots of serum without becoming contaminated. Serum, therefore, did not appear to be responsible for contamination of the tissue cultures.

Since the tissue cultures were contaminated with *Mycoplasma* strains that were serologically indistinguishable, the possibility of cross contamination was investigated.

Mycoplasma colonies were detected on two of four plates exposed to the air next to the pour-off bucket. However, the colonies were confined to circumscribed areas, which suggested that a droplet had splashed on the plates. The Andersen air sampler failed to detect the presence of *Mycoplasma* in the air of the work area during the feeding of infected tissue cultures.

Four bottles of infected Henle intestine cells were trypsinized, and the air within the bottle was immediately sampled. The air from one bottle yielded over 400 *Mycoplasma* colonies on the petri dishes in the sampler; the others yielded 255, 92, and 19 colonies. Two additional bottles were sampled 3 and 6 min after aspiration and yielded two and one colonies, respectively. This experiment was repeated with an infected Chang liver cell line. The bottle sampled immediately after aspiration produced 153 colonies, and the bottles sampled after 1, 2, 3, and 5 min produced 56, 7, 1, and 0 colonies, respectively. It was, therefore, demonstrated that aerosols were not present in the air above the work area but were produced in the tissue-culture bottles during trypsinization.

The population of *Mycoplasma* in broth cultures or fluids from infected tissue cultures was determined by the two methods described above (*Enumeration of Mycoplasma*). The results of these measurements by plate count and titration experiments are shown in Table 2. The populations calculated by the two methods were compared, both on cultures containing heavy growth and on cultures with light growth (Table 2, experiments 1 and 2); the results of the two methods agreed closely. Broth cultures of *Mycoplasma* strains (24 E, HeLa, and *M. hominis* type 1) used as inocula contained 10^5 to 10^8 organisms per ml (experiments 1, 2, 6, and 7). The population of *Mycoplasma* in supernatant fluids of the two tissue cultures examined, embryonic lung and Henle intestine (experiments 3, 4, and 5), was approximately 10^5 to 10^7 organisms per milliliter as measured both by plate count and by titration.

The mechanism of transfer of *Mycoplasma* from one tissue culture to another was investigated. Monkey kidney cell plaque bottles became contaminated with *Mycoplasma* when fed from the same burette used to add medium to infected, freshly trypsinized cultures of Chang liver cells (Table 3). All of the controls inoculated with supernatant fluid from the infected Chang liver bottles became infected. In experiment 1, 75% (6 of 8) of the monkey kidney cultures were infected in the process of feeding; in experiment 2, 50% (4 of 8) of the monkey kidney cultures were infected. Unopened bottles of monkey kidney cells remained free from *Mycoplasma*.

Mycoplasma strain 2-L was examined serologically by the Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases. Results obtained from complement-fixation tests indicated that this strain was not one of the taxonomically defined human *Mycoplasma*

TABLE 3. *Transfer of Mycoplasma by feeding of noninfected tissue cultures after infected tissue cultures*

Culture	Infected cultures/total cultures	
	Expt 1	Expt 2
MK* + Chang liver supernatant fluid	4/4	4/4
Unopened MK	0/2	0/2
MK fed from burette†	6/8	4/8

* MK = plaque bottle cultures of African Green monkey kidney cells.
† Burette used previously to feed infected Chang liver cells.

species. It did, however, cross-react with an unclassified *Mycoplasma* strain isolated from the throat of a child with severe pharyngitis.

DISCUSSION

The high-titered cross-reactions (titers of 1,024 to 2,048) among the tissue culture isolates tested against the four antisera indicate that these *Mycoplasma* strains are closely related serologically. These cross-reactions cannot be due simply to antigenic components from the medium (HIB-HoS-YE), since two antigens (*M. hominis* type 1, and *M. salivarium*) which gave titers of only 64 to 256 were grown in the same medium. Moreover, antiserum prepared from strain 31-K grown in HIB-HoS-YE gave the identical titer (2,048) when tested against antigen 31-K, whether grown in HIB-HoS-YE or in HIB-SF-YE.

The serological similarity of the *Mycoplasma* strains isolated in this investigation suggests a common source of infection. No *Mycoplasma* strains were found in serum used to feed the cells or in the air above the working area. Evidence was obtained, however, that aerosols were generated during the trypsinization of tissue cultures.

The concentration of organisms in each sample of broth culture or supernatant fluid, as calculated from titration in tissue cultures, agreed with calculations from plate counts. Both the titration and the plate count methods of calculating populations of microorganisms are based on the ability of a single organism to multiply until its presence can be detected either by isolation procedures or by the cytopathogenic effect it produces in cell cultures. Since the population of *Mycoplasma*, as determined by titration in tissue cultures, agreed with determinations from plate counts, it was concluded that the same number of *Mycoplasma* is responsible for the formation of a colony on agar as is required to establish an infection in a tissue culture, i.e., one organism.

Although personnel would not knowingly produce aerosols in a tissue-culture laboratory, many technicians are unaware of the potential danger of blowing out the last drop from a pipette. Observations in several laboraties indicate that it is a common practice to blow out a pipette when aspirating cell suspensions. The addition of medium to these freshly trypsinized bottles displaces air containing aerosolized *Mycoplasma*, contaminating the lip of the bottle or the burette used to dispense fresh medium. The next bottle that is opened or fed from the same dispenser is, therefore, exposed to contamination.

Although experimental proof that the 11 human embryonic cell strains were originally *Mycoplasma*-free was not available, attempts to isolate *Mycoplasma* from tissues of two intact human embryos were negative (R. C. O'Connell, *unpublished data*). In addition, Kraemer et al. (1963) reported the absence of *Mycoplasma* in primary human cell cultures and in organ extracts of human fetuses. It is, therefore, probable that the cell strains were uncontaminated in their early passages and only later became contaminated.

The isolated strains cross-reacted serologically only with an unclassified isolate from the human throat. For this reason, it is likely that the original source of the contamination was from the throat of a technician. However, if contamination from technicians was the sole source of *Mycoplasma* in tissue cultures, the presence of the common human oral *Mycoplasma* would also be expected. The absence of the commonly recognized oral species indicated that this was not the principal source of the contamination.

It was concluded that *Mycoplasma* contamination within the laboratory studied was the result of cross-contamination by a single strain of *Mycoplasma* from one cell culture to another via aerosols contaminating the dispenser of fresh medium. Although a burette was used to demonstrate the transfer of *Mycoplasma* from one cell culture to another, the lip of a bottle of tissue-culture medium could function in the same manner if the medium was poured directly into the tissue-culture bottle. The widespread nature of *Mycoplasma* contamination of tissue cultures is probably the result of sporadic contamination being communicated to other cell cultures by failure to adhere rigidly to aseptic techniques.

ACKNOWLEDGMENTS

The authors wish to thank Walter James (National Institute of Allergy and Infectious Diseases) for providing the serological information on *Mycoplasma* 2-L. This work was supported by Public Health Service research grant AI-02332 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

ANDERSEN, A. A. 1958. New sampler for the collection, sizing, and enumeration of viable airborne particles. J. Bacteriol. **76**:471–484.

BARILE, M. F., W. F. MALIZIA, AND D. B. RIGGS. 1962. Incidence and detection of pleuropneumonia-like organisms in cell cultures by fluorescent antibody and cultural procedures. J. Bacteriol. **84**:130–136.

BROWNSTEIN, B., AND A. F. GRAHAM. 1961. Interaction of Mengo virus with L cells. Virology **14**:303–311.

CHANOCK, R. M., L. HAYFLICK, AND M. F. BARILE. 1962. Growth on artificial medium of an agent associated with atypical pneumonia and its identification as a PPLO. Proc. Natl. Acad. Sci. U.S. **48**:41–49.

KENNY, G. E. 1961. The effect of pleuropneumonia-like organisms (*Mycoplasma*) on growth of mammalian cells *in vitro*. Ph.D. Thesis, University of Minnesota, Minneapolis.

KRAEMER, P. M., V. DEFENDI, L. HAYFLICK, AND L. A. MANSON. 1963. *Mycoplasma* (PPLO) strains with lytic activity for murine lymphoma cells *in vitro*. Proc. Soc. Exptl. Biol. Med. **112**:381–387.

MCCARTY, K. S., B. WOODSEN, M. AMSTEY, AND O. BROWN. 1964. Arginine as a precursor of pyrimidines in strain L-929 fibroblasts infected with pleuropneumonia-like organisms. J. Biol. Chem. **239**:544–549.

NELSON, J. B. 1960. The behavior of murine PPLO in HeLa cell cultures. Ann. N.Y. Acad. Sci. **79**:450–457.

O'CONNELL, R. C. 1963. A study of factors contributing to widespread contamination of tissue cultures with pleuropneumonia-like organisms. Ph.D. Thesis, University of Maryland, College Park.

O'CONNELL, R. C., R. G. WITTLER, AND J. E. FABER. 1961. Serological study of a pleuropneumonia-like organism and an associated *Corynebacterium* species. Antonie van Leeuwenhoek J. Microbiol. Serol. **27**:321–331.

POLLOCK, M. E., G. E. KENNY, AND J. T. SYVERTON. 1960. Isolation and elimination of pleuropneumonia-like organisms from mammalian cell cultures. Proc. Soc. Exptl. Biol. Med. **105**:10–15.

POWELSON, D. M. 1961. Metabolism of animal cells infected with *Mycoplasma*. J. Bacteriol. **82**:288–297.

ROUS, H. C., AND V. H. BONIFAS. 1962. Depletion by pleuropneumonia-like organisms of arginine required for adenovirus plaque formation. Bacteriol. Proc., p. 147.

WEIBULL, C., AND B.-M. LUNDIN. 1962. Size and shape of pleuropneumonia-like organisms grown in liquid medium. J. Bacteriol. **84**:513–519.