THE PRODUCTION OF MUTANTS, GENERALIZED TRANSDUCTION, AND THE LYTIC REACTION IN SALMONELLA GALLINARUM PULLORUM

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ABSTRACT

THE PRODUCTION OF MUTANTS, GENERALIZED TRANSDUCTION, AND THE LYTIC REACTION IN SALMONELLA GALLINARUM PULLORUM

by Roy William Snyder

A study of the nature of transfer of genetic information in *Salmonella gallinarum pullorum* has been performed. Temperate *S. gallinarum pullorum* bacteriophage was found to transduce a streptomycin resistant marker between strains of *S. gallinarum pullorum*. Classical methods were used to produce mutants which were resistant to streptomycin as well as mutants which lacked the ability to utilize certain hexoses and pentoses; also modifications of the penicillin technique were integrated to facilitate selection of absolute nutritionally dependent mutants. Generalized transduction reactions were performed by bacteriophage produced from strain 38 and used to transduce all three types of markers between different cells of *S. gallinarum pullorum* 53. An interaction, termed the lytic reaction, was discovered between bacteriophage 38 and sensitive strains of *S. gallinarum pullorum*. Here, 20-30% of the cells infected by phage 38 produced colonies exhibiting a distinct change in color on selective media and containing a high concentration of free bacteriophage. In some cells from such colonies, phage 38 evidently existed in an unintegrated state in the cytoplasm of the cell. It did not require a helper-phage and was not present in lysogenic cells normally containing complete phage. Preliminary evidence indicated that the kinase enzyme level was reduced in mutants which were deficient in the ability to ferment different carbohydrates, and that the enzyme level increased in cells containing unintegrated

phage 38. It was hypothesized that infecting-phage 38 mated with defective phage-sensitive strains to produce a recombined phage which, while multiplying in the cytoplasm, altered or superimposed a genetic control over cell morphology and carbohydrate metabolism.

THE PRODUCTION OF MUTANTS, GENERALIZED TRANSDUCTION, AND THE LYTIC REACTION IN SALMONELLA GALLINARUM PULLORUM

By

Roy William Snyder

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Dedication

The author wishes to dedicate this thesis to his wife who richly deserves a P.H.T. degree.

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TABLE OF CONTENTS

TABLE OF CONTENTS - Continued

LIST OF TABLES

LIST OF FIGURES

APPENDIX TABLES

APPENDIX TABLES - Continued

INTRODUCTION

Beadle (1957, 1958) has emphasized that the basic problem in biology was the "breaking" of the genetic code of the nucleic acid base pair sequence in DNA and RNA. Jacob (1961) has pointed out that the genes of animal and plant alike existed at their simplest level of organization in the viruses. Luria (1959) has indicated that viruses, particularly bacteriophage, should provide the best materials for deciphering the nucleic acid code that translates DNA or RNA structure into the molecular structure of proteins by correlating genetic structure and function.

A potential system for correlating such genetic structure and function exists in *Salmonella gallinarum pullorum*. Evidence will be presented that genetic control of carbohydrate metabolism may exist at an intermediate level of organization in *S. gallinarum pullorum*, and that infection by a specific bacteriophage may superimpose genetic control at this level. The determination of the manner in which the viral genome may effect such control is a basic challenge in this system.

An advantage of the *S. gallinarum pullorum* system is that it affords an opportunity to study basic processes by both *in vitro* classical methods and *in vivo*. Ravin (1960) has emphasized that *in vitro* studies have an increased significance when applied to a natural ecological environment. Schoenhard (1958) observed that a culture prepared from a mixture of living non-virulent, phage resistant *S. gallinarum pullorum* cells, and dead, virulent, phage sensitive cells of the same species was pathogenic to young chickens. It is the purpose of the author to indicate in an *in vitro* study that a specific *S. gallinarum pullorum* bacteriophage when reproducing in a vegetative state may not only control or affect carbohydrate metabolism and cell morphology of sensitive *S. gallinarum pullorum* bacteria, but that it may also in turn effect an *in vivo* change of virulence of the *S. gallinarum pullorum* for its host.

Study of virus-cell interaction may have many practical applications. Rubin (1959) has hypothesized that "the maintenance of the malignant properties of the Rous Sarcoma is the result of close integration of virus and cell genome" and that "whether cellular alteration itself is due to a specific genetic change in the cell which is directly associated with integration of the virus genome, or is a by-product of restricted virus multiplication, is a completely open question." It is possible that study of the mechanism of interaction between phage 38 and sensitive _S. gallinarum pullorum_ strains may indicate how a vegetatively reproducing virus may disrupt genetic control at the cellular level to produce autonomously reproducing cells as are found in "cancer."

In order to derive a hypothesis concerning the nature of the observed _in vivo_ change in virulence, it seemed desirable to study first the system of transfer to genetic information _in vitro_. Since transduction has been demonstrated in other strains of Salmonella it seemed reasonable to determine whether gene transfer by transduction might be generally more operative than conjugation and transformation in _S. gallinarum pullorum_. Consequently, this thesis was developed from work to determine if transduction occurred. Bacteria with streptomycin-resistant markers were obtained and successful transduction was performed. Bacteria with other markers were then produced and these were transferred by transduction. In the process an unusual interaction between infecting bacteriophage and host bacteria, termed the lytic reaction, was discovered.

This thesis is composed of four sections: Methods, Results, Diccussion, and Summary. Each, therefore, deals with newly discovered methods, their development and use in the production of genetic mutants; the use of these mutants in generalized transduction, conjugation and transformation; and finally a study of the lytic reaction developed to indicate that high frequency transduction is not involved in the reaction but rather that the infecting phage 38 may recombine with defective phage within the host to produce a vegetatively reproducing phage 38 that affects carbohydrate metabolism and other cell functions.

METHODS AND MATERIALS

A. Materials and Production of Mutants

Media

Minimal broth and agar of the formula of Schoenhard (1954) and modified by Brock (1958) (Table 1)[1] with and without supplementation of eighteen amino acids to fiftieth molar concentration were used to select for mutants.

Minimal agar for recombination studies was supplemented with 0.2 mg. streptomycin/ml., leucine to M/50 concentration/ml. 0.04% eosin Y and 0.065% methylene blue/ml.

Eosin methylene blue (EMB) agar of the formula of Lederberg (1950) (Table 2) unsupplemented and supplemented with 0.5% of different carbohydrates was used to show gal+ transduction and to indicate the lytic reaction. Levine's EMB agar (Difco Manual, 1953) was used to attempt to indicate the lytic reaction.

Difco Purple broth and agar unsupplemented and supplemented with 0.5% carbohydrate were used to detect acid production of mutant cells following infection. To prepare the agar, 5 g. galactose and 5 g. K_2HPO_4 were added to one liter of Difco purple broth base; pH was adjusted to 7.0 ± 0.2; 5 g. of Difco agar was added and the medium was melted and autoclaved; and 20 ml. dispensed per petri dish.

Streptomycin agar at 0.15 and 0.5 mg./ml. in BHI agar was used as well as BHI agar overlayed agar with streptomycin in the same concentrations.

Seven-tenths percent soft BHI agar was used for stock culture maintenance and phage production. Seven-tenths percent Bacto agar was also used in phage production.

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[1] Tables in arabic numerals are located in the Appendix.

Difco Brain Heart Infusion (BHI) broth and agar were used to produce and maintain bacterial and viral growth.

Synthetic F buffer of Adams (1949) consisting of 1.0 g. NH_4CL, 0.1 g. $MgSO_4$, 3.5 g. NA_2HPO_4, and 1000 ml. H_2O was used to concentrate and maintain phage 38 for use in enzymatic studies.

Cultures

A number of strains of S. gallinarum pullorum were used (Table 3). Of these, phage from strains 33, 34, 35, 38, 53, and 54 grown on strains 53 or 35, were used to infect strains 7, 13, 14, 20, 25, 33, 34, 35, 37, 38, 47, 51, 53, and 54.

Production of Mutants

Auxotrophs

Modifications of the penicillin technique of Davis and Mingoli (1950) were used to produce auxotrophs.

Penicillin technique and supplement modification

1. The initial observations were performed with cells from a culture grown on BHI agar plates and harvested in saline after 6 to 10 hours incubation at 37° C. depending on the strain and mutant used.
2. Cells were refrigerated from 2 to 12 hours, washed in 0.5% saline, diluted to 10^8/ml. and 7 to 8 ml. were irradiated, at a distance of 80 cm. from a Hanovia ultraviolet lamp turned on 15 minutes previously, in a gently agitated plastic petri dish at room temperature. Irradiation was performed for 22 to 24 seconds to produce about 99% kill (Table 4).
3. Cells were incubated at 37° C. for three hours under conditions to prevent photoreactivation.

4. Aliquot portions of serially diluted irradiated cells were then added to tubes containing 5 ml. of minimal medium. In later experiments, the minimal medium in this step and steps 7 and 8 was supplemented with amino acids, purines, and pyrimidines, omitting the amino acid, purine or pyrimidine for which a mutant was being selected. The cultures were then allowed to refrigerate overnight at 4° C. In this way it was assumed that partial synchronization would occur.

5. Penicillin at a concentration of 300 units/ml. was added to the cold cultures and they were incubated at 37° C. for 24 hours.

6. The tube containing the highest initial concentration of cells that were inhibited by the penicillin was used to select for mutants. Additional aliquots of culture at concentrations above and below the first tube remaining clear were sometimes made to further sharpen the end-point.

7. One-tenth ml. aliquots of cultures were removed from the end-point tube, spread on BHI agar, incubated at 37° C. for 24 hours and replicated to well-dried minimal agar and BHI agar plates. Replica plates were incubated at 37° C. for 48 hours.

8. The colonial growth on minimal agar was compared with that on BHI agar. Minimal agar plates with only a trace or no growth were compared with minimal agar plates containing selected amino-acids or with other supplements. Colonies which grew only on complete and supplemented medium were then selected and they were purified by sequentially suspending in saline and replating on minimal medium to which the growth requirement was added. This process was repeated three times.

<u>Post incubation modification</u> (Strauss 1959, Haas and Doudney 1957)

1. Cells irradiated to 99% kill, as above, were inoculated into minimal medium containing the 19 amino acids and were allowed to incubate with shaking at 37° C. for 30 minutes.

2. The culture was then washed, the pellet resuspended to 1-3 x 10^8 cells/ml. and refrigerated for 30 minutes. These cells were then added to penicillin medium, as above.

Pre- and post-incubation modification (Doudney and Haas 1960)

1. Cultures incubated for 8 hours on BHI agar were washed with 5 ml. of saline/culture, centrifuged and resuspended in saline. An inoculum was added to a 500 ml. Erlenmeyer flask containing 125 ml. of minimal medium supplemented with 19 M/50 amino acids and 0.1 mg./ml. of cytosine and uracil.

2. This culture was incubated with moderate shaking for 3 hours, centrifuged and the pellet was resuspended with saline to 1-3 x 10^8 cells/ml. by direct count. This cell suspension was then placed in the refrigerator for 15 to 30 minutes.

3. Seven to 8 ml. of the cold cell suspension were placed in petri dishes and the cells were then irradiated to about 99% kill, as above.

4. As above the irradiated cells were then subjected to post-incubation and then added to supplemented penicillin medium.

Antibiotic Resistant Mutants

The gradient agar technique of Szbalski (1952) was employed in producing streptomycin, azide and chloromycetin resistant mutants. Gradient plates were prepared by allowing solidification of antibiotic containing agar in a slanted position, and then adding sufficient non-antibiotic agar to produce a level surface. Mutants resistant to 0.5 mg./ml. of Merck streptomycin were also obtained by adding 10^8 cells to melted streptomycin agar at 45°C., pouring and incubating them for 48 hours at 37° C.

1. Young cells, both irradiated and non-irradiated, were streaked on multiple plates from low to higher concentrations of the

antibiotic at 24 hour intervals. It is desirable to begin with gradient plates of about 0.3 mg./ml. maximum streptomycin concentration; about M/300 sodium azide (use M/350, M/300 and M/250), and from 2 to 10γ/ml. of chloromycetin.

2. Growth obtained at maximum antibiotic concentration at a low level was serially transferred to higher concentrations, e.g., from 0.3 mg. to 0.5 mg. streptomycin/ml., from M/300 to M/250 sodium azide/ml., from 2 to 10γ/ml. of chloromycetin. Chloromycetin resistance to 100γ/ml. were obtained in this manner.

Fermentation Mutants

1. Both unirradiated and irradiated cells at concentrations of 100 to 300 cells/ml. were spread on EMB galactose agar and examined for white colonies after 48 hours incubation at 37° C.

2. The technique of Morse (1956) was also employed. Here 300 to 500 cells/0.1 ml. were inoculated into soft agar and poured on the surface of EMBG agar. This procedure produced discrete colonies of uniform size permitting examination of larger numbers of colonies per plate than by the preceding method.

B. Transduction, Conjugation and Transformation

Transduction Procedure

Production of Filterable Agent (F.A.)

1. About 10^6 actively growing donor cells were spread on BHI agar plates and allowed to incubate 8 to 12 hours at 37° C.

2. Growth was suspended in 5 ml. saline/plate, centrifuged at 5,000 rpm. for 15 minutes and resuspended to 1-3 x 10^8 cells/ml.

3. One-tenth ml. aliquots of donor cells were added to a series of soft agar tubes and infected at a multiplicity of infection (m.o.i) of 10^{-4} with 0.1 ml. of phage suspension, prepared by standard procedure of Adams (1959).

4. Cultures were mixed and poured on warm BHI plates with gentle agitation, allowed to solidify for 5-15 minutes, and incubated for 12 hours at 37° C.

5. Plates showing almost confluent lysis were harvested by adding 3-5 ml. of BHI broth or F. buffer, shredding with a loop, and after allowing plates to set for 3-5 minutes, centrifuging at 5,000 rpm. for 15 minutes.

6. In some instances large volumes of phage supernatant fluid were concentrated by centrifuging for two hours at about 25,000 x g (max. setting of Servell centrifuge), decanting supernatant fluid and resuspending phage pellet in a 3-5 ml. volume. Also, phage have been concentrated in F buffer to 10^{10}-10^{12} phage/ml. and they have been found to hold titer for over a month's time in it.

7. The supernatant was carefully collected and filtered through a small sterile 0.45 mm. dia. Millipore filter. A 0.1 ml. aliquot was spread on a BHI agar plate and incubated at least 12 hours to test for sterility. Filterable agent was then titered by standard procedure (Adams, 1959).

Generalized transduction techniques

Enrichment technique

1. Recipient cells were harvested from BHI agar plates (or broth) at 8 hours, centrifuged and resuspended in saline to a known concentration of 1-3 x 10^8 cells/ml. as determined by direct count using a Petroff-Hauser chamber.

2. Cells were sometimes suspended in medium containing 0.05 M calcium chloride (Luria and Steiner, 1954). One ml. of cell suspension was mixed with one ml. F. A. suspended at a suitable concentration, usually 1-3 x 10^7 phage (m.o.i. 0.1).

3. The F. A.-bacterium suspension was incubated in an agitated water bath at 37° C. for 15-30 minutes.

4. One-tenth ml. of the undiluted mixture was then spread on BHI agar and allowed to incubate for 8 hours to allow for possible phenotypic lag, nuclear segregation, delayed integration, and cell division and stabilization (Adelberg, 1960; Witkin, 1956). Barer (1951) has found that expression of streptomycin transduction was delayed 5 hours or more in E. coli.

5. Cells were resuspended in saline, diluted to 1-3 x 10^8 by direct count, and either spread on agar containing 0.15 mg. streptomycin/ml. or on enriched minimal medium deficient in threonine. Plates were incubated 48-52 hours at 37° C.

Classical technique (Zinder, 1954, 1955, 1958)

Steps 1, 2, and 3 of enrichment technique, above, were used.

4. Aliquots of the filter agent-bacterium mixture, usually 0.1 ml. were spread either on BHI agar, or on supplemented minimal medium deficient in threonine, and on EMBG or BCPG agar.

5. BHI agar plates were allowed to incubate 3 hours at 37° C. to allow for phenotypic expression (above) and then streptomycin in soft agar was over-layed on these plates to a concentration of 0.15 mg./ml. These plates were incubated 48-52 hours at 37° C.

6. Cultures on enriched minimal agar deficient in threonine were incubated 48-52 hours at 37° C.

7. When 0.1 ml. aliquots of the F. A.-bacterium mixture were spread on EMB or BCP agar usually supplemented with galactose, incubation was continued for 5 days to allow selective growth of transductants against a background of untransduced cells. In transduction of the gal^+ marker, aliquots were also diluted to produce a concentration of 100-300 colonies on each of about 100 plates and incubated 48-52 hours at 37° C.

8. As a control on the frequency of background mutation, 10^7 uninfected cells were spread on threonine deficient, streptomycin

containing, and EMBG or BCPG agar plates and incubated as above. Uninfected cells diluted to produce a concentration of 100-300 colonies/plate, were sometimes spread on BHI agar to serve as a control on the degree of phage-bacterium lysis.

Special Streptomycin Transduction Technique

1. Filterable agent was prepared as above using strain 53 cells resistant to 0.5 mg. streptomycin/ml.

2. Colonies of strain 53 and 54 cells resistant to 0.15 mg. streptomycin/ml. were suspended respectively in 1 ml. saline, spread on 1-3 BHI agar plates and incubated 8-10 hours at 37° C.

3. Growth was harvested in 5 ml. saline per plate, centrifuged at 15,000 rpm for 15 minutes and the pellet of cells resuspended usually to 1-3 x 10^8 cells.

4. One-tenth ml. containing about 10^7 cells was mixed with 0.05 ml. of phage usually at m.o.i. 0.1, diluted in saline to produce about 50-100 calonies/plate, spread on well dried BHI agar plates, containing 0.15 mg. streptomycin/ml. and allowed to incubate for 24 hours at 37° C.

5. Colonial growth was then replica-plated to BHI agar plates containing 0.5 mg. streptomycin/ml. (technique of Lederberg, 1952) and incubated 12-18 hours at 37° C.

6. Colonial growth on replica plates was compared with that on original plates using a standard colony counter.

Identity of mutants and transductants

1. Mutants were identified by Gram stain, sugar fermentation pattern and reaction in citrate, urea and soft nutrient agar media. Some mutants were checked by cross-agglutination and transduction, e.g., Gal_1^- gave a slide-agglutination test with a 10^{-4} dilution of strain 53 antisera.

2. Transduced clones were checked for lysogenization by routinely placing a drop of a suspension of cells on a lawn of recipient cells and

observing for lysis after 12-16 hours incubation at 37° C. Transductants were also checked by Gram stain, fermentation pattern, citrate utilization and urea hydrolysis.

Preparation of antisera

Antiserum against bacteriophage and bacteria was prepared by modification of the methods of Staub and Tinelli (1959).

1. Adjuvants usually of 10^{10} *Corynebacterium xerosis* cells/ml. were ruptured by exposing to maximum setting of an ultra high frequency sonicator for 15 minutes and heat killed at 60° C. for 30 minutes, 0.1/ml. of the sonicate was injected intraperitoneally in young rabbits.

2. Adjuvant injection was repeated after 3 days using 0.5 ml.

3. After one week, 0.1 ml. of BHI broth containing about 10^8 phage 38, was injected intravenously into the marginal vein of a rabbit. Five-tenths ml. was injected every 3 days for three additional injections.

4. Fifty ml. was bled from the heart after an additional three day period. The blood was allowed to clot at 4° C. in 15 ml. amounts on the flat surface of a dilution bottle for a few hours; the clot was loosened aseptically and syneresis allowed to continue for 16-24 hours. The clear sera was removed aseptically.

5. A similar procedure was followed for respective cultures of cells which were washed, suspended at about 10^9/ml. in saline and heat killed at 60° C. for 30 minutes. An initial injection of 0.1 ml. was made subcutaneously in the abdomen 1 week after adjuvant treatment. This was followed by injection of 0.1 ml. intravenously and then 0.5 ml. at 3 day intervals over a three week period. Fifty ml. heart blood was withdrawn by cardiac puncture, allowed to clot and serum removed.

Conjugation Technique Procedure
(Technique of Jacob and Wollman, 1958)

1. Single colonies of thr^-L^s 54 cells and gal^-S^r 53 cells, respectively, were suspended in saline, spread on 3-5 BHI agar places

and incubated for 8-10 hours at 37° C.

2. Growth was harvested in 5 ml. portions of saline per plate and centrifuged at 5,000 rpm. for 15 minutes. Pellets were resuspended to 1-3 x 10^9 cells/ml. with saline.

3. One-tenth ml. aliquots containing 10^7 and 10^8 thr$^-$L^S 54 cells were mixed, respectively, with 0.1 ml. 10^7 and 10^8 containing 10^8 gal$^-$S^r 53 cells and spread on a dozen plates containing minimal agar supplemented with 0.15 mg. streptomycin/ml., and with 17 amino acids, but deficient in threonine. Cultures were incubated for 48 and 96 hours at 37° C.

Transformation Control Technique

1. Gal$_1^-$ 53 colony was suspended in 1 ml. saline, spread on 5 BHI plates and incubated 8-10 hours.

2. Growth was harvested in 5 ml. saline/plate, centrifuged at 5,000 rpm. for 15 minutes and the pellet resuspended in 10 ml. of saline to a concentration of about 3 x 10^8 cells/ml.

3. One hundred mg. crystalline DNA-ase was dissolved in 3 ml. distilled water.

4. One ml. of the phage 38, previously grown on gal$^+$ 53 cells, was added at m.o.i. 0.1 to 1 ml. of the DNA-ase gal$_1^-$ cell mixture and incubated at 37° C. for 30 minutes, diluted to produce a concentration of 100-300 colonies/ml., spread on 8-12 EMBG agar plates and incubated 48-52 hours at 37° C.

5. 10^8 cells were infected, plated and incubated as above but in the absence of DNA-ase. Uninfected cells were also similarly diluted and spread on EMBG plates.

C. The Lytic Reaction and Related Techniques

Lytic Reaction Technique

Steps 1 through 3 of enrichment technique were followed.

4. Cells were diluted to produce a concentration of 100-300 colonies/ plate and spread usually on EMB agar supplemented with or without galactose and on BCP agar supplemented with or without galactose. Cultures were incubated 48-52 hours.

5. Uninfected cells diluted to produce 100-300 colonies per plate were spread on 3-5 BHI agar plates to determine the degree of lysis in bacteria-phage mixtures and usually on 8-12 EMBG agar plates as a control against infection. Cultures were also incubated for 48-52 hours at 37° C.

Technique for the Effect of Acriflavin on Lytic Reaction (Hirota 1957, 1958)

1. Gal_1^- cells were subcultured on BHI agar plates, grown from 8-10 hours on BHI agar plates, harvested, washed and resuspended to 2×10^8/ml. as above.

2. One and five-tenths ml. cells were infected at m.o.i. 0.1 with 1.5 ml. phage 38, previously grown on gal^+ 53 cells, incubated for 15 minutes at 37° C. in a water bath.

3. A one ml. aliquot of infected cells was added to 10 ml. of BHI broth and another aliquot of BHI broth containing 20γ/ml. of acriflavin. One ml. of uninfected cells also was added to 10 ml. BHI broth containing 20γ/ml. of acriflavin. Cultures were incubated on a rotary shaker for 24 hours at 37° C.

4. Cells for all three cultures, respectively, were diluted to produce a concentration of 100-300 colonies/ml. and spread on 8-12 EMBG plates and incubated for 48-52 hours at 37° C.

5. Also one-tenth ml. aliquots of uninfected cells from acriflavin cultures were spread on EMBG and BHI agar plates and incubated 12 hours to show phage induction.

Beta Galactosidase Assay Technique (Based on Lederberg, 1950).

1. The initial technique consisted of growing S. gallinarum pullorum for 8-10 hours on BHI agar plates, harvesting in 5 ml. saline/plate, centrifuging at 5,000 rpm for 15 minutes, and resuspending in saline containing 1% melibiose to a concentration of about 5 x 10^9 cells/ml.

2. These washed cells were added to 500 ml. Erlenmeyer flasks containing 100 ml. of melibiose-saline. These cultures were incubated at 37° C. with shaking for two hours to insure any β galactosidase induction.

3. Cultures were centrifuged and cells resuspended in saline to a concentration of about 10^{10}/ml. One ml. of undiluted, "induced" cells was added to each of 3 small test tubes, a drop of tolulene added to each tube, and the tubes were incubated in a water bath at 30° C. for 30 minutes. Three-tenths ml. H_2O and 0.7 ml. saturated Na_2CO_3 was added to tube 1. Three-tenths ml. of M/100 α-nitrophenyl-β-galactoside (ONPG) in M/4 Na phosphate buffer at pH 7.3 was added to tubes 2 and 3. Seven-tenths ml. of Na_2CO_3 was added immediately to tube 2. Tubes were incubated at 30° C. for 10 minutes and 0.7 ml. Na_2CO_3 was added to tube 3. Tubes were inspected visually.

Modified β-galactosidase Technique (Kuby and Lardy 1953)

1. In this procedure S. gallinarum pullorum cells were grown and harvested as above but this time washed cells were inoculated into a 500 ml. Erlenmeyer flask containing 100 ml. of minimal media supplemented with amino acids and 1% lactose. The initial cell concentration was about 1 x 10^7/ml. This culture was incubated at 37° C. with shaking for 12 hours, the cells suspension was washed twice with saline and the pellet adjusted to a cell concentration of about 10^{10}/ml.

2. One drop of benzene was added to each of 3 tubes and one ml. cell suspension added to each tube. Tubes were incubated at 37° C. for 30 minutes.

3. Three-tenths ml. H_2O and 0.7 ml. Na_2CO_3 were added to tube 1.

4. Three-tenths ml. of M/75 ONPG in M/4 phosphate (pH 7.3) was added to tubes 2 and 3 and 0.7 ml. Na_2CO_3 added immediately to tube 2. Tubes were incubated at 37° C. for 10 minutes and read.

Galactokinase Determination Technique (Klein, 1953; Scott and Melvin, 1953)

1. An inoculum of 300 ml. of 10^7 cells/ml. of wild-type 53, gal_1^-, and infected gal_1^- cells respectively, was added to a flask containing 3 liters of minimal medium supplemented with 18 amino acids. The culture was incubated for 14 hours at 37° C., and the respective cells were collected by centrifugation using a Spinco-angle-centrifuge.

2. Pellets were resuspended respectively in 20 ml. cold phosphate buffer and exposed to sonic vibration at a maximum setting in a 250 watt Raytheon Sonic oscillator for 15 minutes.

3. The culture sonicates then were centrifuged at 15,000 rpm for 5 minutes and 2 ml. aliquots of cell free supernatant dispensed in plastic tubes. All but two samples were frozen at -20° C.

4. One ml. of each enzyme preparation was added to two tubes containing respectively, 0.5 ml. of galactose (5 micro-moles), 0.2 ml. of ATP (10 micro-moles), 0.2 ml. Mg^{++} (10 micro-moles) and 1.1 ml. of phosphate buffer (pH 7.0) (30 micro-moles).

5. Each tube was mixed by inversion and tube 2 was placed in a 30° C. water bath for 30 minutes.

6. Using the method of Somogi (1945), five-tenths ml. of sample was withdrawn from tube 1 at zero time and added to a tube containing 1 ml. of 0.3 N $Ba(OH)_2$. One ml. of 5% $ZnSO_4 \cdot 7H_2O$ was quickly added

to neutralize the $Ba(OH)_2$ and the tube mixed well by inversion and kept in an ice-water bath.

7. Tube 2 was removed after 30 minutes incubation and treated with $Ba(OH)_2$ and $ZnSO_4$ and also kept in an ice-water bath.

8. Two ml. of neutralized initial and final sample were added respectively to 8 ml. stoppered tubes of distilled H_2O and mixed well by inversion. These tubes were chilled to below 10° C.

9. Five ml. of respective samples was carefully overlayed on 10 ml. anthrone (0.2%). Control tubes of 10 ml. of anthrone were prepared with 1 ml. containing 10 micro-moles and 1 ml. containing 50 micro-moles of galactose. Tubes were stoppered, thoroughly mixed by inversion, and the tubes held in ice-water during the process.

10. Samples were brought to room temperature and placed in a 90° C. water bath for 16 minutes, cooled to room temperature and read in a Beckman colorimeter at a wave-length of 625 milli-microns.

RESULTS

A. Production of Mutants

Auxotrophs

Aspects of the penicillin technique of Adelberg and Meyers (1952) were used to modify the technique of Davis and Mingoli (1950) in selecting for nutrient dependent mutants (auxotrophs). Synchronization of growth was obtained upon incubation at 37° C. of serially diluted inocula of irradiated cells in penicillin-minimal medium following overnight refrigeration. A clear-cut end-point was produced after 24 hours incubation. Tubes below the end-point became turbid and permitted massive growth of wild-type cells when aliquots were plated on complete medium; at the end-point, a minimum effective concentration of penicillin permitted growth of only a few wild-type colonies when 1 ml. aliquots were plated; and above the end-point growth of both wild-type and potential auxotrophs were inhibited for all but the peripheral areas of a plate. However, attempts to select for absolute mutants e.g., arginine were unsuccessful. Since *S. gallinarum pullorum* requires arginine for optimum growth, attempts were also made to select for mutants by replicating colonies directly from complete to minimal medium, without success. One hundred and seven colonies showing poor or no initial growth on minimal medium were passed through four cycles of purification and in all instances growth was obtained on minimal medium.

Since the principle of selective supplementation had been used successfully by Snyder (1955) to obtain absolute auxotrophs in the basidiomycete *Schizophylum commune* Beltsville and by Woodward, De Zeeuw, and Srb (1954) with the ascomycete *Neurospora crassa,* it was tried with

S. gallinarum pullorum. All but one amino acid, purine or pyrimidine were added to the minimal-penicillin medium, and auxotrophs were again obtained successfully. By this method an absolute mutant dependent on threonine was found in about 2-4 x 10^8 cells and potential adenine and thymine dependent mutants in 7-10 x 10^6 cells. Arginine, threonine, adenine, thymine, and leucine sensitive mutants were produced in this manner. In order to determine whether post-irradiation incubation in an amino acid rich medium produced mutants by mutation stabilization as has been postulated by Doudney and Haas (1960) and by Strauss (1959), three experiments were performed using their techniques. However, only partially deficient ("leaky") threonine and proline dependent mutants were obtained. The approximate frequencies with which the auxotrophs were found are indicated in Table 5.

A threonine dependent clone of S. gallinarum pullorum 53 was obtained at a frequency between one in 10^2 to 10^3 cells by growing phage 38 on thr^- strain 54 and using the resultant phage to infect strain 53 at a m.o.i. of 0.1. It is possible that this clone was obtained by a process of infection (see gal. mutants below).

Antibiotic Resistant Mutants

Twenty-three mutants of S. gallinarum pullorum 53 and 54 resistant to 0.5 mg. streptomycin per ml. were obtained largely by the gradient plate technique and a few by direct plating in streptomycin agar for transduction purposes. From these mutants one was selected which produced maximum growth at 0.5 mg. streptomycin per ml. and was used to obtain galactose markers. Six mutants were also obtained for each strain which were resistant to intermediate levels of streptomycin to be used in a special transduction experiment. Chloromycetin and azide resistant mutants were also similarly produced by the gradient plate technique for eventual comparison with streptomycin by transduction, and for other studies.

Fermentation Mutants

Three galactose negative mutants of S. gallinarum pullorum 53 were obtained after examining about 15,000 colonies produced from irradiated cells plated on EMB galactose agar. Irradiated cells suspended in soft agar and poured on EMB plates after the method of Morse (1956) produced discrete colonies and permitted examination of greater numbers of colonies per plate. One galactose mutant (gal_1^-) proved satisfactory for inter- and intra-strains transduction experiments. This gal_1^- mutant does not ferment galactose, rhamnose, mannose, mannitol, ribose and xylose (Table III, page 35).

Galactose negative colonies may have been obtained by an infection process. When gal^+ 53 cells were infected at m.o.i. 0.1, with phage 38 grown on gal^+ 53, in addition to a number of partially lysed colonies (termed the "lytic reaction" below), galactose negative colonies which did not contain phage were produced at an approximate frequency of 1 in 10^4 cells in three experiments. Also in preliminary experiments in which phage 38 was grown on gal^+ strain 35 and used to infect gal^+ 35 at m.o.i. 0.1, gal^- colonies were produced at a frequency of 1 in 10^3 cells in addition to the lytic colonies. These gal^- colonies did not contain bacteriophage. Only galactose positive colonies were present on uninfected control plates. The gal_1^- 35 mutant ferments all carbohydrates fermented by wild-type except galactose which contrasts the multiple negative fermentations of gal^- 53 mutants (Table III, page 35).

B. Transduction, Conjugation and Transformation

Generalized Transduction

Fermentation

In three experiments in which EMBG spread plates initially containing 10^7 infected cells were examined, intra-strain transduction was obtained for galactose fermentation from gal^+ strain 53 to gal^- 53

on BCP galactose after frequencies varying from one in 6 to 16 x 10^4 plaque-forming phage (Table 6). The average frequency of transduction at m.o.i. 0.1 was 1.4 x 10^{-5} (Table I). The transduced galactose fermenting colonies appeared large and yellow at 3 days of incubation and after five days of incubation they developed a blue color. The background of growth remained white. In experiments in which 10^3 infected cells were spread on EMB agar one transduction in about 3 x 10^4 cells was observed. Here, the transduced colonies appeared large and yellow and the medium around the colony for several centimeters distance appeared yellow in contrast to the blue background. The m.o.i. was varied from 5 to 0.0001 plaque-forming phage per bacterium and the optimum was found at m.o.i. 0.1 (Table 7). At m.o.i. 0.1 these colonies did not contain phage. These transduction frequencies are in sharp contrast to the mutation frequencies of one in 2 to 3.5 x 10^8 bacteria (Table 6--0.0 m.o.i.).

Auxotroph

Inter-strain transduction from $thr^-gal^-S^r$ strain 53 to $thr^-gal^+S^s$ strain 54 was obtained in three experiments using the technique of infected cell enrichment (see Methods). The frequency of transduction varied from one in 2.1 to 2.9 x 10^5 phage at an optimum m.o.i. of 0.5 whereas, at m.o.i. 0.1, the transduction frequency varied from one in 4.0 to 5.1 x 10^5 phage (Table 8). The average frequency of transduction at m.o.i. 0.5 was 2.3 x 10^{-6} (Table I). By contrast the background mutation frequency of transduction was from one in 3 to 10 x 10^8 bacteria (Table 8--0.0 m.o.i.).

Antibiotic resistance

Using the technique of infected cell enrichment (see Methods), inter-strain transduction at m.o.i. 0.1 was obtained between 53 S^r and 54 S^s at a frequency of one in 8 x 10^5 phage when plated on agar

Table I. Average Frequencies of Transduction in *Salmonella gallinarum pullorum*.

	Experiments		
	1	2	3
1. Galactose fermentation			
Frequency--one in:	1.6×10^5	7.4×10^4	6.0×10^4
Average frequency:	one in $9.8 \times 10^4 = 1.0 \times 10^{-5}$ plaque-forming phage.		
2. Threonine independence			
Frequency--one in:	5.1×10^5	4.0×10^5	4.0×10^5
Average frequency:	one in $4.4 \times 10^5 = 2.3 \times 10^{-6}$ plaque-forming phage.		
3. Streptomycin resistance			
Frequency--one in:	8.3×10^5	4.5×10^5	
Frequency at 0.1 mg. strep./ml.:	one in $4.5 \times 10^5 = 2.2 \times 10^{-6}$ plaque-forming phage.		

supplemented with 0.5 mg./ml. streptomycin. Using 0.1 mg./ml. streptomycin agar, a frequency of one in 4.5 x 10^5 phage was observed and one in 2.4 x 10^5 phage at m.o.i. 0.5 (Table 9). The average frequency of transduction was 4 x 10^{-6} (Table I). The frequency of background mutation was one in 5 x 10^9 bacteria (Table 9-.0 m.o.i.)

Experiments were also performed to transduce markers from high (0.5 mg./ml.) to intermediate levels (0.15 mg./ml.) of streptomycin resistance (Table 10). Phage 38 was grown on strain 53 cells resistant to 0.5 mg./ml. streptomycin, and these phage were mixed and incubated at m.o.i. 0.1 respectively with 53 and 54 cells resistant to low but not high levels of streptomycin.

Following enrichment (above), infected cells were spread-plated on BHI agar and colonies were replicated to plates containing 0.5 mg./ml. streptomycin. Growth on all plates was interpreted as being due to the high cell concentration of the replicated colony which reduced the concentration of streptomycin per cell. With such large inocula some selection for mutant cells may also have occurred. It may be seen (Table 10) that for both strain 53 and 54 the frequency of resistant colonies from uninfected cells was higher than from infected. Since lysis was not appreciable at m.o.i. 0.1, an explanation from these results was not apparent. Two additional experiments were performed with decreasing m.o.i. using wild-type cells sensitive to streptomycin. It was observed that as the number of wild-type cells spread on control plates was increased, the number of clones resistant to streptomycin increased proportionately, but as the number of infected cells were increased per plate, the number of resistant clones did not increase.

Each of 25 resistant colonies from infected plates at m.o.i.'s of 0.001 was found to be lysogenized,i.e., produced phage when a drop of respective colonies was placed on a lawn of sensitive cells ("spotted")

and incubated; whereas, colonies from control plates were uniformly sensitive, i. e., capable of being infected and not producing phage. These lysogenized colonies were presumably produced by transduction and have been observed similarly by Morse (1959) working with *Staphylococcus aureus.*

Several streptomycin and threonine dependent transduction reactions have been performed subsequently with negative results, using classical transduction techniques and relatively more lytic phage, i. e., selected either from heat-killed strain 38 cells or by soft agar technique at 45° C. with negative results. In two instances transductions were obtained at about 1 in 10^7 cells, whereas, a background mutation rate of about 1 in 10^8 cells was observed. In view of the enrichment technique used for the early transduction of threonine and streptomycin markers and the early technique for selection of phage, it would seem that the known high degree of lysis at m. o. i. 1 and above for the more lytic phage used subsequently, obscured transduction by classical methods. Hartman (1961) has stated that low frequencies of transduction will arise if virulent or semi-virulent phages are used. Variability in transduction efficiency with conditions of selection of temperate phage also have been reported by Hartman (1961), Campbell (1958), and Fraser (1957).

Conjugation

Ten million and one hundred million $thr^+leuc^+S^rgal^-$ strain 53 cells were mixed respectively with 10^8 $thr^-leuc^sS^sgal^+$ 54 cells and plated on synthetic EMB medium containing 0.2 mg./ml. streptomycin, galactose and leucine but deficient in threonine. Negative results were obtained on 12 plates for both experiments. These results imply that recombination does not generally occur in *S. gallinarum pullorum* 53 since 6-8 recombinants per plate are generally found with *S. typhimurium* in four factor crosses (Lederberg, 1947).

Transformation

Galactose transduction and the lytic infection reaction were obtained in the presence of DNA-ase without detectable alteration in the frequency of the reactions. Under the conditions of these experiments one would expect destruction of any transforming principle which otherwise might affect these phenotypes.

C. The Lytic Reaction

When *S. gallinarum pullorum* was infected by phage 38, various patterns of partially-lysed, galactose-fermenting colonies were produced at a high frequency. Using cells of different genetic composition, e.g., infection of gal$^-$ strain 53 at m.o.i. 0.1 by phage 38 previously grown on gal$^+$ 53 cells (gal$^-$ 53^{38+}), produced pink-centered, red-ringed and all red lytic colonies when plated on EMB galactose (EMBG) agar and incubated from 52 to 60 hours at 37° C. All blue transduced colonies also were observed but infrequently on the same EMBG plates. On brom-cresol-purple galactose agar, light yellow colonies appeared (Figure 1) that corresponded to the pink-centered colonies grown on EMBG agar (upper right of Figure 2). These yellow colonies were observed to exhibit craters of lysis more clearly than the pink-centered colonies. The yellow color also confirmed the galactose fermenting character of the lytic reaction.

A composite distribution of colony types observed is indicated in Table II. Only about 15 to 25% of the cells exposed to phage 38 at m.o.i. 0.1 remained uninfected and sensitive. Forty to 60% of exposed cells were infected, lysogenized white colonies exhibited no apparent phenotypic change from uninfected colonies. The lytic reaction ranged from 1 to 45% of exposed cells but in early experiments averaged 20% (Table 11). Initial variations in technique as well as by chance, of

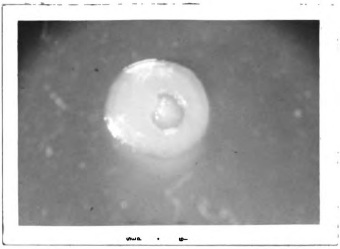


Figure 1. Colony of Gal$^-$ 53^{38+} Cells Showing Lysis.

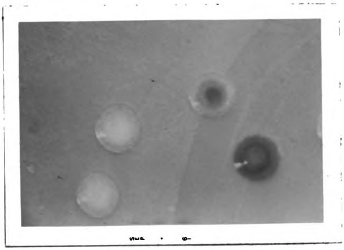


Figure 2. Colonies of Gal$^-$ 53^{38+} Cells Showing Fermentation on EMBG Agar.

Upper right - "pink-centered" colony.
Lower right - "red-ringed" colony.

Table II. Percent Colony Distribution of Gal$^-$ Cells Exposed to Phage 38.*

	Infected (75 - 85%)			Uninfected
	Lytic (10 - 30%)	White lysogenized	Blue transd.	White sensitive
Pink-centered	5-15%	40-60%	0.001%	15-25%
Red-ringed	5-15%			
All-red	1- 4%			

*All percentages are parts of the total 100%.

course, account for some variation in reaction. From 20 to 35% existed with variously derived phage suspensions. Phage selected for transducing ability routinely gave frequencies between 10 and 20% lytic reaction whereas more lytic phage of high titer routinely produced 20 to 30% reaction. For a given preparation of phage, reactions were usually about 2 to 5% higher in the first week. A similar observation was reported by Wilson (1960). Using cells of the same genetic composition (isogenetic) phage 38 was grown on gal^+ 53 cells (wild-type) and used to infect gal^+ 53 cells (wild-type), reaction frequencies of 30 to 35% were obtained (Table 12) (see below).

The pink-centered and red-ringed colonies (upper and lower right colonies, Figure 2) occurred in about equal numbers e.g. of 405 lytic colonies, 205 were red-ringed and 200 pink-centered. The all-red colonies appeared extremely rough when observed under the dissecting microscope. They were found in large groups on about 2% of the plates, sometimes in the absence of pink-centered and red-ringed colonies.

Since transduction to and from identical gal^- cells should produce only gal^- colonies, the pink-centered, red-ringed and all-red colonies obtained when phage 38 was grown on gal^- 53 and used to infect members of the same gal^- clone, clearly shows that transduction was not involved. Even wild-type cells which would be expected to contain the same alleles and hence show only dark blue colonies with a sheen when phage 38 was grown on wild-type 53 cells and used to infect wild-type 53 cells, produced distinctly lytic red-blue ringed and centered colonies when examined under the dissecting microscope after 24 hours incubation.

It is known that decrease in temperature of incubation increases the frequency of lysogenization (Bertani, 1958). The results in Table 13 showed that the frequency of the lytic reaction was not decreased at 24° C. but in fact were slightly increased as compared to incubation at 37° C. It would seem therefore that the lytic colonies contain phage which did not integrate.

Study of the pink-centered colonies revealed that the pink, lytic centers contained phage at a very high titer of about 10^8 phage per ml. The peripheral white cells from such colonies were similar in appearance to the large number of white infected cell clones and indeed produced phage at a similar low titer on spot plate tests. Normally lysogenized cells are known to produce phage spontaneously at a low titer of about 10^3 per ml. (Bertani, 1958). The non-lytic but infected white colonies remained stably lysogenic and did not segregate lytic pink-centered, red-ringed or all-red colonies. By contrast, cells from the lytic pink center, from the red-rings and from the all-red colonies, were highly unstable. These cells produced only about 10% lytic colonies when subcultured on EMBG agar (Table 14). In turn cells from the first subculture lytic colonies produced only about 2% lytic colonies on EMBG agar (Table 14). Such results implied that the lytic colonies contained some phage which did not integrate, but multiplied vegetatively in the cytoplasm to produce a high titer of phage. Fukazawa (1961) has similarly observed that E. coli cells infected with P22 produced 20% blue colonies containing 10^8 phage on alarizin-yellow soluble-blue selective medium, whereas infected lysogenized colonies remained white. He found similarly that these white colonies remained stably lysogenic and did not segregate blue colonies. He also found similarly that cells from the blue colonies segregated only 10% blue colonies and concluded that phage in the blue colonies existed in an unintegrated state.

Campbell (1958) has shown that,in specialized transduction with lambda phage, a helper phage was necessary,since a plot of the efficiency of transduction with decreasing m.o.i. instead of producing a straight line, produced a curve which leveled off at a m.o.i. of 0.03 phage per bacterium. At this point the probability of both helper phage and defective phage infecting the same bacterium became zero. By contrast, in several of my experiments, a linear relation has been obtained

between the efficiency of the lytic reaction and decreasing m.o.i. to below m.o.i.'s of 0.01. In one experiment a straight line relation was found to below m.o.i. 0.0001 (Figure 3, Table 15). Hence, it would seem that a single phage is capable of eliciting the lytic response.

The fact that the lytic reaction was obtained on strains 13, 20, 25, 35, and 53 which were all sensitive strains and not on strains 14, 33, 34, 47 or 54 which were normally lysogenic, contained an implication. Yamamoto and Anderson (1961) and Zinder (1958) have found recombination of infecting phage with other defective phage contained within the host. Since all sensitive bacterial strains suspected of carrying defective phage have been found to be able to produce phage particles carrying a defective genome when a superinfecting normal phage introduces the missing function (Arber and Kellenberger, 1958) and since defective phage are thought to be present in all sensitive strains, it would seem reasonable that phage 38 might induce defective genomes in the sensitive strains above, and by recombining with them produce defective phage 38 incapable of integrating in the particular bacterium involved. Possible recombination of phage 38 with defective phage 53 could explain how a single infecting phage 38 could produce cells containing unintegrated presumably defective phage in "sensitive" bacteria as has been observed above. Recombination could also explain how cells from the white bacterial colonies, stably lysogenized with phage 38 (above), were superinfected by phage 38 to produce the typical lytic reaction. The morphology and frequency were in no way altered from the lytic reaction produced in sensitive bacteria. In addition, recombination could explain in part how different lytic phenotypes were produced when phage 38 presumably recombined with different defective phage in various sensitive strains e.g. in strain 25 lytic colonies were produced of only one phenotype, dark red with white centers. In normally

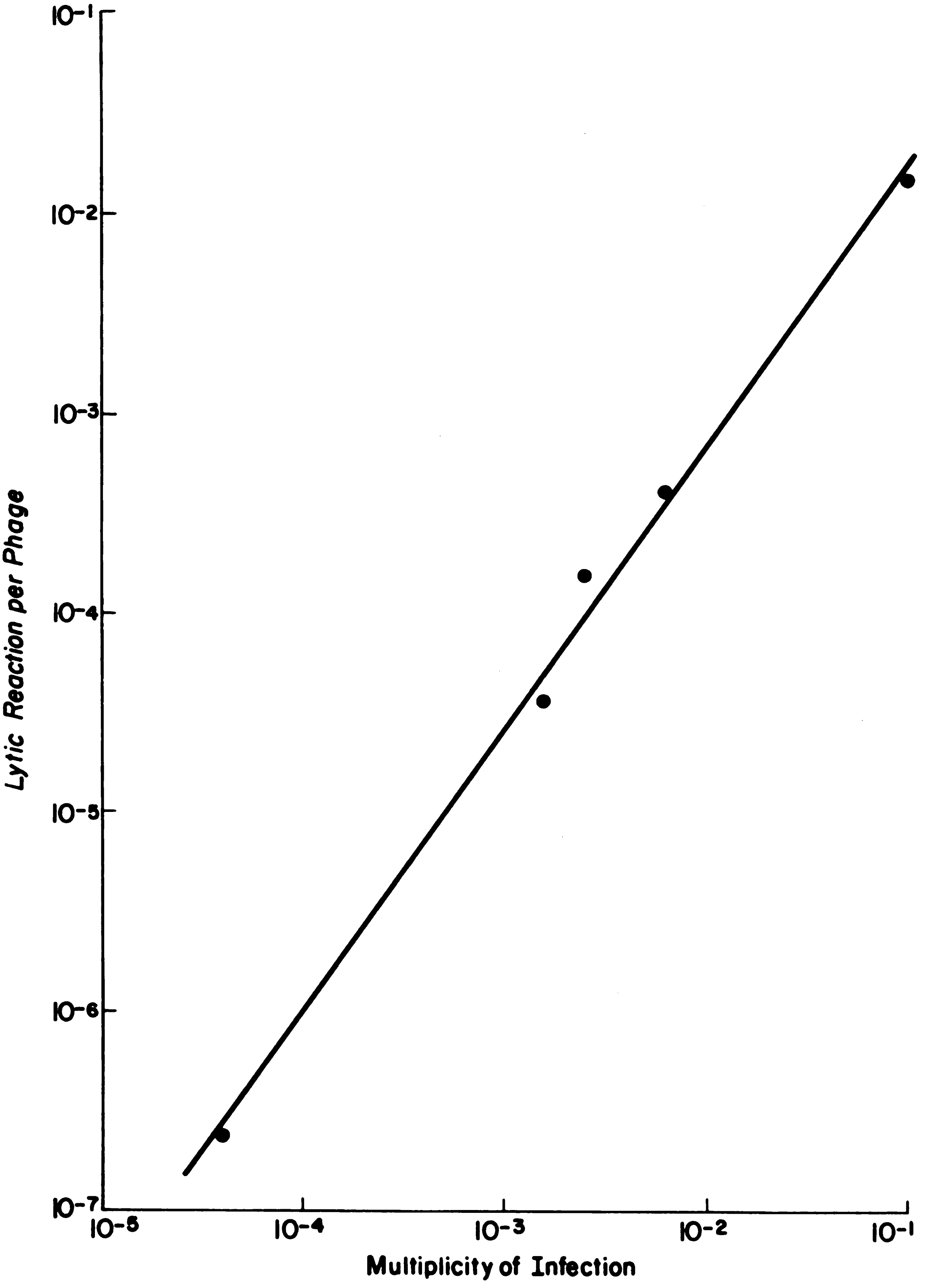


Figure 3. Efficiency of the lytic reaction as a function of the multiplicity of infection.

lysogenic strains, phage 38 recombination would be expected to produce only "complete" phage capable of integration as has been found.

Additional support for the recombination concept exists with the results from acriflavin treatment. Wanatabe and Fukasawa (1961) hypothesize that acriflavin is capable of inducing integrated material to become unintegrated. Uninfected gal$^-$ 53 cells exposed to acriflavin after the technique of Hirota (1958) and plated at a concentration of about 10^8 per plate, produced plaques after 12 hours incubation at 37° C., whereas untreated cells did not. In preliminary experiments, ultraviolet irradiation of 53 cells before infection has similarly been found to produce plaques. Also, infection of a lawn of strain 53 at a m.o.i. of 10^{-5} by phage 38, grown on wild type 53 cells, occasionally produces small, faint plaques termed "petites" in addition to the large turbid and small clear plaque types normally found for phage 38 on different indicator strains. Phage from 40 to 50 petite plaques, 5 to 10 acriflavin and u.v. induced plaques respectively, all produced plaques in a lawn of 10^7 cells but at only a titer of about 10^2 per ml. One to 3 plaques of phage 38, produced by standard procedure, and plaques from lytic centers of pink-centered colonies respectively produce a titer of about 10^8 phage/ml. It would seem therefore that strain 53 does not contain defective phage of the type found in specialized transduction (above), but rather of a type which always produces a few active phage (Kellenberger, 1959). Furthermore, the induction of such defective phage seems to be correlated with an increase in frequency of the lytic reaction. Twenty-four hour incubation of phage and bacterium in liquid medium containing acriflavin after the method of Hirota (1958) produced a 20% lytic reaction. In the absence of acriflavin, only a 1% lytic reaction was obtained. Similar results were obtained on repeated experiments (Table 16). Similarly, in preliminary experiments, irradiated cultures which produced phage 53 at a low titer, also showed a definite increase in frequency of

the lytic reaction as compared with unirradiated cell infection. It would seem in these cases that acriflavin or ultraviolet irradiation as well as infection by phage 38 induced defective phage 53 to multiply vegetatively and to recombine with vegetatively multiplying phage 38. In this manner, a greater number of defective phage 53 would be produced with an increased probability of recombining with infecting phage 38 to produce an increased frequency of the lytic reaction.

Further support for the recombination concept exists in the pattern and segregation behavior of the lytic colonies. Each of the red-ringed, pink-centered and all-red colonies were observed to develop at different time intervals and with differing times of expression of the lytic reaction. It is known that phage DNA exists in a two-strand state in most phage (Sager and Ryan, 1951), and that as many as five cyles of mating(Doerman, Chase and Stahl, 1955), usually one for temperate phage (Stent, 1958), may occur before maturation begins. It would thus be possible for infecting phage 38 to recombine with different portions of the defective phage to produce all-red, red-ringed, pink-centered, and normally lysogenized colonies. Similarly phage from each lytic pattern would be capable of recombining in daughter clones to produce the other lytic patterns observed i.e., each lytic colony type segregated out the other types.

Zinder (1960) has described a red color change along the line of intersection of F$^-$ (fertility factor negative) strains of Salmonella and Escherichia coli when cross-streaked with F$^+$ E. coli K12 on EMB agar in the absence of carbohydrate. He attributed this color change as due to some surface to surface interaction sufficient to damage F$^-$ cells allowing eosin to penetrate the cells and stain the acid cytoplasm. When gal$^-$ 53 cells were infected with phage 38 and plated on the complete EMB agar of Lederberg (Table 2) in the absence of carbohydrate, the pink-centered and red-ringed colonies were still observed. However, when similarly infected cells were inoculated into unsupplemented brom-cresol-purple broth or on to purple agar, no color change was noted.

Here, the brom-cresol-purple agar was also buffered to an alkaline pH of 7.6 so that the presence of a slight amount of acid would be expected to effect a color change. When galactose was added to purple broth and agar, infected gal$^-$ cells produced acid and gas within 6 to 10 hours in broth and yellow colonies on agar after suitable incubation. It would seem, therefore, that the pink-centered and red-ringed colonies might be due to an eosin dye staining effect since no acid change was observed in brom-cresol-blue indicator medium supplemented with carbohydrate. Furthermore, preliminary experiments have indicated that a genetic change affecting fermentation has occurred in the lytic reactions. Weismeyer (1961) has indicated that induction of lambda phage in *E. coli* in the absence of carbohydrate has resulted in an increase in galactokinase activity. One of his interpretations of these results was that an operon controlling carbohydrate metabolism may have been freed of a repressor gene upon phage induction. It seemed desirable,therefore, to attempt to determine whether a similar induction of enzyme activity might occur in *S. gallinarum pullorum* upon infection with phage 38. Using a modification of the technique of Scott *et al.* (1953), galactokinase activity has been indicated in wild-type strain 53 with no detectable activity in the gal$^-$ mutants, but also none was detectable in the infected gal$^-$ cells. An aliquot of cells 15 minutes after infection produced a 30% lytic reaction when plated on EMBG agar as compared to less than 1% when plated 14 hours after infection and incubation. In order to maintain or selectively increase the proportion of lytic colonies, casein hydrolysate supplemented minimal medium was supplied with galactose. A distinctly increased turbidity was noted with infected gal$^-$ cells as compared to uninfected cells and an aliquot of these infected cells showed a 35% lytic reaction frequency when plated on EMBG agar and incubated. Since casein hydrolysate is not satisfactory for enzymatic studies and good growth as well as the lytic reaction had previously been obtained

with minimal medium supplemented with amino acids, the supplemented minimal medium was used. In an initial experiment using a new supply of amino acids no growth even of wild-type cells was obtained. However, these results would indicate that a genetic change produced a decrease in galactokinase activity in gal$^-$ cells and that infection altered or superimposed a different genetic control.

Koibong, Barksdale, and Garmise (1961) have reported that infection of carrier strains of *Shigella dysentariae* with virulent T 7 *E. coli* phage produced a non-specific fermentation of lactose, mannose and maltose sugars not fermented by wild-type cells. They report that this change in fermentation is due to decryptification of Shigella by a change in permeability of the cell wall due to endolysin. Using the technique of Lederberg (1950) and a modification of the technique of Kuby (1953), I performed assays for the presence of β-galactosidase in *S. gallinarum pullorum* 53 using melibiose and lactose as inducers. No β- galactosidase was detected. Infected *S. gallinarum pullorum* cells have been incubated in the presence of lactose, maltose, salicin, d-arabinose, lyxose, saccharose, dulcitol, adonital and arabitol respectively, all carbohydrates not fermented by wild-type cells, and with negative fermentation results. In the absence of positive results to the contrary, it seems desirable to pursue proof of the concept of a change in genetic control in *S. gallinarum pullorum* cells infected with phage 38.

Examination of Table III reveals that the gal$^-$ mutants are each deficient in several fermentation characters as compared to wild-type cells. Gal$_1^-$ 53 does not ferment galactose, mannose, mannitol, rhamnose, xylose or ribose. Gal$_2^-$ 53 does not ferment rhamnose, fructose or galactose. The extremely low frequency with which one would expect to obtain such multiply deficient mutants almost precludes the nature of these mutants as being a series of single unrelated gene mutations. Moreover, gal$_2^-$ N which differs from gal$_2^-$ by fermenting rhamnose and fructose and not

Table III. Fermentation Spectrum of Salmonella gallinarum pullorum Strains.

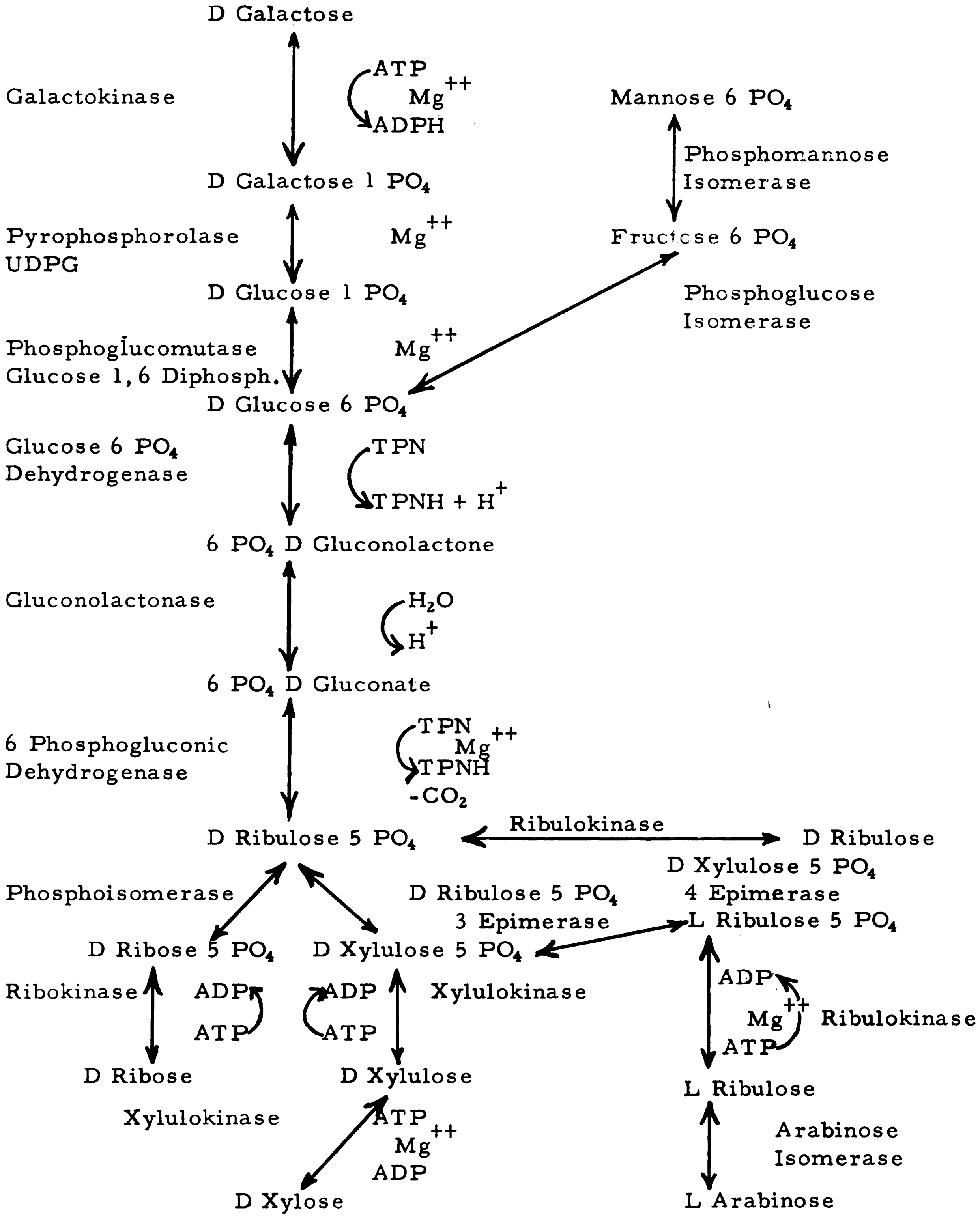
Carbohydrate	Wild-type			Mutants			
	35	53	54	Gal_1^- 35	Gal_1^- 53	Gal_2^- 53*	Gal_2^- 53N
1 Lactose	NC	NC	NC	NC	NC	NC	NC
2 Maltose	NC	NC	NC	NC	NC	NC	NC
3 Galactose	YG	YG	YG	NC x	NC x	NC x	NC x
4 Mannose	YG	YG	YG	YG	NC x	YG	YG
5 Mannitol	YG	YG	YG	YG	NC x	YG	YG
6 Rhamnose	YG	YG	YG	YG	NC x	NC x	YG
7 l Arabinose	YG	YG	YG	YG	YG	YG	NC x
8 Fructose	YG	YG	YG	YG	YG	NC x	YG
9 d Arabinose	NC	NC	-	NC	NC	NC	NC
10 Lyxose	NC	NC	-	NC	NC	NC	NC
11 Dextrose	YG	YG	YG	YG	YG	YG	YG
12 Saccharose	NC	NC	NC	NC	NC	NC	NC
13 Salicin	NC	NC	NC	NC	NC	NC	NC
14 Xylose	YG	YG	YG	YG	NC x	YG	YG
15 Ribose	YG	YG	-	YG	NC x	YG	YG
16 Dulcitol	NC	NC	NC	NC	NC	NC	NC
17 Adonitol	NC	NC	-	NC	NC	NC	NC
18 Arabitol	NC	NC	-	NC	NC	NC	NC

*Mutant obtained by Richard Vaughn, Dept. Micro. Pub. Health, Michigan State University. NC = no change; YG = yellow acid and gas; x = mutant change from wild-type; - = sugar not tested (not available).

fermenting l-arabinose, was discovered on subculturing gal$_2^-$ several times during a two-week period. This mutant indicates that these multiply deficient mutants were probably not produced by gene deletion since wild-type fermentation abilities have been restored. Based on these observations it would seem that multiply deficient mutants were the result of a single gene mutation that simultaneously affects the utilization of a number of carbohydrates. Study of the known pathways of fermentation of the carbohydrates involved (Figure 4) reveals that the absence of no one single enzyme could account for the pattern of negative fermentations observed, e.g. the absence of d-ribulose 5 phosphate 3 epimerase does not account for inability to ferment xylose. Nevertheless, Fukasawa (1960) and others have observed the presence of a number of carbohydrates in the cell wall and that a single mutation will change both the spectrum and amount of carbohydrate present, e.g. where wild-type *S. typhimurium* cells contain galactose, glucose, mannose, rhamnose, and abequose, an "M" mutant contained large amounts of glucose but lacked galactose, mannose, rhamnose and abequose. Perhaps then, genes exist probably at an intermediate level of integration, e.g. as an operon (Jacob and Wollman, 1960), also suggested by Weismeyer above, which can mutate or otherwise be affected to alter complex metabolic sequences presumably involved in cell-wall synthesis and as yet not determined.

Gal$_1^-$ 53 cells infected with phage 38, grown on wild-type 53 cells, (gal$_1^{-38+}$) and plated on EMB agar supplemented with xylose instead of producing pink-centered, red-ringed and all-red colonies, yield only blue-centered colonies (Figure 5). Similarly, cells infected with phage 38, grown on wild-type 53 cells (gal$_2^{-38+}$), and plated on EMB fructose yield small blue-ringed colonies (Figure 6). Varying color intensity and morphological change are also found respectively on mannose, mannitol, rhamnose, ribose and l-arabinose supplemented EMB agar.

Figure 4. Metabolic Paths Involving Galactose Mutants.*



*Adapted from Fruton and Simmonds (1958).

Figure 5. Gal$_1^-$ 53^{38+} Cells on EMB Xylose Agar.

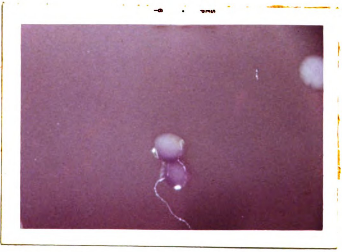


Figure 6. Gal$_2^-$ 53^{38+} Cells on EMB Fructose Agar.

Reproduction of phage components may well depend on the level of energy available which in turn depends on the different source of carbohydrate. Hence, the rate of phage production and consequently the pattern of lysis may depend on the carbohydrate source utilized, as has been observed.

Aliquots of a suspension from a gal$_1^{-38+}$ lytic colony with a relatively high segregation value were plated respectively on galactose, rhamnose, mannose, mannitol, ribose and xylose supplemented EMB agar. Lytic colonies were obtained on all supplements with a small variation in frequency attributable to random distribution and technique of observing colonies at different stages of growth (Table 17). These results suggest a simultaneous induction of the enzymes involved in the utilization of these carbohydrates may have occurred.

Variation in phenotype was also observed as influenced by the genetic constitution of the host cell. Pink-centered, red-ringed and all red colonies are observed among gal$_2^{-38}$ yellow colonies but the phenotype is much fainter and the colony characteristics altered. It has already been mentioned above that the phenotype of the lytic reaction is characteristically different with different strains of *S. gallinarum pullorum*. These differences may reflect an interaction between the superimposed genetic control of the phage and the kind and amount of nutrient, e.g. amino acids, present in the cell due to different although related genes in the host cell. One would expect the identical nutrient complement in recipient cells as was present in the cells on which phage 38 was grown in the lytic reactions of isogenetic material (gal$_1^-$ 38 on gal$_1^-$). This identical complement may facilitate the vegetative multiplication of the unintegrated phage and account for the routinely higher frequency of the lytic reaction observed with isogenetic material (30 to 35%).

It was also previously mentioned that the lytic reaction was observed on the complete EMB medium of Lederberg in the absence of carbohydrate. However, no lytic reaction was observed on the EMB medium of Levine which does not contain casein hydrolysate. This observation led the author to check for the lytic reaction on minimal medium supplemented with the 19 amino acids. A 20% yield of lytic colonies was obtained when gal_1^{-38} cells were incubated for 72 hours. In this instance the colonies obtained appeared quite convex and almost mucoid in smoothness. When cystine was removed from the growth medium the lytic reaction was not obtained. It would seem desirable to attempt to correlate a possible change in virulence in chickens with the colonies obtained as the nutrient supply of the infected gal^- cells is varied.

Smooth mucoid-like tan colonies were also observed when about 5000 or more units of anti-phage 38 sera were overlayed on gal_1^{-38} cell spread-plates. Although Mallman (1932) reported a transient change in colony morphology of S. gallinarum pullorum to smooth in the presence of anti-serum, it would seem desirable to check the infected anti-serum treated cells for a change in virulence. Apparent "curing" of cells was also obtained in the presence of homologous antiserum since occasional sectors of white cells were obtained which did not yield phage on spot-plate tests. Here, the antiserum might tie-up and prevent infection of cells which if they contained only unintegrated phage from recombination assortment, may have outgrown the cytoplasmically reproducing phage to become sensitive or failed to receive phage within the cell by a random distribution.

"Curing" of stably lysogenized 53 cells was obtained for about 20% of cells incubated in the presence of 0.2% sodium citrate. When gal_1^- cells were incubated at 37° C. for 10 minutes in the presence of phage 38 (m.o.i. 0.1) and sodium citrate was added to a concentration

of 0.2% and incubation continued for 30 minutes, a 60% reduction in frequency of the lytic reaction was observed as compared to untreated cells (Table 18). The mechanism producing "curing" and reduction in frequency of the lytic reaction is not apparent although Adams (1949) has shown that sodium citrate interfers with adsorption of phage.

DISCUSSION

A. Production of Mutants

Haas and Doudney (1957) showed that incubation at 4-6° C. for 2 hours or longer produced simultaneous growth and division of a majority of *E. coli* cells when they were subsequently incubated at 37° C. The sharpened end-point of *S. gallinarum pullorum* growth response in penicillin medium was probably due to such synchronous growth. Here an increased proportion of cells would be just beginning to grow and would be sensitive to penicillin. Hence, less cell growth would occur and less metabolites and waste products would be present to permit "feeding" of potential anxotrophs (see below). In addition, less penicillin would be required to prevent growth, so that, at the end-point a lower concentration of penicillin would be present. Thus, there would be less likelihood of the penicillin inhibiting growth of auxotrophs when the penicillin concentration was further diluted by plating aliquots from the penicillin medium on complete medium.

That the combination of synchronized growth conditions with the use of a penicillin medium supplemented with all but one selected amino acid, purine or pyrimidine, may have increased the efficiency of the penicillin technique at least for *S. gallinarum pullorum*, is attested to by obtaining threonine, arginine and other potential mutants with relative ease by comparison with the lack of success in unsupplemented penicillin medium and by previous experience using *E. coli* and *S. typhinurium.* Lederberg (1949) has shown that a mixture of absolute auxotrophs supplied with 10% of their nutrient requirement will grow sufficiently in liquid medium to accumulate or produce products enabling normal mutual growth. Such cross-feeding is called growth by syntrophism. Based on the findings of Lederberg, one might hypothesize an increase in efficiency upon

selective supplementation with growth requirements of the penicillin medium, as due to elimination of all potential auxotrophs except one. Here, all but one potential auxotroph would begin to grow and would become sensitive to penicillin. Again, in the absence of potential auxotroph growth the level of metabolites and waste products would be reduced and possible feeding would be less likely. Doudney and Haas (1960) and Strauss (1959), have postulated that post-irradiation incubation in a complex amino acid medium stabilizes mutation and consequently increases the yield of the entire spectrum of mutants. Although Adelberg (1961) has stated that the hypothesis of mutation stabilization is not generally applicable, it was found in this study that under conditions of post-irradiation incubation, a definite increase in the yield of potential mutants was observed in each of three experiments with *S. gallinarum pullorum*. The fact that no absolute auxotrophs were obtained may merely be due to a chance distribution of the increased number of leaky mutants, but it would seem more likely that synchronized growth in supplemented penicillin medium may have been more efficient in selecting for absolute mutants. It would seem desirable to continue a comparison of these methods.

The apparent increase in frequency of production of gal^- mutants upon infection of gal^+ 53 and 35 cells with phage, suggests the production of some type of mutagenic agent in the infected cell. It is known that the purine and pyrimidine base pool may be altered qualitatively and quantitatively upon infection, e.g., hydroxy-methyl-cytosine is produced upon phage T_2 infection of *E. coli* (Hershey, Dixon and Chase, 1953). Since Doudney and Haas (1960) have shown that merely an increase in the level of cytosine in *S. typimurium*, followed by post-irradiation incubation, will increase the proportion of mutants obtained, it is possible that some similar type of process may occur on infection of *S. gallinarum pullorum* by phage 38. It is possible but would seem very unlikely that

recombination of gal-sites in transduction could account for production of the gal^- character. Since the mutation rate for gal^- and gal^+ cells would be at least as infrequent as 1 in 10^7 cells (back mutation of gal^- to gal^+ is 10^7-10^8), it would seem unlikely that gal^- cells would be obtained by chance variation at an apparent rate of 1 in 10^3 to 10^4 cells. Nevertheless, it would be desirable to expand a study of the production of such mutants by plating larger numbers of infected cells and by using more adequate controls of uninfected cells than were used in order to derive a conclusion of higher statistical validity.

B. Generalized Transduction

In general, transduction in *S. gallinarum pullorum* is similar to that found in other bacteria. The average number of transductions for thr^+ and S^r markers is 2.3×10^{-6}. These frequencies are somewhat higher than the frequencies of 10^{-5} to 10^{-8} reported for other bacteria (Braun, 1953). The fact that difficulty was encountered in transducing thr^+ and S^r markers when classical technique was used, is very likely due to the virulent nature of the temperate phage. Hartman (1961) has pointed out that virulent phage may obscure the frequency of transduction of markers normally transduced at a low frequency. It has been observed routinely that at m.o.i. 1.0 there is a 60 to 100 fold decrease in the number of cells in the transduction-mixture attributable to lysis by the phage. Support for the lower frequency of transduction with the *S. gallinarum pullorum* system was observed with respect to S^r transduction at an approximate frequency of 10^{-7} at m.o.i. 0.1 on two occasions where lysis was less than half a log unit using classic technique. This frequency is typical of that found in *S. typhimurium*. An indication that lysis is also affecting transduction of the gal^+ marker is indicated in Table 9. Here the greatest number of transductions was recorded at

m. o. i. 5.0, although the highest frequency of transduction/phage was observed at m. o. i. 0.1 where the degree of lysis is reduced considerably. It is very likely that even with a high frequency of transduction as exists for the gal^+ marker, the higher absolute frequency of transduction expected at m. o. i. 5.0 is reduced due to a marked degree of lysis of transduced as well as other cells. The effect of lysis may and should be corrected for by using recipient cells which are resistant to phage 38 (Zinder 1955).

The average frequency of 1×10^{-5} for transduction of the gal^+ marker in S. gallinarum pullorum compares closely with that of 10^{-4} to 10^{-5} found for lactose transduction in Shigella dysenteriae (Luria, 1960). The absolute frequency using classical technique for transduction of gal^+ to gal^- cells is probably somewhat higher in S. gallinarum pullorum as has been indicated in several instances where large numbers of isolated infected colonies were examined.

Again, as found in other organisms at m. o. i.'s below 1 (Adams and Luria, 1958), transduced clones for both thr^+ and gal^+ markers were found to be free of phage, i. e., the same phage which transduced was not capable of lysogenizing (called "defective," Adelberg, 1961). In contrast, transduced S^r clones were found to produce phage and were presumably lysogenized even at m. o. i.'s of 0.01 as found by Morse (1959). Such results led Morse to hypothesize an association of donor DNA and phage genome at least for streptomycin transduction. Wanatabe and Fukasawa (1961a), have found that streptomycin resistance may be transferred by conjugation and with other evidence they hypothesize that streptomycin markers may either be stably or unstably integrated in the bacterial chromosome. Since they also hypothesize that acriflavin may induce integrated material to become unintegrated it would seem of interest to determine the affect of acriflavin on streptomycin transduction in S. gallinarum pullorum.

Demerec (1948) has postulated that streptomycin resistance is controlled by a multigene S^r locus which differs from the multigene control of penicillin resistance. Watanabe and Watanabe (1959) have performed transductions from high to intermediate levels of resistance and found that a separate control exists for the high (indifferent) resistance of a locus removed from the multiple gene locus for intermediate resistance. In similar independently conceived initial experiments a high level S^r marker was transduced to strains 53 and 54 resistant to different intermediate levels of resistance. However, the phage-bacterium mixtures were diluted and plated on complete medium and individual infected colonies were replica-plated to high-level streptomycin agar (0.5 mg.ml.). Different frequencies of colonies resistant to 0.5 mg./ml. of streptomycin were obtained, suggesting that mutants resistant to different intermediate levels of streptomycin "throw-off" mutants resistant to a high level of streptomysin at different rates. It would seem that further study in S. gallinarum pullorum should be performed of the frequency of transduction from high to intermediate levels of streptomycin resistance and from intermediate to high levels of resistance, coupled with a study of the mutation frequencies at which intermediate level resistant cells mutate to high level resistance and high level resistant cells mutate to low level resistance. Such a study may cast more light on how multigene action for streptomycin control differs from that of penicillin. It has been observed in two instances of streptomycin-resistance transduction that when the number of cells was increased from 10^8 to 10^{10}/ml. in the presence of 10^7 F.A./ml., about the same number of resistant clones was observed; whereas, a marked increase in resistant clones was obtained when the number of uninfected cells was increased from 10^8 to 10^{10}/ml. It is possible that infection may block the production of enzyme or cell-wall components necessary for resistance to streptomycin. If these observations are substantiated, the question as to how presumed transduction of

S^r genetic material could control this process and prevent cell lysis should be investigated possibly by comparing the carbohydrate composition of cell extracts of uninfected, infected and transduced cells first by chromotography and then by serological methods.

C. The Lytic Reaction

On the basis of experimental findings, the hypothesis has been advanced that in the lytic infection reactions, phage 38 infects sensitive strains of *S. gallinarum pullorum*, induces and recombines with defective phage to produce a defective phage 38, incapable of integrating in the host cell. Thus defective phage 38 would then multiply vegetatively in the host cell and either superimpose genetic control on the host cell genome or modify its function with respect to carbohydrate metabolism, cell morphology and perhaps cell virulence and other functions.

The results of this study indicate that the high frequency of colonies fermenting galactose and other carbohydrates is not due to high frequency transduction as reported by Morse (1956) in *E. coli*. Infection of *S. gallinarum pullorum* gal$^-$ cells by phage 38 grown on members of the same gal$^-$ clone still produce galactose fermentation, whereas no galactose fermentation could be expected by transduction. Also, Campbell (1958) has shown that in *E. coli* a helper phage is necessary for high frequency transduction since transductions were not found below a m.o.i. of 0.03 where it would be improbable that transducing and helper phage might infect the same cell. By contrast, the lytic infection reaction continued in a linear relation with decreasing m.o.i. to below m.o.i. 0.0001, indicating that a single infecting phage was capable of producing the lytic reaction. The highly unstable segregation pattern of lytic colonies, the lack of change in frequency of the lytic reaction with decrease in temperature of incubation, the unaltered ability of superinfecting phage 38 to elicit the lytic reaction, and the high titer of phage in lytic colonies as compared to infected non-lytic colonies, all indicate that a phage 38 exists in an unintegrated state in the host cell. A low titer phage 53 has been induced from bacterial strain 53 by infection with phage 38, by acriflavin treatment and by ultra-violet irradiation. Since infection with phage 38 and treatment either with acriflavin or ultra-violet is correlated with an apparent increase in frequency of the lytic reaction, it would seem that the lytic reaction is dependent upon the induction of phage 53.

Because of this correlation and since the titer of phage produced in lytic colonies is about 10^8/ml., which is similar to the titer of phage from plaques of phage 38 produced on an indicator strain, it would seem reasonable to assume that a recombination had occurred between infecting phage 38 and defective phage contained in the host cell to produce phage 38 incapable of integration. The lytic reaction does not occur in lysogenic strains containing complete phage. Yamamoto and Anderson (1961) have indicated that infecting phage may recombine in whole or in part with defective phage at the prophage site. Thus it would seem that infecting phage 38 may combine with different portions of defective phage 38 to produce different defective phage 38 responsible for the pink-centered, red-ringed and all-red colony phenotypes observed. (In segregation reactions, free defective phage 38 could be expected to superinfect gal^- 53 cells, and recombine). It has also been observed that gal^- 53 cells, stably lysogenized with phage 38, may be superinfected with phage 38 at m.o.i. 0.1 without alteration in frequency of the lytic reaction. Hence, in segregation reactions, free "recombined" phage 38 could be expected to superinfect gal^- 53 cells and recombine again with defective phage 53.

Since Schoenhard and Robinson (1961) have evidence that some six strains of _S. gallinarum pullorum_ may be polylysogenic, it would seem possible that strain 53 may contain several defective phage types. Here, phage 38 would recombine with the three different defective phage to produce the three lytic colony phenotypes observed. Similarly phage 38 would combine with one defective phage in strain 25 to produce the one lytic colony phenotype observed.

Another possible operating mechanism would have strain 38 be polylysogenic and phage of at least three genotypes corresponding to the three plaque types of phage 28 occasionally observed on indicator strains.

In order to determine more exactly the nature of the phage-host cell interaction and to support the recombination hypothesis it would be desirable to show first that infecting phage 38 differs from lytic phage 38. One may check for a converting antigen either present or absent in lytic colonies as compared to lysogenized colonies using slide-agglutination and other classical techniques as has been done by Fukazawa (1961) for the similar high titer phage colonies produced by infection of certain strains of *S. typhimurium* with P22. One may check for antigenic differences between intact phage 38 and defective phage 38 or between the products of lysis from a lytic colony as compared to a lysogenic colony using the immunochemical technique of Koibong *et al.* (1961). Also one may check for differences in morphology, using the electron microscope, as has been done for recombinant phage from a complete infecting phage and a contained defective phage by Yamamoto and Anderson (1961). Secondly, it would be desirable to similarly show a common characteristic between defective phage 53 and lytic phage 38, not present between defective phage 53 and infecting phage 38. Other indirect supporting experiments could also be performed.

That the internal environment of the bacterium plays a role in the lytic reactions is indicated by alteration of the response in the absence and presence of different carbohydrates. In the absence of carbohydrate a color change is observed for lytic colonies plated on complete EMB agar but not on complete BCP agar. Zinder (1960) notes a color change in F^-

Salmonella when cross-streaked with F$^+$ cells. One hundred percent of the infected cells exhibit the color change and Zinder has hypothesized that cell damage has permitted entrance of the eosin dye. It would seem possible therefore, that cell damage may have occurred sufficiently for the eosin-dye to have entered and stained the cells or that sufficient cell lysis had occurred for the dye to have stained the proteins liberated.

In the presence of carbohydrate, lytic colonies produce acid on BCPG agar and acid and gas in BCPG broth. Apparently the source of energy plays a role in determining the pattern of lysis as only dark blue-centered colonies are formed by gal$^-$ 38$^+$ cells on BCP xylose agar, whereas, pink-centered, red-ringed and all-red colonies are found on EMBG agar. Koibong, Barksdale and Garmise (1961) report a reddening of Shigella colonies on EMB agar supplemented with lactose and maltose sugars which are not normally fermented by Shigella. They attribute this change as due to cell damage produced by a phage associated endolysin within the infected carrier cell permitting entrance of these carbohydrates (decryptification). It is possible again that cell damage has occurred in *S. gallinarum pullorum* permitting carbohydrate to enter the infected cell. However, only mutant cells have been observed to exhibit a change in fermentation upon infection and the multiple negative fermentation character of the mutant suggests that an operon complex may be involved. Such an operon may be responsible for cell-wall synthesis. Horaker (1960) has shown that a specific gene-controlled permease is involved in galactose transport in *E. coli* and similarly Weismeyer (1959) has shown a specific permease for maltose transport. Since the permease systems are under single gene control it is unlikely that the cell wall structures controlled are part of this system. Preliminary study indicates that galactokinase is present in wild-type cells, absent in gal$_1^-$ and that it is produced in infected cells grown in the presence of galactose. Since gal$_1^-$ exhibits a multiply negative fermentation pattern and since

Wiesmeyer (1961) has indicated that induction of the hypothesized operon complex operative in *E. coli* simultaneously produces an increase in several different enzymes in the absence of substrate, it would be interesting to determine if an increase in the kinase enzymes for xylose and rhamnose exists in the absence of substrate in *S. gallinarum pullorum* gal_1^- 38^+. In conjunction with such enzymatic study it would be well to correlate any change in carbohydrate composition of the cell-wall from wild-type to mutant or to infected mutant. This could be accomplished by chromatograming cell-wall lysates using the technique of Fukasawa and Nikaido (1960). Should the study to determine alteration of genetic control upon infection yield negative results one might proceed to check for cell wall damage (Zinder, 1960, Koibong, *et al.*, 1961) permitting entrance of carbohydrate in lytic cells using a labeled carbohydrate source.

The phenotype of the lytic reaction is also altered depending on the amino acid environment of the cell. In the absence of casein hydrolysate no reaction occurs. On minimal medium supplemented with 18 amino acids, lytic colonies which are observed after 72 hours incubation are convex and almost mucoid in appearance. Presumably cells from such colonies may exhibit an altered virulence for chickens. Removal of cystine from the amino acid pool prevents the lytic reaction. It would seem desirable to correlate any change in virulence of lytic colonies with alteration of the amino acid and perhaps purine and pyrimidine composition of the growth medium.

SUMMARY

Mutants have been obtained for antibiotic resistance, for inability to ferment different carbohydrates, and dependent for growth upon specific amino acids, purines or pyrimidines. Several modifications of the basic penicillin technique for selection of auxotrophs were used. One modification was developed which apparently produced a better yield of absolute mutants by using an inoculum of synchronously growing cells in a synthetic medium. This medium was supplemented with 17 amino acids but was selectively deficient in one. It also contained penicillin in various concentrations. An increase in the appearance of mutants lacking the ability to ferment various carbohydrates may occur in cells infected with bacteriophage 38.

Generalized transduction was obtained for streptomycin resistance, galactose fermentation, and threonine dependent markers. The absolute frequencies of transduction of these markers were probably obscured using classical techniques because of the semi-virulent nature of the phage employed. Provision has been made to clarify further these frequencies. Transduced clones for threonine independence and galactose fermentation were found to be free of phage at m.o.i.'s below one, whereas, transduced clones for streptomycin resistance at the same m.o.i. contained phage. These results paralleled those found in other bacteria. Possible interference by the bacteriophage with the process of mutation to streptomycin resistance in infected cells has been suggested from the results of streptomycin transduction at reduced m.o.i.'s.

When sensitive strains of *S. gallinarum pullorum* were infected with *S. gallinarum pullorum* bacteriophage 38 under variable conditions, various patterns of partially lysed colonies were produced at a high

frequency on selective media. The lytic colonies were not produced by transduction, since phage grown on cells from a clone might be used to infect other cells from the same clone to produce the lytic reaction. Infecting-phage 38 is not defective in the sense that it required a helper-phage, since a lytic reaction was produced at m.o.i.'s far below where a helper phage might simultaneously infect recipient cells. In fact, the lytic reaction did not occur in the presence of complete phage as are found in lysogenic strains. However, phage 38 evidently existed in a cytoplasmic state in some cells of lytic colonies and this phage 38 was defective in integration capability.

Acriflavin treatment, ultra-violet irradiation and infection with phage 38 have been found to induce a low titer of presumably defective phage from sensitive strain 53. Infection with phage 53 and either acriflavin treatment or ultra-violet irradiation seemed to markedly increase the frequency of the lytic reaction. Hence, it appears reasonable to assume that infecting-phage 38 may have recombined with the defective phage contained in strain 53 to produce a recombined phage 38 incapable of integration although it was present in lytic colonies in high titer.

Various methods have been suggested to clarify further the host cell-phage complex and to support the hypothesis of phage recombination.

In *S. gallinarum pullorum* apparently a single mutational event produced cells which were incapable of fermenting a number of different carbohydrates. This fact suggested that a gene had been affected at an intermediate level of control, probably an operon for cell wall synthesis. Although it was possible that such a "mutant" operon might affect cell wall structure so that a number of specific carbohydrates might not enter the cell, and that infection with phage 38 might damage certain cells so that now these carbohydrates might enter, preliminary evidence indicated that the operon which controls the kinase enzymes might be affected. Initial experiments indicated that the galactokinase enzyme level was reduced in mutant cells, and that it was increased in infected cells in which phage 38 was reproducing in a vegetative state.

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APPENDIX

Table 1. Minimal Medium for Growth of *Salmonella gallinarum pullorum*

Nutrient	Weight/Liter	
	Gm.	Mg.
NH_4Cl	5.00	
NH_4NO_3	5.00	
Na_2SO_4	2.00	
K_2HPO_4	3.82	
KH_2PO_4	2.18	
$KHCO_3$	2.00	
Carbohydrate	5.00	
L-leucine		87
L-cystine		200
L-arginine		43
$MgSO_4H_2O$		100

Note: Add distilled water to nutrients to bring to a volume of 1000 ml.

Table 2. Eosin Methylene Blue Galactose Agar.

Nutrient	Gms. /Liter
Galactose	5.000
Casein Digest	8.000
Yeast Extract	1.000
NaCl	5.000
K_2HOP_4	2.000
Eosin Y	0.400
Methylene Blue	0.065
Agar	14.000

Note: Nutrients added to 1000 ml. water.

Table 3. Source and Number of Salmonella gallinarum pullorum Strains

MSU Strain Number*	Source	Source Number
7	New York	51-46
13	California	3230
14	California	3082
20	Pennsylvania	BU7324
25	Massachusetts	1
33	South Carolina	A
34	South Carolina	B
35	South Carolina	C
37	South Carolina	E
38	South Carolina	F
40	Connecticut	A78560
43	Connecticut	A86660
45	Illinois	L58573
47	Michigan	6
51	Michigan	2422
53	Kentucky	CDC3522
54	Kentucky	NE17

*Strains were obtained from a collection of Dr. D. E. Schoenhard, Department of Microbiology, Michigan State University.

Table 4. Death of *Salmonella gallinarum pullorum* 53 After Exposure to Ultraviolet Light

Exposure Time (Seconds)	Percent Death
0	0
10	97.90
20	99.50
30	99.97

Table 5. Frequency of Selection of Auxotrophs

Mutant Selected	Number Plates Observed	 Potential Mutants	 Auxotroph Mutants
Threonine	10	6	1
Threonine*	20	12	9
Proline*	20	18	12
Leucine	25	20	1
Arginine	25	6	1
Thymine	20	48	-
Adenine	20	39	-

*Mutants produced by post-irradiation incubation were all "leaky."

-No attempt was made to purify these mutants.

Experiment	Gal$^+$ colonies/ plate	Av. No. Gal$^+$ col./plate	Cell conc./ plate	1 Gal$^+$ col./ cell conc.	M.O.I.	1 Gal$^+$ col./ phage conc.
1	6, 7, 8, 6, 7	6.4	8.75×10^6	1.35×10^6	1.2000	1.64×10^6
	3, 6, 6, 5, 6, 6, 6, 2	5.0	8.75×10^6	1.75×10^6	1.0000	1.75×10^6
	C, 4, 2, 2	2.7	8.75×10^6	3.24×10^6	0.5000	1.63×10^6
	7, 2, 6, 6, 11, 4, 5, 4	5.6	8.75×10^6	1.56×10^6	0.1000	1.56×10^5
	1, 1, 3, 6, 4, 1, 3, 4	2.9	8.75×10^8	3.00×10^8	0.0010	3.0×10^5
	0, 0, 4, 3, 1, 2, 5, 1, 1, 3	2.0	1.75×10^9	8.75×10^8	0.0000	0.0
2	14, 16, 11, 15, 13, 24, 30, 17, 18, 14	17.2	3.2×10^6	1.85×10^5	5.0000	9.3×10^5
	9, 5, 10, 4, 7, 6, 5, 8, 4, 4, 7	6.3	3.2×10^6	5.1×10^5	1.0000	5.1×10^5
	3, 5, 4, 8, 4, 5, 3, 5 4, 4, 4, 3, 1, 3, 1, 2, 1, 1	4.3	3.2×10^6	7.4×10^5	0.1000	7.4×10^4
	1, 2, 1, 0, 0, 3	1.3	3.2×10^8	2.5×10^8	0.0010	2.5×10^6
	0, 2, 3, 1, 6, 8, 5, 2, 2, 4, 8, 9	4.8	6.4×10^8	1.3×10^8	0.0000	0.0
3	27, 22, 39, 21, 20, 30, 15, 25	25.0	3.5×10^6	1.4×10^5	5.0000	7.0×10^5
	6, 5, 11, 13, 12, 12	10.0	3.5×10^6	3.5×10^5	1.0000	3.5×10^5
	8, 3, 6, 7, 8, 4, 5	5.9	3.5×10^6	6.0×10^5	0.1000	6.0×10^4
	6, 9, 6, 5, 6, 6, 12	7.0	3.5×10^8	5.0×10^7	0.0010	5.0×10^4
	9, 5, 8, 6, 6, 2, 6, 3, 4, 1	5.0	3.5×10^9	7.0×10^8	0.0001	7.0×10^5
	10, 7, 8, 7, 7, 7, 8, 9	7.7	7.0×10^8	1.0×10^7	0.0000	0.0
	16, 23, 22, 18, 14	18.6	7.0×10^9	3.8×10^8	0.0000	0.0

Sample Calculation: $\frac{8.75 \times 1.2}{6.4}$ = 1 trans. in 1.64×10^6 phage.

Table 7. Optimum Multiplicity of Infection for the Lytic Reaction.

	Experiment					
	1		2		3	
M.O.I.	Fract.	Percent	Fract.	Percent	Fract.	Percent
0.600	0	0				
0.300	$\frac{2}{78}$	2.8				
0.100	$\frac{83}{797}$	10.4	$\frac{674}{3034}$	22.2	$\frac{857}{2915}$	29.4
0.033	$\frac{112}{960}$	11.7	$\frac{696}{4104}$	16.9	$\frac{1028}{4860}$	21.4
0.016	$\frac{120}{2208}$	5.3				
0.008	$\frac{166}{3530}$	4.93	$\frac{58}{3192}$	1.8	$\frac{152}{6427}$	2.4
0.004			$\frac{76}{5859}$	1.3	$\frac{43}{4219}$	1.0
0.002			$\frac{144}{7821}$	1.8	$\frac{120}{10826}$	1.1
0.0002			$\frac{74}{9459}$	0.79		

Table 8. Inter-strain Transduction of the Thr$^+$ Marker from *Salmonella gallinarum pullorum*. 53 to 54.

Experiment	Thr$^+$ colonies/ plate	Av. Thr$^+$ col./plate	Cell conc./ plate	1 Thr$^+$ col./ cell conc.	M.O.I.	1 Thr$^+$ col./ phage conc.
1	9, 5, 0, 1, 3, 1	2.4	1.0×10^6	4.1×10^5	0.5	2.1×10^5
	7, 18, 4, 21, 8, 3, 7	9.7	5.0×10^7	5.1×10^6	0.1	5.1×10^5
	0, 3, 3, 1, 0, 0, 0, 0	0.5	5.0×10^8	1.0×10^9	0.0	0.0
2	4, 1, 3, 12, 2, 2, 0, 4	3.5	2.0×10^6	5.7×10^5	0.5	2.8×10^5
	28, 11, 7, 13, 7, 3, 21, 9	12.4	5.0×10^7	4.0×10^6	0.1	4.0×10^5
	1, 1, 0, 0, 0, 0, 0	0.3	1.0×10^9	3.3×10^8	0.0	0.0
3	1, 9, 3	4.3	1.7×10^7	4.0×10^6	0.1	4.0×10^5
	48, 68, 61	59.0	1.7×10^{10}	2.9×10^8	0.0	0.0

Table 9. Inter-strain Transduction of the Streptomycin Marker from *Salmonella gallinarum pullorum* 53 to 54.

Experiment	Conc. Strep.	S^r colonies/ plate	Av. S^r col./ plate	Cell conc./ plate	1 S^r col./ cell conc.	M.O.I.	1 S^r col/ phage conc.
1	0.5 mg./ ml.	3, 7, 8, 6, 5, 7	6	5 x 10^7	8.3 x 10^6	0.1	8.3 x 10^5
			0	1 x 10^9	10^9	0.0	0.0
2	0.1 mg./ ml.	38, 25, 39, 45, 33, 39	36.5	1.7 x 10^7	4.7 x 10^5	0.5	2.4 x 10^5
		26, 24, 19 23, 20, 11	20.5	8.3 x 10^7	4.5 x 10^6	0.1	4.5 x 10^5
		0, 2, 1, 2, 1 0, 1, 1, 0	0.9	5.0 x 10^9	5.7 x 10^9	0.0	0.0

Table 10. Replication of Intermediate Level Streptomycin Resistant Colonies Infected with Phage From High Level Resistance.

	Character of Replicated Colonies on 0.5 mg./ml. Streptomycin		
	Growth Reduced	No Growth	Normal Growth
Uninfected Strain 54			
Plate 1	10	6	26
2	9	4	7
3	19	23	6
4	11	48	11
Total number colonies	49	81	50
Percent dist.	27	45	28
Uninfected Strain 53			
Plate 3	2	0	15
4	3	4	11
5	8	7	6
6	8	7	4
7	4	4	11
8	5	0	19
Total number colonies	30	22	66
Percent dist.	25	19	56
Infected Strain 54			
Plate 1	14	29	1
2	15	23	0
3	23	33	36
4	25	47	31
Total number colonies	77	132	77
Percent dist.	27	46	27
Infected Strain 53			
Plate 1	20	15	1
2	12	9	8
3	5	25	1
4	5	6	21
Total number colonies	42	55	31
Percent Dist.	33	43	24

Table 11. Lytic Response of Gal$^-$ 53 Infected with Phage 38 Grown on Gal$^+$ 53.

Experiment	Lytic/Non-lytic Colonies/Plate						Lytic/Non-lytic col./experiment	Percent lytic col./exp.	Percent lytic col/5 exp.
1	$\frac{75}{215}$	$\frac{13}{71}$	$\frac{11}{68}$	$\frac{46}{106}$			$\frac{145}{460}$	31.5	
2	$\frac{4}{215}$	$\frac{43}{478}$	$\frac{83}{353}$	$\frac{64}{371}$	$\frac{72}{415}$	$\frac{49}{243}$	$\frac{282}{2111}$	13.3	20.06
3	$\frac{112}{1072}$	$\frac{83}{880}$					$\frac{195}{1952}$	10.0	
4	$\frac{7}{173}$	$\frac{134}{170}$	$\frac{10}{58}$	$\frac{11}{22}$			$\frac{62}{423}$	14.6	
5	$\frac{674}{3034}$	$\frac{696}{4104}$	$\frac{857}{2915}$	$\frac{1028}{4804}$			$\frac{3255}{14657}$	22.2	

Table 12. Lytic Response of Gal$^+$ 53 Infected with Phage 38 Grown on Gal$^+$ 53.

Plate	Fractional Response (36 Hours)	Total
1	$\frac{1}{16}$ $\frac{3}{9}$ $\frac{3}{11}$ $\frac{6}{13}$ $\frac{2}{11}$ $\frac{3}{11}$ $\frac{2}{9}$ $\frac{2}{10}$ $\frac{2}{8}$ $\frac{3}{11}$ $\frac{3}{13}$	$\frac{33}{122}$
2	$\frac{3}{16}$ $\frac{5}{15}$ $\frac{1}{8}$ $\frac{4}{7}$ $\frac{3}{9}$ $\frac{1}{5}$ $\frac{1}{11}$ $\frac{2}{9}$ $\frac{4}{12}$ $\frac{2}{9}$ $\frac{1}{6}$	$\frac{31}{119}$
3	$\frac{1}{9}$ $\frac{5}{15}$ $\frac{0}{7}$ $\frac{1}{6}$ $\frac{0}{8}$ $\frac{2}{6}$ $\frac{1}{6}$ $\frac{4}{13}$ $\frac{11}{25}$ $\frac{6}{12}$ $\frac{1}{7}$	$\frac{35}{115}$
4	$\frac{3}{9}$ $\frac{5}{13}$ $\frac{10}{27}$ $\frac{5}{13}$ $\frac{3}{11}$ $\frac{5}{12}$ $\frac{4}{18}$ $\frac{4}{8}$ $\frac{14}{28}$ $\frac{2}{11}$	$\frac{45}{151}$
5	$\frac{4}{8}$ $\frac{8}{25}$ $\frac{8}{23}$ $\frac{6}{18}$ $\frac{3}{6}$ $\frac{5}{8}$ $\frac{3}{11}$ $\frac{4}{16}$ $\frac{4}{13}$ $\frac{7}{13}$	$\frac{52}{141}$
6	$\frac{1}{19}$ $\frac{1}{18}$ $\frac{0}{9}$ $\frac{4}{9}$ $\frac{4}{14}$ $\frac{3}{11}$ $\frac{7}{19}$ $\frac{4}{8}$ $\frac{3}{7}$ $\frac{3}{9}$ $\frac{7}{11}$	$\frac{37}{114}$
7	$\frac{2}{10}$ $\frac{8}{12}$ $\frac{6}{15}$ $\frac{7}{14}$ $\frac{3}{6}$ $\frac{4}{11}$ $\frac{3}{14}$ $\frac{1}{7}$ $\frac{4}{8}$ $\frac{1}{5}$ $\frac{3}{7}$ $\frac{5}{11}$ $\frac{2}{10}$	$\frac{49}{130}$
8	$\frac{0}{6}$ $\frac{5}{8}$ $\frac{5}{12}$ $\frac{3}{6}$ $\frac{5}{6}$ $\frac{2}{6}$ $\frac{2}{7}$ $\frac{4}{10}$ $\frac{4}{14}$ $\frac{8}{21}$ $\frac{10}{17}$ $\frac{3}{17}$ $\frac{12}{29}$	$\frac{63}{157}$
9	$\frac{0}{9}$ $\frac{3}{6}$ $\frac{6}{13}$ $\frac{4}{16}$ $\frac{1}{11}$ $\frac{4}{8}$ $\frac{1}{4}$ $\frac{1}{5}$ $\frac{3}{9}$ $\frac{1}{4}$ $\frac{5}{13}$ $\frac{9}{19}$ $\frac{10}{20}$ $\frac{6}{17}$	$\frac{54}{153}$
10	$\frac{4}{9}$ $\frac{3}{7}$ $\frac{3}{6}$ $\frac{5}{10}$ $\frac{5}{9}$ $\frac{4}{8}$ $\frac{3}{8}$ $\frac{2}{13}$ $\frac{3}{6}$ $\frac{1}{5}$ $\frac{7}{14}$ $\frac{5}{7}$	$\frac{48}{102}$
11	$\frac{6}{10}$ $\frac{3}{9}$ $\frac{2}{12}$ $\frac{2}{8}$ $\frac{3}{10}$ $\frac{4}{9}$ $\frac{2}{8}$ $\frac{5}{8}$ $\frac{1}{7}$ $\frac{4}{9}$ $\frac{4}{7}$ $\frac{10}{19}$ $\frac{1}{10}$ $\frac{3}{11}$ $\frac{5}{10}$ $\frac{2}{4}$ $\frac{5}{11}$ $\frac{1}{8}$ $\frac{3}{10}$ $\frac{3}{11}$	$\frac{69}{191}$
	Grand total	$\frac{513}{1495}$
	Percent	34.35

Table 13. Effect of Temperature on the Lytic Reaction.

Temperature	M.O.I.	Fractional Reaction										Percent Reaction
37° C.	0.1	$\frac{69}{575}$	$\frac{159}{636}$	$\frac{144}{593}$	$\frac{153}{628}$	$\frac{149}{602}$						22.2
	0.33	$\frac{148}{872}$	$\frac{143}{790}$	$\frac{114}{711}$	$\frac{170}{830}$	$\frac{121}{901}$						16.9
	0.004	$\frac{3}{626}$	$\frac{15}{625}$	$\frac{20}{627}$	$\frac{10}{643}$	$\frac{10}{670}$						1.8
	0.002	$\frac{3}{575}$	$\frac{1}{570}$	$\frac{5}{631}$	$\frac{4}{551}$	$\frac{1}{494}$	$\frac{6}{553}$	$\frac{18*}{712}$	$\frac{18*}{762}$	$\frac{7}{417}$	$\frac{13}{594}$	1.3
	0.001	$\frac{29}{926}$	$\frac{30}{999}$	$\frac{37}{1072}$	$\frac{9}{901}$	$\frac{7}{898}$	$\frac{4}{1048}$	$\frac{22}{1030}$	$\frac{6}{947}$			1.8
	0.0002	$\frac{3}{1458}$	$\frac{0}{1136}$	$\frac{14}{1428}$	$\frac{7}{1361}$	$\frac{35}{1393}$	$\frac{8}{1450}$	$\frac{7}{1233}$	$\frac{0}{1058}$			0.8
25° C.	0.1	$\frac{129}{533}$	$\frac{146}{470}$	$\frac{129}{434}$	$\frac{168}{539}$	$\frac{151}{522}$	$\frac{134}{477}$					29.3
	0.33	$\frac{56}{828}$	$\frac{227}{887}$	$\frac{104}{576}$	$\frac{224}{848}$	$\frac{215}{855}$	$\frac{194}{810}$					21.4
	0.004	$\frac{13}{783}$	$\frac{45}{727}$	$\frac{23}{655}$	$\frac{43}{762}$	$\frac{8}{1350}$	$\frac{8}{696}$	$\frac{6}{735}$				2.4
	0.002	$\frac{2}{842}$	$\frac{14}{1306}$	$\frac{1}{482}$	$\frac{9}{526}$	$\frac{4}{326}$	$\frac{4}{449}$	$\frac{9}{288}$				1.0
	0.0001	$\frac{4}{740}$	$\frac{7}{978}$	$\frac{11}{914}$	$\frac{0}{770}$	$\frac{7}{472}$	$\frac{22}{575}$	$\frac{17}{921}$				1.5
	0.0002	$\frac{3}{1284}$	$\frac{0}{1243}$	$\frac{4}{1201}$	$\frac{28}{1306}$	$\frac{6}{1121}$						1.1

Table 14. Segregation Values of Primary and Secondary Subcultures of Lytic Colonies.

Response	Colony							Total Fract.	Response Percent
	Primary Subculture								
Lytic/Non-lytic col./plates	1	2	3	4	5	6	7		
Fraction	$\frac{274}{2169}$	$\frac{223}{896}$	$\frac{56}{554}$	$\frac{64}{829}$	$\frac{32}{2508}$	$\frac{2}{542}$	$\frac{149}{1706}$		
Percent	12.63	24.9	10.1	7.7	1.3	0.4	8.7		
	8	9	10	11	12	13	14		
Fraction	$\frac{1}{212}$	$\frac{12}{118}$	$\frac{6}{729}$	$\frac{171}{516}$	$\frac{117}{538}$	$\frac{133}{1426}$	$\frac{194}{835}$		
Percent	0.5	9.3	0.8	33.1	21.7	9.7	23.2		
	15	16	17	18	19	20	21		
Fraction	$\frac{1}{129}$	$\frac{27}{436}$	$\frac{6}{724}$	$\frac{2}{387}$	$\frac{141}{1175}$	$\frac{19}{274}$		$\frac{1730}{16715}$	10.8
Percent	0.8	6.2	0.8	0.5	12.0	6.9			
	Secondary Subculture								
	1	2	3	4	5	6	7		
Fraction	$\frac{6}{1236}$	$\frac{9}{1929}$	$\frac{3}{833}$	$\frac{12}{240}$	$\frac{14}{1015}$	$\frac{12}{732}$	$\frac{5}{1053}$		
Percent	0.5	0.5	0.5	5.0	1.3	1.6	0.5		
	8	9	10	11	12				
Fraction	$\frac{116}{2486}$	$\frac{1}{167}$	$\frac{2}{196}$	$\frac{1}{219}$				$\frac{180}{9106}$	1.97
Percent	4.2	0.6	1.0	0.5					

Table 15. Efficiency of Lytic Reaction as a Function of M.O.I.

M.O.I.	Percent Reaction Lytic/Non-lytic	Reaction per Phage
1×10^{-1}	15.76*	1.58×10^{-2}
8×10^{-3}	7.47	5.98×10^{-4}
4×10^{-3}	4.55	1.82×10^{-4}
2×10^{-3}	2.82	5.64×10^{-5}
7×10^{-5}	0.52	3.64×10^{-7}

*This result by interpolation of result from duplicate experiment.

Table 16. Effect of Acriflavin on the Lytic Reaction

Concentrat. Acriflavin Percent	Fractional Reaction Lytic/Non-lytic							Percent Reaction
0.0	$\frac{8}{413}$	$\frac{2}{364}$	$\frac{8}{481}$	$\frac{3}{391}$	$\frac{3}{374}$	$\frac{3}{454}$	$\frac{2}{411}$	1.0
1.0	$\frac{42}{244}$	$\frac{35}{176}$	$\frac{41}{229}$	$\frac{38}{215}$	$\frac{34}{223}$	$\frac{33}{181}$		17.6

Table 17. Segregation of Lytic Response of Gal$_1^-$ 53^{38+} on EMB Agar Supplemented with Various Carbohydrates.

Carbohydrate	Lytic/Non-Lytic Response				Total Response	Percent Response
Galactose	$\frac{9}{530}$	$\frac{18}{572}$	$\frac{13}{764}$	$\frac{24}{857}$	$\frac{67}{2903}$	2.3
Rhamnose	$\frac{2}{709}$	$\frac{11}{955}$	$\frac{9}{790}$	$\frac{11}{682}$	$\frac{33}{3136}$	1.1
Mannose	$\frac{18}{937}$	$\frac{15}{725}$	$\frac{12}{629}$	$\frac{13}{783}$	$\frac{58}{3074}$	1.8
Mannitol	$\frac{9}{843}$	$\frac{6}{713}$	$\frac{5}{737}$	$\frac{13}{792}$	$\frac{24}{3085}$	0.8
Ribose	$\frac{1}{464}$	$\frac{7}{528}$	$\frac{3}{418}$	$\frac{1}{378}$	$\frac{12}{1788}$	0.7
Xylose	$\frac{1}{383}$	$\frac{2}{590}$	$\frac{4}{621}$		$\frac{7}{1594}$	0.4

Growth response differed, i.e. galactose, mannose and mannitol were read at 52 hours; ribose and xylose at 58 hours, and rhamnose at 66 hours.

Table 18. Effect of Citrate on the Lytic Reaction.

Citrate Concentration Percent			Fractional Response Lytic/Non-Lytic				Total Fract.	Response Percent
0.0	$\frac{151}{575}$	$\frac{101}{515}$	$\frac{101}{378}$	$\frac{116}{405}$	$\frac{60}{313}$	$\frac{74}{378}$	$\frac{603}{2564}$	23.5
0.2	$\frac{25}{319}$	$\frac{20}{354}$	$\frac{29}{313}$	$\frac{41}{344}$	$\frac{46}{315}$	$\frac{61}{392}$	$\frac{222}{2037}$	10.8