INHIBITION OF *SALMONELLA GALLINARUM* BY D-SERINE AND ITS REVERSAL

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY

M. Louise Brock

1958

INHIBITION OF *SALMONELLA GALLINARUM* BY D-SERINE AND ITS REVERSAL

By

M. Louise Brock

AN ABSTRACT

Submitted to the School of Science and Arts of Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Microbiology and Public Health

1958

Approved by _____

Abstract M. Louise Brock

The growth, nutrition and inhibition of Salmonella gallinarum was studied in a defined synthetic medium using a turbidimetric method and viable cell count. This organism is inhibited by the D isomer of DL-serine which reduces the growth rate. The inhibition is temporary and is reversed noncompetitively by nucleic acid derivatives and amino acids, for which inhibition indices have been determined, and also by RNA, $KHCO_3$ and L-serine.

The metabolism of amino acids and nucleic acids is discussed, and it is suggested that D-serine interferes with the metabolism of several amino acids, particularly where they are required for nucleic acid synthesis.

D-Cycloserine and azaserine also inhibit S. gallinarum but O-carbamyl-D-serine does not.

INHIBITION OF *SALMONELLA GALLINARUM* BY D-SERINE AND ITS REVERSAL

By

M. Louise Brock

A THESIS

Submitted to the School of Science and Arts of Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Microbiology and Public Health

1958

ACKNOWLEDGMENTS

The author wishes to express her appreciation to Dr. D. E. Schoenhard for suggesting this problem and for his guidance and encouragement in the study.

The advice and encouragement of Dr. T. D. Brock, in whose laboratory at Western Reserve University much of the work was performed, is also appreciated.

TABLE OF CONTENTS

INTRODUCTION

Salmonella gallinarum was first described by Klein in 1889 (Breed, Murray, and Smith, 1957). A fermentation variant of S. gallinarum was described by Rettger and Harvey in 1908 and given the name Bacterium pullorum by Rettger in 1909. This was later changed to Salmonella pullorum (Breed et al., 1957), and in the latest edition of Bergey's Manual for Determinative Bacteriology is included within the species Salmonella gallinarum. For the sake of clarity the species name "pullorum" will be retained here in reference to research published prior to this recent revision in nomenclature when it had been distinguished between the species pullorum and gallinarum.

S. gallinarum is pathogenic for fowl, causing the disease known as white diarrhea or fatal septicemia; it is in this regard that it differs mainly from other organisms of the genus Salmonella.

Early attempts to grow S. pullorum in synthetic media met with no success. Koser and Rettger (1919) found it would not grow in an amino acid-salts-glycerol medium and grew only slightly when complex nitrogen sources were added. In another case several strains would not grow on a simple salts medium supplemented with inorganic nitrogen sources and salts of

organic acids (Hajna, 1935).

Beard and Snow (1936) included S. pullorum among the organisms they grew on synthetic media for a study of antigenic characteristics, using a protein-free medium of 21 amino acids, glucose and salts. Of a large number of strains used by Johnson and Rettger (1943) for a nutritional study, most did not require growth factors while some required one or more of the following: nicotinic acid or its amide, glucose, aspartic acid, glutamic acid, leucine, and arginine. Several strains would not grow even when supplemented with 16 amino acids, vitamins, and other factors. All the strains of S. gallinarum they tested could grow without glucose but required thiamine hydrochloride, while some required 10% CO_2 in addition to initiate growth. Lederberg (1947) was able to grow two strains of S. pullorum in a glucose-salts-asparagine medium if leucine and cystine were supplied for one and these plus methionine for the other. Davis and Solowey (1950), however, found that when several amino acids and other organic compounds were added individually to a salts medium, S. pullorum would not grow.

The synthesis of glutamic acid and alanine by several strains of S. pullorum in synthetic media was studied by Jones and Holtman (1953), and Schoenhard and Stafseth (1953) described the culture cycle of the organism grown in several complex media compared with a synthetic medium. This synthetic medium was then modified by Gilfillan, Holtman and Ross (1955)

to decrease the period of time required for maximum growth, and among the modifications is the addition of DL-serine. The latest published work concerning S. pullorum is that of Stokes and Bayne (1957) who were able to increase the colony size and growth rate on complex solid media, but they could not decrease the lag period.

In the process of developing a synthetic medium for the growth of this organism Schoenhard (personal communication) found the racemic mixture of DL-serine to be inhibitory. There have been many reports in the literature of amino acids being inhibitory to the growth of bacteria. The single amino acids threonine, lysine, and cysteine, and the combination of serine and alanine were found to inhibit S. pullorum (Gilfillan, Holtman and Ross, 1955), and urea was also inhibitory (Ross, Holtman and Gilfillan, 1956). Castellani (1953) found that DL-serine had a possible slight inhibition of the growth of this organism in a cream pastry filling, but no further work was done in a synthetic medium.

The D isomer of serine prevents toxin formation by Clostridium tetani (Mueller and Miller, 1949) and interferes with pantothenic acid synthesis in Escherichia coli (Maas and Davis, 1950). DL-Serine inhibits Bacillis anthracis (Gladstone, 1939), mutants of Bacillis subtilis (Teas, 1950), lactobacilli (Teeri and Josselyn, 1953), Aerobacter aerogenes (Dagley, Dawes and Morrison, 1950), Agrobacterium tumefaciens

(Van Lanen, Riker and Baldwin, 1952), Bacterium linens (Friedman, Wood and Nelson, 1953), Mycobacterium tuberculosis var. hominis (Dubos, 1949), and Streptococcus bovis (Prescott et al., 1957). In most of these instances the mechanism of action is not known.

This study was undertaken in an attempt to determine the nature of DL-serine inhibition in S. gallinarum when the organism is grown in a defined synthetic medium; a large number of compounds including amino acids, purines, pyrimidines, nucleic acids, and nucleic acid derivatives were tested for possible reversal of serine inhibition.

The term inhibition is used in this study to describe the fact that a test culture has a turbidity less than that of a control culture at some arbitrary time after inoculation.

The term reversal is used to describe the fact that in the presence of some added compound inhibition by serine does not occur, or occurs to a lesser extent, when the turbidity of a test culture is compared with that of a control culture; it does not imply by what mechanism the inhibition is prevented or counteracted.

The inhibition index is used to evaluate the ability of a compound to relieve serine inhibition. It is obtained by subtracting that concentration of serine in mM/ml at which half-maximum growth of the control culture occurs from the concentration of serine giving half-maximum growth in the presence of a reverser; the ratio of this value to the

concentration of reverser in mM/ml is the inhibition index. The formula for calculating the indices is shown below.

$$\frac{\text{mM/ml DL-serine for ½ max. growth in presence of reverser} - \text{mM/ml DL-serine for ½ max. growth of control}}{\text{mM/ml of reverser}} = \text{Inhibition Index}$$

MATERIALS AND METHODS

Culture methods. Strain 6 of Salmonella gallinarum was isolated from poultry. It conforms to the physiological characteristics of S. gallinarum with the exception of producing a weak alkaline reaction in litmus milk; H_2S is produced but citrate is not utilized. The cells are agglutinated by Salmonella polyvalent O serum (Lederle) and are highly virulent when introduced into day-old chicks.

All the growth and inhibition studies were made using the following synthetic medium:

L-leucine	87 mg	NH_4Cl	5 g
L-cystine	60 mg	NH_4NO_3	1 g
L-arginine·HCl	43 mg	Na_2SO_4	2 g
Thiamine·HCl	1 mg	K_2HPO_4	3.82 g
Calcium pantothenate	2 mg	KH_2PO_4	2.18 g
$MgSO_4 \cdot 7H_2O$	100 mg	Glucose	10 g
Trace elements*	1 ml	$KHCO_3$	2 g
distilled water	1000 ml		

*Trace elements solution (Horowitz and Beadle, 1943):

$Na_2B_4O_7 \cdot 10H_2O$	3.52 mg
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	2.78 mg
$FeCl_3 \cdot 6H_2O$	9.68 mg
$CuSO_4$ anhydrous	2.51 mg
$MnCl_2 \cdot 4H_2O$	0.72 mg
$ZnSO_4 \cdot 7H_2O$	88.05 mg
distilled water	100 ml
dilute 1-100	

The amino acids, vitamins, salts and trace elements were dissolved at 1.5625 times the above concentration in distilled water and autoclaved. The glucose was prepared similarly at 10 times the above concentration and

autoclaved separately, after which it was added to the sterile basal medium; 1 to 5 liter amounts of this medium were made at one time and stored in the refrigerator for future use. The bicarbonate was also prepared at 10 times the above concentration, sterilized by filtration through asbestos, and added to 900 ml or more quantities of the above basal medium containing glucose; this was then refrigerated and used within two weeks. Preliminary experiments showed that refrigeration of the complete concentrated medium containing bicarbonate for this length of time had no effect on the growth of the organism.

After having been inoculated the concentrated medium was dispensed in 4 ml quantities into sterile test tubes containing 1 ml of test solution or, for the growth curve experiments, in 40 ml quantities into sterile dilution bottles containing 10 ml of test solution.

The cultures were maintained on brain heart agar (Difco) slants which were stored in the refrigerator after a 24 hr incubation period at 37 C. For each experiment cells were inoculated into 5 ml amounts of synthetic medium and two-drop amounts transferred at 24 hr intervals. The inoculum was then taken from the third tube of synthetic medium in which the organisms had been grown and used to seed the complete medium at the rate of 1 ml inoculum per liter of 1.25-strength medium.

Compounds to be tested were dissolved in distilled water and autoclaved in test tubes or bottles at 118 C for 20 min.

Estimation of growth. Growth was measured either by visual comparison or by plate count methods. For the visual method the amount of turbidity in test tubes was compared with that in a set of standard $BaSO_4$ tubes (McFarland's nephelometer) (Stafseth, Stockton, and Newman, 1956), numbered 1 through 10. No attempt was made to estimate an amount of growth greater than 10. Preliminary experiments showed this method to be more sensitive than one using a colorimeter to evaluate low turbidities. The turbidities of cell suspensions were compared with the $BaSO_4$ tubes and total cell counts were made using the Petroff-Hauser counting chamber. The results of this experiment are shown in figure 1 which indicates that the $BaSO_4$ units are proportional to the logarithm of the total cell count.

Standard Methods for the Examination of Dairy Products (1948) was followed regarding the selection and counting of plates. Dilutions were made in sterile tap water; nutrient agar (Difco) was used for the plating medium.

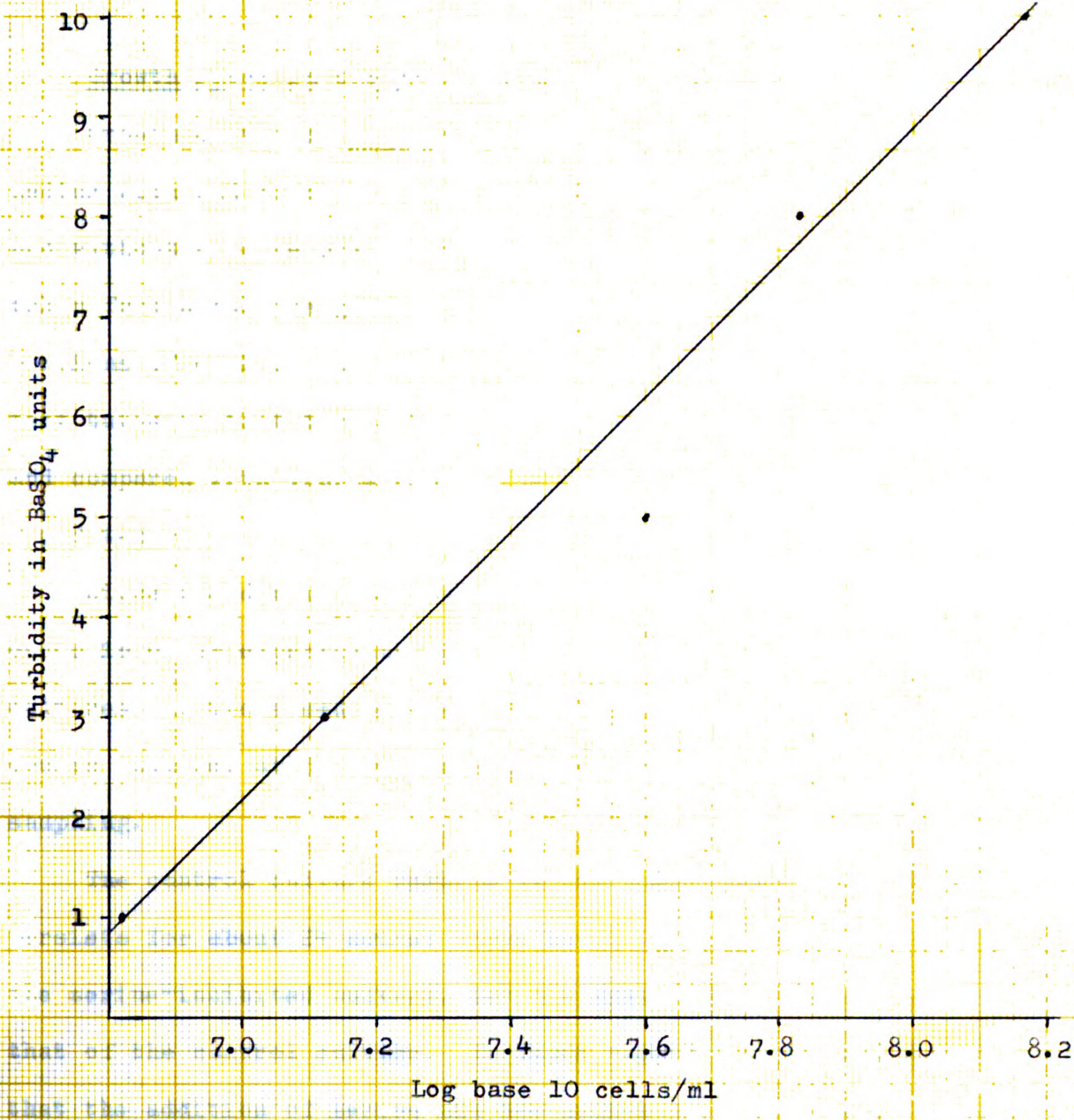
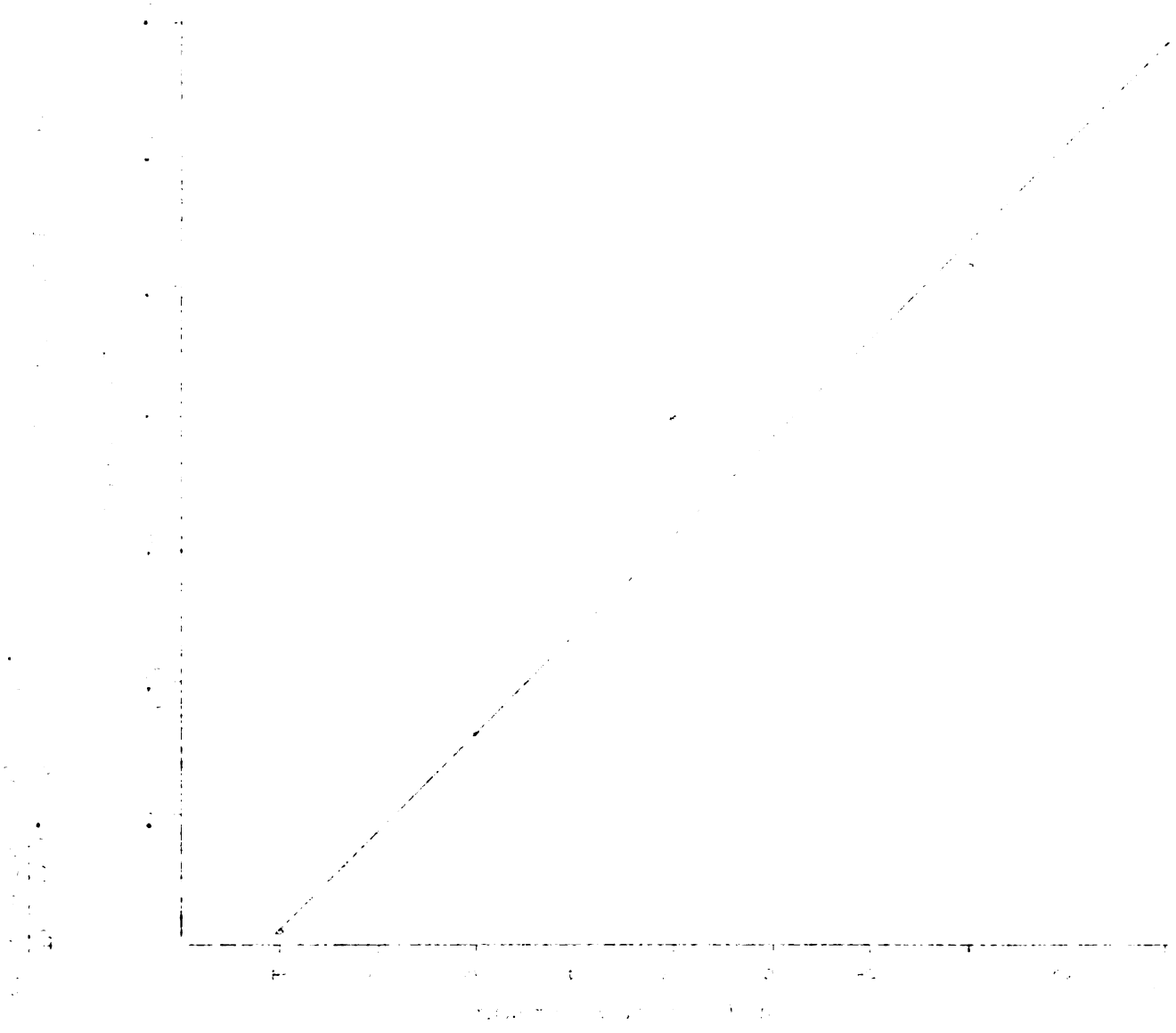


Figure 1. Comparison of total cell count with turbidity measured in $BaSO_4$ units.



RESULTS

Growth rates in the presence of serine and a reverser.

Growth curves of Salmonella gallinarum in the synthetic medium with and without a high concentration of serine and with and without uridine are shown in figure 2. The inoculum was taken from a culture growing in the logarithmic phase. Five ml portions of each of the four cultures used were transferred to test tubes so that turbidity could be estimated in $BaSO_4$ units and compared with viable count; the results are shown in figure 2 and table 1.

Since the dip in the growth curve at 36 hours did not occur in other experiments and since the turbidities of the cultures had increased between 24 and 36 hours, the low cell counts obtained at 36 hours are probably due to errors in sampling.

The control culture remains in the logarithmic phase which persists for about 24 hours; there is no initial lag phase in the serine-inhibited culture, but the growth rate is less than that of the control culture. In other experiments it was found that the addition of serine did not affect the final cell count. The addition of 62.5 ug/ml of uridine completely reversed serine inhibition after 36 hours by enabling the cells to attain approximately the same growth rate as the controls, whereas serine-inhibited cells do not attain the same growth rate as the controls even at 60 hours.

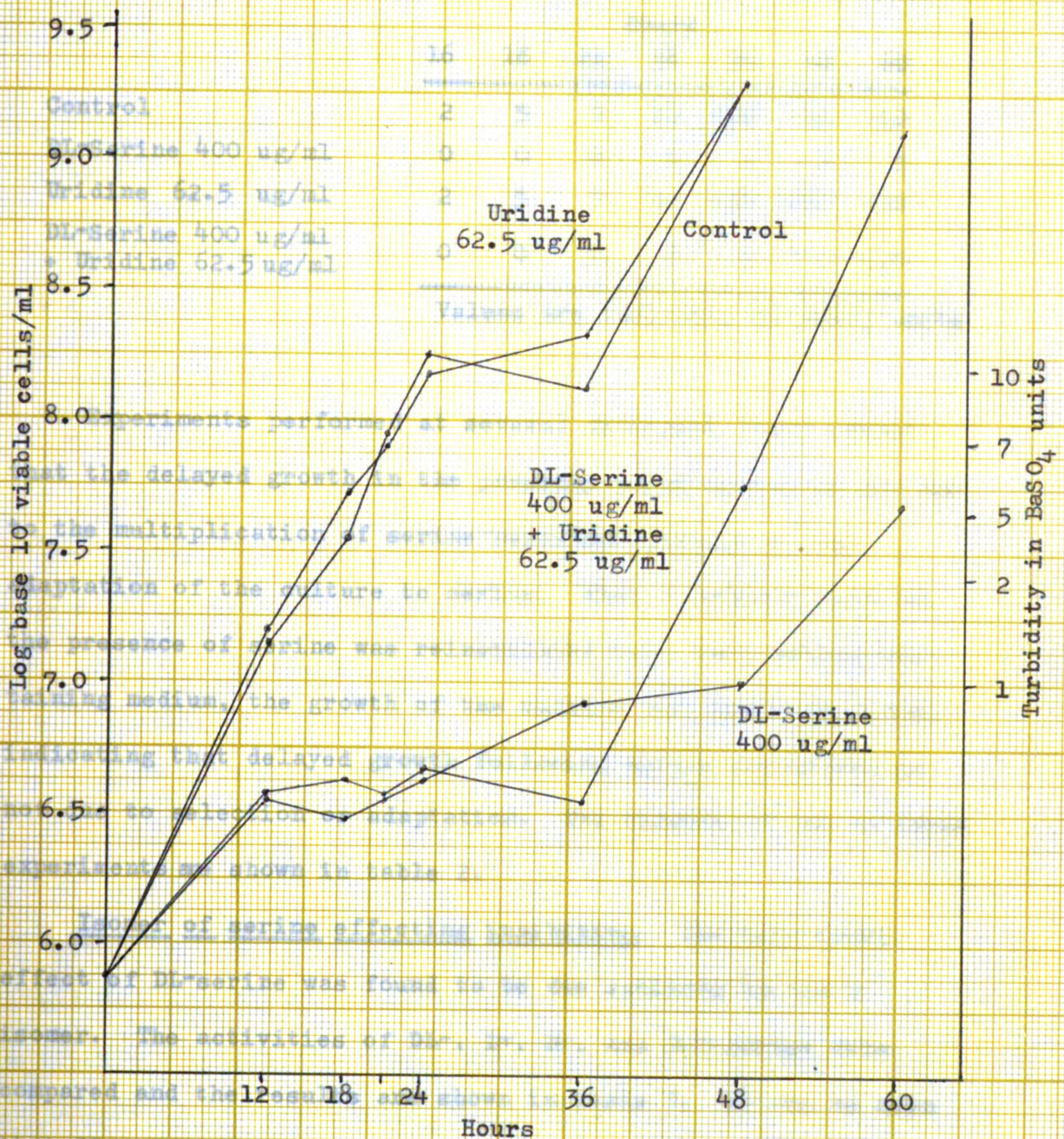


Figure 2. Growth curves of S. gallinarum in a synthetic medium with and without DL-serine and uridine. Viable count compared with turbidity in $BaSO_4$ units.

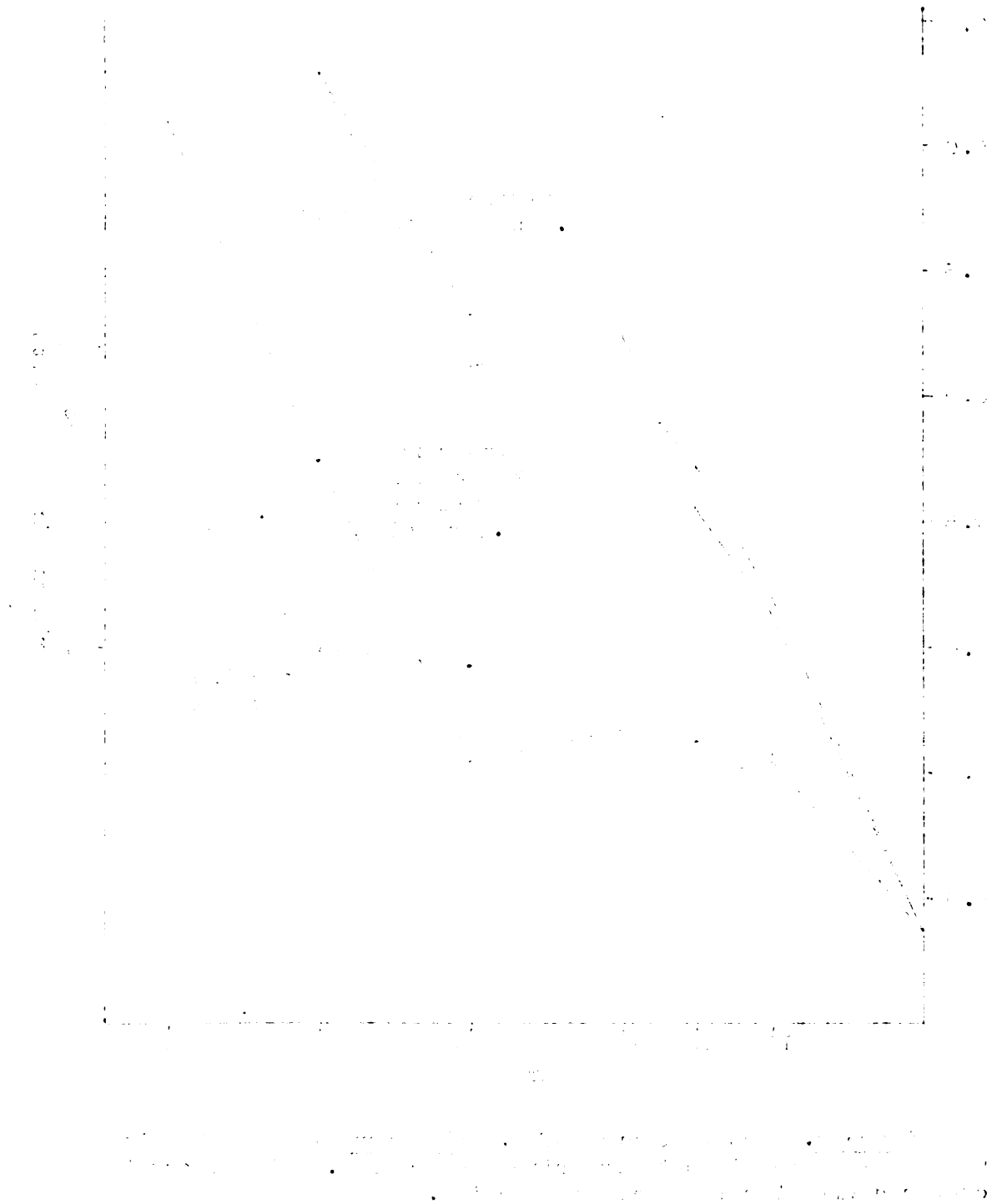


TABLE 1

Effect of DL-serine and uridine on growth of S. gallinarum

	Hours						
	16	18	21	24	36	48	60
Control	2	5	7	10	>10	>10	>10
DL-Serine 400 ug/ml	0	0	0	0	1	1	5
Uridine 62.5 ug/ml	2	5	7	10	>10	>10	>10
DL-Serine 400 ug/ml + Uridine 62.5 ug/ml	0	0	0	0	1	5	>10

Values are turbidity in $BaSO_4$ units

Experiments performed at several different times showed that the delayed growth in the presence of DL-serine is not due to the multiplication of serine-resistant mutants or the adaptation of the culture to serine. When a culture grown in the presence of serine was reinoculated into fresh serine-containing medium, the growth of the culture was again inhibited, indicating that delayed growth following serine inhibition is not due to selection or adaptation. The results of one of these experiments are shown in table 2.

Isomer of serine effecting inhibition. The inhibitory effect of DL-serine was found to be due entirely to the D isomer. The activities of DL-, L-, D-, and D+L-serine were compared and the results are shown in table 3. It can be seen that DL-serine has the same activity as a mixture of equal amounts of D- and L-serine, and that L-serine is essentially

TABLE 2

Effect of DL-serine on growth of S. gallinarum

	Hours			
	18	25	30	42
DL-Serine 100 ug/ml	0	1	3	10
DL-Serine 50 ug/ml	1	7	>10	>10
DL-Serine 25 ug/ml	4	8	>10	>10
Control	7	>10	>10	>10

At 26 hours the culture in the tube containing 50 ug/ml of DL-serine was diluted and re-inoculated into the following tubes:

	Hours			
	24	28	40	48
DL-Serine 800 ug/ml	0	0	0	0
DL-Serine 400 ug/ml	0	0	0	3
DL-Serine 200 ug/ml	0	0	5	>10
DL-Serine 100 ug/ml	0	3	>10	>10
DL-Serine 50 ug/ml	3	6	>10	>10
DL-Serine 25 ug/ml	5	8	>10	>10
DL-Serine 12.5 ug/ml	5	10	>10	>10
Control	5	9	>10	>10

not inhibitory. It can also be seen that, comparing the same concentrations of D- and DL-serine, the D isomer is more than half as active as the racemic mixture, indicating that D-serine is partially reversed by L-serine. In the experiments which follow, DL-serine was used throughout since it is cheaper and reversal by the L component is slight. There are insufficient data available to determine an inhibition index for L-serine.

TABLE 3

Comparison of the effects of DL-, L-, D-, and D+L-serine on growth of S. gallinarum

18 Hours	Total serine concentration in ug/ml											
Isomer	3200	1600	800	400	200	100	50	40	30	20	10	5
DL			0	0	0	0	0	0	1	3	5	7
L	0	0	0	3	5	7	7	8	8	8	8	8
D			0	0	0	0	0	0	0	0	0	1
D+L		0	0	0	0	0		0		3	5	
	Control: 8											

24 Hours	Total serine concentration in ug/ml											
Isomer	3200	1600	800	400	200	100	50	40	30	20	10	5
DL			0	0	0	1	3	5	6	7	9	10
L	0	1	4	7	10	>10	>10	>10	>10	>10	>10	>10
D			0	0	0	0	0	0	0	0	1	4
D+L		0	0	0	0	1		5		7	9	
	Control: >10											

Inhibition by the antibiotics azaserine and D-cycloserine.

Two antibiotics which are derivatives of serine were tested to see if they would affect the growth of S. gallinarum. Although azaserine and D-cycloserine were not tested extensively, several experiments showed that these compounds were inhibitory, while O-carbamyl-D-serine, an unusual amino acid produced by a species of Streptomyces, was not.

Azaserine was tested in the syntnetic medium reported here which contained, in addition, L-asparagine in a final

concentration of 1 mg/ml. Azaserine was highly inhibitory in concentrations from 3 ug/ml to 100 ug/ml, while lower concentrations were less active. The results of this experiment, in which a 48 hour inoculum diluted to approximately 1.5×10^5 cells/ml was used, are shown in table 4.

TABLE 4

Inhibition of S. gallinarum by azaserine

		Hours						
		18	24	28	34	48	60	96
Azaserine ug/ml	100	.008	.078	.005	.012	.002	.012	0
	50	.021	.005	.020	.024	.020	.025	.015
	25	.017	.020	.015	.015	.012	.015	.010
	12.5	.016	.012	.018	.018	.012	.015	.020
	6.25	.028	.017	.030	.030	.023	.025	.025
	3.125	.030	.027	.031	.028	.024	.032	.43
	1.5	.025	.027	.028	.030	.038	.20	.57
	0.75	.030	.025	.058	.22	.53	.22	.48
	0.375	.022	.040	.072	.30	.57	.51	.55
	0	.038	.070	.14	.38	.54	.51	.49

Turbidity in optical density, determined on a Bausch and Lomb Spectronic 20 colorimeter, 525 λ.

Cycloserine and O-carbamyl-D-serine were tested at a single concentration of 250 ug/ml each using a 24 hour old inoculum. O-Carbamyl-D-serine was not inhibitory in the presence of either threonine or asparagine, while cycloserine was inhibitory in the presence of threonine or asparagine or asparagine plus alanine. The results of this experiment are shown in table 5.

TABLE 5

Effect of D-cycloserine and O-carbamyl-D-serine on growth of *S. gallinarum*

	Hours					
	18	23	28	41	69	92
O-Carbamyl-D-serine 250 ug/ml + DL-Threonine 1 mg/ml	1	1	3	>10	>10	>10
O-Carbamyl-D-serine 250 ug/ml + L-asparagine 1 mg/ml	1	3	5	>10	>10	>10
D-Cycloserine 250 ug/ml + DL-Threonine 1 mg/ml	0	0	0	0	0	0
D-Cycloserine 250 ug/ml + L-Asparagine 1 mg/ml	0	0	0	0	0	0
D-Cycloserine 250 ug/ml + L-Asparagine 1 mg/ml + DL-α alanine 1 mg/ml	0	0	0	0	0	0
DL-Threonine 1 mg/ml	0	3	6	10	>10	>10
Control	0	0	2	10	>10	>10

Effect of constituents of the medium on growth and serine inhibition. During the preliminary work on this study of serine inhibition a synthetic medium was used which was identical to the one reported here except that it contained L-asparagine in a final concentration of 1.5 mg/ml. Serine inhibition was irregular and the results of some of the experiments could not be duplicated. In attempting to reverse serine inhibition it was found that when the asparagine content of the medium was doubled asparagine itself was inhibitory and that this inhibition was reversed by 50 ug/ml of DL-serine. Consequently an experiment was set up in which various concentrations of asparagine

were tested for their effect on serine inhibition. It was discovered that asparagine in low concentrations was able to reverse serine inhibition, and this explained the irregular results in asparagine-containing medium. Since asparagine was not required for growth, it was omitted, and more reproducible results were obtained. Schoenhard (personal communication) had previously found that aspartic acid could also reverse serine inhibition.

In order to determine what effect the other constituents in the medium had on growth some of these were eliminated singly and some in combination from the synthetic medium. Serial transfers of 2 drops from each tube were carried over at 24 hour intervals into the various deficient media. To obtain continuous growth in the synthetic medium cystine, leucine, and glucose were found to be essential, while there was some need for arginine and inorganic nitrogen. Thiamine and pantothenic acid were nonessential under these conditions of a large inoculum.

When the constituents were then added back to the various deficient media in graded concentrations with and without serine to determine the effect of concentration of these ingredients on serine inhibition, a small inoculum was again used. It was found that the concentrations of cystine, leucine, glucose, arginine, and inorganic nitrogen were not critical for growth, nor did these compounds have any tendency to

reverse serine inhibition. A four-fold increase in amount of glucose, amino acids, or inorganic nitrogen over the concentrations normally present in the complete synthetic medium did not relieve serine inhibition. When the amount of glucose was reduced, growth was also reduced but serine inhibition was unaffected. Reduced amounts of amino acids also gave less growth, and serine was more inhibitory under these conditions. In these experiments histidine could replace arginine with nearly identical results on growth and inhibition. Reduced amounts of inorganic nitrogen did not affect growth or serine inhibition, while increased amounts over that normally present in the complete medium were somewhat inhibitory and potentiated serine inhibition.

When graded concentrations of $KHCO_3$ were added to a bicarbonate-deficient medium with and without serine it was found that the growth attained in 21 hours was independent of the bicarbonate concentration up to 2 mg/ml. At this time growth was reduced slightly in the presence of 4 mg/ml of $KHCO_3$ and markedly by an 8 mg/ml concentration. At 35 hours concentrations from 100 ug/ml to 4 mg/ml gave better growth than no bicarbonate at all, while the 4 mg/ml and 8 mg/ml concentrations potentiated the inhibition caused by 50 ug/ml and 100 ug/ml of DL-serine. Concentrations of bicarbonate from 100 ug/ml to 2 mg/ml had some ability to reverse serine inhibition but there are insufficient data to determine an inhibition index.

In their studies on the nutrition and virulence of S. pullorum in a synthetic medium, Gilfillan, Holtman and Ross (1955) reported that DL-serine was not inhibitory, but their medium also contained glycine and DL-alanine. DL-alanine and glycine were also tested singly by the writer and found not to reverse serine inhibition; the raw data for these experiments are included in tables 23 and 24 of the appendix. The combination of alanine and glycine was not tested here for its effect on growth and serine inhibition.

It should be noted that the medium of Gilfillan, Holtman and Ross (1955) also contained xanthine in a concentration of 5 ug/ml. Since in the work reported here xanthine has been found to be inactive at 12 ug/ml in reversing a serine concentration of 50 ug/ml, it is improbable that the presence of xanthine in their medium would be responsible for the lack of serine inhibition, but their strain would have to be tested before any definite statement to this effect could be made.

Reversal of inhibition by nucleic acid derivatives. Certain nucleosides and ribonucleic acid had considerable ability to relieve serine inhibition. Since no clear-cut line could be drawn between moderately and weakly active reversing compounds, the inhibition index has been used to indicate the relative activities of these compounds, and they are listed in table 6.

TABLE 6

Inhibition indices for several concentrations of nucleic acid derivatives; based on control turbidities of 5 $BaSO_4$ units, between 17 and 24 hours

Molecular Weight	Compound	Concentration of compound in ug/ml					
		10	12.5	25	50	100	200
244	Uridine	14		13	11	>6	
267	Adenosine	18		9	4	3	
283	Cytidine hemisulfate	16		7	5	3	
323	Cytidylic acid (2' and 3')	14		12	6	3	
324	Uridylic acid (2' and 3')	14		8	4	2	
347	Adenylic acid (2' and 3')	12		6	4	4	
263	Deoxycytidine hydrochloride	10		5	3	2	
242	Thymidine	6		6	3	2	
433	Calcium thymidylate·$4H_2O$			6	6	3	
319	Guanosine·$2H_2O$	8		5	2	1	
455	Trisodium guanylate·$4H_2O$ (2' & 3')	8		3	2	1	
251	Deoxyadenosine	6		3	2	1	
307	Deoxycytidylic acid	5		3	2	1	
331	Deoxyadenylic acid (5')	5		2	2	1	
152	Xanthine		1	inh	inh		
267	Deoxyguanosine			2	1	0	
112	Uracil			0	0.1	0	
150 + 215	Ribose + Adenine sulfate				0.1	0.6	0.6
347	Deoxyguanylic acid (5')			0	0	0	
215	Adenine sulfate			inh	inh	inh	
	Ribonucleic acid	+		+	+	+	
	Deoxyribonucleic acid	+			+	inh	

Molecular weight of serine, 105

inh = inhibitory

+ = reversal; index not calculated

Uridine has a more constant index than any of the other compounds tested, around 13, while adenosine and cytidine are the next most active compounds followed by the other nucleic acid derivatives which have indices of 14 or less. Figure 3 shows typical inhibition curves plotted from data obtained with uridine. Although the inhibition indices of uridine indicate that it may possibly reverse serine competitively, the highest concentration tested was 100 ug/ml so no definite conclusions can be drawn. The method used for calculating inhibition indices is shown in table 7.

Although inhibition indices were not determined for RNA and DNA because of unknown molecular weights, RNA was less active than cytidine and more active than cytidylic acid when these compounds are compared on a weight basis. In contrast to the activities of the deoxyribose derivatives and RNA, DNA was actually inhibitory alone but somewhat stimulatory in the presence of serine, depending on the concentrations used.

Since one of the nucleic acid derivatives, adenine sulfate, was completely without ability to relieve serine inhibition, and in fact was somewhat inhibitory while adenosine was very active, ribose and adenine together were tested against serine. This combination was only weakly active in relieving inhibition, while ribose alone was inactive. Deoxyguanylic acid was the only DNA derivative tested which did not show some serine reversal within 24 hours.

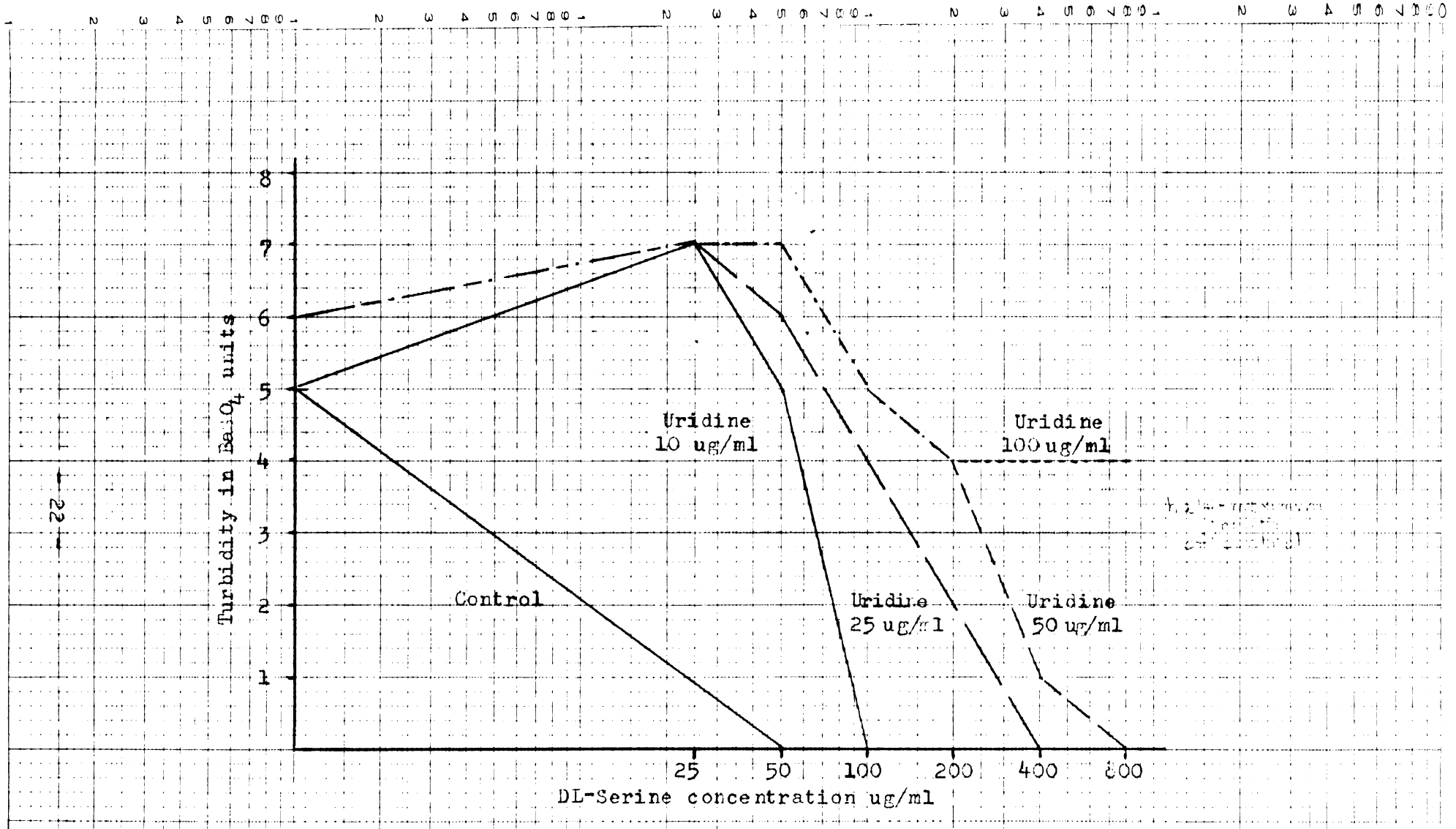


Figure 3. Inhibition curves showing the growth response of S. gallinarum to varying concentrations of DL- serine and uridine at 17 hours.

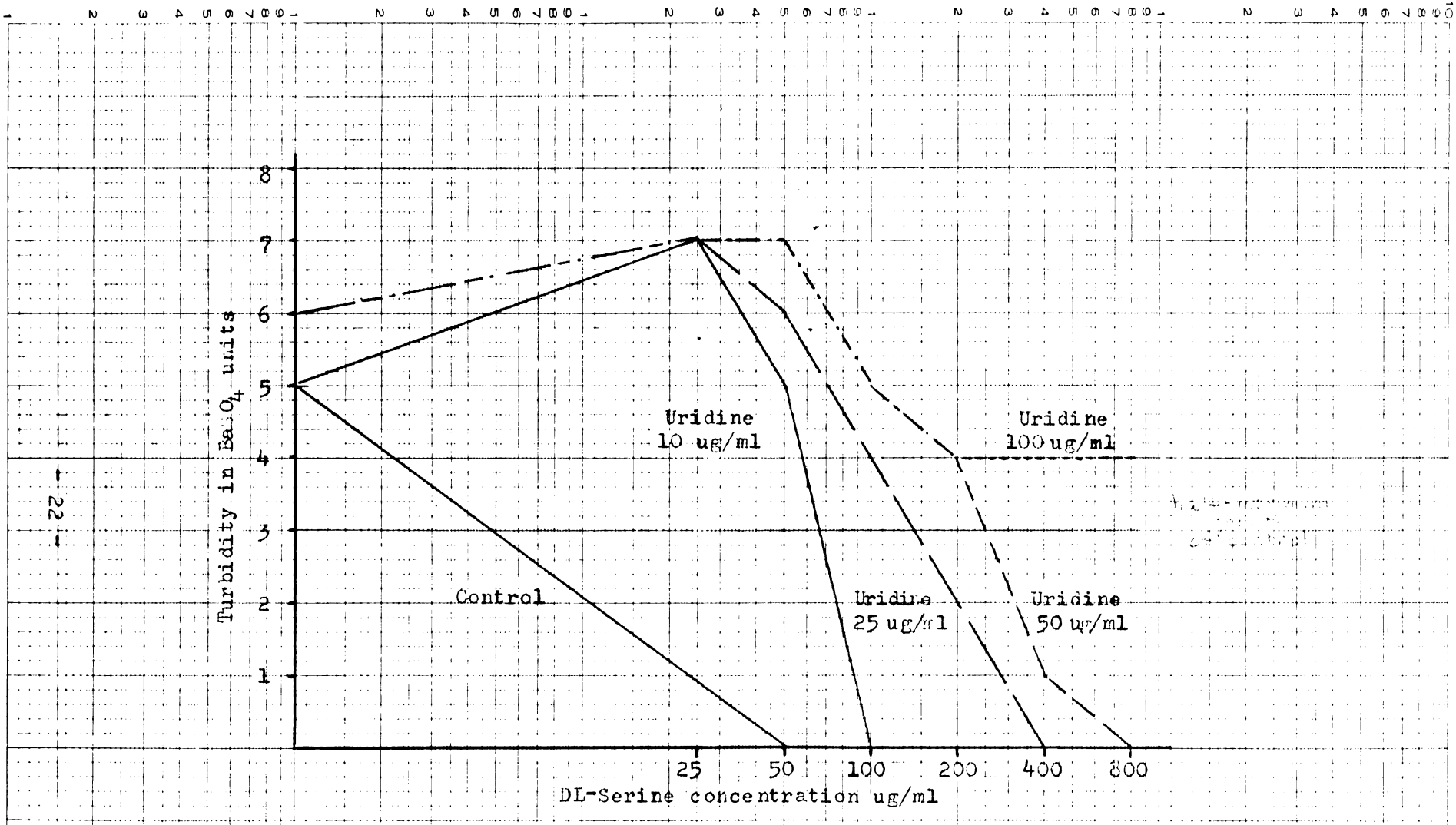


Figure 3. Inhibition curves showing the growth response of S. gallinarum to varying concentrations of DL - serine and uridine at 17 hours.

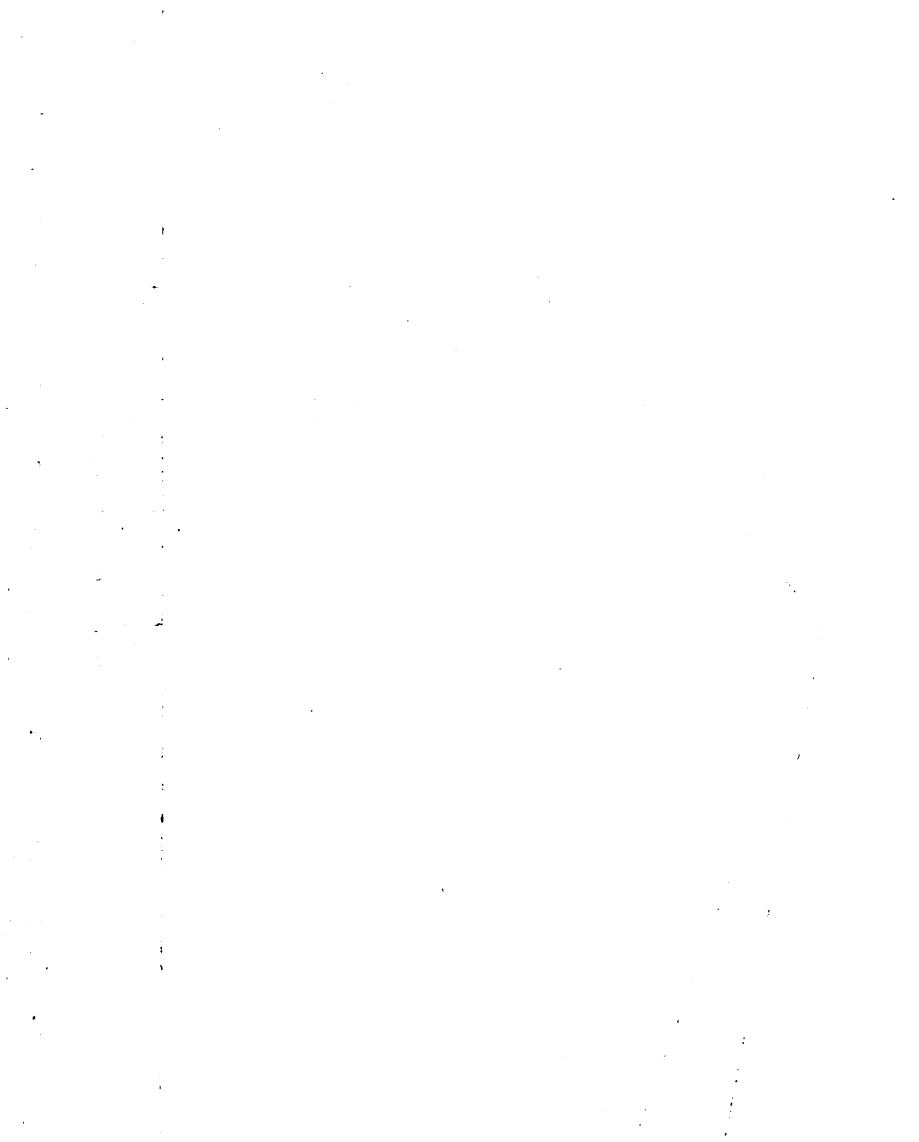


TABLE 7

Method of calculating inhibition indices; based on curves shown in figure 3

Uridine concentration		DL-Serine concentration at ½ maximum growth		Calculations
ug/ml	mM/ml	ug/ml	mM/ml	
0	0	5	0.048	
10	0.041	66	0.63	$\frac{0.63 - 0.48}{0.41} = 14$
25	0.102	140	1.34	$\frac{1.34 - 0.48}{0.102} = 13$
50	0.205	250	2.38	$\frac{2.38 - 0.48}{0.205} = 11$
100	0.410	>250	>2.38	$\frac{>2.38 - 0.48}{0.41} = >6$

The raw data showing the activities of RNA, DNA, and the nucleic acid derivatives tested are shown in tables 25 through 47 in the appendix.

Effect of amino acids, vitamins, and other organic compounds on serine inhibition. Several amino acids, amino acid amides, and urea were found to relieve serine inhibition to varying degrees. The most active of these compounds is L-glutamic acid with an inhibition index of 2, followed by L-aspartic acid, DL-isoleucine, L-glutamine, L-asparagine, L-histidine, and L-proline. The inhibition indices of DL-methionine and urea were also determined but, since these were based on control turbidities greater than 5, these values cannot be compared directly with the others. The inhibition indices are given in tables 8 and 9, and the raw data are included in tables 48 through 56 in the appendix.

TABLE 8

Inhibition indices for several concentrations of amino acids and amino acid amides; based on control turbidities of 5 $BaSO_4$ units, between 18 and 24 hours

Molecular Weight	Compound	Concentration of compound, ug/ml 10	120	125	250	500	1000	2000
147	L-Glutamic acid	2.0	2.0					
133	L-Aspartic acid	1.0	0.1					
131	DL-Isoleucine					0.5	0.4	>0.2
146	L-Glutamine	0.3	0.2					
132	L-Asparagine	0	0.03					
210	L-Histidine					0.06	0.03	0.004
115	L-Proline				0.03	0.02	0.02	0.01

TABLE 9

Inhibition indices for several concentrations of methionine and urea; based on control turbidities of 9 at 24 hours and 7 at 20 hours, respectively

Molecular Weight	Compound	Concentration of compound, ug/ml 10	120	125	250	500	1000	2000
149	DL-Methionine			0.1	0.03	0.02	0.008	0.004
60	Urea			0	inh	0.02	0.05	0.04

inh = inhibitory

The ability of urea to reverse serine inhibition in this strain, although slight, is especially interesting in view of the findings of Ross, Holtman, and Gilfillan (1956) that urea is inhibitory to S. pullorum at a concentration of 60 mg/ml, while a concentration of 15 mg/ml reduced growth somewhat. The writer found that while urea was very slightly inhibitory alone at much lower concentrations, from 250 ug/ml to 2 mg/ml, these amounts of urea were capable of reversing serine inhibition to varying degrees depending on the concentration used.

In an attempt to reverse serine inhibition by adding graded amounts of L-asparagine it was found that high concentrations of this amino acid amide were toxic, and that serine reversed this inhibitory effect of asparagine. Table 10 shows that in the presence of 5 mg/ml of L-asparagine no growth occurs at a concentration of DL-serine less than 2.5 mg/ml by 29 hours.

TABLE 10

Inhibition of growth of S. gallinarum by L-asparagine and its reversal by DL-serine

19 Hours	DL-Serine concentration mg/ml									
	10	5	2.5	1.25	0.625	0.312	0.156	0.078	0.039	0
L-Asparagine 5 mg/ml	7	5	0	0	0	0	0	0	0	0
Control: 7										

29 Hours	DL-Serine concentration mg/ml									
	10	5	2.5	1.25	0.625	0.312	0.156	0.078	0.039	0
L-Asparagine 5 mg/ml	>10	>10	3	0	0	0	0	0	0	0
Control: >10										

As in the case of serine inhibition, asparagine inhibition is temporary and the cultures eventually develop a high turbidity, by approximately 65 hours in this experiment. Other compounds were not tested for this effect.

Interesting results were obtained with L-cysteine* and glutathione*. Gilfillan, Holtman, and Ross (1955) reported that a concentration of 60 ug/ml of cysteine was inhibitory to their strain of S. pullorum. As is shown in table 11, cysteine is inhibitory to S. gallinarum in the absence of serine, depending on the concentration used. Cysteine potentiates the inhibition caused by DL-serine for 30 hours or more, but by 42 hours after inoculation cysteine reverses the serine inhibition. A similar set of results of potentiation of inhibition followed by reversal was obtained with glutathione (L-γglutamyl-L-cysteyl-glycine). Since glycine alone neither stimulated growth nor reversed serine inhibition, while L-glutamic acid reversed serine but did not reduce growth, the activity of glutathione is probably due to the cysteine component. The complete data for cysteine and glutathione are included in tables 57 and 58 of the appendix.

A number of amino acids, vitamins, and other compounds were found to be unable to reverse within 48 hours the inhibition caused by a DL-serine concentration of 120 ug/ml. These

*autoclaved only 5 minutes

TABLE 11

Effect of L-cysteine and DL-serine on growth of S. gallinarum

18 Hours		DL-Serine ug/ml							
		800	400	200	100	50	25	12.5	0
L-Cysteine ug/ml	2000	0	0	0	0	0	0	0	0
	1000	0	0	0	0	0	0	2	4
	500	0	0	0	0	0	0	4	4
	250	0	0	0	0	0	0	0	6
	125	0	0	0	0	0	2	5	7
	0	0	0	0	0	1	4	6	7

25 Hours		DL-Serine ug/ml							
		800	400	200	100	50	25	12.5	0
L-Cysteine ug/ml	2000	0	0	0	0	0	0	0	1
	1000	0	0	0	0	2	5	8	8
	500	0	0	0	0	0	1	10	10
	250	0	0	0	0	1	6	6	>10
	125	0	0	0	0	5	7	10	>10
	0	0	0	0	1	7	8	10	>10

30 Hours		DL-Serine ug/ml							
		800	400	200	100	50	25	12.5	0
L-Cysteine ug/ml	2000	0	0	0	0	0	0	5	5
	1000	0	0	0	0	9	>10	>10	>10
	500	0	0	0	0	3	5	>10	>10
	250	0	0	0	0	7	>10	>10	>10
	125	0	0	0	0	9	>10	>10	>10
	0	0	0	0	3	>10	>10	>10	>10

42 Hours		DL-Serine ug/ml							
		800	400	200	100	50	25	12.5	0
L-Cysteine ug/ml	2000	10	5	5	1	1	0	10	10
	1000	>10	>10	>10	9	>10	>10	>10	>10
	500	0	0	1	9	>10	>10	>10	>10
	250	0	0	0	7	>10	>10	>10	>10
	125	0	0	0	7	>10	>10	>10	>10
	0	0	0	0	10	>10	>10	>10	>10

compounds in the highest concentrations tested are listed in table 12. A combination of the eight vitamins in a total concentration of 1 ug/ml was also ineffective in relieving inhibition.

TABLE 12

Compounds which did not reverse a DL-serine concentration of 120 ug/ml within 48 hours

Compounds in which the highest concentration tested was 1 mg/ml	
DL- Threonine	L-Tyrosine
DL-Homoserine	Hydroxyproline
DL-Ornithine	Glycine*
DL-Valine	Sodium acetate
DL-αAlanine	Thiourea
DL-βAlanine	Allylthiourea*
DL-Lysine	Succinic acid
DL-Tryptophane	Sodium formate
L-Tryptophane	Methionine sulfoxide
DL-Norleucine	

Compounds in which the highest concentration tested was 1 ug/ml	
Riboflavin	Thiamine hydrochloride
Folic acid	ρAminobenzoic acid
Inositol	Pyridoxine hydrochloride
Nicotinic acid	

Compound in which the highest concentration tested was 1 ug/l
Biotin

* inhibitory

DISCUSSION

The concept of inhibition and limitations of inhibition studies. An inhibitor is a chemical which decreases the rate of a reaction. Inhibitors may react preferentially with particular groupings on enzyme or protein molecules, although enzymes or proteins are not necessarily involved. Inhibition may result from the affinity of a compound structurally similar to the substrate for an enzyme or prosthetic group, or the inhibitor may have no structural similarity to the substrate. Inhibition may be due also to the removal of a required substrate, as in the case of a chelating agent removing ions necessary for a particular reaction.

The term inhibition has been applied in other literature to an increase in the duration of the lag phase, a reduction in amount of total growth, and also to a decrease in growth rate. These types of inhibitory actions are clearly distinguishable and are discussed more fully by Moore and Boylen (1952).

Reversal of inhibition may be accomplished in several ways and can sometimes be used to determine the nature and specificity of the inhibition. This can be done by removing the inhibitor or adding a substance which will combine preferentially with the inhibitor, but since these methods may

present difficulties when the growth of cells is being studied, the following methods are more useful in demonstrating specificity: adding increased amounts of the inhibited metabolite or its precursor, supplying the product of the inhibited reaction or an effective substitute for the product, or supplying precursors of the blocked enzyme or prosthetic group.

The action of inhibitors can be studied best with the use of isolated enzymes; nevertheless, actively growing cells can be used although the results must be interpreted with caution. In living organisms the penetration of the inhibitor and reverser must be considered, as well as the ability of the cells to alter these compounds. If the inhibitor is able to affect several different processes, a single reverser may not be effective in relieving each of these inhibitions to the same degree. Therefore inhibition of growth may be the result of a number of complex interactions and must be considered accordingly.

The inhibition index proposed by McIlwain (1942) to evaluate the effectiveness of a reversing agent and used extensively by Shive (1950) to trace metabolic pathways is the ratio of the inhibitor concentration to the reverser concentration, both on a weight basis, at a point of marked inhibition or half-maximum growth. The inhibition index the writer used is based on the difference in inhibitor concentration between the control value and that obtained in the presence of a

reverser, both at a point of half-maximum growth, and is the ratio of this value to the concentration of reverser; it is not based on merely a total amount of inhibitor. This corrects for the amount of metabolite which may be synthesized within the cell (Woolley, 1952) and also corrects for the variations obtained in the control tubes from one experiment to the next. It is also based on a concentration expressed in moles rather than in grams. In other words, the inhibition index used here indicates the number of molecules of serine that are reversed, which is more meaningful for intact cells than a simple inhibitor/reverser ratio based on weight alone.

An inhibition may be reversed competitively or non-competitively. If competitive, the inhibition index will remain constant over a large range of concentrations of inhibitor and reverser, but if non-competitive, the index is not constant but will change as the concentrations of inhibitor and reverser are changed. Perhaps the most widely known worker in the field of inhibition analysis is Shive (1950) who has been able to trace several steps in a series of metabolic transformations by studying competitive inhibitions.

The conclusion that a substance which overcomes an inhibition may be directly or indirectly involved in a blocked reaction is not necessarily correct in each case, as has been pointed out by Davis (1956). He showed that while valine and isoleucine could relieve the inhibition of growth of E. coli

caused by homoserine, these amino acids acted not by enabling an inhibited reaction sequence to proceed but by preventing the penetration of homoserine.

It has also been pointed out by Cohen and Monod (1957) that in the competitive reversal of norleucine inhibition by methionine in the growth of E. coli, norleucine does not inhibit the utilization of methionine, nor does norleucine inhibit a possible function of methionine in the biosynthesis of valine, leucine or isoleucine as had been postulated. Instead, norleucine has been found to substitute for methionine, resulting in the synthesis of "false proteins," but norleucine does not impair the synthesis of methionine.

Inhibitory action of D-serine. The D isomer of DL-serine inhibits S. gallinarum by reducing the growth rate, and indirect evidence indicates that the degree of reduction is dependent on the concentration of DL-serine used. Serine does not reduce the amount of total growth.

Although only broad generalizations can be made from the limited growth curve data, it appears that the serine-inhibited culture, shown in figure 2, page 11, grows at a fairly constant rate. The apparent lag following an initial growth of about 2 generations may reflect the actual growth pattern of the serine-inhibited cultures. On the other hand, errors in sampling may have resulted in high cell counts obtained at 12 hours, in which case the serine-inhibited

cultures would continue to grow logarithmically from the time of inoculation. The latter interpretation is the simpler one. If further experiments showed that a lag period does exist after approximately 12 hours, it may result from the cells having used up some product of a serine-inhibited reaction, after which inhibition by the D isomer would be manifested by a period of negligible growth.

The delay of reversal activity in the presence of uridine, also shown in figure 2, suggests that uridine may be converted to some other compound before complete reversal occurs. Since growth curves were not determined for other compounds, no conclusions can be drawn concerning the direct effects on growth of compounds other than uridine.

Because none of the constituents of the synthetic medium in the concentrations normally present have any ability to relieve serine inhibition, serine does not appear to inhibit growth by preventing the incorporation of an essential nutrient. Although it is not known if D-serine is actually taken up by the cells, indirect evidence indicates that any interference it has with metabolism occurs within the boundaries of the cell. It has been shown that the inhibitory effect of DL-serine is due to the D isomer. But because the organism does not require an exogenous supply of L-serine, D-serine would not inhibit by interfering with the uptake of L-serine but could possibly interfere with the endogenous utilization of its

isomer or some other compound synthesized within the cell. D-serine does not interfere with the utilization of L-serine in Pasteurella pestis (Levine, 1954). D-serine could be substituting for its L isomer in a reaction, for example where serine acts as a one-carbon donor through folic acid.

Composition of the synthetic medium. In the study of the organic compounds, inorganic nitrogen, and bicarbonate requirements of S. gallinarum and their effects on serine inhibition it was found that concentrations of the individual compounds in the medium could be varied over a wide range, and that some of the concentrations of compounds in the original medium were not optimum. Since it was intended merely to show that the medium itself was not masking serine inhibition, the medium was not modified for further work. In order to reduce the time required for maximum growth, and possibly to obtain a greater amount of growth, it is suggested that in further studies the amount of cystine be increased two- to four-fold and the amounts of inorganic nitrogen compounds be halved. The medium could be simplified by the omission of thiamine and calcium pantothenate if a large inoculum is used.

Relationship of serine inhibition to amino acid metabolism. Since D-serine is an amino acid it might be interfering with the metabolism of other amino acids. Serine has been found to be present in the cell walls of S. pullorum along with 13 other amino acids including glutamic acid, aspartic acid, proline,

arginine, and methionine; asparagine and glutamine were not present (Salton, 1952). Several of these compounds are interconvertible by reactions involving transamination and amidation.

In the urea cycle citrulline and aspartic acid combine to form arginosuccinic acid from which arginine is derived; the hydrolysis of arginine yields ornithine and urea. Aspartic acid is also a precursor of carbamylaspartic acid from which pyrimidines are synthesized, and it is involved in purine synthesis along with the bicarbonate ion. Since arginine is required for the growth of S. gallinarum but was not demonstrated to reverse serine inhibition, nor was ornithine, the function of urea in relieving serine inhibition does not seem to involve this cycle if it is operative in this organism.

Ferguson and Hook (1943) tested 75 strains of Salmonella including 1 strain of Salmonella gallinarum and found none of them to have urease activity, although they suggest that it would be not at all surprising to find a variant in view of the fact that some Salmonella organisms digress from the typical metabolic reactions. It is possible that strain 6 of S. gallinarum used here possesses a small amount of urease activity, undetectable by the usual method, which can hydrolyze urea to ammonia and CO_2, both of these compounds then being available for other reactions. The ammonia may be incorporated into glutamic acid or aspartic acid or, a more interesting speculation, it might recombine with CO_2 in the presence of

ATP to form carbamyl phosphate required in pyrimidine synthesis. Carbamyl phosphate may also be formed from free amino groups derived from glutamine, asparagine, and amino acids. Glutamic acid also functions as a constituent of the folic acid co-enzymes which are required in purine synthesis.

The methyl carbon of methionine is probably derived from the β hydroxymethyl group of serine in E. coli (Gibson, 1952). The role of methionine in nucleic acid metabolism is not clear, but it is known to function as a methyl donor to folic acid enzymes and is able to relieve inhibitions of folic acid and RNA synthesis in E. coli and other microorganisms. Some interesting interrelations of methionine, serine, and CO_2 are found in E. coli and Streptococcus bovis. Gibson (1952) reported that methionine synthesis in E. coli was inhibited by a 20% concentration of CO_2 and was reversed by DL-serine, while Prescott, Ragland, and Stutts (1957) found that DL-serine inhibition in S. bovis was reversed by a 0.0002% concentration of CO_2 supplied as $NaHCO_3$.

In some experiments it was found that low concentrations of DL-serine, from 5 to 25 ug/ml, occasionally stimulated growth. This type of a stimulatory effect has been reported to occur with low concentrations of many inhibitors. Some bacteria are able to utilize D-amino acids, many of which occur naturally in bacterial cells, and it has been postulated by Work (1957) that these D-amino acids may function in the

osmotic barrier of the cell. S. gallinarum may possess a limited ability to racemize or oxidatively deaminate D-serine to its L isomer, although no reports to this effect have been found.

Inhibition by antibiotics containing serine. In connection with serine inhibition it is interesting to note that two serine-containing antibiotics produced by Streptomyces sp. inhibit the growth of S. gallinarum. Azaserine, an L-serine containing analog of L-glutamine, was highly active, while D-cycloserine was also inhibitory. O-Carbamyl-L-serine is also an analog of L-glutamine but was not available for testing; however, its D isomer, O-carbamyl-D-serine which is also produced by Streptomyces was found not to be inhibitory at a concentration of 250 ug/ml. Azaserine and O-carbamyl-L-serine have been shown in other bacteria to interfere with a step in purine synthesis where glutamine acts as an amino donor.

Reversal of inhibition by nucleic acid derivatives. The fact that the nucleic acid derivatives were more active in relieving serine inhibition than any other compounds tested would seem to indicate that serine interferes, directly or indirectly, with nucleic acid metabolism. In each case the nucleoside was more active than the corresponding nucleotide or free base in relieving inhibition, and the adenine, guanine, and cytosine ribosides were more active than the corresponding deoxyribosides.

It is not known whether or not these compounds must enter the cell to be active in relieving serine inhibition, but Cohen and Barner (1956) found that free pyrimidines and pyrimidine ribosides could be incorporated by E. coli mutants requiring thymine. The fact that adenine sulfate is not only inhibitory to S. gallinarum but potentiates serine inhibition indicates that this compound is either able to penetrate into the cells or it interferes with an extracellular reaction, possibly the incorporation of some other substance into the cells. Since Harkins and Freiser (1956) have shown that the acidic NH group of adenine (position 7 on the imidazole ring) forms metal complexes with the divalent ions copper, nickel, and cobalt, it may be that adenine inhibits by forming chelates with essential metals. They reported that adenosine does not behave in quite the same manner as adenine, but it and ribose can also react with copper. No attempts were made to reverse the inhibitory effect of adenine by increasing the concentration of metals in the medium. Inhibition did not occur with the other two free bases tested, uracil and xanthine.

Since it is not known whether the nucleic acid derivatives which reverse serine actually penetrate the cell as intact molecules or are converted to some other active products inside or outside the cell, no conclusions can be drawn concerning the mechanism by which these compounds reverse the inhibition.

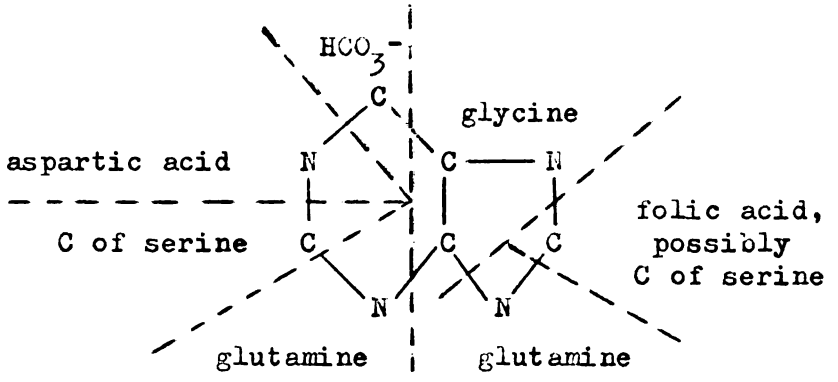
Because both precursors and products of a blocked reaction may relieve an inhibition non-competitively, and the compounds considered here were non-competitive, these compounds may be either precursors or products of a possible blocked reaction or may be involved in more than one reaction. It is also possible that they are not related to a blocked reaction.

The inhibition indices are higher for most of the nucleic acid derivatives than for the amino acids or amino acid amides tested, many of which are known to function in nucleic acid synthesis. From this one could conclude that the nucleic acid derivatives are either more closely associated with the blocked reaction(s) than the amino acids or their amides, or that they penetrate the cells more easily. Figure 4 illustrates the origins of carbon and nitrogen in the structures of the purine and pyrimidine rings and points out the importance of the amino acids discussed here in relation to nucleic acid synthesis.

Because the inhibition indices were obtained using growing cells and the compounds did not relieve serine inhibition competitively, further conclusions regarding at what step(s) serine inhibits are not warranted, and one can only speculate beyond this point.

This discussion indicates that there are a number of complex interactions which may involve serine and other compounds. However, in a study of this sort where the growth of cells is the sole criterion to evaluate the effect of a number

Purine



Pyrimidine

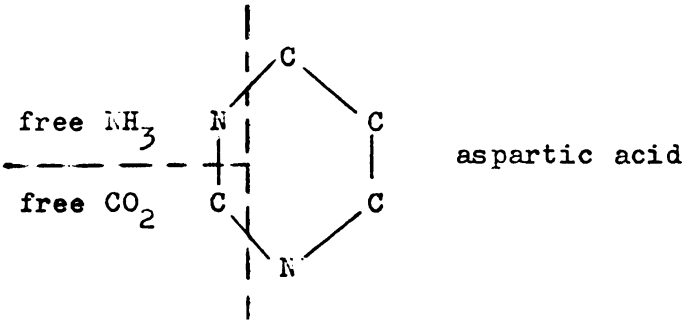


Figure 4. Origin of carbon and nitrogen in purine and pyrimidine rings.

of different kinds of compounds, the results are entirely dependent on the ability of the organisms to utilize in some manner the compounds supplied. The inhibition index as a tool in evaluating the activities of amino acids and nucleic acid derivatives is limited, and the values are only relative and subject to change depending on the conditions used. They show only the growth response to a particular compound in the presence of serine, and since this response is dependent on permeability of the compounds before or after any possible conversion that may occur, the growth response may reflect only the degree of permeability rather than the "true" activity of a particular compound. For example, assuming equal penetrability, uridylic acid might be incorporated into R.A much more easily than uridine, but since in this study these compounds were supplied exogenously, the organisms seem to be able to take up uridine more readily than the phosphorylated compound, and hence the riboside appears to have the greater activity.

A number of possible areas for the interference of serine with metabolic processes have been suggested, but cell-free extracts will probably have to be used in order to confirm or deny these hypotheses. It is hoped that these experiments will stimulate further research on this problem.

SUMMARY

DL-Serine inhibits the growth of Salmonella gallinarum in a synthetic medium by reducing the growth rate. The inhibition, due to the D isomer, is temporary and is reversed by nucleic acid derivatives, particularly uridine, adenosine and cytidine, and amino acids, particularly glutamic acid and aspartic acid. DL-Serine inhibition is also reversed by L-serine, $KHCO_3$ and RNA, while adenine sulfate is inhibitory; DNA is inhibitory alone but is capable of reversing low concentrations of serine. It is suggested that D-serine interferes with the metabolism of several amino acids, particularly where they are required for nucleic acid synthesis.

This organism is also inhibited by azaserine and D-cycloserine but not by O-carbamyl-D-serine.

Some miscellaneous observations on the nutrition of S. gallinarum and the effect of varying the concentrations of the constituents of the synthetic medium have been noted.

BIBLIOGRAPHY

Beard, P. J. and Snow, J. E. 1936 Antigenic characteristics of related organisms after cultivation on synthetic medium. J. Infectious Diseases , 59, 40-42.

Breed, R. S., Murray, E. G. D., and Smith, N. R. 1957 Bergey's manual of determinative bacteriology, 7th ed. Williams and Wilkins Co., Baltimore.

Castellani, A. G. 1953 Inhibiting effects of amino acids and related compounds upon the growth of enterotoxigenic micrococci in cream pastry. Applied Microbiology, 1, 195-199.

Cohen, G. N. and Monod, J. 1957 Bacterial permeases. Bacteriol. Revs., 21, 169-194.

Cohen, S. S. and Barner, H. D. 1956 Studies on the induction of thymine deficiency and on the effects of thymine and thymidine analogues in Escherichia coli. J. Bacteriol., 71, 588-597.

Dagley, S., Dawes, E. A., and Morrison, G. H. 1950 Inhibition of growth of Aerobacter aerogenes: the mode of action of phenols, alcohols, acetone, and ethyl acetate. J. Bacteriol., 60, 369-379.

Davis, B. D. 1956 Relations between enzymes and permeability (membrane transport) in bacteria. in Enzymes: units of biological structure and function, pp. 509-522. Edited by O. H. Gabler. Academic Press, N. Y.

Davis, F. and Solowey, M. 1950 The utilization of some organic compounds by one strain each of Salmonella anatum, Salmonella oranienburg, and Salmonella pullorum. J. Bacteriol, 59, 361-366.

Dubos, R. J. 1949 Toxic effects of DL-serine on virulent human tubercle bacilli. Am. Rev. Tuberc., 60, 385.

Ferguson, W. W. and Hook, A. E. 1943 Urease activity of Proteus and Salmonella organisms. J. Lab. Clin. Med., 28, 1715-1720.

Friedman, M. E., Wood, W. A., and Nelson, W. O. 1953 The influence of various metabolites upon the p-aminobenzoate-pantothenate interaction in Bacterium linens. J. Bacteriol., 66, 568-571.

Gibson, F. 1952 Methionine synthesis by Escherichia coli. Congr. intern. biochim., Résumés communs., IIe Cong., Paris, 80-81.

Gilfillan, R. F., Holtman, D. F., and Ross, R. T. 1955 A synthetic medium for propagation and maintenance of virulent strains of Salmonella pullorum. Poultry Sci., 34, 1283-1287.

Gladstone, G. P. 1939 Inter-relationships between amino acids in the nutrition of E. anthracis. Brit. J. Exptl. Pathol., 20, 189-200.

Hajna, A. A. 1935 Decomposition of salts of organic acids by bacteria of the genus Salmonella. J. Bacteriol., 29, 253-258.

Harkins, T. R. and Freiser, H. 1958 Adenine-metal complexes. J. Am. Chem. Soc., 80, 1132-1135.

Horowitz, N. H. and Beadle, G. N. 1943 A microbiological method for the determination of choline by use of a mutant of Neurospora. J. Biol. Chem., 150, 325-333.

Johnson, E. A. and Rettger, L. F. 1943 A comparative study of the nutritional requirements of Salmonella typhosa, Salmonella pullorum, and Salmonella gallinarum. J. Bacteriol., 45, 127-135.

Jones, A. W. and Holtman, D. F. 1953 Synthesis of glutamic acid and alanine by Salmonella pullorum. J. Bacteriol., 66, 147-149.

Koser, S. A. and Rettger, L. F. 1919 Studies on bacterial nutrition. The utilization of nitrogenous compounds of definite chemical composition. J. Infectious Diseases, 24, 301-321.

Lederberg, J. 1947 The nutrition of Salmonella. Arch. Biochem., 13, 287-290.

Levine, H. B., Weimberg, R., Dowling, J. H., Evenson, M., Rochenmacher, M., and Wolochow, H. 1954 The oxidative dissimilation of serine by Pasteurella pestis. J. Bacteriol., 67, 369-376.

Maas, W. K. and Davis, B. D. 1950 Pantothenate studies. I. Interference by D-serine and L-aspartic acid with pantothenate synthesis in Escherichia coli. J. Bacteriol., 60, 733-745.

McIlwain, H. 1942 Bacterial inhibition by metabolite analogues. 3. Pantoyltaurine. The antibacterial index of inhibitors. Brit. J. Exptl. Pathol., 23, 95-102.

Moore, A. M. and Boylen, J. B. 1952 Patterns of growth inhibition. J. Bacteriol., 64, 315-324.

Mueller, J. H. and Miller, P. A. 1949 Inhibition of tetanus toxin formation by D-serine. J. Am. Chem. Soc., 71, 1865-1866.

Prescott, J. M., Ragland, R. S., and Stutts, A. L. 1957 Effects of carbon dioxide on the growth of Streptococcus bovis in the presence of various amino acids. J. Bacteriol., 73, 133-138.

Rettger, L. F. 1909 Further studies on fatal septicemia in young chickens, or "white diarrhea." J. Med. Research, 21, 115-123.

Rettger, L. F. and Harvey, S. C. 1908 Fatal septicemia in young chickens, or "white diarrhea." J. Med. Research, 18, 277-290.

Ross, R. T., Holtman, D. F., and Gilfillan, R. F. 1956 Alterations in the nitrogen excretion pattern of chicks infected with Salmonella pullorum. J. Bacteriol., 71, 521-524.

Salton, M. R. J. 1953 Studies of the bacterial cell wall. IV. The composition of the cell walls of some Gram-positive and Gram-negative bacteria. Biochim. et Biophys. Acta, 10, 512-523.

Schoenhard, D. E. and Stafseth, H. J. 1953 Growth curves of Salmonella pullorum in different media. J. Bacteriol., 65, 69-74.

Shive, W. 1950 The utilization of antimetabolites in the study of biochemical processes in living organisms. Ann. N. Y. Acad. Sci., 52, 1212-1234.

Stafseth, H. J., Stockton, J. J., and Newman, J. P. 1956 A laboratory manual for immunology. Burgess Publishing Co., Minneapolis.

Standard methods for the examination of dairy products, 10th ed. 1953 Am. Pub. Health Assoc, Inc., New York.

Stokes, J. L. and Bayne, H. G. 1957 Growth rates of Salmonella colonies. J. Bacteriol., 74, 200-206.

Teas, H. J. 1950 Mutants of Bacillus subtilus that require threonine or threonine plus methionine. J. Bacteriol., 59, 93-104.

Teeri, A. E. and Josselyn, D. 1953 Effect of excess amino acids on growth of certain lactobacilli. J. Bacteriol., 66, 72-73.

Van Lanen, J. M., Riker, A. J., and Baldwin, I. L. 1952 The effect of amino acids and related compounds upon the growth, virulence, and enzyme activity of crown gall bacteria. J. Bacteriol., 63, 723-734.

Woolley, D. W. 1952 A study of antimetabolites, pp. 66-67. John Wiley and Sons, New York.

Work, E. 1957 Biochemistry of the bacterial cell wall. Nature, 179, 841-847.

APPENDIX

TABLE 13

Effect of uridine and serine on growth of *Salmonella gallinarum*

	Time in hours	Log cells/ml	Turbidity in $BaSO_4$ units
Control	0	5.88	0
	12	7.14	0
	16		2
	18	7.54	5
	21	7.94	7
	24	8.24	10
	36	8.11	>10
	48	9.28	>10
	60		>10
DL-Serine 400 ug/ml	12	6.56	0
	16		0
	18	6.48	0
	21	6.56	0
	24	6.62	0
	36	6.83	1
	48	7.0	1
	60	7.67	5
Uridine 62.5 ug/ml	12	7.20	0
	16		2
	18	7.73	5
	21	7.90	7
	24	8.18	10
	36	8.32	>10
	48	9.29	>10
	60		>10
DL-Serine 400 ug/ml + Uridine 62.5 ug/ml	12	6.58	0
	16		0
	18	6.63	0
	21	6.59	0
	24	6.69	0
	36	6.54	1
	48	7.75	5
	60	9.11	>10

TABLE 14

Effect of serine isomers on growth of Salmonella gallinarum

15 Hours	Total serine ug/ml											
Isomer	3200	1600	800	400	200	100	50	40	30	20	10	5
DL			0	0	0	0	0	0	0	1	3	5
L	0	0	1	1	3	5	5	5	6	6	6	6
D			0	0	0	0	0	0	0	0	0	0
D+L		0	0	0	0	0		0		1	3	
	Control: 6											

18 Hours	Total serine ug/ml											
Isomer	3200	1600	800	400	200	100	50	40	30	20	10	5
DL			0	0	0	0	0	0	1	3	5	7
L	0	0	0	3	5	7	7	8	8	8	8	8
D			0	0	0	0	0	0	0	0	0	1
D+L		0	0	0	0	0		0		3	5	
	Control: 8											

24 Hours	Total serine ug/ml											
Isomer	3200	1600	800	400	200	100	50	40	30	20	10	5
DL			0	0	0	1	3	5	6	7	9	10
L	0	1	4	7	10	>10	>10	>10	>10	>10	>10	>10
D			0	0	0	0	0	0	0	0	1	4
D+L		0	0	0	0	1		5		7	9	
	Control: >10											

28 Hours	Total serine ug/ml											
Isomer	3200	1600	800	400	200	100	50	40	30	20	10	5
DL			0	0	0	1	7	9	10	10	>10	>10
L	0	5	9	10	>10	>10	>10	>10	>10	>10	>10	>10
D			0	0	0	0	0	0	0	0	3	9
D+L		0	0	0	0	1		9		10	>10	
	Control: >10											

41 Hours	Total serine ug/ml											
Isomer	3200	1600	800	400	200	100	50	40	30	20	10	5
DL			0	0	0	3	>10	>10	>10	>10	>10	>10
L	0	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
D			0	0	0	0	0	0	0	0	10	>10
D+L		0	0	0	0	10		>10		>10	>10	
	Control: >10											

64 Hours	Total serine ug/ml											
Isomer	3200	1600	800	400	200	100	50	40	30	20	10	5
DL			>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
L	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
D			10	10	>10	>10	9	9	9	>10	>10	>10
D+L		>10	>10	>10	>10	>10		>10		>10	>10	
	Control: >10											

TABLE 15

Effect of omitting organic and inorganic constituents of synthetic medium on the growth of *Salmonella gallinarum*

Compound omitted	Number of times transferred						
	1	2	3	4	5	6	7
L-Leucine	>10	>10	>10	>10	>10	10	0
L-Cystine	7	0	0				
L-Arginine	>10	>10	>10	>10	>10	>10	9
Thiamine	>10	>10	>10	>10	>10	>10	>10
Pantothenic acid	>10	>10	>10	>10	>10	>10	>10
Arginine + Leucine	>10	>10	10	10	10	7	10
Pantothenic acid + Thiamine	>10	>10	>10	>10	>10	>10	>10
Inorganic nitrogen	>10	>10	10	10	10	5	
Glucose	10	1	0				
Control - nothing omitted	>10	>10	>10	>10	>10	>10	>10

Turbidity in $BaSO_4$ units at 24 hour intervals, except for tube 7 at 48 hours
Transfers of 2 drops to each successive tube except from tube 6 to tube 7 when 2 drops of a 1:1000 dilution was transferred

TABLE 16

Effect of variation of arginine concentration on growth and serine inhibition of Salmonella gallinarum

42 Hours		DL-Serine ug/ml		
		100	50	0
L-Arginine ug/ml	160		5	5
	80	0	5	5
	40	0	5	5
	20	0	6	5
	10	0	5	5
	4	0	5	5
	2	0	4	4
	0	0	3	4

48 Hours		DL-Serine ug/ml		
		100	50	0
L-Arginine ug/ml	160		>10	>10
	80	9	>10	>10
	40	9	>10	>10
	20	9	>10	>10
	10	9	>10	>10
	4	9	>10	>10
	2	7	>10	>10
	0	6	10	>10

67 Hours

All tubes >10

TABLE 17

Effect of variation of histidine concentration on growth and serine inhibition of Salmonella gallinarum

42 Hours		DL-Serine ug/ml		
		100	50	0
L-Histidine ug/ml	160	0	3	5
	80	0	3	6
	40	0	4	6
	20	0	3	7
	10	0	3	6
	4	0	3	5
	2	0	3	5
	0	0	0	4

48 Hours		DL-Serine ug/ml		
		100	50	0
L-Histidine ug/ml	160	7	10	>10
	80	7	10	>10
	40	9	10	>10
	20	7	10	>10
	10	7	10	>10
	4	7	10	>10
	2	7	10	>10
	0	6	10	>10

67 Hours

All tubes >10

TABLE 18

Effect of variation of cystine concentration on growth and serine inhibition of Salmonella gallinarum

42 Hours		DL-Serine ug/ml		
		100	50	0
L-Cystine ug/ml	240	0	0	>10
	120	0	0	7
	60	0	0	5
	30	0	0	4
	15	0	0	3
	6	0	0	3
	3	0	0	3
	0	0	0	0

67 Hours		DL-Serine ug/ml		
		100	50	0
Cystine ug/ml	240	3	10	>10
	120	5	>10	>10
	60	5	>10	>10
	30	5	>10	>10
	15	3	>10	>10
	6	0	10	>10
	3	0	5	>10
	0	0	0	0

48 Hours		DL-Serine ug/ml		
		100	50	0
L-Cystine ug/ml	240	0	0	>10
	120	0	0	>10
	60	0	0	>10
	30	0	0	10
	15	0	0	9
	6	0	0	9
	3	0	0	9
	0	0	0	0

96 Hours		DL-Serine ug/ml		
		100	50	0
Cystine ug/ml	240	>10	>10	>10
	120	>10	>10	>10
	60	>10	>10	>10
	30	>10	>10	>10
	15	>10	>10	>10
	6	>10	>10	>10
	3	>10	>10	>10
	0	0	0	0

TABLE 19

Effect of variation of leucine concentration on growth and serine inhibition of *Salmonella gallinarum*

42 Hours		DL-Serine ug/ml		
		100	50	0
L-Leucine ug/ml	360	0	0	5
	180	0	0	6
	90	0	0	6
	45	0	0	6
	22.5	0	0	6
	9	0	0	5
	4.5	0	0	5
	0	0	0	0

67 Hours		DL-Serine ug/ml		
		100	50	0
Leucine ug/ml	360	3	>10	>10
	180	4	>10	>10
	90	3	>10	>10
	45	3	>10	>10
	22.5	0	3	>10
	9	0	3	>10
	4.5	0	0	>10
	0	0	0	>10

48 Hours		DL-Serine ug/ml		
		100	50	0
L-Leucine ug/ml	360	0	1	>10
	180	0	3	>10
	90	0	3	>10
	45	0	0	>10
	22.5	0	0	>10
	9	0	0	>10
	4.5	0	0	>10
	0	0	0	3

96 Hours		DL-Serine ug/ml		
		100	50	0
Leucine ug/ml	360	>10	>10	>10
	180	>10	>10	>10
	90	>10	>10	>10
	45	>10	>10	>10
	22.5	>10	>10	>10
	9	>10	>10	>10
	4.5	0	>10	>10
	0	0	0	>10

TABLE 20

Effect of variation of glucose concentration on growth and serine inhibition of Salmonella gallinarum

42 Hours		DL-Serine ug/ml		
		100	50	0
D-Glucose mg/ml	40	0	0	3
	20	0	0	6
	10	0	0	5
	5	0	0	5
	2.5	0	0	5
	1	0	0	3
	0.5	0	0	3
	0	0	0	1

67 Hours		DL-Serine ug/ml		
		100	50	0
Glucose mg/ml	40	0	0	>10
	20	0	0	>10
	10	0	0	>10
	5	0	0	>10
	2.5	0	0	>10
	1	0	0	>10
	0.5	0	0	>10
	0	0	0	3

48 Hours		DL-Serine ug/ml		
		100	50	0
D-Glucose mg/ml	40	0	0	10
	20	0	0	>10
	10	0	0	>10
	5	0	0	>10
	2.5	0	0	>10
	1	0	0	10
	0.5	0	0	10
	0	0	0	3

96 Hours		DL-Serine ug/ml		
		100	50	0
Glucose mg/ml	40	>10	>10	>10
	20	>10	>10	>10
	10	0	1	>10
	5	0	0	>10
	2.5	0	0	>10
	1	0	0	>10
	0.5	0	0	>10
	0	0	0	3

TABLE 21

Effect of variation of inorganic nitrogen concentration on growth and serine inhibition of Salmonella gallinarum

21 Hours

NH_4Cl	NH_4NO_3	DL-Serine ug/ml 100	50	0
20 mg/ml	4 mg/ml	0	0	0
10 mg/ml	2 mg/ml	0	0	3
5 mg/ml	1 mg/ml	0	3	5
2.5 mg/ml	0.5 mg/ml	0	3	7
1.25mg/ml	0.25mg/ml	0	3	7
625 ug/ml	125 ug/ml	0	3	7
312.5 ug/ml	62.5 ug/ml	0	3	7
0	0	0	0	3

35 Hours

NH_4Cl	NH_4NO_3	DL-Serine ug/ml 100	50	0
20 mg/ml	4 mg/ml	5	7	>10
10 mg/ml	2 mg/ml	6	>10	>10
5 mg/ml	1 mg/ml	>10	>10	>10
2.5 mg/ml	0.5 mg/ml	>10	>10	>10
1.25mg/ml	0.25mg/ml	>10	>10	>10
625 ug/ml	125 ug/ml	>10	>10	>10
312.5 ug/ml	62.5 ug/ml	>10	>10	>10
0	0	>10	>10	>10

96 Hours

All tubes >10

TABLE 22

Effect of variation of bicarbonate concentration on growth and serine inhibition of Salmonella gallinarum

21 Hours		DL-Serine ug/ml		
		100	50	0
$KHCO_3$ mg/ml	8	0	0	3
	4	0	1	5
	2	0	1	7
	1	0	1	7
	0.5	0	1	7
	0.25	0	1	7
	0.1	0	1	7
	0	0	1	7

35 Hours		DL-Serine ug/ml		
		100	50	0
$KHCO_3$ mg/ml	8	0	0	3
	4	0	1	>10
	2	5	>10	>10
	1	>10	>10	>10
	0.5	>10	>10	>10
	0.25	>10	>10	>10
	0.1	>10	>10	>10
	0	3	7	10

TABLE 23

Effect of glycine and serine on growth of Salmonella gallinarum

24 Hours

		DL-Serine ug/ml				
		400	200	100	50	0
Glycine mg/ml	4	0	0	0	0	0
	2	0	0	0	0	3
	1	0	0	0	3	5
	0	0	0	0	5	5

38 Hours

		DL-Serine ug/ml				
		400	200	100	50	0
Glycine mg/ml	4	0	0	0	0	6
	2	0	0	0	8	10
	1	0	0	0	10	>10
	0	0	0	10	>10	>10

48 Hours

		DL-Serine ug/ml				
		400	200	100	50	0
Glycine mg/ml	4	0	0	0	0	10
	2	0	0	0	>10	>10
	1	0	0	0	>10	>10
	0	0	0	>10	>10	>10

TABLE 24

Effect of glycine, DL-α-alanine and low concentrations of DL-serine on growth of S. gallinarum

20 Hours		DL-Serine ug/ml							
		20	10	7.5	5	2.5	1.25	0.625	0
Glycine ug/ml	1000	1	2	3	4	5	5	5	5
	100	2	3	3	5	5	5	5	5
	10	2	3	3	5	5	5	5	5
	1	2	3	3	5	5	5	5	5
	0	2	3	3	5	5	5	5	5

		DL-Serine ug/ml							
		20	10	7.5	5	2.5	1.25	0.625	0
DL-α-alanine ug/ml	1000	1	1	1	1	1	1	1	1
	100	2	2	2	2	2	1	1	1
	10	2	3	3	3	3	3	3	3
	1	2	3	3	5	5	5	5	5
	0	2	3	3	5	5	5	5	5

41 Hours All tubes >10

TABLE 25

Effect of uridine and serine on growth of Salmonella gallinarum

17 Hours

		DL-Serine ug/ml						
		800	400	200	100	50	25	0
Uridine ug/ml	100	4	4	4	5	6	7	6
	50	0	1	4	5	7	7	6
	25	0	0	2	4	6	7	6
	10	0	0	0	0	5	7	5
	1	0	0	0	0	0	5	5
	0	0	0	0	0	0	1	5

26 Hours

		DL-Serine ug/ml						
		800	400	200	100	50	25	0
Uridine ug/ml	100	>10	>10	>10	>10	>10	>10	>10
	50	0	0	>10	>10	>10	>10	>10
	25	0	0	>10	>10	>10	>10	>10
	10	0	6	6	7	>10	>10	>10
	1	0	0	0	0	7	>10	>10
	0	0	0	0	0	0	6	>10

40 Hours

		DL-Serine ug/ml						
		800	400	200	100	50	25	0
Uridine ug/ml	100	>10	>10	>10	>10	>10	>10	>10
	50	0	0	>10	>10	>10	>10	>10
	25	0	0	>10	>10	>10	>10	>10
	10	0	>10	>10	>10	>10	>10	>10
	1	0	0	0	>10	>10	>10	>10
	0	0	0	0	2	>10	>10	>10

72 Hours — Growth in all tubes

TABLE 26

Effect of adenosine and serine on growth of *Salmonella gallinarum*

18 Hours		DL-Serine ug/ml						
		800	400	200	100	50	25	0
Adenosine ug/ml	100	0	2	2	4	7	10	9
	50	0	0	1	3	7	9	7
	25	0	0	1	3	7	7	7
	10	0	0	0	3	7	7	7
	1	0	0	0	0	4	4	7
	0	0	0	0	0	1	4	5

24 Hours		DL-Serine ug/ml						
		800	400	200	100	50	25	0
Adenosine ug/ml	100	0	1	7	>10	>10	>10	>10
	50	0	1	8	>10	>10	>10	>10
	25	0	0	4	10	>10	>10	>10
	10	0	0	3	9	>10	>10	>10
	1	0	0	0	4	9	>10	>10
	0	0	0	0	0	7	10	>10

48 Hours		DL-Serine ug/ml						
		800	400	200	100	50	25	0
Adenosine ug/ml	100	>10	>10	>10	>10	>10	>10	>10
	50	>10	>10	>10	>10	>10	>10	>10
	25	>10	>10	>10	>10	>10	>10	>10
	10	>10	>10	>10	>10	>10	>10	>10
	1	3	4	>10	>10	>10	>10	>10
	0	0	0	3	>10	>10	>10	>10

TABLE 27

Effect of cytidine and serine on growth of Salmonella gallinarum

17 Hours

		DL-Serine ug/ml						
		800	400	200	100	50	25	0
Cytidine ug/ml	100	0	0	1	4	7	7	6
	50	0	0	0	3	5	7	5
	25	0	0	0	1	5	7	5
	10	0	0	0	0	5	5	5
	1	0	0	0	0	0	5	5
	0	0	0	0	0	0	1	5

26 Hours

		DL-Serine ug/ml						
		800	400	200	100	50	25	0
Cytidine ug/ml	100	0	0	2	>10	>10	>10	>10
	50	0	0	10	>10	>10	>10	>10
	25	0	0	0	10	>10	>10	>10
	10	0	0	0	7	>10	>10	>10
	1	0	0	0	2	7	>10	>10
	0	0	0	0	0	2	6	>10

40 Hours

		DL-Serine ug/ml						
		800	400	200	100	50	25	0
Cytidine ug/ml	100	0	0	>10	>10	>10	>10	>10
	50	0	>10	>10	>10	>10	>10	>10
	25	0	2	0	>10	>10	>10	>10
	10	0	0	>10	>10	>10	>10	>10
	1	0	0	0	>10	>10	>10	>10
	0	0	0	0	8	>10	>10	>10

72 Hours

Growth in all tubes except tube in column 3, row 3

TABLE 28

Effect of cytidylic acid and serine on growth of *Salmonella gallinarum*

17 Hours

		DL-Serine ug/ml						
		800	400	200	100	50	25	0
Cytidylic acid ug/ml	100	0	0	0	3	5	6	5
	50	0	0	0	3	5	5	5
	25	0	0	0	3	5	5	5
	10	0	0	0	0	3	5	5
	1	0	0	0	0	0	5	5
	0	0	0	0	0	0	1	5

26 Hours

		DL-Serine ug/ml						
		800	400	200	100	50	25	0
Cytidylic acid ug/ml	100	>10	0	5	>10	>10	>10	>10
	50	0	0	0	>10	>10	>10	>10
	25	0	0	0	>10	>10	>10	>10
	10	0	0	0	5	>10	>10	>10
	1	0	0	0	0	6	10	>10
	0	0	0	0	0	0	6	>10

40 Hours

		DL-Serine ug/ml						
		800	400	200	100	50	25	0
Cytidylic acid	100	>10	0	>10	>10	>10	>10	>10
	50	>10	0	5	>10	>10	>10	>10
	25	0	5	5	>10	>10	>10	>10
	10	0	0	0	>10	>10	>10	>10
	1	0	0	0	10	>10	>10	>10
	0	0	0	0	2	>10	>10	>10

72 Hours Growth in all tubes

TABLE 29

Effect of uridylic acid and serine on growth of Salmonella gallinarum

17 Hours		DL-Serine ug/ml						
		800	400	200	100	50	25	0
Uridylic acid ug/ml	100	0	0	0	0	5	6	5
	50	0	0	0	0	5	5	5
	25	0	0	0	0	5	5	5
	10	0	0	0	0	3	5	5
	1	0	0	0	0	0	5	5
	0	0	0	0	0	0	1	5

26 Hours		DL-Serine ug/ml						
		800	400	200	100	50	25	0
Uridylic acid ug/ml	100	0	0	1	10	>10	>10	>10
	50	0	0	0	10	>10	>10	>10
	25	0	0	6	6	>10	>10	>10
	10	0	0	0	0	>10	>10	>10
	1	0	0	0	0	6	10	>10
	0	0	0	0	0	0	6	>10

40 Hours		DL-Serine ug/ml						
		800	400	200	100	50	25	0
Uridylic acid ug/ml	100	0	0	5	>10	>10	>10	>10
	50	0	0	2	>10	>10	>10	>10
	25	0	0	>10	>10	>10	>10	>10
	10	0	0	0	10	>10	>10	>10
	1	0	0	0	10	>10	>10	>10
	0	0	0	0	2	>10	>10	>10

72 Hours — Growth in all tubes

TABLE 30

Effect of adenylic acid and serine on growth of *Salmonella gallinarum*

18 Hours		DL-Serine ug/ml						
		800	400	200	100	50	25	0
Adenylic acid ug/ml	100	0	0	2	4	7	10	8
	50	0	0	1	3	7	9	8
	25	0	0	0	2	5	9	7
	10	0	0	0	1	5	9	7
	1	0	0	0	0	3	4	5
	0	0	0	0	0	1	4	5

24 Hours		DL-Serine ug/ml						
		800	400	200	100	50	25	0
Adenylic acid ug/ml	100	0	1	4	>10	>10	>10	>10
	50	0	1	9	>10	>10	>10	>10
	25	0	0	9	10	>10	>10	>10
	10	0	0	3	7	>10	>10	>10
	1	0	0	0	3	9	>10	>10
	0	0	0	0	0	7	10	>10

48 Hours		DL-Serine ug/ml						
		800	400	200	100	50	25	0
Adenylic acid	100	>10	>10	>10	>10	>10	>10	>10
	50	>10	>10	>10	>10	>10	>10	>10
	25	>10	>10	>10	>10	>10	>10	>10
	10	7	7	>10	>10	>10	>10	>10
	1	0	0	3	>10	>10	>10	>10
	0	0	0	2	>10	>10	>10	>10

TABLE 31

Effect of deoxycytidine and serine on growth of Salmonella gallinarum

22 Hours

Deoxycytidine ug/ml	DL-Serine ug/ml 800	400	200	100	50	25	0
100	0	0	0	5	10	10	9
50	0	0	0	3	9	9	7
25	0	0	0	0	9	9	6
10	0	0	0	0	7	7	5
1	0	0	0	0	5	6	5
0	0	0	0	0	0	5	5

28 Hours

Deoxycytidine ug/ml	DL-Serine ug/ml 800	400	200	100	50	25	0
100	0	0	0	>10	>10	>10	>10
50	0	0	0	8	>10	>10	>10
25	0	0	0	3	>10	>10	>10
10	0	0	0	0	>10	>10	10
1	0	0	0	0	10	>10	10
0	0	0	0	0	6	10	10

42 Hours

Deoxycytidine ug/ml	DL-Serine ug/ml 800	400	200	100	50	25	0
100	0	0	0	>10	>10	>10	>10
50	0	0	0	>10	>10	>10	>10
25	0	0	0	>10	>10	>10	>10
10	0	0	0	0	>10	>10	>10
1	0	0	0	0	>10	>10	>10
0	0	0	0	0	>10	>10	>10

66 Hours — Growth in all tubes

TABLE 32

Effect of thymidine and serine on growth of Salmonella gallinarum

22 Hours		D L-Serine ug/ml						
		800	400	200	100	50	25	0
Thymidine ug/ml	100	0	0	0	4	9	9	6
	50	0	0	0	3	8	8	6
	25	0	0	0	3	7	8	6
	10	0	0	0	0	4	7	5
	1	0	0	0	0	3	5	5
	0	0	0	0	0	0	5	5

28 Hours		DL-Serine ug/ml						
		800	400	200	100	50	25	0
Thymidine ug/ml	100	0	0	0	9	>10	>10	>10
	50	0	0	0	8	>10	>10	>10
	25	0	0	0	8	>10	>10	>10
	10	0	0	0	0	10	>10	10
	1	0	0	0	0	8	>10	10
	0	0	0	0	0	6	10	10

42 Hours		DL-Serine ug/ml						
		800	400	200	100	50	25	0
Thymidine ug/ml	100	0	0	0	>10	>10	>10	>10
	50	0	0	0	>10	>10	>10	>10
	25	0	0	0	>10	>10	>10	>10
	10	0	0	0	0	>10	>10	>10
	1	0	0	0	0	>10	>10	>10
	0	0	0	0	0	>10	>10	>10

66 Hours — Growth in all tubes

TABLE 33

Effect of thymidylic acid and serine on growth of Salmonella gallinarum

24 Hours		DL-Serine ug/ml				
		400	200	100	50	0
Thymidylic acid ug/ml	100		0	5	5	5
	50	0	0	5	5	5
	25	0	0	3	5	5
	0	0	0	0	5	5

38 Hours		DL-Serine ug/ml				
		400	200	100	50	0
Thymidylic acid ug/ml	100		8	>10	>10	8
	50	0	8	>10	>10	>10
	25	0	0	>10	>10	>10
	0	0	0	10	>10	>10

48 Hours		DL-Serine ug/ml				
		400	200	100	50	0
Thymidylic acid ug/ml	100		>10	>10	>10	>10
	50	0	>10	>10	>10	>10
	25	0	3	>10	>10	>10
	0	0	0	>10	>10	>10

TABLE 34

Effect of guanosine and serine on growth of Salmonella gallinarum

18 Hours

Guanosine ug/ml	DL-Serine ug/ml 800	400	200	100	50	25	0
100	0	0	0	1	5	7	8
50	0	0	0	1	4	7	7
25	0	0	0	1	4	5	5
10	0	0	0	0	4	5	5
1	0	0	0	0	2	4	5
0	0	0	0	0	1	4	5

24 Hours

Guanosine ug/ml	DL-Serine ug/ml 800	400	200	100	50	25	0
100	0	0	0	4	>10	>10	>10
50	0	0	0	4	>10	>10	>10
25	0	0	0	4	>10	>10	>10
10	0	0	0	4	10	>10	>10
1	0	0	0	0	8	10	>10
0	0	0	0	0	7	10	>10

48 Hours

Guanosine ug/ml	DL-Serine ug/ml 800	400	200	100	50	25	0
100	3	3	10	>10	>10	>10	>10
50	3	3	3	>10	>10	>10	>10
25	3	3	3	>10	>10	>10	>10
10	2	3	>10	>10	>10	>10	>10
1	0	0	1	>10	>10	>10	>10
0	0	0	0	3	>10	>10	>10

TABLE 35

Effect of guanylic acid and serine on growth of **Salmonella gallinarum**

18 Hours		DL-Serine ug/ml						
		800	400	200	100	50	25	0
Guanylic acid ug/ml	100	0	0	0	1	3	4	5
	50	0	0	0	1	3	4	5
	25	0	0	0	0	3	4	5
	10	0	0	0	0	3	4	5
	1	0	0	0	0	1	4	5
	0	0	0	0	0	1	4	5

24 Hours		DL-Serine ug/ml						
		800	400	200	100	50	25	0
Guanylic acid ug/ml	100	0	0	0	3	8	10	>10
	50	0	0	0	3	8	10	>10
	25	0	0	0	0	7	10	>10
	10	0	0	0	0	7	10	>10
	1	0	0	0	0	7	10	>10
	0	0	0	0	0	7	10	>10

48 Hours		DL-Serine ug/ml						
		800	400	200	100	50	25	0
Guanylic acid ug/ml	100	5	5	5	>10	>10	>10	>10
	50	3	3	10	>10	>10	>10	>10
	25	>10	3	10	>10	>10	>10	>10
	10	3	3	8	>10	>10	>10	>10
	1	0	0	1	>10	>10	>10	>10
	0	0	0	0	3	>10	>10	>10

TABLE 36

Effect of deoxyadenosine and serine on growth of *Salmonella gallinarum*

22 Hours		DL-Serine ug/ml						
		800	400	200	100	50	25	0
Deoxyadenosine ug/ml	100	0	0	0	1	8	9	4
	50	0	0	0	0	8	8	4
	25	0	0	0	0	7	8	4
	10	0	0	0	0	4	7	4
	1	0	0	0	0	3	7	5
	0	0	0	0	0	0	5	5

28 Hours		DL-Serine ug/ml						
		800	400	200	100	50	25	0
Deoxyadenosine ug/ml	100	0	0	0	6	>10	>10	9
	50	0	0	0	5	>10	>10	9
	25	0	0	0	3	>10	>10	9
	10	0	0	0	0	10	>10	9
	1	0	0	0	0	9	>10	9
	0	0	0	0	0	5	10	10

42 Hours		DL-Serine ug/ml						
		800	400	200	100	50	25	0
Deoxyadenosine ug/ml	100	0	0	5	>10	>10	>10	>10
	50	0	0	6	>10	>10	>10	>10
	25	0	0	5	>10	>10	>10	>10
	10	0	0	1	10	>10	>10	>10
	1	0	0	0	1	>10	>10	>10
	0	0	0	0	0	>10	>10	>10

66 Hours — Growth in all tubes

TABLE 37

Effect of deoxycytidylic acid and serine on growth of *Salmonella gallinarum*

22 Hours

Deoxycytidylic acid ug/ml	DL-Serine ug/ml 800	400	200	100	50	25	0
100	0	0	0	0	3	6	5
50	0	0	0	0	4	6	5
25	0	0	0	0	4	6	5
10	0	0	0	0	3	5	5
1	0	0	0	0	3	5	5
0	0	0	0	0	0	5	5

28 Hours

Deoxycytidylic acid ug/ml	DL-Serine ug/ml 800	400	200	100	50	25	0
100	0	0	0	0	8	>10	10
50	0	0	0	0	10	>10	10
25	0	0	0	0	10	>10	10
10	0	0	0	0	8	>10	10
1	0	0	0	0	8	10	10
0	0	0	0	0	5	10	10

42 Hours

Deoxycytidylic acid ug/ml	DL-Serine ug/ml 800	400	200	100	50	25	0
100	0	0	0	0	>10	>10	>10
50	0	0	0	10	>10	>10	>10
25	0	0	0	0	>10	>10	>10
10	0	0	0	0	>10	>10	>10
1	0	0	0	0	>10	>10	>10
0	0	0	0	0	>10	>10	>10

66 Hours — Growth in all tubes

TABLE 38

Effect of deoxyadenylic acid and serine on growth of *Salmonella gallinarum*

22 Hours

Deoxyadenylic acid ug/ml	DL-Serine ug/ml 800	400	200	100	50	25	0
100	0	0	0	0	5	7	4
50	0	0	0	0	4	7	4
25	0	0	0	0	3	7	4
10	0	0	0	0	3	7	4
1	0	0	0	0	0	5	5
0	0	0	0	0	0	5	5

28 Hours

Deoxyadenylic acid ug/ml	DL-Serine ug/ml 800	400	200	100	50	25	0
100	0	0	0	0	10	>10	8
50	0	0	0	0	10	>10	8
25	0	0	0	0	9	>10	8
10	0	0	0	0	9	>10	10
1	0	0	0	0	3	10	10
0	0	0	0	0	5	10	10

42 Hours

Deoxyadenylic acid ug/ml	DL-Serine ug/ml 800	400	200	100	50	25	0
100	0	0	3	10	>10	>10	>10
50	0	0	0	10	>10	>10	>10
25	0	0	0	10	>10	>10	>10
10	0	0	0	5	>10	>10	>10
1	0	0	0	0	>10	>10	>10
0	0	0	0	0	>10	>10	>10

66 Hours — Growth in all tubes

TABLE 39

Effect of xanthine and serine on growth of Salmonella gallinarum

24 Hours

		DL-Serine ug/ml				
		400	200	100	50	0
Xanthine ug/ml	50	0	0	0	1	1
	25	0	0	1	3	3
	12.5	0	0	1	5	5
	0	0	0	0	5	5

38 Hours

		DL-Serine ug/ml				
		400	200	100	50	0
Xanthine ug/ml	50	0	6	8	>10	>10
	25	0	1	>10	>10	>10
	12.5	0	8	>10	>10	>10
	0	0	0	10	>10	>10

48 Hours

		DL-Serine ug/ml				
		400	200	100	50	0
Xanthine ug/ml	50	0	>10	>10	>10	>10
	25	0	>10	>10	>10	>10
	12.5	0	>10	>10	>10	>10
	0	0	0	>10	>10	>10

TABLE 40

Effect of deoxyguanosine and serine on growth of *Salmonella gallinarum*

24 Hours		DL-Serine ug/ml				
		400	200	100	50	0
Deoxyguanosine ug/ml	100	0	0	0	5	5
	50	0	0	1	5	5
	25	0	0	1	5	5
	0	0	0	0	5	5

38 Hours		DL-Serine ug/ml				
		400	200	100	50	0
Deoxyguanosine ug/ml	100	0	0	>10	>10	>10
	50	0	0	>10	>10	>10
	25	0	5	>10	>10	>10
	0	0	0	10	>10	>10

48 Hours		DL-Serine ug/ml				
		400	200	100	50	0
Deoxyguanosine ug/ml	100	0	3	>10	>10	>10
	50	0	5	>10	>10	>10
	25	0	>10	>10	>10	>10
	0	0	0	>10	>10	>10

TABLE 41

Effect of uracil and serine on growth of Salmonella gallinarum

24 Hours		DL-Serine ug/ml				
		400	200	100	50	0
Uracil ug/ml	100	0	0	1	5	5
	50	0	0	1	5	5
	25	0	0	0	5	5
	0	0	0	0	5	5

38 Hours		DL-Serine ug/ml				
		400	200	100	50	0
Uracil ug/ml	100	0	1	10	>10	>10
	50	0	5	10	>10	>10
	25	0	5	10	>10	>10
	0	0	0	10	>10	>10

48 Hours		DL-Serine ug/ml				
		400	200	100	50	0
Uracil ug/ml	100	0	>10	>10	>10	>10
	50	0	>10	>10	>10	>10
	25	0	>10	>10	>10	>10
	0	0	0	>10	>10	>10

TABLE 42

Effect of ribose, adenine and serine on growth of *Salmonella gallinarum*

24 Hours		DL-Serine ug/ml				
D-Ribose*	Adenine sulfate*	400	200	100	50	0
100 ug/ml	100 ug/ml	0	0	5	7	5
50 ug/ml	50 ug/ml	0	0	3	0	5
25 ug/ml	25 ug/ml	0	0	1	5	5
0	0	0	0	0	5	5

38 Hours		DL-Serine ug/ml				
D-Ribose	Adenine sulfate	400	200	100	50	0
100 ug/ml	100 ug/ml	0	10	>10	>10	>10
50 ug/ml	50 ug/ml	0	10	>10	5	>10
25 ug/ml	25 ug/ml	0	10	10	>10	>10
0	0	0	0	10	>10	>10

48 Hours		DL-Serine ug/ml				
D-Ribose	Adenine sulfate	400	200	100	50	0
100 ug/ml	100 ug/ml	0	>10	>10	>10	>10
50 ug/ml	50 ug/ml	10	>10	>10	>10	>10
25 ug/ml	25 ug/ml	0	>10	>10	>10	>10
0	0	0	0	>10	>10	>10

*Autoclaved together 118°C 20 minutes

TABLE 43

Effect of deoxyguanylic acid and serine on growth of Salmonella gallinarum

24 Hours		DL-Serine ug/ml				
		400	200	100	50	0
Deoxyguanylic acid ug/ml	100	0	0	0	5	5
	50	0	0	0	5	5
	25	0	0	0	5	5
	0	0	0	0	5	5

38 Hours		DL-Serine ug/ml				
		400	200	100	50	0
Deoxyguanylic acid ug/ml	100	0	0	>10	>10	>10
	50	0	0	>10	>10	>10
	25	0	0	10	>10	>10
	0	0	0	10	>10	>10

48 Hours		DL-Serine ug/ml				
		400	200	100	50	0
Deoxyguanylic acid ug/ml	100	0	3	>10	>10	>10
	50	0	0	>10	>10	>10
	25	0	0	>10	>10	>10
	0	0	0	>10	>10	>10

TABLE 44

Effect of adenine and serine on growth of Salmonella gallinarum

24 Hours		DL-Serine ug/ml				
		400	200	100	50	0
Adenine sulfate ug/ml	100	0	0	0	5	3
	50	0	0	0	5	3
	25	0	0	0	5	4
	0	0	0	0	5	5

38 Hours		DL-Serine ug/ml				
		400	200	100	50	0
Adenine sulfate ug/ml	100	0	0	0	8	6
	50	0	0	8	>10	10
	25	0	0	8	>10	10
	0	0	0	10	>10	>10

48 Hours		DL-Serine ug/ml				
		400	200	100	50	0
Adenine sulfate ug/ml	100	0	0	0	>10	>10
	50	0	0	>10	>10	>10
	25	0	0	>10	>10	>10
	0	0	0	>10	>10	>10

TABLE 45

Effect of ribose and serine on growth of Salmonella gallinarum

24 Hours		DL-Serine ug/ml				
		400	200	100	50	0
D-Ribose ug/ml	100	0	0	0	5	5
	50	0	0	0	5	5
	25	0	0	0	5	5
	0	0	0	0	5	5

38 Hours		DL-Serine ug/ml				
		400	200	100	50	0
D-Ribose ug/ml	100	0	0	8	>10	>10
	50	0	0	9	>10	>10
	25	0	0	10	>10	>10
	0	0	0	10	>10	>10

48 Hours		DL-Serine ug/ml				
		400	200	100	50	0
D-Ribose ug/ml	100	0	0	>10	>10	>10
	50	0	8	>10	>10	>10
	25	0	8	>10	>10	>10
	0	0	0	>10	>10	>10

TABLE 46

Effect of ribonucleic acid and serine on growth of Salmonella gallinarum

22 Hours		DL-Serine ug/ml						
		800	400	200	100	50	25	0
Ribonucleic acid ug/ml	100	0	0	3	8	10	10	5
	50	0	0	1	7	10	10	5
	25	0	0	0	5	10	10	5
	10	0	0	0	1	7	9	5
	1	0	0	0	0	3	5	5
	0	0	0	0	0	0	5	5

28 Hours		DL-Serine ug/ml						
		800	400	200	100	50	25	0
Ribonucleic acid ug/ml	100	0	4	7	>10	>10	>10	9
	50	0	0	3	>10	>10	>10	9
	25	0	0	0	>10	>10	>10	9
	10	0	0	0	4	>10	>10	10
	1	0	0	0	0	8	>10	10
	0	0	0	0	0	6	10	10

42 Hours		DL-Serine ug/ml						
		800	400	200	100	50	25	0
Ribonucleic acid ug/ml	100	>10	>10	>10	>10	>10	>10	>10
	50	>10	>10	>10	>10	>10	>10	>10
	25	10	10	10	>10	>10	>10	>10
	10	0	0	6	>10	>10	>10	>10
	1	0	0	0	0	>10	>10	>10
	0	0	0	0	0	>10	>10	>10

66 Hours — Growth in all tubes

TABLE 47

Effect of deoxyribonucleic acid and serine on growth of Salmonella gallinarum

21 Hours		DL-Serine ug/ml			
		120	60	12	0
Deoxyribonucleic acid ug/ml	100	0	0	0	0
	50	0	7	6	3
	10	0	7	7	5
	0	0	5	5	5

36 Hours		DL-Serine ug/ml			
		120	60	12	0
Deoxyribonucleic acid ug/ml	100	0	1	3	1
	50	10	>10	>10	5
	10	>10	>10	>10	8
	0	7	>10	>10	10

64 Hours		DL-Serine ug/ml			
		120	60	12	0
Deoxyribonucleic acid ug/ml	100	0	>10	>10	>10
	50	>10	>10	>10	>10
	10	>10	>10	>10	>10
	0	>10	>10	>10	>10

TABLE 48

Effect of glutamic acid and serine on growth of *Salmonella gallinarum*

17 Hours		DL-Serine ug/ml					
		120	90	60	30	10	0
L-Glutamic acid ug/ml	120	0	0	0	2	5	7
	10	0	0	0	0	5	7
	0	0	0	0	0	0	5

26 Hours		DL-Serine ug/ml					
		120	90	60	30	10	0
L-Glutamic acid ug/ml	120	3	5	7	9	10	>10
	10	3	3	5	7	10	>10
	0	0	0	0	0	5	>10

40 Hours		DL-Serine ug/ml					
		120	90	60	30	10	0
L-Glutamic acid ug/ml	120	>10	>10	>10	>10	>10	>10
	10	>10	>10	>10	>10	>10	>10
	0	0	0	5	10	>10	>10

72 Hours — Growth in all tubes

TABLE 49

Effect of aspartic acid and serine on growth of *Salmonella gallinarum*

17 Hours

		DL-Serine ug/ml					
		120	90	60	30	10	0
L-Aspartic acid ug/ml	120	0	0	0	0	5	7
	10	0	0	0	0	4	6
	0	0	0	0	0	0	5

26 Hours

		DL-Serine ug/ml					
		120	90	60	30	10	0
L-Aspartic acid ug/ml	120	1	1	5	7	10	>10
	10	0	0	1	6	8	>10
	0	0	0	0	0	5	>10

40 Hours

		DL-Serine ug/ml					
		120	90	60	30	10	0
L-Aspartic acid ug/ml	120	10	>10	>10	>10	>10	>10
	10	1	10	>10	>10	>10	>10
	0	0	0	5	10	>10	>10

72 Hours — Growth in all tubes

TABLE 50

Effect of isoleucine and serine on growth of Salmonella gallinarum

24 Hours		DL-Serine ug/ml				
		400	200	100	50	0
DL-Isoleucine mg/ml	2.0	4	5	7	7	7
	1.0	3	5	7	7	7
	0.5	1	5	7	7	7
	0	0	0	0	5	5

38 Hours		DL-Serine ug/ml				
		400	200	100	50	0
DL-Isoleucine mg/ml	2.0	>10	>10	>10	>10	>10
	1.0	>10	>10	>10	>10	>10
	0.5	5	>10	>10	>10	>10
	0	0	0	10	>10	>10

48 Hours		DL-Serine ug/ml				
		400	200	100	50	0
DL-Isoleucine mg/ml	2.0	>10	>10	>10	>10	>10
	1.0	>10	>10	>10	>10	>10
	0.5	>10	>10	>10	>10	>10
	0	0	0	>10	>10	>10

TABLE 51

Effect of glutamine and serine on growth of Salmonella gallinarum

17 Hours		DL-Serine ug/ml					
		120	90	60	30	10	0
L-Glutamine ug/ml	120	0	0	0	2	5	7
	90	0	0	0	2	5	7
	60	0	0	0	1	5	6
	30	0	0	0	0	4	6
	15	0	0	0	0	2	5
	10	0	0	0	0	2	5
	5	0	0	0	0	0	5
	0	0	0	0	0	0	5

26 Hours		DL-Serine ug/ml					
		120	90	60	30	10	0
L-Glutamine ug/ml	120	5	5	7	10	10	>10
	90	5	5	7	10	10	>10
	60	3	5	5	8	10	>10
	30	0	1	3	6	9	>10
	15	0	0	0	4	8	>10
	10	0	0	0	3	7	>10
	5	0	0	0	3	6	>10
	0	0	0	0	0	5	>10

40 Hours		DL-Serine ug/ml					
		120	90	60	30	10	0
L-Glutamine ug/ml	120	>10	>10	>10	>10	>10	>10
	90	>10	>10	>10	>10	>10	>10
	60	>10	>10	>10	>10	>10	>10
	30	>10	>10	>10	>10	>10	>10
	15	9	10	>10	>10	>10	>10
	10	1	9	>10	>10	>10	>10
	5	0	5	10	>10	>10	>10
	0	0	0	5	10	>10	>10

72 Hours — Growth in all tubes

TABLE 52

Effect of asparagine and serine on growth of *Salmonella gallinarum*

17 Hours		DL-Serine ug/ml					
		120	90	60	30	10	0
L-Asparagine ug/ml	120	0	0	0	0	2	6
	10	0	0	0	0	0	5
	0	0	0	0	0	0	5

26 Hours		DL-Serine ug/ml					
		120	90	60	30	10	0
L-Asparagine ug/ml	120	0	0	0	4	7	>10
	10	0	0	0	0	5	>10
	0	0	0	0	0	5	>10

40 Hours		DL-Serine ug/ml					
		120	90	60	30	10	0
L-Asparagine ug/ml	120	1	10	>10	>10	>10	>10
	10	0	0	1	>10	>10	>10
	0	0	0	5	10	>10	>10

72 Hours — Growth in all tubes

TABLE 53

Effect of histidine and serine on growth of Salmonella gallinarum

24 Hours		DL-Serine ug/ml				
		400	200	100	50	0
L-Histidine mg/ml	2.0	0	0	1	5	5
	1.0	0	0	1	7	7
	0.5	0	0	1	7	10
	0	0	0	0	5	5

38 Hours		DL-Serine ug/ml				
		400	200	100	50	0
L-Histidine mg/ml	2.0	8	>10	>10	>10	>10
	1.0	0	>10	>10	>10	>10
	0.5	0	0	>10	>10	>10
	0	0	0	10	>10	>10

48 Hours		DL-Serine ug/ml				
		400	200	100	50	0
L-Histidine mg/ml	2.0	>10	>10	>10	>10	>10
	1.0	0	>10	>10	>10	>10
	0.5	3	3	>10	>10	>10
	0	0	0	>10	>10	>10

TABLE 54

Effect of proline and serine on growth of Salmonella gallinarum

18 Hours

L-Proline mg/ml	DL-Serine ug/ml 800	400	200	100	50	25	12.5	0
2.0	0	0	0	1	2	4	6	8
1.0	0	0	0	1	2	5	5	7
0.5	0	0	0	0	1	3	5	7
0.25	0	0	0	0	1	3	5	6
0.125	0	0	0	0	1	3	5	5
0	0	0	0	0	0	1	5	5

24 Hours

L-Proline mg/ml	DL-Serine ug/ml 800	400	200	100	50	25	12.5	0
2.0	3	3	3	5	7	9	>10	>10
1.0	3	3	3	5	6	9	>10	>10
0.5	0	1	3	3	6	7	9	>10
0.25	0	0	1	3	5	7	9	>10
0.125	0	0	1	3	5	7	9	10
0	0	0	0	0	0	3	9	9

48 Hours

L-Proline mg/ml	DL-Serine ug/ml 800	400	200	100	50	25	12.5	0
2.0	>10	5	>10	>10	>10	>10	>10	>10
1.0	3	5	>10	>10	>10	>10	>10	>10
0.5	1	3	>10	>10	>10	>10	>10	>10
0.25	1	3	5	>10	>10	>10	>10	>10
0.125	3	5	>10	>10	>10	>10	>10	>10
0	0	0	0	7	>10	>10	>10	>10

52 Hours

L-Proline mg/ml	DL-Serine ug/ml 800	400	200	100	50	25	12.5	0
2.0	>10	>10	>10	>10	>10	>10	>10	>10
1.0	10	>10	>10	>10	>10	>10	>10	>10
0.5	7	10	>10	>10	>10	>10	>10	>10
0.25	5	9	>10	>10	>10	>10	>10	>10
0.125	10	>10	>10	>10	>10	>10	>10	>10
0	0	0	3	>10	>10	>10	>10	>10

TABLE 55

Effect of methionine and serine on growth of *Salmonella gallinarum*

24 Hours		DL-Serine ug/ml							
		800	400	200	100	50	25	12.5	0
DL-Methionine mg/ml	2.0	0	0	0	3	5	6	8	10
	1.0	0	0	0	0	5	6	8	10
	0.5	0	0	0	0	5	6	8	9
	0.25	0	0	0	0	5	6	8	9
	0.125	0	0	0	3	6	7	8	9
	0	0	0	0	0	3	5	7	9

28 Hours		DL-Serine ug/ml							
		800	400	200	100	50	25	12.5	0
DL-Methionine mg/ml	2.0	1	4	6	7	9	>10	>10	>10
	1.0	1	4	5	6	9	10	>10	>10
	0.5	1	4	5	6	8	10	>10	>10
	0.25	1	4	5	7	8	10	>10	>10
	0.125	1	4	5	7	10	10	>10	>10
	0	0	0	4	1	7	8	>10	>10

40 Hours		DL-Serine ug/ml							
		800	400	200	100	50	25	12.5	0
DL-Methionine mg/ml	2.0	>10	>10	>10	>10	>10	>10	>10	>10
	1.0	10	>10	>10	>10	>10	>10	>10	>10
	0.5	9	>10	>10	>10	>10	>10	>10	>10
	0.25	7	>10	>10	>10	>10	>10	>10	>10
	0.125	5	>10	>10	>10	>10	>10	>10	>10
	0	0	0	>10	>10	>10	>10	>10	>10

48 Hours		DL-Serine ug/ml							
		800	400	200	100	50	25	12.5	0
DL-Methionine mg/ml	2.0	>10	>10	>10	>10	>10	>10	>10	>10
	1.0	>10	>10	>10	>10	>10	>10	>10	>10
	0.5	>10	>10	>10	>10	>10	>10	>10	>10
	0.25	>10	>10	>10	>10	>10	>10	>10	>10
	0.125	9	>10	>10	>10	>10	>10	>10	>10
	0	0	0	>10	>10	>10	>10	>10	>10

TABLE 56

Effect of urea and serine on growth of Salmonella gallinarum

20 Hours		DL-Serine ug/ml						
		800	400	200	100	50	25	0
Urea mg/ml	2.0	0	0	4	6	6	6	6
	1.0	0	0	0	5	6	6	6
	0.5	0	0	0	1	4	5	6
	0.25	0	0	0	0	4	4	6
	0.125	0	0	0	0	0	4	7
	0	0	0	0	0	0	4	7

30 Hours		DL-Serine ug/ml						
		800	400	200	100	50	25	0
Urea mg/ml	2.0	0	5	9	10	10	10	9
	1.0	0	0	8	10	10	>10	>10
	0.5	0	0	3	9	10	>10	>10
	0.25	0	0	0	5	9	>10	>10
	0.125	0	0	0	3	8	>10	>10
	0	0	0	0	1	7	>10	>10

45 Hours		DL-Serine ug/ml						
		800	400	200	100	50	25	0
Urea mg/ml	2.0	0	>10	>10	>10	>10	>10	>10
	1.0	0	3	>10	>10	>10	>10	>10
	0.5	0	3	9	>10	>10	>10	>10
	0.25	0	3	5	>10	>10	>10	>10
	0.125	0	0	3	>10	>10	>10	>10
	0	0	0	3	10	>10	>10	>10

55 Hours		DL-Serine ug/ml						
		800	400	200	100	50	25	0
Urea mg/ml	2.0	7	>10	>10	>10	>10	>10	>10
	1.0	5	9	>10	>10	>10	>10	>10
	0.5	4	8	>10	>10	>10	>10	>10
	0.25	3	8	9	>10	>10	>10	>10
	0.125	4	5	7	>10	>10	>10	>10
	0	3	3	10	>10	>10	>10	>10

TABLE 57

Effect of cysteine and serine on growth of Salmonella gallinarum

18 Hours

		DL-Serine ug/ml							
		800	400	200	100	50	25	12.5	0
L-Cysteine mg/ml	2.0	0	0	0	0	0	0	0	0
	1.0	0	0	0	0	0	0	2	4
	0.5	0	0	0	0	0	0	4	4
	0.25	0	0	0	0	0	0	0	6
	0.125	0	0	0	0	0	2	5	7
	0	0	0	0	0	1	4	6	7

25 Hours

		DL-Serine ug/ml							
		800	400	200	100	50	25	12.5	0
L-Cysteine mg/ml	2.0	0	0	0	0	0	0	0	1
	1.0	0	0	0	0	2	5	8	8
	0.5	0	0	0	0	0	1	10	10
	0.25	0	0	0	0	1	6	6	>10
	0.125	0	0	0	0	5	7	10	>10
	0	0	0	0	1	7	8	10	>10

30 Hours

		DL-Serine ug/ml							
		800	400	200	100	50	25	12.5	0
L-Cysteine mg/ml	2.0	0	0	0	0	0	0	5	5
	1.0	0	0	0	0	9	>10	>10	>10
	0.5	0	0	0	0	3	5	>10	>10
	0.25	0	0	0	0	7	>10	>10	>10
	0.125	0	0	0	0	9	>10	>10	>10
	0	0	0	0	3	>10	>10	>10	>10

42 Hours

		DL-Serine ug/ml							
		800	400	200	100	50	25	12.5	0
L-Cysteine mg/ml	2.0	10	5	5	1	1	0	10	10
	1.0	>10	>10	>10	9	>10	>10	>10	>10
	0.5	0	0	1	9	>10	>10	>10	>10
	0.25	0	0	0	7	>10	>10	>10	>10
	0.125	0	0	0	7	>10	>10	>10	>10
	0	0	0	0	10	>10	>10	>10	>10

54 Hours

		DL-Serine ug/ml							
		800	400	200	100	50	25	12.5	0
L-Cysteine mg/ml	2.0	>10	>10	>10	>10	>10	8	>10	>10
	1.0	>10	>10	>10	>10	>10	>10	>10	>10
	0.5	7	7	8	>10	>10	>10	>10	>10
	0.25	7	7	8	>10	>10	>10	>10	>10
	0.125	7	7	7	>10	>10	>10	>10	>10
	0	6	6	7	>10	>10	>10	>10	>10

TABLE 58

Effect of glutathione and serine on growth of Salmonella gallinarum

18 Hours		DL-Serine ug/ml							
		800	400	200	100	50	25	12.5	0
Glutathione mg/ml	2.0	0	0	0	0	0	0	0	0
	1.0	0	0	0	0	0	0	0	0
	0.5	0	0	0	0	0	0	0	0
	0.25	0	0	0	0	0	1	3	6
	0.125	0	0	0	0	0	1	3	7
	0	0	0	0	0	0	0	3	5

24 Hours		DL-Serine ug/ml							
		800	400	200	100	50	25	12.5	0
Glutathione mg/ml	2.0	0	0	0	0	0	0	0	0
	1.0	0	0	0	0	0	0	0	1
	0.5	0	0	0	0	0	0	1	7
	0.25	0	0	0	0	0	3	7	10
	0.125	0	0	0	0	3	7	9	10
	0	0	0	0	0	0	3	6	9

42 Hours		DL-Serine ug/ml							
		800	400	200	100	50	25	12.5	0
Glutathione mg/ml	2.0	3	3	3	3	6	8	10	10
	1.0	0	0	0	0	5	7	10	>10
	0.5	0	0	0	0	10	10	>10	>10
	0.25	0	0	0	10	7	>10	>10	>10
	0.125	0	0	3	10	>10	>10	>10	>10
	0	0	0	0	5	>10	>10	>10	>10

52 Hours		DL-Serine ug/ml							
		800	400	200	100	50	25	12.5	0
Glutathione mg/ml	2.0	9	9	9	>10	>10	>10	>10	>10
	1.0	3	3	5	7	>10	>10	>10	>10
	0.5	0	0	2	7	>10	>10	>10	>10
	0.25	0	3	9	>10	>10	>10	>10	>10
	0.125	3	3	5	>10	>10	>10	>10	>10
	0	0	0	1	>10	>10	>10	>10	>10

68 Hours		DL-Serine ug/ml							
		800	400	200	100	50	25	12.5	0
Glutathione mg/ml	2.0	>10	>10	>10	>10	>10	>10	>10	>10
	1.0	10	>10	>10	>10	>10	>10	>10	>10
	0.5	5	7	>10	>10	>10	>10	>10	>10
	0.25	10	>10	>10	>10	>10	>10	>10	>10
	0.125	>10	>10	>10	>10	>10	>10	>10	>10
	0	>10	>10	7	>10	>10	>10	>10	>10