Studies on the Nutrition and Growth Requirements of *Mycoplasma gallisepticum*

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SUMMARY

A complex tissue culture medium supplemented with swine serum and peptone supported optimal growth of *Mycoplasma gallisepticum* strain 293. Media lacking any of these components supported little or no growth. However, when 5'-monophosphate nucleotides replaced the peptone growth was supported. The minimal nucleotides necessary to support good growth were adenylic, cytidylic, guanylic and thymidylic acids. In some cases the addition of the four ribonucleotides and the four deoxyribonucleotides in place of peptone improved growth; the four ribonucleotides alone supported poor growth. Thymidylic acid seemed essential for growth, and uridylic acid appeared to be inhibitory.

The mixture of ribonucleosides and deoxyribonucleosides, but not of purines and pyrimidines, when substituted for the peptone, also supported good growth. The concentrations of nucleosides and nucleotides had a significant effect on growth. Although the nutritional factors of swine serum were not defined, the effects of different sera on growth were investigated. Rabbit, horse, turkey and swine serum supported optimum growth; human serum supported less, whereas PPLO serum fraction (Difco) or bovine serum supported poor growth. Dog serum did not support growth.

INTRODUCTION

The nutritional requirements of some *Mycoplasma* species have been reported by Edward (1947, 1953, 1954), Smith & Morton (1951), Smith, Lecce & Lynn (1954), Smith (1955), Chalquest & Fabricant (1960), Rodwell (1960), Razin & Knight (1960*a, b*), Razin (1962), and Razin & Cohen (1963). It is apparent from these studies that the pathogenic as well as the saprophytic species have complex nutritional requirements. The main purpose of the present work was to evolve a defined medium which would support the growth of the avian pathogen *Mycoplasma gallisepticum* (Edward & Kanarek, 1960) strain 293 and to determine its nutritional requirements. A partially defined medium described by Razin & Knight (1960*a*), or modifications of it, did not support growth of the test organism. However, excellent growth was supported by a medium containing tissue culture (TC) medium 199 (Morgan, Morton & Parker, 1950), swine serum and Bacto-peptone. This medium was selected as a basis for further work to replace the undefined components with defined ones. Media without the serum did not support

growth. However, growth of the test organism did occur when the 5'-monophosphate ribonucleotides and deoxyribonucleotides, or the ribonucleosides and deoxyribonucleosides replaced the peptone component.

METHODS

Organism. *Mycoplasma gallisepticum* strain 293 was selected as the test organism. This strain was originally isolated from a field case of chronic respiratory disease in chickens by Dr J. R. E. Taylor in 1957; its morphological and physiological characteristics were described by Fabricant *et al.* (1959). Since isolation the organism has been maintained in continuous subculture on the same laboratory medium.

Media. Adler's overlay medium (Adler, Yamamoto & Bankowski, 1954) was used for maintaining stock cultures and for growing the organism for inocula in the nutritional experiments.

Difco PPLO broth without crystal violet, enrichments or inhibitors was used as diluent in preparation of serial dilutions for colony counts. Difco PPLO agar enriched with 10 % (v/v) pooled swine serum, 1000 units penicillin/ml. and 0.05 % (w/v) thallium acetate was used for colony counts. The pooled swine serum was obtained from a local slaughtering plant and sterilized by passage through a Seitz EK filter. All batches of serum were checked for ability to support growth of several *Mycoplasma* species and then stored at -25° until needed.

The medium used for the nutritional experiments was prepared from Difco TC medium 199 (based on the tissue culture medium of Morgan *et al.* 1950) liquid ten-fold concentrate. This medium was diluted to double concentration with sterile glass-distilled water, buffered with 0.3 % (w/v) K_2HPO_4 , rather than with sodium bicarbonate. After the addition of 10 % (v/v) pooled swine serum, and various supplements, the appropriate volume of distilled water was added to adjust the TC medium 199 to single concentration; when necessary, this was readjusted to pH 7.6–7.8 with 10 % (w/v) KOH. Hereafter the TC medium 199 + swine serum but without other supplements will be referred to as the basal medium. The control medium used in all nutritional experiments consisted of basal medium to which had been added 1 % (w/v) Bacto-peptone.

Except for Adler's overlay medium all media, including all reagents and supplements, were prepared with glass-distilled water. Pyrex glassware was used in all experiments. Before use it was cleaned with chromic + sulphuric acid mixture and thoroughly rinsed with distilled water.

All nucleic acid derivatives and coenzymes were sterilized by passage through sintered glass filters. Undegraded DNA preparations were sterilized with a few drops of chloroform and then decanted from the chloroform. All other inorganic reagents and media used for colony counts were sterilized by autoclaving at 120° for 15 min.

Conditions of growth. Five ml. of basal medium with various supplements were dispensed in 13×100 mm. tubes with stainless steel closures. These were inoculated with 0.05 ml. of a 48 hr stock culture. The cultures were incubated statically in air at 37° . Colony counts showed that the inoculum contained about 10^6 organisms/ml. medium.

Assessment of growth. The estimation of growth by turbidimetric methods or acid titration was unreliable under our experimental conditions; the basal medium

was too turbid and there was no consistent correlation between acid production and colony counts. Therefore, growth was measured by the colony count technique described by Fabricant, VanDemark & Fabricant (1962). All plates for colony counts were incubated in candle jars with excess moisture at 37°.

Chemicals. Undegraded DNA, the purines and pyrimidines adenine, cytosine, guanine, uracil and thymine, and the 5'-monophosphate deoxyribonucleotides, deoxyadenylic acid disodium salt.3H₂O (dAMP), deoxycytidylic acid.2H₂O (dCMP), deoxyguanulate.NH₄ (dGMP), and thymidylic acid sodium salt (TMP) were products of the California Corporation for Biochemical Research (Los Angeles, California). The 5'-monophosphate ribonucleotides muscle adenylic acid (AMP), guanylic acid sodium salt (GMP), cytidylic acid sodium salt (CMP), and uridylic acid disodium salt (UMP) were products of the Pabst Laboratories (Milwaukee, Wisconsin) or the Sigma Chemical Company (St Louis, Missouri). The 5'-triphosphate nucleotides of adenosine, disodium salt (ATP), cytidine, sodium salt (CTP), guanosine, sodium salt (GTP), and uridine, sodium salt (UTP) were also products of the Pabst Laboratories. The ribonucleosides adenosine, cytidine, guanosine, uridine, and the deoxyribonucleosides, deoxyadenosine, deoxycytidine.HCl, deoxyguanosine, and thymidine were products of the Nutritional Biochemical Corporation (Cleveland, Ohio).

RESULTS

The partially defined medium described by Razin & Knight (1960*a*) for a strain of *Mycoplasma laidlawii* did not permit survival of *Mycoplasma gallisepticum* strain 293. Attempts to improve this medium by addition of PPLO serum fraction (Difco), 10 % (v/v) swine serum, increased amounts of glucose, or biotin and folic acid also failed and further work with it was abandoned.

Initial attempts to grow the test organism in TC medium 199 with or without 10 % (v/v) swine serum also failed. Since this medium changed from pH 7.6 to 8.9 within 3 hr of static incubation at 37°, the medium was subsequently buffered with 0.3 % (w/v) K₂HPO₄ rather than with sodium bicarbonate as recommended. Inocula of the test organism survived better in this medium, without serum and buffered with K₂HPO₄, than in media buffered with sodium bicarbonate or tris buffer (pH 7-9; Sigma).

However, minimal growth of strain 293 occurred when the TC medium 199 was supplemented with 10 % (v/v) swine serum (basal medium). Addition of 0.1-0.4 % (w/v) of glucose to this basal medium did not improve growth.

Other studies indicated that the basal medium lacked some essential nutritional factor, or factors, which could be supplied by Difco PPLO broth (without crystal violet). The factor, or factors, could be supplied equally by 1 % (w/v) Bacto-peptone, one of the PPLO broth ingredients (see Table 1), and to a lesser degree by beef heart for infusion, the other PPLO broth ingredient.

The basal medium supplemented with 1 % (w/v) Bacto-peptone was used as the control medium in all later experiments. Optimal growth of strain 293 was obtained only in the control medium. No growth occurred in media when either TC medium 199 or serum were omitted.

Optimal growth of the test organism also occurred in media where the concentration of peptone (in the control medium) was altered from 0.4 to 4 % (w/v).

Concentration of 6, 0.2 and 0.1 % peptone yielded less growth, while concentrations above 6 % were inhibitory.

Essentially the same amount of growth occurred in media where rabbit, horse, or turkey serum was substituted for one lot of swine serum in the control medium (see Table 2). Human serum supported moderate growth, Difco PPLO serum fraction or bovine serum supported poor growth, while dog serum did not support growth. Other experiments indicated that some batches of swine serum were superior to horse serum in supporting growth of strain 293 in the control medium. Neither tryptic digests of casein nor peptone prepared according to the method of Roberts & Snell (1946), when substituted for the peptone of the control medium, allowed growth of strain 293.

Table 1. *The growth of Mycoplasma gallisepticum strain 293 in TC medium 199 + swine serum (basal medium) supplemented with Difco PPLO broth, Bacto-peptone, or beef heart for infusion (Difco)*

Media*	Time (days)			
	1	2	3	4
	Counts†/ml. medium ($\times 10^6$)			
PPLO broth + swine serum‡	81	139	124	380
Basal medium	7	5	5	3§
Basal medium + PPLO broth	3	10	1255	240
Basal medium + 1 % (w/v) peptone	180	1555	0	0
Basal medium + 5 % (w/v) beef heart for infusion	65	196	90	44

* Since the inoculum did not survive in any of the above mixtures of medium components without serum, these counts are not given in the Table.

† The inoculum contained 4×10^6 viable particles/ml. medium. All counts have been rounded off to the nearest 10^6 figure.

‡ Swine serum was added in 10 % (v/v) to all media.

§ Marked decrease in all counts after initial growth was due to acid production.

Table 2. *The growth of Mycoplasma gallisepticum strain 293 in TC medium 199 supplemented with peptone and various pooled sera or PPLO serum fraction (Difco)*

Media* supplement	Time (days)			
	1	2	3	4
	Counts†/ml. medium ($\times 10^6$)			
Rabbit serum	295	855	3‡	0
Horse serum	795	500	< 1	0
Swine serum	705	530	< 1	0
Turkey serum	605	< 1	0	0
Human serum	105	230	< 1	< 1
Bovine serum	22	6	10	6
PPLO serum fraction	9	6	8	7
Dog serum	< 1	< 1	< 1	< 1

* TC medium 199 was buffered with 0.3 % (w/v) K_2HPO_4 supplemented with 1 % (w/v) peptone and 10 % (v/v) of the various sera or 1 % (v/v) of Difco PPLO serum fraction.

† The inoculum contained 7×10^6 viable particles/ml. medium; all counts have been rounded off to the nearest 10^6 figure.

‡ Marked decrease in counts after initial growth was due to acid production.

Further work involved attempts to replace the serum and peptone of the control medium with chemically defined factors. However, none of the factors tested were capable of replacing the serum and only data concerning replacement of the peptone will be included here.

Media in which the peptone of the control medium was replaced by the 5'-monophosphate ribonucleotides or ribonucleosides, where these were added in the same concentration as the free purine and pyrimidine bases already present in TC medium 199, supported slight growth of the test organism. The addition of coenzyme A, diphosphopyridine nucleotide (DPN), flavin adenine dinucleotide (FAD) and α -lipoic acid (added in 0.25, 0.7, 0.1, 0.001 mg./100 ml. medium, respectively) to these media did not stimulate growth. Nor did the addition of reduced glutathione and L-cysteine.HCl.H₂O (100 mg. each/100 ml. medium) improve the growth in these media.

Table 3. *The influence of ribonucleotides AMP, CMP, GMP, deoxyribonucleotide TMP, and DNA on the growth of Mycoplasma gallisepticum strain 293 in the basal medium*

Media*	Time (days)				
	1	2	3	4	5
	Counts $\dagger$ /ml. medium ($\times 10^6$)				
(Basal medium $\dagger$ supplemented with:)					
1 % (w/v) peptone (control medium)	320	600	34	24	—
No supplements added	71	3	2	4	—
AMP, CMP, GMP, TMP§	4	8	92	48	—
DNA§	1	30	53	140	29
AMP, CMP, GMP, TMP, DNA	71	8	75	350	42

* Media combinations without serum have been omitted since they did not support growth.

$\dagger$ Basal medium contained TC medium 199 buffered with 0.3 % (w/v) K₂HPO₄ and 10 % (v/v) swine serum.

$\ddagger$ The inoculum contained 13×10^6 viable particles/ml. medium. All counts have been rounded off to the nearest 10^6 figure.

§ Nucleotides and undegraded DNA each added in 1 mg./100 ml. medium.

|| Marked decrease in all counts after initial growth was due to acid production.

However, as shown in Table 3, growth was stimulated when the 5'-monophosphate ribonucleotides AMP, CMP, GMP and the same isomer of deoxyribonucleotide TMP were added in 1 mg./100 ml. basal medium (nucleotides replacing the peptone of the control medium). As further shown in Table 3, growth was markedly stimulated when DNA (1 mg./100 ml. medium) was added to the basal medium supplemented with these four nucleotides.

In view of the growth of strain 293 in basal medium + DNA only, and the stimulation of growth by DNA in the medium containing the nucleotides, AMP, CMP, GMP, and TMP, the four 5'-monophosphate deoxyribonucleotides were substituted for the DNA to determine their comparative effects on growth. Table 4 shows that the basal medium supplemented with the four ribonucleotides alone supported the least growth. The preparation of DNA or the nucleotides AMP, CMP, GMP, UMP and TMP supplementing the basal medium supported the same amount of growth. Better growth was supported in the medium containing AMP, CMP, GMP, and TMP, while slightly less growth occurred in the medium containing both the

ribonucleotides and the deoxyribonucleotides. The medium containing the deoxyribonucleotides alone supported growth intermediate between the last two media named.

As also shown in Table 4, the addition of the 5'-triphosphate nucleotide ATP to the medium containing the eight monophosphate nucleotides, further stimulated growth of strain 293. The addition of the four triphosphate nucleotides ATP, CTP, GTP and UTP to this medium stimulated only slightly more growth than ATP alone. In this experiment the triphosphate nucleotides were prepared and stored frozen for 1 week before use. In other experiments freshly prepared ATP did not show the same growth stimulation.

Table 4. *The influence of 5'-monophospho-ribonucleotides, deoxyribonucleotides, DNA and triphosphonucleotides on the growth of Mycoplasma gallisepticum strain 293 in the basal medium*

Media (basal medium† supplemented with:)	Time (days)			
	1	2	3	4
	Counts*/ml. medium (× 10 ⁶)			
I. 1 % (w/v) peptone (control medium)	190	640	480	54¶
II. AMP, CMP, GMP, UMP‡§	6	18	18	2
III. AMP, CMP, GMP, UMP, TMP	10	26	47	< 1
IV. AMP, CMP, GMP, TMP	28	56	77	< 1
V. AMP, CMP, GMP, TMP, DNA	8	101	8	< 1
VI. DNA‡	8	22	48	39
VII. dAMP, dCMP, dGMP, TMP‡	18	56	60	< 1
VIII. AMP, CMP, GMP, UMP + DNA	12	69	115	< 1
IX. AMP, CMP, GMP, UMP, dAMP, dCMP, dGMP, TMP	9	67	50	< 1
X. Medium IX with ATP‡	23	190	4	< 1
XI. Medium IX with ATP, CTP, GTP, UTP	12	215	10	< 1

* Inoculum contained 13×10^6 organisms per ml. of medium. All counts have been rounded off to the nearest 10^6 figure.

† Basal medium contained TC medium 199 buffered with 0.3 % (w/v) K_2HPO_4 and 10 % (v/v) swine serum.

‡ All ribo- and deoxyribonucleotides and DNA were added in 1 mg. each/100 ml. of medium. The triphosphonucleotides were added in 0.1 mg. each/100 ml. of medium.

§ The 5'-monophosphoribonucleotides.

|| The 5'-monophosphodeoxyribonucleotides.

¶ Marked drop in all counts after initial growth was due to acid production.

Further effects on the growth of strain 293 in the basal medium supplemented with various mixtures or concentrations of nucleotides are illustrated in Table 5. The results indicate that the concentration of the eight monophosphate nucleotides significantly influenced the amount of growth. Growth was essentially doubled when the concentration of these nucleotides was halved, whereas doubling their concentration supported approximately half as much growth. In this experiment the medium containing the eight monophosphate nucleotides supported slightly more growth than that containing only AMP, CMP, GMP and TMP. The omission of UMP from the medium containing the seven other nucleotides yielded more growth than the medium including UMP. In this same experiment, a week-old preparation of ATP had a stimulatory effect on growth in the medium containing the eight monophosphate nucleotides, whereas adenosine diphosphate (ADP) seemed to have an inhibitory effect. When ATP and ADP were added together, the resulting growth was essentially the same as that in the medium where ATP was added alone.

Table 6 compares the effects of growth of strain 293 in the basal medium supplemented with the eight monophosphate nucleotides, the corresponding eight nucleosides, or the free purine and pyrimidine bases. The concentrations of the nucleosides

Table 5. *The influence of various combinations and concentrations of 5'-monophosphonucleotides and the adenine diphospho- and triphosphonucleotides ADP, ATP) on growth of Mycoplasma gallisepticum strain 293 in the basal medium*

Media (basal medium* supplemented with:)	Time (days)		
	1	2	3
	Counts†/ml. medium (× 10 ⁶)		
I. 1 % (w/v) peptone (control medium)	435	745	80
II. AMP, CMP, GMP, UMP, dAMP, dCMP, dGMP, TMP‡	15	66	7
III. Medium II without UMP	38	94	3
IV. AMP, CMP, GMP, TMP	13	51	43
V. Medium II with nucleotides at 1/2 concentration	32	114	2
VI. Medium II with nucleotides at 2 × concentration	28	37	1
VII. Medium II plus ATP§	22	102	9
VIII. Medium II plus ADP§	23	18	23
IX. Medium II plus ADP and ATP	33	99	29

* Basal medium contained TC medium 199 buffered with 0.3 % (w/v) K₂HPO₄ and 10 % (v/v) swine serum.

† The inoculum contained 12 × 10⁶ viable particles/ml. of medium. All counts have been rounded off to the nearest 10⁶ figure.

‡ The first four listed are the 5'-ribonucleotides, the others are the 5'-deoxyribonucleotides; each was added in 1 mg./100 ml. of medium. (Monophosphate nucleotides).

§ ADP and ATP were each added in 0.1 mg./100 ml. of medium.

|| Slight increase in growth at 3 days was followed in four days by a drop to less than 1 × 10⁶. Marked decrease in all counts after initial growth was due to acid production.

and the purine and pyrimidine bases added were calculated to approximate the concentrations of the nucleotides, each of which was added at 1 mg./100 ml. medium. Growth was slightly greater in the medium containing nucleosides than that containing nucleotides. Although growth was increased in both media when the concentration of nucleosides or nucleotides was halved, considerably more growth resulted in the medium with the lower concentration of nucleosides. However, it was noted in the course of colony counts, that subcultures to plating agar from media containing nucleosides resulted in a large number of atypical rough colonies. Atypical colonies were rarely, if ever, noted in subcultures during the growth phase of strain 293 from media containing nucleotides. The substitution of purines and pyrimidines for the peptone of the control medium resulted in minimal growth. It was further noted that all colonial growth on subcultures to plating agar was extremely rough and atypical.

DISCUSSION

Since the tissue culture medium (TC medium 199) of Morgan *et al.* (1950) contains a large complement of amino acids, vitamins, purines, pyrimidines, inorganic salts and cholesterol, it was selected as the base medium from which to evolve a defined medium capable of supporting growth of *Mycoplasma gallisepticum* strain 293.

Table 6. *The relative influence of the 5'-monophospho-, ribo- and deoxyribonucleotides, the corresponding nucleosides, or the free purine and pyrimidine bases on growth of Mycoplasma gallisepticum strain 293 in the basal medium*

Media (basal medium* supplemented with:)	Time (days)			
	1	2	3	4
	Counts†/ml. medium (× 10 ⁶)			
I. 1 % (w/v) peptone (control medium)	630	705	120§	—
II. AMP, CMP, GMP, UMP, dAMP, dCMP, dGMP, TMP‡	59	81	12	< 1
III. Medium II with nucleotides at 1/2 concentration	53	105	62	< 1
IV. Nucleosides‡	54	100	6	< 1
V. Medium IV with nucleosides at 1/2 concentration	86	167	8	< 1
VI. Purines and pyrimidines‡	5	12	12	< 1
VII. Medium VI with purines and pyrimidines at 1/2 concentration	8	9	12	2

* Basal medium contained TC medium 199 buffered with 0.3 % (w/v) K₂HPO₄ and 10 % (v/v) swine serum.

† The inoculum contained 10 × 10⁶ organisms/ml. of medium. All counts have been rounded off to the nearest 10⁶ figure.

‡ The first four listed are the ribonucleotides, the others are the deoxyribonucleotides; each was added in 1 mg./100 ml. of medium. Calculated equivalent concentrations of the corresponding nucleosides (adenosine, cytidine, guanosine, deoxyadenosine, deoxycytidine, deoxyguanosine, and thymidine) and purines and pyrimidines (adenine, cytosine, guanine, uracil and thymine) were added to media IV and VI respectively.

§ Marked drop in all counts after initial growth was due to acid production.

This medium, however, was found to lack essential nutrients which could be supplied by adding serum + Bacto-peptone. Optimal growth of strain 293 occurred only in media containing the TC medium 199 + serum + peptone, omission of any of these three components resulted in little or no growth. It is likely that all the components of TC medium 199 are not essential for growth of strain 293. The present work and the report of Razin (1962) suggest that the purines and pyrimidines might be eliminated. It is also possible that the essential ingredients of this medium are not at optimal concentrations. However, it was felt that until defined reagents were found capable of adequately replacing the serum and peptone parts of the control medium, it would be unprofitable to determine the effects of the individual components of TC medium 199 on growth of strain 293.

The reports of Edward (1954), Chalquest & Fabricant (1960) and Razin & Knight (1960*b*) suggested that nucleic acid precursors or other known protein derivatives might replace either or both undefined components (serum, peptone) of the control medium. Professor R. W. Holley suggested (personal communication) that the preformed ribonucleosides or the 5'-monophosphate ribonucleotides of adenine, cytosine, guanine and uracil, rather than purines and pyrimidines might be essential for growth of organisms with a limited content of enzymes. Therefore these ribonucleosides or ribonucleotides were substituted for either or both serum and peptone of the control medium. Initial studies indicated that the ribonucleotides, rather than the ribonucleosides, would partially replace the peptone, but not the serum, in supporting growth of strain 293. However, better growth resulted when

peptone was replaced by the ribonucleotides AMP, CMP, GMP and the deoxy-ribonucleotide (5'-monophosphate isomer) thymidylic acid (TMP). It was found that the best growth occurred when each of these nucleotides was added in the same concentration. These findings parallel those of Razin (1962) in which he reported that mixtures of the 5'-monophosphate ribonucleotides were active in growth promotion of *Mycoplasma laidlawii* strain A.

Comparison of growth in media where the 5'-monophosphate nucleotides of RNA, or DNA or mixtures of these were substituted for the peptone (each nucleotide added to 1 mg./100 ml. medium) showed that the four RNA nucleotides, AMP, CMP, GMP and UMP, gave the poorest growth. The four DNA nucleotides, dAMP, dCMP, dGMP and TMP, or the RNA and DNA nucleotides together gave about four times as much growth as the RNA nucleotides alone. However better growth was given by the nucleotides AMP, CMP, GMP and TMP than by the four DNA nucleotides only. The experimental evidence indicates that in these media TMP may be essential for growth while UMP may be somewhat inhibitory.

In one experiment when undegraded DNA replaced the peptone in the control medium there was more growth than in the media containing nucleotides. However, in a later experiment various mixtures of nucleotides (supplementing the basal medium) were superior to undegraded DNA. Further work may resolve this discrepancy. The effect of ATP on growth of strain 293 in basal medium supplemented with nucleotides also needs clarification. Week-old preparations of ATP had a stimulatory effect on growth, while fresh preparations did not. The difference did not seem to be due to the possible breakdown of ATP to ADP, since ADP seemed to have an inhibitory effect on growth in one trial.

Comparison of growth of *Mycoplasma gallisepticum* strain 293 in the basal medium supplemented by the RNA and DNA nucleosides, 5'-monophosphate nucleotides or purines and pyrimidines, parallels in part the results reported by Razin (1962) for *M. laidlawii* strain A. He found that purines and pyrimidines would not stimulate growth of *M. laidlawii* strain A in his partially defined medium. The minimal growth of strain 293 in our basal medium supplemented with purines and pyrimidines was probably due to the basal medium alone. Furthermore, the markedly rough and atypical colonial growth from these media subcultured on plating agar, suggests that purines and pyrimidines may disrupt typical colonial development of strain 293. Growth of *M. gallisepticum* strain 293 was better in media containing nucleosides than in those containing nucleotides; similar results were reported by Razin (1962) for *M. laidlawii*. However, we noted that a large proportion of colonies plated from media with nucleosides was rough and atypical. Atypical colonies were not noted during the growth phase of strain 293 in media containing nucleotides. Since the only difference between the nucleosides and nucleotides was the phosphate linkage of the latter it is possible that the preformed nucleotides may be better utilized by strain 293 for subsequent typical colonial growth.

Further work is needed to establish the optimum concentrations and mixtures of nucleosides or nucleotides for growth of strain 293 in the basal medium. The concentration of 0.5 mg. of each nucleoside or nucleotide/100 ml. medium supported more growth than double this concentration. Further increase in concentration of nucleotides (and possibly nucleosides) was inhibitory.

We found that the minimal nucleotides which supported good growth of *Mycoplasma gallisepticum* strain 293 were adenylic, cytidylic, guanylic and thymidylic acids (5'-monophosphates). Future work may establish that the minimal nucleoside requirements of strains 293 may be adenosine, cytidine, guanosine and thymidine. This would parallel the minimal nucleoside requirements for growth of *M. laidlawii* strain A as reported by Razin (1962) in his partially defined medium.

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