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REPORT NUMBER 2

THE RELATIONSHIP BETWEEN MYCOPLASMA SPECIES AND SELECTED RESPIRATORY VIRUSES (ADENOVIRUS, INFLUENZA VIRUS AND RHINOVIRUS)

ANNUAL REPORT

By

Donald P. Fletcher

Department of Microbiology
School of Dental Medicine
University of Pittsburgh
Pittsburgh, Pennsylvania 15213

December 1970

Life Sciences Division
Army Research Office
3045 Columbia Pike
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SUMMARY

The effect of Mycoplasma pneumoniae on rhinovirus (type 1A, strain 2060) nucleic acid synthesis in KB cells was studied. Rhinovirus RNA synthesis, measured by tritiated-uridine uptake in the presence of actinomycin D, demonstrated that viral RNA synthesis was stimulated throughout the entire period of observation in cells inoculated with M. pneumoniae (Antimicrobial Agents and Chemotherapy, p. 196, 1969).

If M. pneumoniae and PPLO growth medium were inoculated on KB monolayer cell systems, stimulation of viral RNA synthesis was greater than in the presence of PPLO alone. The medium components, yeast extract and PPLO broth, stimulated viral RNA synthesis, whereas agar, phenol red or dextrose inhibited this synthesis (Folia Microbiologica 15:325, 1970).

Following these observations, emphasis was placed on the interaction of mycoplasma and adenoviruses, which are associated with respiratory disease in military recruits. M. pneumoniae apparently stimulates ^{3}H-thymidine uptake (DNA synthesis) of adenovirus type 4 in L-132 cell monolayer systems, when the cell-systems were treated with mycoplasma at 48 and 24 hours prior to the virus inoculum. If thymidine-uptake values of the virus-infected systems are considered to be 100 per cent, the virus incorporated thymidine in minus 48 hour mycoplasma-treated systems ranged from 145 to 159 per cent. The TCA-soluble fractions (nucleotides phosphorylated) from the same samples (adenovirus-mycoplasma) showed 127 to 168 per cent stimulation, when compared to the virus cell systems. Studies with a 30 minute pulse label also showed increased ^{3}H-thymidine uptake in the virus-mycoplasma infected L-132 and KB cell systems. There appeared to be slight increases of adenovirus yields in

the mycoplasma-treated cells compared to untreated virus-cell systems measured by both tube end-point and fluorescent antibody titrations.

For the purpose of comparison with another DNA virus, Herpes simplex virus, was selected. M. pneumoniae, added at 48 and 24 hours before herpes virus inoculations, appeared to enhance the viral-produced CPE in rabbit kidney monolayers. In contrast, if KB cell monolayers were pre-treated (minus 24 and 48 hours) with herpes virus instead of mycoplasma and subsequently infected with rhinovirus 2060, the rhinovirus RNA synthesis was partially or completely inhibited, respectively. Pre-treatment (24 hours or less) of KB cell systems with adenovirus type 4, did not appear to effect rhinovirus RNA synthesis.

Finally, the interaction of M. pneumoniae and influenza A/PR8 virus was observed in 1,308 mice. These combinations of mycoplasma and influenza appeared to produce earlier symptoms and deaths than observed in mice infected with virus alone.

INTRODUCTION

Interactions of respiratory viruses and Mycoplasma pneumoniae, have been investigated. It has been determined that rhinovirus ribonucleic acid synthesis is increased by M. pneumoniae and that this increase is further magnified by certain components of mycoplasma growth medium.

During the past year (1970), a study of the effect of mycoplasma on influenza virus and adenovirus type 4 was undertaken. Influenza A/PR8 virus-produced hemagglutination appeared to be enhanced when the virus was grown in the presence of M. pneumoniae-infected monkey kidney cells. Comparable studies in mice showed a similar synergistic effect.

Because of the interest in adenoviruses, the effect of M. pneumoniae on adenovirus type 4 was examined in detail. These studies showed an increased rate of DNA synthesis in virus-mycoplasma-inoculated cell systems which will be described below.

MATERIALS AND METHODS

ADENOVIRUS: Adenovirus type 4 was initially purchased from the American Type Culture Collection (strain RI-67, passage level Human Trachea/4, HeLa/17, KB/2). The ATCC received the culture from Dr. M.R. Hilleman, Merck Institute, West Point, Pennsylvania. Virus stock cultures were prepared in KB cell monolayers or in L-132 monolayers contained in 32 oz prescription bottles in the presence of Earle's BME supplemented with 2% calf serum. After the cell systems were infected, they were incubated at 37C until a 3-4+ CPE was observed. The virus-cell systems were subjected to 6 cycles of freezing and thawing (-60C to 37C), followed by centrifugation at 5000 RPM for 10 minutes at 4C. The

supernatant fluids were then titered on L-132 cell monolayers ($TCID_{50}$= $10^{5.5}$ to 10^{6}/ml), and stored at -60C.

INFLUENZA VIRUS: The influenza A/PR8 virus was obtained from Lederle Laboratories, Pearl River, N.Y., and was cultivated in Rhesus monkey kidney cell monolayers. This type A/PR8 virus was titered ($TCID_{50}$=$10^{7.7}$/ml), and stored at -60C.

HERPES VIRUS: For the purpose of comparing results obtained with adenovirus type 4, another DNA virus (Herpes simplex) was selected. Herpes simplex virus (herpes-virus hominis type 2) was procured from the American Type Culture Collection (ATCC VR No. 540, Lot No 1-D). The virus was passed in sheep choroid plexus/?, HeLa/?, PrRK/7 and our virus stock was prepared and titered ($TCID_{50}$=10^{5} to 10^{6}/ml) in primary rabbit kidney cell monolayers. This virus stock was also stored at -60C.

MYCOPLASMA: Mycoplasma pneumoniae (Eaton agent) was procured from the American Type Culture Collection (ATCC #15293) and was propagated in 70% Difco PPLO broth, 20% horse serum (GIBCO) and 10% fresh yeast extract (GIBCO),supplemented with 0.5% dextrose (w/v) and 0.004% phenol red (w/v). These procedures were described in detail in Annual Report Number 1, 1969 , The Relationship Between Mycoplasma Species and Selected Respiratory Viruses.

The mycoplasma stock (MP/G) attached to glass surfaces facilitating the removal of the PPLO growth medium from the colonies by draining and washing with Earle's BME solution containing 10% calf serum and 0.85% $NaHCO_3$ (three wash cycles). The adherent colonies were resuspended in the aforementioned rinse solution by 1 cycle of freeze and thaw, and titered at 10^{6} to 10^{7} acid forming units/ml in PPLO liquid medium. The MP/G stock was employed throughout these studies because of the

stimulatory effect of PPLO growth medium on rhinovirus replication (Fletcher, Milligan and Albertson, 1970, Folia Microbiol. 15(5): 325-329).

TISSUE CULTURE: The L-132 (Human Embryonic Lung, Davis) and the KB (Human Carcinoma of Nasopharynx, Eagle) cell monolayers were purchased from Flow Laboratories, Rockville Maryland in 16 x 125 mm screwcap tubes. These cells were used as a source of starter cells for preparing monolayers in 32oz prescription bottles and also for assaying viral DNA synthesis. Media for growing the cells consisted of Earle's cell culture medium (BME) with glutamine, supplemented with 10% calf serum. Similar medium with 2% calf serum was utilized to maintain cell cultures during virus replication. Neither the growth nor maintenance media contained antibiotics. The cells were tested routinely for the presence of PPLO contamination.

VIRAL DNA SYNTHESIS: The interaction of M. pneumoniae and adenovirus type 4 was studied by measuring the rate of ^{3}H-thymidine uptake in L-132 and KB cell monolayer systems. Cell monolayer systems containing 0.9 ml of BME (2% calf serum) were divided into 4 groups: 1 and 2 were inoculated with 0.1 ml of M. pneumoniae stock (10^{6} acid forming units) at minus 48, 24, 6 and 2 hours or at other selected times; 3 and 4 were inoculated with 0.1 ml of BME (10% calf serum). After the appropriate incubation period, the tubes were drained and washed 2 times with BME, and groups 1 and 3 were infected with 0.5 ml of adenovirus stock ($TCID_{50}$= 0.5 x 10^{6}/ml) and 0.5 ml of BME was added to groups 2 and 4. All tubes then received 0.5 ml of ^{3}H-thymidine (5uc), and incubation was continued at 37C. This procedure was used for continuous labeling experiments with ^{3}H-thymidine. Pulse label experiments for 2 hr, 1 hr and 30 min were also conducted. The 30 min pulse label was the ideal time of exposure to the

labeled thymidine (The reason for using a 30 min pulse is due to a linear rate of incorporation of ^{3}H-thymidine during this time period. After 40 mins, the rate of thymidine incorporation decreases). Finally, test systems were removed for processing at indicated hours post-virus infection. Processing was accomplished as follows: triplicate tubes of each group were drained, washed 3 times with 4 ml of 0.85% NaCl solution, drained and washed with two 6 ml volumes of trichloracetic acid (TCA). These TCA soluble fractions were collected and measured for ^{3}H-thymidine that had been phosphorylated. The TCA insoluble fractions were drained and solubilized in 0.5 ml of hydroxide of hyamine and placed in 10 ml of scintillation solution 2,5 diphenyloxazole (PPO), 4 gm; 1,4-bis-[2-(4-methyl-5-phenyloxazolyl)]-benzene (Dimethyl POPOP), 200 mg; toluene, 950 ml; and absolute ethanol, 50 ml).

The TCA soluble fractions (1 ml each) were counted in a Triton-X scintillation solution (PPO, 5.5 gm; Dimethyl POPOP, 0.1 gm, triton X-100, 333 ml; and toluene, 667 ml).

Activity/sample was measured in a Packard Tri-Carb scintillation counter, model 3320.

ANIMAL STUDIES: Influenza A/PR8-M. pneumoniae studies were conducted using 3 week old Swiss-Webster mice (male and female, approximately 15 gms each). The mice were first inoculated, while under slight ether anesthesia, by the intranasal instillation of 0.05 ml volumes of M. pneumoniae or BME. These inoculations were made in separate groups of mice at 4, 3, 2, and 1 days before the virus inoculation and in some cases in combination with the virus. Each mouse was infected, while under slight ether anesthesia, by the intranasal instillation of 0.05 ml volumes of appropriate dilutions of influenza virus stock.

HERPES SIMPLEX-RESPIRATORY AGENTS: Herpes simplex virus was added to KB cell monolayer cultures at 48 and 24 hours before rhinovirus inoculations. The rhinovirus inoculations were made in the presence of 10µg/ml actinomycin D (actinomycin D inhibits DNA dependent RNA synthesis of Herpes simplex virus and KB cells), and pulse-labeled for 1 hour with 2µc ^{3}H-uridine at selected times during the rhinovirus replication. These radioactive samples were processed as described in Annual Report Number 1, 1969, The Relationship Between Mycoplasma Species and Selected Respiratory Viruses.

RESULTS

It has been described in detail, Annual Report Number 1, 1969, The Relationship Between Mycoplasma Species and Selected Respiratory Viruses, that rhinovirus 2060 RNA synthesis was greater in Mycoplasma pneumoniae inoculated KB cells than in PPLO-free cell systems. In that study, M. pneumoniae was grown on glass to eliminate PPLO medium. However, if PPLO medium was added to the M. pneumoniae inoculum, stimulation of viral-RNA synthesis was greater than in the presence of PPLO alone.

Further observations in this area have shown, that with increasing multiplicities of infection (0.4, 4, and 40) of rhinovirus 2060, there are increasing levels of ^{3}H-uridine incorporation into acid-precipitable material. ^{3}H-uridine incorporation into acid-precipitable material (i.e. viral RNA) was observed at all MOI's tested. At MOI's of 40 and 4, the peak level of incorporation was at 9 hours post-infection in continuous labeling experiments, whereas with a MOI of 0.4 a definite peak was not observed. A plateau of maximum incorporation at a MOI of 0.4 was attained at 7 and 8 hours post-infection.

Similar experiments were performed to determine differences in acid-soluble radioactivity of rhinovirus-infected cells. Only at a MOI of 40 was there a striking difference in acid-soluble counts. The acid-soluble ^{3}H-uridine counts (representing nucleotide precursors taken into the cell and phosphorylated but not incorporated into RNA) increased at all MOI's as a function of time post-infection. There was generally a much greater percentage of acid-soluble counts in tissue controls and cells infected with a MOI of 4 or 0.4, and relatively low levels in cells infected at a multiplicity of infection of 40. It, therefore, appears that at the higher MOI's the nucleotide precursors of the viral RNA are rapidly phosphorylated and incorporated, whereas in tissues infected at the lower MOI's,phosphorylation increases in the absence of rapid incorporation. This later relationship is born out by plotting the ratio of acid-precipitable to acid-soluble counts (i.e.nucleotides phosphorylated and incorporated/nucleotides phosphorylated) versus time post-infection. This ratio is, therefore, directly proportional to the MOI (i.e. the greater the MOI the greater the ratio).

Following this study, emphasis was placed on a study to determine the effect of mycoplasma on adenovirus. The adenovirus selected for these investigations is the type 4 strain, which is one of the major causative agents of respiratory disease in military recruits. Initially, the effect of M. pneumoniae on the ability of adenovirus 4 to synthesize DNA was investigated (Table 1). Viral-stimulation was observed when the tissue culture systems were treated with mycoplasma at 48 and 24 hours prior to the virus inoculum. If thymidine-uptake values of the virus infected systems are considered to be 100 per cent, the virus-incorporated thymidine in 48 hour mycoplasma-treated systems ranged from 145 to 159

Table 1. ^{3}H-Thymidine uptake of adenovirus type 4 in M. pneumonia inoculated and uninoculated L-132 monolayer cell systems.

Adenovirus type 4	*Exposure time to M. pneumoniae (hrs)	COUNTS PER MINUTE									
		TCA Insoluble					TCA Soluble				
		exposure time to thymidine-^{3}H (hours)									
		0-14	14-16	14-18	18-20	18-22	0-14	14-16	14-18	18-20	18-22
+	48	284,602	25,540	36,955	24,705	35,698	15,728	13,393	13,196	12,846	14,786
+	24	227,394	25,607	32,717	23,458	36,991	15,761	10,844	11,878	11,056	14,304
+	6	127,363	18,802	27,498	16,673	23,148	9,727	11,514	12,763	8,807	12,565
+	2	145,385	9,373	17,409	17,940	19,194	9,103	2,281	8,664	10,571	11,762
+	NONE	195,960	17,085	23,435	15,541	24,245	9,346	9,698	13,256	10,114	13,351
0	48	844,670	---	679,137	---	---	19,962	---	56,119	---	---
0	24	776,982	290,924	628,209	414,130	598,223	22,447	86,603	56,000	79,758	27,518
0	6	677,305	312,991	771,282	380,794	524,694	16,150	101,948	82,177	99,737	60,236
0	2	538,532	258,833	689,630	282,002	523,041	19,370	105,240	67,313	78,960	60,988
0	NONE	799,318	289,420	676,407	323,297	549,275	19,335	101,701	74,386	97,338	63,947

*Exposure of L-132 tissue monolayer to M. pneumoniae prior to adenovirus inoculation.

---not tested

per cent. The TCA-soluble fractions (nucleotides phosphorylated) from the same samples (adenovirus-mycoplasma) ranged from 127 to 168 per cent stimulation, compared to the virus alone. In general, under these conditions mycoplasma-tissue uptake of thymidine was no higher than in tissue controls.

L-132 cell monolayers were pre-inoculated (minus 24 hrs) with M. pneumoniae, subsequently infected with adenovirus, and later pulse-labeled for 30 minutes with ^{3}H-thymidine (Table 2). Thymidine incorporation for the mycoplasma-virus systems ranged from 81% to 132% compared to the appropriated virus-infected controls. The cell-monolayers inoculated only with mycoplasma had a similar rate of thymidine incorporation as the untreated-cell controls.

In KB monolayer cell systems pulse-labeled for 30 minutes with tritiated thymidine, the virus-mycoplasma combination showed thymidine incorporation of 118% to 234% from 10 to 24 hrs post-infection (Table 3). Virus-cell samples were designated as 100% for the purpose of comparison. Post-infection times for adding ^{3}H-thymidine were selected to correspond with the cessation of host DNA synthesis (approximately 10 hrs) and the period of viral DNA synthesis (approximately 11-21 hrs). Uptake of thymidine by the mycoplasma-cell systems was high compared to the untreated tissue, possibly indicating preferential growth of M. pneumoniae in KB cells, and not in the L-132 cells. Apparently, M. pneumoniae is favored in KB cells, or mycoplasma stimulate KB cell DNA synthesis, based on the high thymidine incorporation of mycoplasma-cell systems compared to the untreated KB cells. Because adenovirus infection inhibits host cell DNA synthesis before these labeling times, the increased thymidine incorporation of the combination (adenovirus + mycoplasma) is

Table 2. ^{3}H-Thymidine (30 minute pulse-label) uptake of adenovirus type 4 in M. pneumoniae inoculated and uninoculated L132 monolayer cell systems.

Adenovirus, type 4	M. pneumoniae	[a]Time (hours), Post-virus Infection									
		16		18		20		22		24	
		[b]CPM	[c]%	CPM	%	CPM	%	CPM	%	CPM	%
+	+	38,913	120	33,907	93	31,157	103	15,078	81	30,166	132
+	0	32,375	100	36,404	100	30,074	100	18,572	100	22,854	100
0	+	35,539	110	24,278	67	30,960	103	15,731	85	22,044	97
0	0	37,597	116	22,636	62	30,211	101	18,696	100	26,943	118

[a] ^{3}H-Thymidine added 30 minutes prior to time indicated and samples processed on the hour.

[b] counts per minute

[c] virus-cell systems were designated 100%.

Table 3. ^{3}H-Thymidine (30 minute pulse-label) uptake of adenovirus type 4 in M. pneumoniae inoculated and uninoculated KB monolayer cell systems.

Adenovirus, type 4	M. pneumoniae	[a]Time (hours), Post-virus Infection															
		10		12		14		16		18		20		22		24	
		[b]CPM	[c]%	CPM	%	CPM	%	CPM	%	CPM	%	CPM	%	CPM	%	CPM	%
+	+	68,593	131	22,441	159	38,383	234	42,883	118	47,454	161	53,042	165	61,342	149	69,073	165
+	0	52,185	100	14,148	100	16,410	100	36,339	100	29,534	100	32,075	100	41,127	100	41,755	100
0	+	69,366	133	62,411	441	46,874	286	63,932	176	59,187	200	87,424	273	97,087	236	97,403	233
0	0	48,185	92	31,205	221	33,001	201	54,613	150	63,017	213	59,711	186	88,595	215	85,241	204

[a]^{3}H-Thymidine added 30 minutes prior to time indicated, e.g. 9 1/2 hrs radioactive isotope added, and samples processed at 10 hrs, etc.

[b]counts per minute

[c]virus-cell systems were designated 100%.

considered significant. Density gradient studies are in progress to definitely establish which DNA (host, mycoplasma or virus) incorporated tritiated-thymidine.

As can be seen in Table 1, the stimulation of DNA synthesis is greatest in the virus-mycoplasma systems when the mycoplasma was added at 24 and 48 hours before the virus infection.

As stated in the Annual Report Number 1, influenza A/PR8 hemagglutination titers appeared to be enhanced in M. pneumoniae infected Rhesus monkey kidney monolayer systems. Since this report, a study was conducted in 1,308 mice to observe if a similar synergistic effect could be demonstrated. Mice infected with the combination, influenza A/PR8 and M. pneumoniae, have shown: (1) earlier symptoms, (2) earlier deaths and (3) in general, a greater number of deaths, than mice infected with influenza alone. These investigations are being expanded to consider the effect of different mycoplasma titers, and the duration of mycoplasma treatment on influenza viral replication in vivo. Preliminary trials with germ-free mice show no apparent differences from the results seen in conventional animals. Mycoplasma inoculated at 4, 3, 2 and 1 day prior to the virus infection appeared to produce a similar effect. M. pneumoniae inoculations (without virus) did not produce deaths in mice tested.

For the purpose of comparing adenovirus-mycoplasma results with another DNA virus, the Herpes simplex virus was selected. Synergistic or antagonistic effects of M. pneumoniae and rhinovirus were tested in combination with this agent. Herpes simplex virus was added to KB cell systems at 48 and 24 hours before rhinovirus inoculations. The rhinovirus

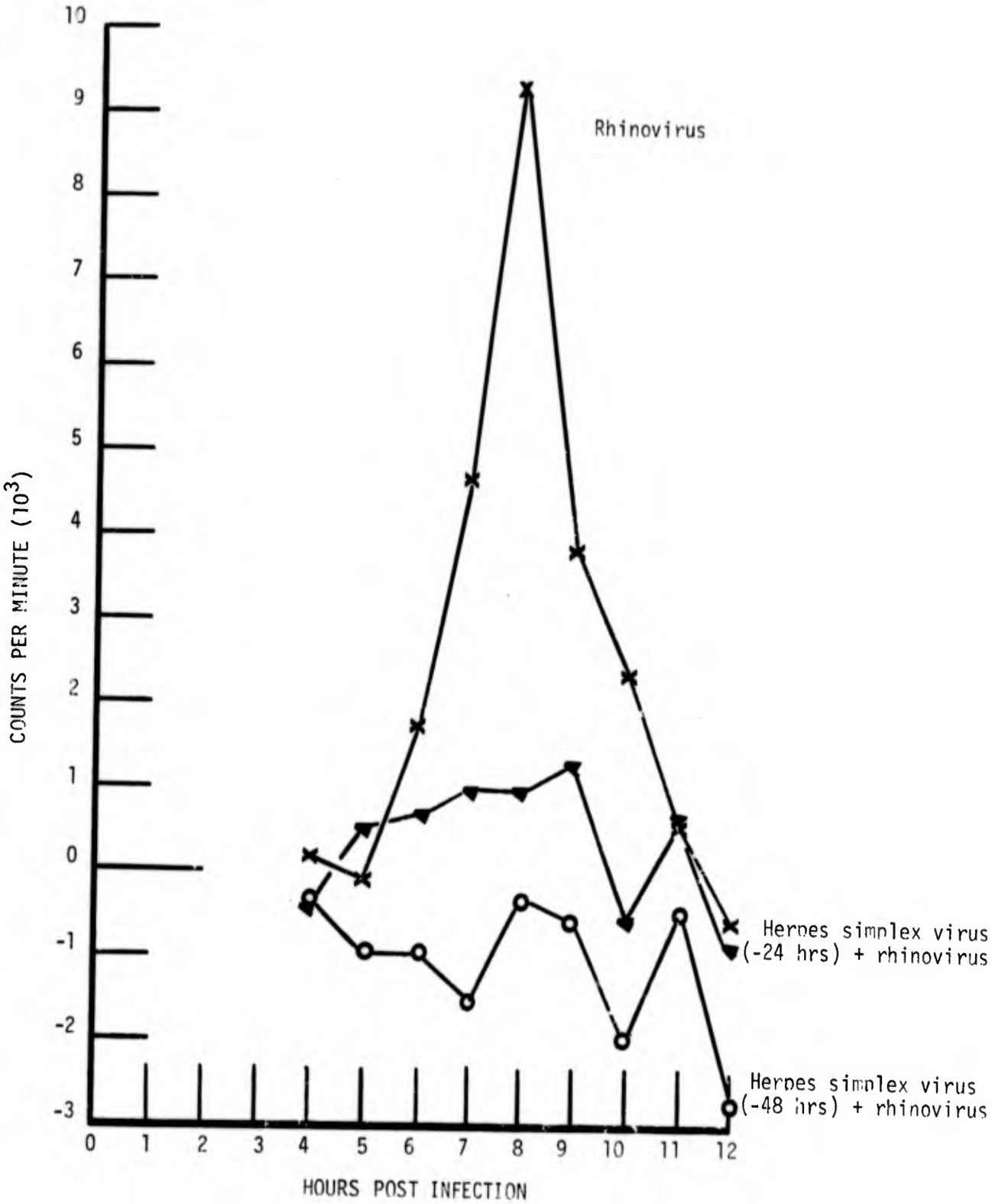
inoculations were made in the presence of 10 µg/ml actinomycin D (actinomycin D inhibits DNA-dependent RNA synthesis of Herpes simplex virus and KB cell systems), and pulse-labeled for 1 hour with 2µc ^{3}H-uridine at selected times during the rhinovirus replication (Fig. 1). All CPM reported were corrected, minus appropriate tissue or tissue-herpes control. Herpes simplex virus added at minus 48 hours completely inhibited rhinovirus RNA synthesis and when added at minus 24 hours inhibited rhinovirus RNA synthesis by 90 per cent. Similar studies conducted with adenovirus type 4 pre-treated (minus 24 hours) KB-cell-monolayer systems did not show inhibition of rhinovirus RNA synthesis.

In contrast, M. pneumoniae added at 48 and 24 hours before Herpes simplex virus inoculations appeared to enhance the viral produced CPE in rabbit kidney cell monolayers. The herpes virus yield titered two logs higher in the 48 and 24 hour mycoplasma pre-treated cells than in the cells pre-treated with mycoplasma at minus 6 hours and in the untreated cells.

DISCUSSION

Because of the enhancement of rhinovirus RNA synthesis by mycoplasma, a similar interaction with other respiratory viruses was studied. The viruses selected were adenovirus type 4 and influenza A/PR8. In the rhinovirus studies, the host cell and mycoplasma RNA synthesis could be inhibited by actinomycin D, which allowed direct measurement of viral ^{3}H-uridine uptake (viral-RNA synthesis). In these studies, actinomycin D was not employed because it not only inhibits the host cell DNA-dependent RNA synthesis, but also RNA synthesis of adenovirus or influenza virus.

Figure 1. Inhibition of rhinovirus-RNA synthesis (^{3}H-uridine untake) by Herpes simplex virus in KB cell-monolayer cultures.



As a result, host cells were not inhibited except after adenovirus infection e.g. host-cell DNA is inhibited by adenovirus type 5 at approximately 10 hours post-infection, whereas viral DNA synthesis occurs between 10 and 21 hours post-infection (Ginsberg, Bello, and Levine, in The Molecular Biology of Viruses (Colter and Paranchych, eds), Academic Press, N.Y. 1967, p. 547). In adenovirus-mycoplasma studies ^{3}H-thymidine was added after host DNA synthesis was predicted to be inhibited. Tissue controls and mycoplasma-treated cell systems showed high thymidine incorporation compared to the lower levels of DNA synthesis observed in the mycoplasma-virus and virus infected systems. In general, the mycoplasma-adenovirus incorporation of thymidine was higher than in the virus infected systems (no mycoplasma treatment). The increased DNA synthesized is probably viral, as supported by the fact viral end-point titers in KB or L132 cells were greater in the presence of mycoplasma. In addition, great numbers of virus infected cells were observed in mycoplasma-cell cultures, compared to untreated cells, as demonstrated by fluorescent antibody techniques. Confirmation of which DNA (host cell, adenovirus or mycoplasma) contains the highest concentration of tritiated-thymidine is in progress using a cesium chloride density gradient. The guanine-cytosine (GC ratio is 53-57% for adenovirus 3, 4 and 7 (Pina and Green, 1965, Proc. Natl. Acad. Sci. U.S., 54:547); 39% for M. pneumoniae (Neimark, 1967, Ann. N.Y. Acad. Sci. 143:31); and 42-44% for KB cells (H. Fraenkel-Conrat in Molecular Basis of Virology, Reinhold Book Corporation, N.Y., p. 398, 1968). These ratios are sufficiently different to allow separation of viral DNA from the DNA of host cell and mycoplasma. Separated bands of DNA will be collected and measured for radioactivity (^{3}H-thymidine incorporation).

As mentioned, Influenza A/PR8 virus infections of mice allowed for a measure of viral pathogenicity in the presence and absence of M. pneumoniae. These investigations are being expanded to consider the effect of different mycoplasma titers and the duration of mycoplasma treatment on influenza viral replication in vivo. Also, serum from mice will be collected and tested for the presence of interferon, which, if present, could partially mask the virus infection.

Herpes virus inhibited rhinovirus-RNA synthesis appeared to be a typical viral interference that was dependent on time of inoculation and the input virus MOI. Pre-infection of the KB cell monolayer systems with adenovirus did not appear to effect rhinovirus RNA synthesis until the cells detached from the glass surface (cell sheets showing a 4+ cytopathic effect).

During 1971, an attempt will be made to elucidate the mode-of-action of the stimulatory effect of M. pneumoniae on virus replication. As enhancement appears to occur at an early stage in the virus infection cycle, the rate of viral adsorption and penetration will be measured in the presence and absence of M. pneumoniae. Because mycoplasma enhanced virus nucleic acid synthesis results in only a slight increase, if any, in total virions, the possibility of an increased number of defective virions will be considered.

CONCLUSIONS

Mycoplasma pneumoniae and certain PPLO growth medium components, PPLO broth and fresh yeast extract, stimulate rhinovirus (type 1A, strain 2060) RNA synthesis. In addition, the higher the input multiplicities of virus the greater the RNA incorporation of tritiated-uridine (acid-

precipitable), and the lower the tritiated-uridine content of phosphorylated fraction (acid-soluble).

Adenovirus type 4 and M. pneumoniae interactions measured by tritiated-thymidine uptake showed greater DNA synthesis, as compared to adenovirus infected cells alone. The positive identity of this labeled DNA is unknown, but is presently under investigation.

In mice, influenza A/PR8 virus produced a more severe infection in animals pre-treated with M. pneumoniae than in those infected with virus alone. M. pneumoniae alone produced no apparent effect on mice.

Pre-treatment of KB cell monolayer systems with Herpes simplex virus interfered with rhinovirus-RNA synthesis. There was complete inhibition of rhinovirus nucleic acid synthesis when the herpes virus was added at minus 48 hours. In contrast, pre-treatment (minus 48 and minus 24 hours) of rabbit kidney cells with M. pneumoniae appeared to stimulate Herpes simplex virus infections.

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I also wish to acknowledge the technical assistance given by Dr. Chuinrudee Jayavasu and Mr. Roger Johnson.

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Fletcher, R.D. 1969. The Relationship Between Mycoplasma Species and Selected Respiratory Viruses (Adenovirus, Influenza Virus and Rhinovirus). Annual Report Number 1, Life Science Division, Army Research Office, Arlington, Virginia 22204.

Milligan III, W.H. and R.D. Fletcher. 1969. The effect of Mycoplasma pneumoniae on rhinovirus-RNA synthesis in KB cells. Antimicrobial Agents and Chemotherapy-1969: 196-199.

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13 ABSTRACT Because of the importance of adenovirus and M. pneumoniae infections in military personnel, emphasis was placed on a study of these interactions. These investigations have shown that M. pneumoniae apparently stimulates ^{3}H-thymidine uptake (DNA synthesis) by adenovirus type 4 in L-132 cell monolayer systems, when cell systems were treated with mycoplasma at minus 48 and minus 24 hours prior to the virus inoculum. If thymidine-uptake values of the virus-infected systems are considered to be 100 per cent, the virus incorporated thymidine in 48 hour mycoplasma-treated systems ranged from 145 to 159 per cent. There appeared to be slight increases of adenovirus yields in mycoplasma-treated cells compared to virus-cell systems measured by both tube end-point and fluorescent antibody titrations.

For the purpose of comparison, another DNA virus, Herpes simplex virus was selected. M. pneumoniae, added at 48 and 24 hours before herpes virus inoculations, appeared to enhance the viral-produced CPE in rabbit kidney monolayers. In contrast, if KB cell monolayers were pre-treated (minus 24 and minus 48 hours) with herpes virus, instead of mycoplasma, and subsequently infected with rhinovirus, the rhinovirus RNA synthesis was partially or completely inhibited, respectively. In a comparable study, pre-treatment (24 hrs or less) of KB cell systems with adenovirus type 4, did not appear to effect rhinovirus RNA synthesis.

Finally, the interaction of M. pneumoniae and influenza A/PR8 virus was observed in 1,308 mice. These combinations of mycoplasma and influenza appeared to produce earlier symptoms and deaths than observed in mice infected with virus alone.

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