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The Effect of Certain Drugs on the Lysogenic System

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THE EFFECT OF CERTAIN DRUGS ON THE LYSOGENIC SYSTEM

by

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LIFE

Demetrios George Halkias was born in Kosma, Kynourias, Greece, August 6, 1932. He was graduated from the Leonidion Gymnasium, Leonidion, Kynourias, Greece, in July, 1950, and later attended the Superior School of Commerce and Economic Sciences, Athens, Greece until October, 1951.

In December, 1951 he came to the United States and attended the Jackson Elementary School and Lindbloom High School, Chicago, Illinois.

He entered the University of Illinois, February, 1953 and graduated in June, 1957 with the Degree of Bachelor of Science.

The author began his graduate studies at Loyola University in September 1957 and in June, 1959 graduated with the Degree of Master of Science in Microbiology. He continued his graduate studies at Loyola University and from September of 1959 till present he was the recipient of a Standard Oil Fellowship, a Royal E. Cabell Fellowship, a number of Teaching and Graduate Assistanships and in 1960 he was a fellow for the Louisiana State University Interamerican Program in Tropical Medicine and Parasitology. As Fellow of the above program he visited Panama, Costa Rica, El Salvador, Guatemala and Mexico.

In June 1962, he was married to Evangeline Paraskevas. They have a son, George. Demetrios George Halkias is a member of the

Illinois Society for Microbiology, the American Society for Microbiology, and since May 1963, an associate member of the Society of SIGMA XI.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

The relationship between a parasite and its host has been and is a fascinating problem to men of science. Among higher animals and plants this problem has been investigated to a great extent by various scientists. Although a number of discoveries have been made and a great number of questions have been answered by the investigators working on this problem a great amount of information is needed. The lack of this knowledge is due to the difficulties that one faces working with higher plants, animals and their parasites.

Studies on viruses (plant, animal and bacterial viruses) has opened new fields for research. Research with bacteriophages (d'Herelle 1917) and their specific hosts has been vigorously followed in many laboratories. This research can be done at less cost, under closer observation and under better controlled conditions. The study of the bacterium-bacteriophage relationship has attracted not only bacteriologists, but also geneticists, virologists, immunologists, biochemists, etc.

Bacteriophages, are submicroscopic entities capable of being introduced into specific living bacterial cells and of reproducing inside such cells only. Inside the bacterial cell

the bacteriophage behaves as a cell constituent. The bacterial cell is not a simple, but a very complicated entity. All the cell's constituents are not thrown into a cell at random, but they are set in a definite pattern and constitute an organized entity. The main problem in the study of intracellular behavior of bacteriophages is that of their reproductive mechanisms.

Clarification of these mechanisms would give scientists a better understanding of the nature of bacteriophages and their relationships, and perhaps help to control their propagation. Information on the mechanics of bacteriophage reproduction may throw some light on the central problem of biology which is the reproduction of individual specific biological elements such as genes. It has been accepted that bacteriophages carry specific genetic material (Lederberg 1953).

Although certain details of the bacteriophage bacterium interaction have been answered, our investigation has been the study of the host-parasite relationship as it exists in the bacteriophage infected bacterial cell. Specifically this investigation is the study of the lysogenic system in Escherichia coli, strain K12 and the effect of certain drugs on the lysogenic system.

A number of purine and pyrimidine analogues and other antimetabolite compounds were used in this investigation. The effect of these drugs on the bacterium bacteriophage combination will be studied by the paper disc method I.N.Asheshov et, al.(1955) Drugs that do show some effect will be studied further for their

effect on bacteriophage lambda; for their effect on E. coli W1485; their effect on the bacterium bacteriophage interaction by the one step growth experiment of Ellis and Delbruck (1939) and finally the ability of these drugs to induce E. coli K12 to produce lambda phage will be studied.

Another aspect of this study will be the conversion of E. coli W1485 cells into spheroplasts by the method of Jeynes ((1957), and the infection of these spheroplasts with lambda bacteriophage.

CHAPTER II

REVIEW OF RELATED LITERATURE

The first descriptions of bacterial viruses (bacteriophages) were published by Twort (1915) and d'Herelle (1917). Twort (1915) speculated on the nature of the bacteriophage in many ways. He thought that bacteriophage could be (1) an ultramicroscopic virus; (2) an enzyme with the power of growth; (3) a stage in the life history of the micrococcus; (4) an enzyme produced by the micrococcus itself and leading to its own destruction and the production of more enzyme. Twort favored the view that the "material" was produced by the coccus and pointed out the connection with the problem of cancer of this "apparent spontaneous production of a self destroying material which, when started, increases in quantity.

D'Herelle (1921) believed bacteriophage to be a parasite, a virus which penetrates into sensitive bacteria, develops and lyses its bacterial host. This concept of bacteriophage presents the correct picture of the relationship between a sensitive bacterium and a virulent bacteriophage, but does not apply to lysogeny. The concept of lysogeny was introduced by Gildenmeister, Bail, Otto, Hunter and Bordet (Lwoff 1953) who speculated that bacteriophage was produced by certain bacteria in the absence of

phage. This theory led people to believe that bacteriophages could be produced by bacteria without infection. Thus Gildemeister and Herzberg (1924) clearly conceived of lysogeny as a property of bacteria which could be maintained in the absence of free phage.

Bail (1925) and Bordet (1925) independently reported the existence of strains of bacteria in which each bacterium gives rise to a bacteriophage producing clone, this lysogenic property being maintained in the absence of free bacteriophage.

Burnet and Mcfie (1929) attempted to explain lysogeny in terms of the "anlage". After treating lysogenic bacteria with d distilled water and observing that approximately 0.1% of the bacteria contained bacteriophage, they concluded that lysogenic bacteria contained in their hereditary constitution a unit potentially capable of liberating bacteriophage. This lytic principle "anlage" is a normal constituent of lysogenic phage which is incorporated into the hereditary constitution of the bacterium. Bacteriophage particles are liberated only if this "anlage" is activated.

Lwoff and Gutmann (1950) observed that lysogenic bacteria grow and divide without liberating bacteriophage. Only a small fraction of the lysogenic bacteria in a lysogenic population produces bacteriophage and this process of bacteriophage production is lethat to the bacterium.

From this brief historical review we can see that bacteriophage may be classified into two kinds: "Virulent" and

"Temperate" depending on the response they elicit upon infecting bacteria.

"Virulent" bacteriophages, composed of a core of desoxyribosenucleic acid (DNA) and a protein coat, when coming in contact with the proper bacterial cell adsorb onto the surface of the bacterium. The DNA of the bacteriophage enters the cell(infection) whereas most if not all of the protein coat remains outside apparently without performing any other actin. The DNA is thus the carrier of the genetic specificity of bacteriophage. Multiplication of the phage DNA begins soon after infection ('productive infection' Lwoff 1953). During this time interaction may occur between the genetic elements formed leading to the production of genetic recombinants of the phage. New bacterion phage protein thus begins to be synthesized and the mature phage particles appear. Still later the cell is lysed liberating into the growth medium a large number of phage particles. This occurs with the "virulent" bacteriophage.

In the case of the "temperate" bacteriophages after they come in contact and adsorb onto the proper bacterial cell a variable number of bacteria may be lysed, but some bacteria survive and give rise to lysogenic progeny ('reductive infection' Lwoff 1953). In lysogenic bacteria the ability to produce phage is believed due to a prophage which appears to be part of the hereditary material of the bacterium and is located on the bacterial chromosome (Lederberg and Lederberg 1953); Lennox (1955) and Jacob (1955). Lysogeny then is the hereditary property of

producing bacteriophage without infection by external particles. A lysogenic bacterium possesses and transmits to its progeny the capacity to produce bacteriophage (Adams 1959).

The evidence for the concept of the location of the prophage on the chromosome and its genetic role was brought to light by Murray (1953). He showed that infection leading to lysogenicity produces characteristic transient morphological changes in the host chromatin. The presence of prophage in each of the daughter cells suggests that it divides in harmony with some nuclear structure.

Lederberg and Lederberg (1953) demonstrated that prophage lambda is linked with a specific gene which controls the fermentation of galactose in E. coli K12.

How can bacteriophage be produced by cultures of lysogenic bacteria? It has been found that bacteriophage production from lysogenic strains of bacteria can be spontaneous or induced.

Using single bacteria isolated by a micromanipulator, Lwoff and Gutmann (1950) demonstrated that bacteriophages are not secreted by living and multiplying bacteria, but are released by lysis of a small number of bacteria. The constant ratio between free bacteriophage particles and the bacteria in growing cultures indicates that in each generation a given fraction of the population is lysed and bacteriophage is released. The ratio observed depends on the burst size and the frequency of lysis. The factors which determine spontaneous bacteriophage production in lysogenic cultures are not all known.

Induction of prophage to produce bacteriophages was first discovered by Lwoff, Siminovitch, and Kjeldgaard (1950). They found that irradiation of lysogenic cultures with small doses of ultra-violet light was followed by bacteriophage formation in almost the entire population; forty-five minutes after irradiation the bacteria lysed and liberated large numbers of bacteriophages. This led to the discovery of other inducing agents.

Latarjet (1951) found that X-rays can also induce bacteriophage formation. In addition several chemical compounds including such reducing compounds as thiomallic acid, reduced glutathione and ascorbic acid (Lwoff et al., 1952) were found to have an inducing effect on certain lysogenic bacteria.

Tertio-butyl peroxide also induces the development of prophage in Bacillus megatherium and in Pseudomonas pyocyanea. These bacteria are induced also by butadiene-1:3:diepoxide as well as by ethyleneimine (Lwoff and Jacob 1952). Nitrogen mustard is also an inducer on Pseudomonas pyocyanea (Jacob 1952) and on Salmonella thompson (Williams Smith 1953). Bacteriophage formation is also induced in some lysogenic strains of Salmonella thompson by sulfathiazole, the effect of which is suppressed by para-aminobenzoic acid (Williams Smith 1953).

Gots, Bird and Mudd (1955) described the action of the antibiotic L-Azaserine as an inducing agent for the development of bacteriophage in the lysogenic E. coli K12.

Otsuji et. al., (1959) showed Mitomycin C to be another inducer for bacteriophage production in the lysogenic E. coli K12.

Further work along this line has revealed several other chemical inducing agents. In our investigation, as it is stated in the introduction, we have tested a number of purine , pyrimidine and amino acid analogues and other antimetabolite compounds for their effect on the bacterium-bacteriophage relationship and also for their ability to induce bacteriophage formation in E. coli K12. These drugs showing ability in our screening procedure were studied more extensively in an effort to determine their site of action. The use of these drugs in the study of the lysogenic system has certain advantages over the use of ultraviolet irradiation, X-ray irradiation and nitrogen mustards. With these latter inducing agents precise control of dosage is not as easily achieved as with L-azaserine, mitomycin C and other compounds.

Bacteriophage lambda was originally isolated by Lederberg and Lederberg (1953) from E. coli K12. The adsorption of bacteriophage lambda to its bacterial host cells is rather poor under conditions (Physiologically young cells) which are most suitable to the adsorption of most other bacteriophages; it is quite good, however, with starved cells in the presence of Mg (Kaiser 1955). Under optimal conditions the latent period for bacteriophage lambda is forty-five minutes and the burst size is eighty to one hundred and thirty. Other workers (Lieb 1953) reported that lambda bacteriophage gives a burst size of thirty-five to one hundred particles after a latent period of thirty-five minutes. Bacteriophage lambda has been studied by the electron microscope

(Kellenberger 1954) and was found to be morphologically very similar to bacteriophage T_5. Serologically bacteriophage lambda is not related to any of the T-bacteriophages (Weigle and Delbruck 1951).

The nucleic acid of bacteriophage lambda has been analyzed by J.D.Smith and L. Siminovitch (quoted by Lwoff, 1953): its bases were found to be adenine, thymine, cytosine, and guanine; 5-hydroxy-methylcytosine was not detected.

Lambda phages used in different laboratories, although, all derived from E. coli K12, often differ genetically to some extent. This is probably due to the fact that lambda phages are usually obtained by inducing E. coli K12 with ultra-violet irradiation which is mutagenic and also to the fact that many derivatives of E. coli K12 were irradiated at one time or another in order to produce mutants for studies of bacterial genetics.

Another aspect of our problem is the preparation of E. coli W1485 spheroplasts and the infection of these spheroplasts with temperate bacteriophage. We hope that if these spheroplasts could be infected with lambda bacteriophage to make a more detailed study of the spheroplast bacteriophage relationship.

Bacterial protoplasts have been prepared by various procedures. It has been proposed to call the fragile structures that are produced from gram positive bacteria after the removal of their cell wall protoplasts, and those from the gram negative bacteria protoplasts in quotes or protoplasts like, (Weibull 1953). In our literature review we shall use this nomenclature.

Weibull (1953) showed that protoplasts can be prepared by treating cells of Bacillus megatherium with egg white lysozyme in the presence of suitable concentration of glucose or polyethylene glycol.

Lester (1953) and Beljanski (1954) working idependently showed that protoplasts of Micrococcus lysodeikticus likewise were produced with egg white lysozyme.

Mitchell and Moyle (1956) prepared protoplasts of Sarcina lutea with lysozyme.

Mitchell and Moyle (1957) in trying to prepare protoplasts of Staphylococcus aureus by lysozyme made the interesting observation that under certain conditions in the absence of lysozyme, suspensions of Staphylococcus aureus became sensitive to osmotic shock and appeared to have been converted to protoplast like bodies by autodigestion of part of the cell wall. Egg white lysozyme has been also used in preparation of "protoplasts" of gram negative bacteria.

Zinder and Arnt (1956) described the conversion of E. coli cells to osmotically fragile bodies by the action of lysozyme alone, provided that the medium was adjusted to either pH 5.0 or to between pH 8.0 and pH 9.0.

Repaske (1956) found that cells of E. coli, Aerobacter vinelandii and Pseudomonas aeruginosa, can be lysed by lysozyme at pH 7,6 to pH 8.0 if ethylenediaminetetracetic acid (EDTA) was present in the reaction mixture.

Spizizen (1957) prepared E. coli "protoplasts" with egg

white lysozyme.

Panijel and Huppert (1957) found that osmotically shocked T2r bacteriophage preparations were able to convert E. coli, strain B to protoplast like structures. Bacteriophage D4 was active on Salmonella enteriditis. Sucrose solution (20% W/V) was used as stabilizing agent and EDTA was necessary in the case of Salmonella enteriditis.

Lederberg (1956) and Hahn and Ciak (1957) idependently showed that penicillin-inhibited growth of Proteus vulgaris and E. coli could lead to spherical, osmotically sensitive protoplast like structure.

Jeynes (1957) used 3% W/V amino acetic acid (glycine) as inducing agent for protoplast formation.

Tabor (1961) reported the use of quinacrine hydrochloride as stabilizing agent.

Growth of bacteriophage in bacterial protoplasts has been reported by various investigators. Salton and McQuillen (1955), Brenner and Stent (1955), Brenner (1955) and McQuillen (1956) have reported that protoplasts of Bacillus megatherium retain the ability to support growth of bacteriophage. In their experiments, cells of Bacillus megatherium were infected with bacteriophage and then a portion of the cells was converted into protoplasts by lysozyme. Cells and protoplasts later lysed producing phage.

Zinder and Arndt (1956) infected E. coli, strain B with T4 bacteriophage and after adsorption was completed lysozyme was added. Protoplasts thus produced by lysozyme were capable of

producing T_4 bacteriophage in titer comparable to that produced by intact cells.

Lederberg and St. Clair (1958) have reported that penicillin spheroplasts (protoplasts with cell wall parts left on them) of E. coli could be infected with T_3, T7 and T_5 bacteriophages.

In our experiments we shall attempt to produce E. coli W1485 spheroplasts by the method of Joynes (1957) and if these spheroplasts show ability to adsorb lambda bacteriophage further experiments will be planned.

CHAPTER III

MATERIALS AND METHODS

Bacterial strains and bacteriophage used

Three strains of Escherichia coli were used in all of our experiments: E. coli K12, E. coli C and E. coli W1485. Two of the cultures were furnished by Dr. Thomas J. Bird, and E. coli W1485 was obtaine from the American Type Culture Collection. Their identification was based on a number of biochemical reactions as is indicated in Table II (page 33).

The temperate bacteriophage lambda from E. coli K12 was used throughout our experiments. Lambda phages were prepared by irradiating lysogenic E. coli K12 with ultra-violet light, incubating the irradiated culture in the dark for 2½ hours and then centrifuging to remove the nonlysed bacteria and debris. The supernatant was mixed with chloroform, shaken for 3o minutes to kill the remaining bacteria and centrifuged again. The clear supernatant was assayed for lambda phage with E. coli W1485, the phage sensitive indicator strain. Lysates prepared by this method gave us 10^3-10^5 plaque forming units per ml.

Phage assays or titers mentioned thoughout this work were obtained by the agar layer method Gratia (1936). Briefly, the phage suspension is diluted serially in exactly the same manner

as are bacterial suspensions. A measured volume of the diluted suspension, usually 0.1 ml, is placed into about 3.0 ml of 0.75% Agar in Brain Heart Infusion Broth (Difco) which has been previously melted and placed in a 45-47°C water bath. The agar has also been inoculated with approximately 10^8-10^9 bacteria per ml, which are susceptible to the bacteriophage. This mixture of agar, bacteria, and phage is poured onto a petri-dish containing about 25ml of Nutrient Agar (difco). The bacteria grow in the top layer and would give a confluent film of bacteria except for the action of phages, which lyse circular clearings called plaques. The number of plaques on a plate multiplied by the dilution factor of the original suspension gives the assay of phages as number which has been shown by the electron microscope to be the same as the number of phage particles (Luria, Williams, and Backus 1951).

High titer stocks were prepared by the agar layer method (Adams 1959). Titers of 10^7-10^8 bacteriophage particles per ml were obtained by this method. Further concentration of phages was achieved by lyophilization (Adams 1959) or carbowax adsorption.

Screening of chemical compounds

The chemical compounds (drugs) used in this work are listed in Table I, (pages 30-32). The number following each chemical compound denotes the source from which it was obtained. Number 1 indicates that the drug was obtained from Nutritional Biochemicals Corporation, 2 from California Corporation for Biochemical Research, 3 from Mann Research Laboratories, 4 from Bios Laboratory and 5 that the chemical was obtained from elsewhere.

The screening of the effect of the drugs on the bacteriophage bacterium interaction was studied using the paper disc method described by Asheshov, Hall, and Flon (1955). Filter paper discs ¼inch diameter (Schleicher&Schuell, # 740 E) of highly adsorbent paper were saturated with a solution of the test substance and placed on the surface of prepared bacteriophage-bacteria agar plates. A mixture of bacteria and bacteriophage producing approximately 300 plaques was spread over a nutrient agar plate. The agar plates were allowed to dry for 30 minutes and the discs containig the proper concentration of the drug were placed on the surface of the agar plate. Four different concentrations were used for each chemical compound (drug): 50mg/ml, 25mg/ml, 15mg/ml and 5mg/ml. The plates were then incubated overnight at 37^{0}C and the results were recorded as is shown in Table I (pages 30-32). From the drugs that showed some effect by this screening method a number were selected and studied more extensively for their effect on free lambda bacteriophage, bacteria, bacteria-bacteriophage combination by the one step growth experiment Ellis and Delbruck (1939) and for their ability to induce lambda bacteriophage formation in E. coli K12.

Effect of chemical compounds on free bacteriophage

The effect of the following chemical compounds on free lambda bacteriophage was studied:

L-Arterenol Bitartrate Hydrate

DL-Beta-Phenyllactic Acid

Benzimidazole

Bisulfan

Dichloracetyl-DL-Serine Na

Dichloracetyl-L-Serine

DL-Para-Fluorophenylalanine

Pentamidine

Propamidine

N-CBZ-DL-Serine

Stilbamidine

DL-BEta-3-Thienylalanine

The above chemical compounds were dissolved in cold or hot water, but for uniformity all the above chemical compounds were heated to 85°-90°C before use.

The titer of the lysate used was 2.4×10^8 plaque forming units per milliliter. Nine-tenths ml of the lysate was dispensed into test tubes. To this was added 0.1ml of the proper dilution of the chemical compound. The concentrations used for each drug tested were: 5mg/ml, 2.5mg/ml, 1.5mg/ml, and 0.5mg/ml. Controls were run concurrently. Assays for lambda bacteriophage were run after one hour, three hours and twenty-four hours of incubation. The agar layer plate method of Gratia (1936) was used for these assays.

Effect of chemical compounds on Escherichia coli W1485

E. coli W1485 was grown in nutrient broth (Difco) at 37°C for twenty-four hours. One tenth ml of this culture was inoculated into 100ml of nutrient broth and was incubated with aeration in a 37°C water bath for and one half hours. The 2½ hour aerated

culture was centrifuged and the supernatant was discarded. The bacterial cells were resuspended in saline, centrifuged and again resuspended in saline. Plate counts done in dublicate showed that the bacterial culture contained 2.4×10^8 bacterial cells per ml. From this bacterial culture a dilution tube was prepared containing 2.4×10^6 bacterial cells per ml. From this 0.1ml was inoculated into 1.0ml of nutrient broth containing the appropriate chemical compound at the proper concentration, thus the bacterial culture was diluted from 2.4×10^6 to 2.4×10^5 bacterial cells per ml. Controls were run concurrently. The concentrations of each chemical compound used were: 50mg/ml, 25mg/ml, 15mg/ml, and 5.0mg/ml.

The above bacterial-chemical compound mixture and the controls were incubated in a 37°C water bath and aliquots were removed at zero time, after one, three, five and twenty-four hours. Plate counts were made for each aliquot taken. The inhibitory, n non-inhibitory or the stimulating effect of these chemical compounds upon the growth of E. coli W1485 was studied. Three such experiments were performed for each chemical compound tested.

Additional experiments of this type were performed using only one concentration (1mg/ml) of each chemical compound. The chemical compounds thus tested were dissolved in phosphate buffer. Aliquots were drawn at zero time after two, four, six hours and after overnight incubation at 37°C. One experiment for each chemical compound was performed.

Effect of chemical compounds on the intracellular development of lambda bacteriophage

The effect of each chemical compound on the intracellular development of lambda bacteriophage was studied by the "one step growth" experiment described by Delbruck and Luria (1942).

The intracellular development of lambda phage was also studied by a method described by Asheshov et al., (1955) which is a modification of the "one step growth" experiment. By this method the adsorption tube is diluted 1:50,000 and assays are made after one, two, three, and five hours of incubation of the bacterial-bacteriophage chemical compound combination.

Ability of chemical compounds to induce lambda formation in Escherichia coli K12

The ability of a chemical compound to induce the growth and development of active bacteriophage from the prophage state in lysogenic bacteria has been correlated with mutagenic and carcinogenic activities Lwoff (1953). In our investigation we tested a number of chemical compounds which had shown some effect on the bacteria-bacteriophage interaction by the paper disc method.

The effect of these chemical compounds on the maturation of the prophage in the lysogenic E. coli K12 was studied with the described by Gots, Bird, and Mudd (1955) in their experiment with L-Azaserine.

Preparation of E. coli W1485 spheroplasts Infection of spheroplasts with lambda

E. coli W1485 spheroplasts were prepared by the method of Joynes (1957). The spheroplasts wer examined microscopically and

their highly fragile nature was observed when they came in contact with water.

Infection of E. coli W1485 spheroplasts with lambda phage was accomplished in the following manner. One ml of an 18-20 hour culture of spheroplasts was centrifuged and the supernatant was discarded. The pellet of spheroplasts was dispersed into two milliliters of water containing 0.001M/liter of quinacrine hydrochloride which has been reported by Tabor (1961) to be a good stabilizer for E. coli spheroplasts. To this tube lambda phage was added and adsorption took place for 6.0-12.0 minutes at 37°C. Assays for adsorbed and non-adsorbed bacteriophage particles by spheroplasts were compared with assays obtained with whole cells of E. coli W1485.

CHAPTER IV

RESULTS

SCREENING OF CHEMICAL COMPOUNDS

The screening of the chemical compounds studied was done by the filter paper disc method as described in materials and methods. The results obtained by this method were recorded in Table I (pages 30-32). as B, P, BP, and O.

1). B: Antibacterial activity: Is characterised by a zone of inhibition around the disc, Figure No. 1 (page 50). A zone of complete bacterial inhibition is visible around the disc and this is surrounded by a zone of lighter bacterial growth.

2). P: Antiphage activity: Is characterized by an area around the disc in which although bacterial growth is visible bacteriophage plaques are lacking. Also an area in which fewer plaques are present is visible. Figure No. II (page 51).

3). BP: Combined antibacterial and antiphage activity: This case is evident in Figure No. III (page 52). A zone of bacterial inhibition is surrounded by another zone of lighter bacterial growth and an area lacking bacteriophage plaques.

4). O: No effect whatsoever: Figure No. IV (page 53).

One more phenomenon not shown in the figures should be mentioned here. This phenomenon may be called stimulation of

bacteriophage. It appeared in very few cases. A greater number of bacteriophage plaques was observed close to the disc than in the rest of the plate.

Effect of chemical compounds on free bacteriophage

A dozen of chemical compounds were tested for their effect on free lambda bacteriophage. Assays for surviving lambda phage were made after one, three, and twenty-four hours of incubation. The results are shown in Table No. III (page 35).

It can be seen from this table that the chemical compounds DL-Beta-Phenyllactic Acid and Benzimidazole inhibit free lambda phage completely at the concentrations 5, 2.5, and 1.5mg/ml. At the concentration of 0.5mg/ml the inhibition is not complete.

L-Arterenol Bitartrate Hydrate, Bisulfan, Dichloracetyl-L-Serine and N-CBZ-DL-Serine inhibit lambda phage completely at the concentration of 5mg/ml after 24 hours of incubation; L-Arterenol Bitartrate Hydrate inhibits lambda phage after 24 hrs at the 2.5mg/ml concentration and also it is more inhibitory at lower concentrations than the other chemical compounds. Bisulfan in the lower concentration of 0.5mg/ml has no effect on lambda. Pentamidine has a slight inhibitory effect on lambda bacteriophage in all the concentrations used.

Propamidine, Stilbamidine, and DL-Beta-3-Thienylalanine all have a very slight inhibitory effect in the higher concentrations and no effect in the 1.5mg/m and 0.5mg/ml concentrations.

Dichloracetyl-DL-Serine Na has no effect on free lambda phage in any of the concentrations tested.

Effect of chemical compounds on E. coli W1485

The effect of a number of chemical compounds on E. coli W1485 was tested. The inhibitory, non-inhibitory or the stimulating effect of these chemical compounds upon the growth of E. coli w1485 is recorded in Table IV (page 36).

L-Arterenol Bitartrate Hydrate, inhibits the growth of E. coli W1485 at all the concentrations tested after twenty-four hours of incubation. In shorter periods of incubation a gradual reduction of bacterial cell numbers was noted in all the concentrations. (Not recorded in table). After one hour of incubation in the concentration of 50mg/ml the bacterial cell number is reduced form 2.4×10^5 to 1.1×10^4 cells/ml; after three hours of incubation it was reduced to 2.8×10^3, and after twenty-four hours to zero. Similar progressive reduction was noted for the concentrations of 25mg/ml, 15mg/ml, and 5mg/ml of the compound L-Arterenol Bitartrate Hydrate.

Benzimidazole: This compound inhibited partially the growth of E. coli W1485 at all the concentrations after 24 hours of incubation. During incubation for one, and three hours the reduction was slight. Thus after twenty-four hours of incubation the reduction was, for 50mg/ml from 2.4×10^5 to 9×10^4, for 25mg/ml to 7.2×10^4, for 15mg/ml was 7.8×10^4 and for 5mg/ml was 8.9×10^4 cells per milliliter.

Bisulfan at all the concentrations used inhibited the growth of E. coli W1485 completely after one, three, and twenty-

four hours incubation.

Dichloracetyl-DL-Serine Na reduced the number of E. coli W1485 cells from 2.4 $X10^5$ to 2.5 $X10^4$ at the concentration of 50mg/ml after twenty-four hours of incubation; at the other concentrations used there was no reduction during the same period of incubation.

N-Fluoro-DL-Phenylalanine in the concentrations of 50mg/ml and 25mg/ml completely inhibited the growth of E. coli W1485; in the lower concentrations of 15mg/ml and 5mg/ml there was a reduction in the number of E. coli W1485 cells to 13 $X10^5$ and to 20.4 $X10^4$ cells per milliliter.

Dichloracetyl-L-Serine, Pentamidine, Propamidine and N-CBZ-DL-Serine all these chemical compounds inhibited the growth of E. coli W1485 completely after one, three, five and twenty-four hours of incubation at all the concentrations used with the only exception of Dichloracetyl-L-Serine and N-CBZ-DL-Serine which at the lower concentration of 5mg/ml did allow some growth to take place even after twenty-four hours of incubation.

Stilbamidine inhibited the growth of E. coli W1485 in all the concentrations tested to some degree.

Additional experiments of the same type were performed. The concentration of the chemical compounds used was 1mg/ml. Assays were made to determine the number of bacteria growing. The results obtained from these assays are recorded (for zero and one hundred and twenty minutes) in Table V (page 37).

The effect of the chemical compounds upon the generation

time of E. coli W1485 has been calculated and recorded. The calculations were carried out by the use of the two formulae used in the studies of growth of bacteria, (Clifton 1957). The two formulae used are shown below:

$$(1) \qquad n = \frac{\log Bt - \log Bo}{\log 2} \qquad (2) \qquad G \text{ or } g = \frac{t}{n}$$

Where:
- n Number of generations
- t Time
- g Average generation time
- Bo Number of bacteria at the beginning of time t
- Bt Number of bacteria at the end of time

As it can be seen from Table V (page 37) for the chemical compounds Pentamidine and Propamidine, a calculation of the generation time was not attempted since it was evident that both chemicals suppressed the growth of E. coli W1485 at the concentrations tested. As the concentration of these chemical compounds is lowered slight growth takes place. Perhaps both compounds at a lower concentration may increase the generation time thus slowing the overall growth of E. coli W1485.

From examining the generation time of E. coli W1485 in the presence of the various chemical compounds we can see that the compound Bisulfan increases the generation time from 30 minutes to 48.7 minutes. Stilbamidine which in higher concentrations inhibited growth completely, at the concentration of 1mg/ml suppressed the growth by increasing the generation time to 44.5 minutes. L-Arterenol-Bitartrate-Hydrate increased the generation time to 42.8 minutes. Dichloracetyl-L-Serine also increased the generration time to 40 minutes. Benzimidazole and Dichloracetyl-DL-

Serine Na increased the generation time to 33 minutes.

Some of the chemical compounds tested had other effects on the growth of E. coli W1485. DL-Beta-Phenyllactic Acid showed a stimulating effect. The generation time was reduced from 30 to 28 minutes. DL-Para-FluorophenylAlanine and DL-Beta-3-Thienyl-alanine both stimulated growth by reducing the generation time to 26.6 minutes. N-CBZ-DL-Serine also stimulated the growth of E. coli W1485 by reducing the generation time to 25.5 minutes.

Effect of chemical compounds on the intracellular development of lambda phage

The effect of a number of chemical compounds on the intracellular development of lambda phage was studied by the "one step growth" experiment of Delbruck, and Luria (1942) and also by a modification of the above experiment described by Asheshov et al. (1955).

As is indicated in Table VI (page 38) the different chemical compounds varied in their effects on the intracellular development of lambda phage. We can see the effect of each chemical compound on the length of the "latent period" and also the effect on the "burst size". The extent of the latent period varies from 3o minutes which is below the normal of (control) 34 to the high 47 minutes, and the burst size varies from 56 plaque forming units per milliliter (which is far below the 106 plaque forming units per milliliter of the control) to 136 plaque forming units pre ml.

The effect of the same chemical compounds on the intracellular development of lambda phage was also studied by the method of Asheshov et al., (1955). This method was used to determine in a gross way whether any of the chemical compounds had an inhibitory or stimulatory actinn on the intracellular development of lambda phage which was too slight to be observed by the disc screening procedure.

As it can be seen from Table VII (page 39) the chemical compounds were divided into two groups. The number of plaques

showing in each assay performed is shown in this table and also the effect of each compound on the intracellular development of lambda phage has been identified by I: for inhibitory effect, NI: for non-inhibitory effect, and S: for stimulatory effect.

Ability of chemical compounds to induce lambda phage formation in E. coli K12

Fifteen chemical compounds were tested for their ability to induce lambda phage maturation in E. coli K12. The results obtained are shown in Table VIII (page 40). In this table we can see the number of lambda phage particles produced by exposure of E. coli K12 to the various chemical compounds.

DL-Alamyl-DL-Serine, DL-Arterenol HCL, Benzimidazole, Dichloracetyl-L-Serine, 3,4-Dihydroxynorephedrine, Formyl-DL-Phenylalanine and N-CBZ-DL-Serine all induce the formation of lambda bacteriophage from E. coli K12. It is of interest to note that Benzimidazole, which at 5mg/ml concentration inhibits free lambda phage, induces formation of lambda phage at the lower concentration of 100ug or 1mg/ml.

Stilbamidine, Propamidine, Pentamidine, Thiouracil and L-Arterenol Bitartrate Hydrate did not induce lambda phage formation.

DL-Para-Fluorophenylalanine induces lambda phage at the lower concentration of 100ug and the higher concentration of 1mg/ml but the size of the plaques is much smaller than usually and this should be investigated.

Infection of E. coli W1485 spheroplasts with lambda bacteriophage

It was found that lambda phage was adsorbed onto E. coli W1485 spheroplasts at almost the same rate as to the whole cells of E. coli W1485. As it can be seen from Table IX (page 41) the adsorption of lambda phage onto whole cells of E. coli W1485 is much more rapid in the first six minutes and then it becomes slower to the twelveth minute. In contrast adsorption of lambda phage onto E. coli W1485 spheroplasts is less rapid in the first six minutes and more rapid in the twelveth minute. In total percentages the adsorption of lambda phage to E. coli W1485 whole cells and spheroplasts was almost similar for the time of six to twelve minutes. There seems to be a large number of receptor sites for lambda phage remaining on the spheroplasts of E. coli W1485. The results indicate that E. coli W1485 growing in a medium with glycine is converted not into true protoplasts, but into spheroplasts. We have here the same observation that other workers have reported while they were working with other phages. The fact remains that lambda phage infects E. coli W1485 spheroplasts at the same rate as it infects whole cells.

Table I

EFFECT OF COMPOUNDS TESTED
DISC TEST

Compound	Concentrations			
	50mg/ml	25mg/ml	15mg/ml	5mg/ml
DL-Alamyl-DL-Serine[3]	B	B	O	O
DL-Arterenol[1]	B	B	B	B
L-Arterenol Bitartrate Hydrate[2]	B	B	O	O
8-Azaguanine[1]	B	B	O	O
6-Aza Uridine[1]	B	B	B	B
DL-Beta Phenyllactic Acid[1]	B	B	O	O
Benzimidazole[1]	B	B	O	O
Bisulfan[3]	B	B	O	O
Chloracetyl-DL-Phenylalanine[1]	B	B	O	O
Dichloracetyl-DL-Serine Na[3]	B	B	B	B
N-Dichloracetyl-DL-Serine Na[1]	B	B	O	O
Chlorambucil[1]	B	B	O	O
Dichloracetyl-L-Serine[1]	B	B	O	O
D(+)-Dihydroxyphenylalanine[2]	B	B	O	O
D-Epinephrine[2]	B	B	O	O
L-Epinephrine[2]	B	B	O	O
DL-p-Fluorophenylalanine[1]	B	B	B	O
M Fluoro DL Phenylalanine[2]	B	B	O	O
Formyl-DL-Phenylalanine[3]	BP	BP	O	O
5-Iodo-Deoxyuridine[1]	B	B	O	O

Table I

EFFECT OF COMPOUNDS TESTED

DISC TEST

Compound	Concentrations			
	50mg/ml	25mg/ml	15mg/ml	5mg/ml
DL-N-Isopropylarterenol NNR[2]	B	B	O	O
Kinetin[1]	B	B	O	O
Pentamidine[4]	BP	BP	BP	BP
DL-Phenylalanine Benzyl Ester[3]	B	B	B	B
Propamidine[4]	P	P	P	P
Pyridine-3-Sulfonic Acid[1]	B	B	B	B
N-CBZ-DL-Serine[3]	B	B	O	O
Stilbamidine[4]	B	B	O	O
DL-Beta-3-Thienylalanine[1]	P	P	O	O
Thiouracil[1]	BP	BP	O	O
2,5,6-Triamino-4-Hydroxypyrimidine Sulfate[5]	B	B	O	O
L-Tyrosine[2]	B	O	O	O

THE FOLLOWING COMPOUNDS SHOWED NO EFFECT

Acetyl-D-Phenylalanine[1], 3-Acetyl Pyridine[1], Adenine Sulfate, Adeno Pep[5], Adenosine[1], Allyl Glycine[1], Aminoacetic Acid[1], 2-Amino-4-Methyl Pyrimidine[1], Aminopterin[3], L-3Amino Tyrosine DiHcl[1], L-Arginine Monohydrochloride[1], L-Asparagine[1], 8-Aza-Adenine[1], Barbituric Acid[1], Batyl Distearate[2], Benzimidine[1], N-Benzol-DL-Phenylalanine b-naphthol ester[2], 5-Bromo-Uracil[5],

Table I

EFFECT OF COMPOUNDS TESTED
DISC TEST

Compound	Concentrations			
	50mg/ml	25mg/ml	15mg/ml	5mg/ml

N-Dichloracetyl-DL-Serine Na[1], 2 Chloro-4-Aminobenzoic Acid[1], DL-p-Chloro Phenylalanine[1], 6-Chloro Purine[1], 8-Chloro-Xanthine[1], DL-Desthiobiotin[1], Desoxypyridoxine HCL[1], Dicumarol[1], Dihydro-cholesterol[1], 3,4-Dihydroxynorephedrine HCL[2], DL-3,4-Dihydroxy-phenylalanine[2], L-3,4-Dihydroxyphenylalanine[2] (L-DOPA), L-Epinephrine Bitartrate[2], Estradiol[1], DL-Ethionine[1], Gamma-Benzene Hexachloride[1], Glycyl-DL-Phenylalanine[2], Guanidine HCL[1], Guanine Sulfate[1], Homocysteine[5], 5-Hydroxyindole-3-Acetic Acid[2], 3-Hydroxytramine HCL[2], Indole[1], D-Leucine[1], DL-Leucine[1], 2-Mercaptopurine[2], 6-Mercaptopurine Hydrate[2], Methionine Sulfo-xide[1], 7-Methyl-Folic Acid[5], DL Methyl Glutamic Acid[1], DL-Norephedrine HCL[2], DL-a-Phenyl-a-Alanine[2], DL-b-Phenyl Acetic Acid[2], DL-b-Phenyl-b-Alanine[2], Phenylalanine HCL[2], N-CBZ-DL-Phenylalanine[3], DL-Phenylalanine Benzyl Ester[3], L-Phenylephrine HCL[2], a-Picolinic Acid HCL[1], Pteridyl Sulfonamide[5], Pteroyl Aspartic Acid[5], Pyridine-3-Sulfonic Acid Na[1], Pyromycin DiHCL[4], Quercetin[1], Sulfanilamide USP[1], Beta-2-Thienylalanine[1], Beta-2-Thienylserine[1], P-Tosyl-DL-Phenylalanine[3], DL-Tyrosine[2], L-Valine[3].

Table II

BIOCHEMICAL REACTIONS OF ORGANISMS USED

Biochemical Reactions	Organisms Used		
	Escherichia coli, Strains:		
	K12	C	W1485
Adonitol	N	P	N
Aesculin	P	P	N
Arabinose	P	P	P
Galactose	P	P	P
Glucose	P	P	P
Glucose-gas	P	P	P
Inositol	N	N	N
10% Lactose	P	P	P
.5% Lactose	P	P	
Levulose	P	P	N
Maltose	P	P	P
Mannitol	P	"	P
Mannose	p	N	p
Melibiose	P	P	P
Raffinose	N	N	N
Ramnose	P	P	P
Salicin	N	N	N
Sorbitol	P	P	p
Sorbose	N	N	N
Sucrose	N	N	N

Table II

BIOCHEMICAL REACTIONS OF ORGANISMS USED

Biochemical Reactions	Organisms Used		
	Escherichia coli, Strains:		
	K12	C	W1485
Trehalose	P	P	P
Xylose	P	P	P
Urea	N	N	N
Indol	P	P	P
Methyl Red	P	P	P
Voges-Proskauer	N	N	N
Citrate	N	N	N
Gelatin	N	N	N
Motility	N	N	N
Sulfide	N	N	p
HL-of-OX	P	P	P
HL-of-AN	P	P	P

P— Positive Reaction

N— Negative Reaction

p— Weak Positive Reaction

Table III

EFFECT OF DRUGS ON FREE BACTERIOPHAGE

Drugs Tested	Time in Hours	Concentrations Used in mg/ml 5	2.5	1.5	0.5
L-Arterenol Bitartrate Hyd.	1	8*	7	70	810
	24	0	0	10	800
DL-Beta Phenyllactic Acid	1	0	0	3	7
	24	0	0	0	1
Benzimidazole	1	0	.2	6	7
	24	0	0	0	3
Bisulfan	1	.3	60	730	2400
	24	0	60	700	2100
Dichloracetyl-DL-Serine Na	1	2400	2400	2400	2400
	24	2400	2400	2400	2400
Dichloracetyl-L-Serine	1	.2	19	25	26
	24	0	14	17	23
DL-para-Fluorophenylalanine	1	1800	2300	2400	2400
	24	230	2100	2400	2400
Pentamidine	1	120	180	290	610
	24	2	30	27	51
Propamidine	1	1000	1700	1200	1800
	24	38	19	280	1000
N-CBZ-DL-Serine	1	150	360	540	670
	24	0	.04	1	66
Stilbamidine	1	220	300	200	2400
	24	2	210	250	2200
DL-Beta-3-Thienylalanine	1	160	200	460	2400
	24	140	200	460	2400

*All counts representing plaque forming units are expressed as whole numbers X 10^5.

Table IV

EFFECT OF DRUGS ON SURVIVAL OF E. COLI W1485*

Drugs Tested	Concentrations Used in mg/ml 50	25	15	5
	Colony Counts/ml			
L-Arterenol Bitartrate Hyd.	0	0	0	0
Benzimidazole	9	72	78	89
Bisulfan	0	0	0	0
Dichloracetyl-DL-Serine Na	25	5100	6800	8000
Dichloracetyl-L-Serine	0	0	0	0
M-Fluorophenylalanine	0	0	130	204
Pentamidine	0	0	0	0
Propamidine	0	0	0	0
N-CBZ-DL-Serine	0	0	0	220
Stilbamidine	2	2	2	2
Control	13000			

* E. coli W1485 (24 X10^4 cells/ml) incubated in Nutrient Broth with the various concentrations of each drug for 18-24 hours. Colony counts are expressed as whole numbers X 10^3

Table V

EFFECT OF DRUGS ON THE GENERATION TIME OF E. COLI W1485

Drugs Tested	Concentr. Used lmg/ml.	Time in Minutes 0 Dil.	0 Count*	120 Dil.	120 Count	GT in Min
L-Arterenol Bitartrate Hyd.	"	-4	TNTC	-4	TNTC	
		-6	17**	-6	119	42.8
DL-Beta Phenyllactic Acid	"	-4	TNTC	-4	TNTC	
		-5	89	-6	132	28.0
Benzimidazole	"	-3	TNTC	-3	TNTC	
		-5	24	-5	285	33.6
Bisulfan	"	-4	136	-4	TNTC	
		-5	11	-6	60	48.7
Dichloracetyl-DL-Serine Na	"	-3	TNTC	-4	TNTC	
		-5	89	-6	116	32.4
Dichloracetyl-L-Serine	"	-3	478	-4	TNTC	
		-5	89	-6	7	40.0
DL-Para-Fluorophenylalanine	"	-4	389	-4	TNTC	
		-5	69	-6	158	26.6
Pentamidine	"	-2	110	-2	10	
		-4	0	-4	0	
Propamidine	"	-1	90	-1	0	
		-2	0	-2	0	
N-CBZ-DL-Serine	"	-3	TNTC	-4	TNTC	
		-5	49	-6	132	25.5
Stilbamidine	"	-2	118	-2	TNTC	
		-3	11	-3	71	44.5
DL-Beta-3-Thienylalanine	"	-4	360	-4	TNTC	
		-6	11	-6	259	26.6
Control	0	-5	98	-5	TNTC	
		-6	17	-6	160	30.0

* TNTC: Stands for Too Numerous To Count

** Colony counts per milliliter; Average of three plates

GT: Stands for Generation Time

Table VI

EFFECT OF DRUGS ON THE INTRACELLULAR DEVELOPMENT OF LAMBDA BACTERIOPHAGE

Drugs Tested	Concent. Used	Latent Period in Minutes	Burst Size P.F.U.*
L-Arterenol Bitartrate Hyd.	1mg/ml	41	83
DL-Beta-Phenyllactic Acid	"	37	78
Benzimidazole	"	35	80
Bisulfan	"	40	63
Dichloracetyl-DL-Serine Na	"	34	136
Dichloracetyl-L-Serine	"	33	101
DL-Para-Fluorophenylalanine	"	30	122
Pentamidine	"	47	67
Propamidine	"	46	59
N-CBZ-DL-Serine	"	33	128
Stilbamidine	"	45	46
DL-Beta-3-Thienylalanine	"	30	97
Control	0	34	105

* P.F.U. Stands for Plaque Forming Units

Table VII

EFFECT OF DRUGS ON THE INTRACELLULAR DEVELOPMENT OF LAMBDA BACTERIOPHAGE

Drugs Tested*	Time in Hours----Assays 1 Dil.	3 Plaques	5 Counted	Effect**
Group I***	-2	0	7	11	
Dichloracetyl-DL-Serine Na	-2	71	247	345	S
Dichloracetyl-L-Serine	-2	0	9	12	N I
Pentamidine	-2	0	0	0	I
N-CBZ-DL-Serine	-2	89	136	206	S
Group II***	-2	23	110	90	
L-Arterenol Bitartrate Hyd.	-2	34	35	37	I
Benzimidazole	-2	48	152	101	N I
Bisulfan	-2	37	34	22	I
DL-Beta-Phenyllactic Acid	-2	0	0	0	I
DL-Para-Fluorophenylalanine	-2	98	350	325	S
Propamidine	-2	18	0	0	I
DL-Beta-3-Thienylalanine	-2	34	59	89	N I

* Concentration of drugs used (1mg/ml)

** Effect of the drug on the number of Plaque Forming Units

***Two controls were used; one for each Group.

Table VIII

ABILITY OF DRUGS TO INDUCE LAMBDA FORMATION IN E. COLI K12

Drugs Tested	Time in Hours	Concentrations Used in γg/ml 100	200	500	1000
		Plaque Forming Units			
DL-Alamyl-DL-Serine	2	0	14	18	32
	4	7	44	56	380
DL-Arterenol HCL	2	0	0	11	66
	4	0	20	47	423
DL-Beta-Phenyllactic Acid	2	0	12	13	60
	4	11	17	24	448
Benzimidazole	2	14	20	11	196
	4	354	33	15	493
Dichloracetyl-DL-Serine Na	2	7	0	26	59
	4	20	140	109	222
Dichloracetyl-L-Serine	2	18	10	259	68
	4	12	16	1966	323
3,4-Dihydroxynorephedrine HCL	2	22	11	13	56
	4	14	43	23	289
DL-Para-Fluorophenylalanine	2	25	19	18	130
	4	355	49	98	1236
Formyl-DL-Phenylalanine	2	10	27	15	150
	4	40	22	23	387
N-CBZ-DL-Serine	2	0	10	17	127
	4	21	33	22	467
L-Arterenol Bitartrate Hyd.	2	10	11	12	11
	4	12	26	16	21
Pentamidine	2	4	12	47	2
	4	24	31	67	23
Propamidine	2	10	9	3	13
	4	15	13	17	15
Stilbamidine	2	6	8	11	16
	4	34	42	15	29
Thiouracil	2	10	2	15	37
	4	11	12	22	41
Control	2	17			
	4	38			

Table IX

ADSORPTION OF LAMBDA PHAGE ONTO SPHEROPLASTS OF E. COLI W1485

Adsorption Tubes		Time in Minutes	Percent Adsorption*
E. coli W1485		6	34.15
Spheroplasts/ml	$25X10^6$	8	23.75
Lambda Phage/ml	$24X10^6$	10	11.25
		12	16.00
			Total 85.15
E. coli W1485		6	45.10
Whole Cells/ml	$60X10^7$	8	25.50
Lambda Phage/ml	$43X10^7$	10	11.50
		12	4.55
			Total 86.65

*The same pattern of adsorption was seen in a number of similar experiments.

CHAPTER V

DISCUSSION

For a considerable time after bacteriophage was discovered by Twort (1915) and d'Herelle (1921) the possibility that bacteriophages might prove to be the ideal agent for treating diseases caused by bacterial infection was recognized and efforts were undertaken to explore this possibility.

Bacteriophage is not an agent for treatment of bacterial infections and interest in this aspect has diminished. Most of the investigations in our times are undertaken not beaause of therapeutic importance of bacteriophages, but because in contrast to the animal and plant viruses these bacterial viruses are easy culture and to handle and so present a simple means of acquiring fundamental knowledge of virus behavior. There are differences in the characters of viruses which affect such a great number of hosts but there are also many similarities and so discoveries made in the study of bacteriophages point the way to the solution of some of the problems encountered in the investigation of animal and plant viruses.

With this in mind we decided to study the host parasite relationship as it exists in the bacteriophage-infected bacterial cell. The approach that we used in studying the lysogenic system

in E.coli K12 was essentially the screening of a number of drugs and determining their effect on the lysogenic system.

One hundred purine, pyrimidine, amino acid analogues, and other antimetabolite compounds were screened by the paper disc method for their effect on the bacterial cell-bacteriophage interaction. By this method more than two dozens of these chemical compounds showed some effect. We were interested in the host parasite relationship and attempted to select a variety of drugs ranging from antiparasitic to substituted amino acids and study their effect further.

Stilbamidine, Propamidine and Pentamidine have been used as chemotherapeutic drugs against mycoses, leishmanial infections and trypanosomiasis. In all these diseases the infectious agent is an intracellular parasite and the drug acts either by interference with aerobic glucose or nucleic acid metabolism within the organisms. These drugs had shown an effect in our system as demonstrated by the disc method.

Among the purine and pyrimidine analogues tested, Benzimidazole had an interesting effect. Benzimidazole, which is a part of the Vitamin B12 molecule has an inhibitory effect on vaccinia virus in tissue culture Thompson (1947); MEF-1-poliomyelitis virus in tissue culture Brown (1952); inhibited the cytopathogenic effects on the following enteroviruses: Coxsackie A9; Coxsackie B 1 to 6; and ECHO virus types 1 to 9, 11 to 21, and 24 to 27. (Eggers and Tamm, 1961).

Another group of compounds that were tested further were

the halogenated amino acids both aliphatic and aromatic. Several amino acid analogues have been reported to inhibit multiplication of bacteriophage, but most of these have an inhibitory effect on the growth of uninfected bacterial cells and none brings about the "cure" of an infected cell. (Matthews and Smith 1955).

Bisulfan, which resembles nitrogen mustard at least to the extent that it is an alkylating agent, was selected because of its effects on myelogenous leukemia, and its effect against other bacterium-bacteriophage combinations.

L-Arterenol Bitartrate Hydrate, was also investigated, because of its structural similarity to substituted amino acids and its effect in our system as demonstrated by the disc method.

When we are working with a chemical compound (drug) a number of things may take place. The drug may affect the stability of free bacteriophage, or the latent period and the burst size. It may prevent lysis altogether, or it may affect the bacterial growth. In the case of a temperate phage such as lambda the chemical compound may also cause a change in the number of cells being lysogenized.

DL-Beta-Phenyllactic acid, Benzimidazole, Bisulfan and N-CBZ-DL-Serine inhibited free lambda phage at the concentrations used. This could have been expected since we know that compounds of the described nature have inhibited other free phages (Matthews and Smith 1955). Phage lambda was resistant to the other drugs tested as are animal and plant viruses.

Another possible mode of action of the drugs tested is

their effect on the indicator strain E. coli W1485. Most of the drugs inhibited E. coli W1485 at the high concentration of 50, 25, 15, and 5mg/ml while at the low concentration of 1mg/ml some of the drugs suppressed or increased the growth of E. coli W1485 as was determined from calculating the generation time of E. coli W1485 in the presence of the drug. Here the antiparasitic drugs greatly suppressed the growth of E. coli W1485 as also did Bisulfan, L-Arterenol Bitartrate Hydrate and Dichloracetyl-L-Serine. On the other hand most of the halogenated amino acids stimulated the growth of E. coli W1485.

Aside from the effect of these drugs on free phage and E. coli W1485 their effect on the intracellular development of lambda phage was more important from our point of view. The events of bacteriophage development and multiplication are very important and they should be mentioned here. The addition of lambda phage or any phage to a sensitive bacteria culture it is followed by a period during which phage disappears being adsorbed to the bacterial host, and a period of intracellular multiplication without liberation of bacteriophage, the latent period, terminated by the lysis of the bacterium and release of phages ,the burst size. Lambda phage may in addition be established in the lysogenic state.

Lambda phage developed normally in the presence of DL-Beta-3-Thienylalanine and Dichloracetyl-L-Serine. In the presence of the remaing drugs a number of things were observed.

The mode of action of these drugs is difficult to explain.

We know that the main constituents of the phage particle are protein and DNA; also we know that phage itself is devoid of any independent metabolic activity, and that it needs a bacterial host to replicate. Phage DNA injected into its bacterial host is committed to two principal tasks:

1). Hundredfold replication of this specific DNA to generate the genetic substance which the progeny is to be endowed, and

2). The manufacture of several hundred replicas of the phage protein to provide heads, tails and internal protein for the completion of mature infectious phage parieles.

Agents which prevent the synthesis of protein and nucleic acids may be operating by interfering with nospecific energy yielding reactions, preventing synthesis or polymerization of the essential building blocks, or by interfering with the synthesis or factors of specific cofactors. Chemical interference with amino acid metabolism and hence protein synthesis would be expected to result in the cessation of phage formation. This might be the case here with the antiparasitic drugs and perhaps with DL-Beta-Phenyllactic Acid, Bisulfan and the purine analogue Benzimidazole. Benzimidazole seems to interfere with some step in the intracellular development of lambda, The halogenated amino acids stimulated the intracellular development of lambda as was determined by the one step growth experiment. Para-Fluoro-phenylalnine which in our experiments stimulated the intracellular development of lambda has been reported by Zimmerman and Schafer (1960) to inhibit the growth of fowl plague virus in chick embryo

fibroblasts. They reported that the effect is a result of inhibition of protein synthesis and the inhibition is reversible by phenylalanine. A possible explanation for this contradiction is that fowl plague virus is an RNA virus while lambda phage is a DNA virus. Also here our system is different, we are working with bacteria and they are working with chick embryo fibroblasts.

Although we can study the effect of these drugs on the intracellular development of lambda phage by the one step growth experiment we should not overlook the possible production of immature or non-infective bacteriophage. With some of the drugs a reduction in the burst size of infective particles can be accompanied by the liberation of non-infective mature phage (in case of 5-Bromouracil) or of non-infective immature particles (in case of Proflavine) Matthews and Smith (1965). This possible production of immature phages can be detected by examining the lysates in the electron microscope and by comparing the number of infective units determined by plaque counts to the visible number of particles in the electron microscope. The production of immature bacteriophage could be also studied by serological methods, total nitrogen determinations and phage neutralization with specific antisera. In our experiment the production of immature or non-infective bacteriophage was not studied..

Another factor that should be investigated, before we can say for certain that what the effect of the drugs on the intracellular development of lambda might be, is the determination of the number of bacterial cells becoming lysogenized. Perhaps the

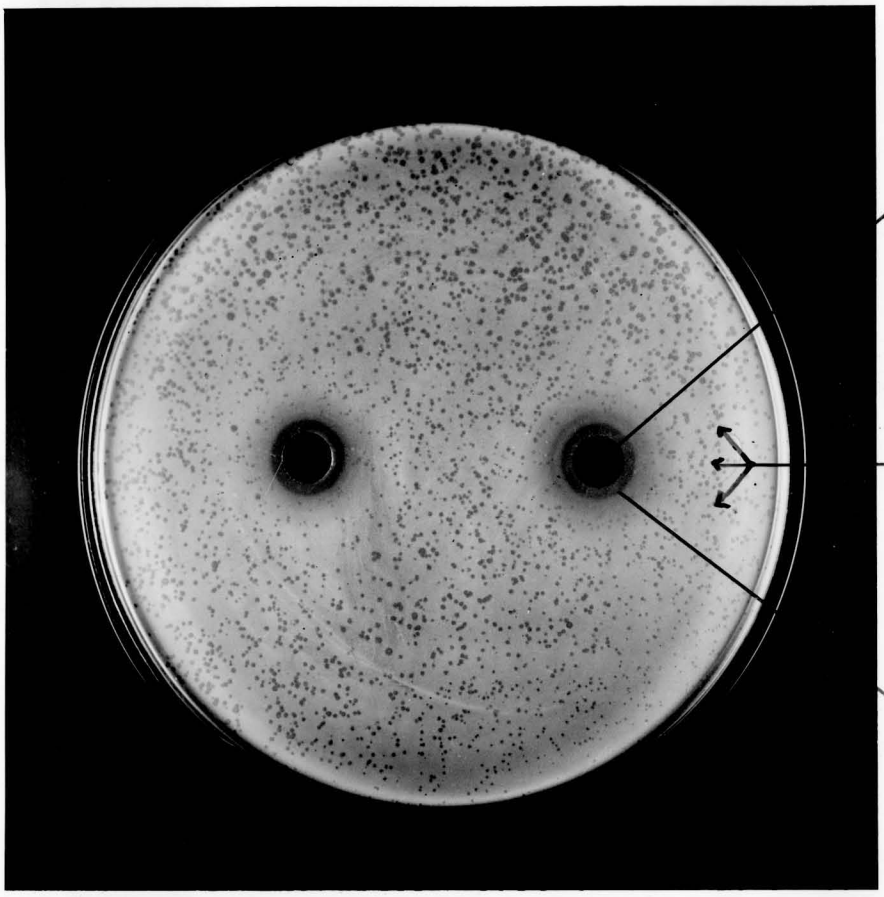
burst size that was obtained in our assays was not the true burst size due to a change in the proportion of the cells becoming lysogenized. The % of lysogenized cells can be studied by the use of the galactose marker "conversion" Lederberg (1956) or by identification of the changes in the bacterial host resulting from lysogenization.

Some of the compounds we used in our studies have been also screened for their mutagenic or carcinogenic action in studies of cancer. Since mutagenic or carcinogenic properties of a substance can be correlated with induction of phage maturation, we attempted to see if any of the chemical compounds tested had the ability to induce the development of active phage from the prophage state in the lysogenic E. coli K12. DL-Para-Fluoro-phenylalanine, 3,4-Dihydroxynorephedrine HCL, N-CBZ-DL-Serine, DL-Beta-Phenyllactic Acid, Formyl-DL-Phenylalanine, DL-Arteremol HCL, Dichloracetyl-L-Serine and Benzimidazole can be added to the other inducing agents such as ultra-violet irradiation, X-rays, nitrogen mustards, Azaserine, etc. Dichloracetyl-L-Serine showed a greater number of phage particles to be produced at the low concentration of 100ug and a lower number of phage particles at the high concentration of 1mg/ml. A possible explanation for this might be the effect of Dichloracetyl-L-Serine on free phage. Dichloracetyl-L-Serine inhibits free phage and here it seems that the smaller number of phage particles that we are getting with the high concentration is the result of the inhibitory effect of the drug. The variety of compounds listed above with inducing activity

may be useful in determining sensitive sites in the lysogenic system. It would be interesting to investigate the mode of action of these inducing agents.

Infection of E. coli W1485 spheroplasts with lambda phage was accomplished. Lambda phage was adsorbed onto E. coli W1485 spheroplasts at the same rate as onto whole cells.

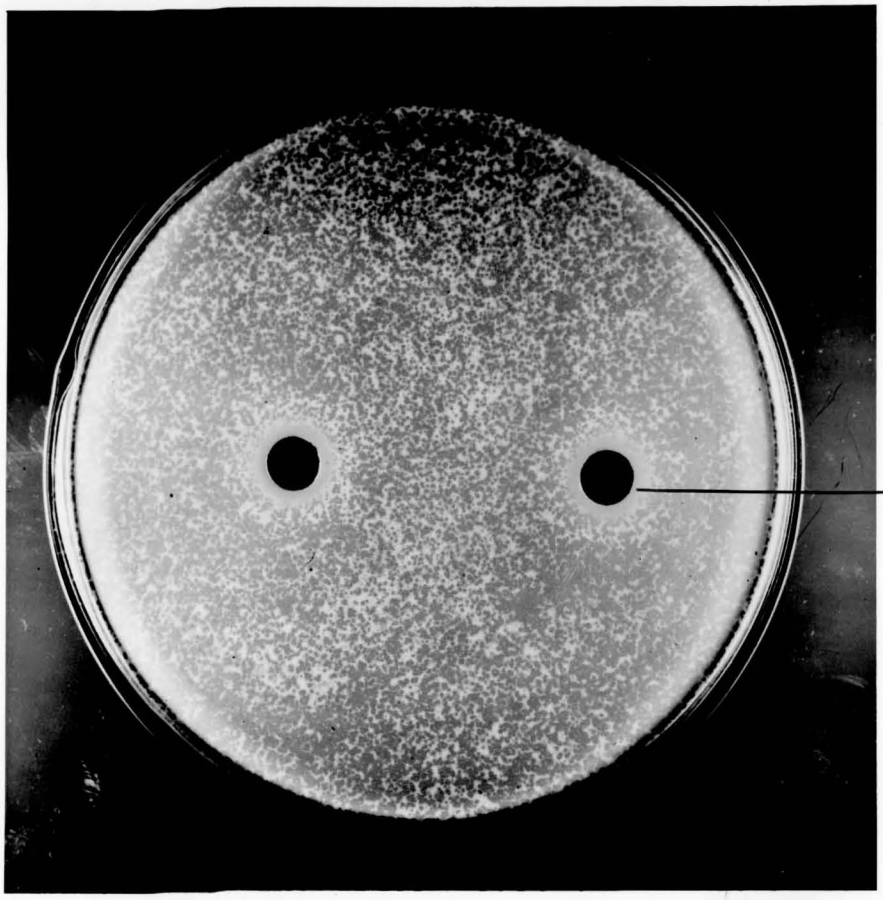
Figure No. I



ANTIBACTERIAL ACTIVITY

Stilbamidine 15mg/ml

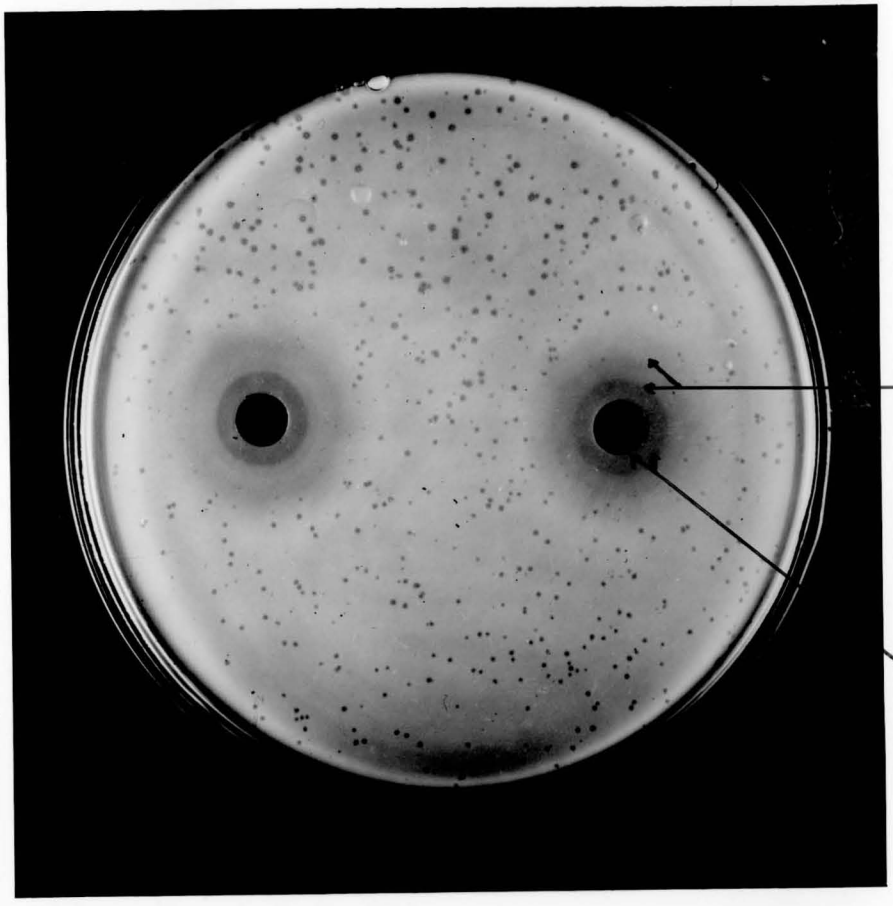
Figure No. II



ANTIPHAGE ACTIVITY

Propamidine 15mg/ml

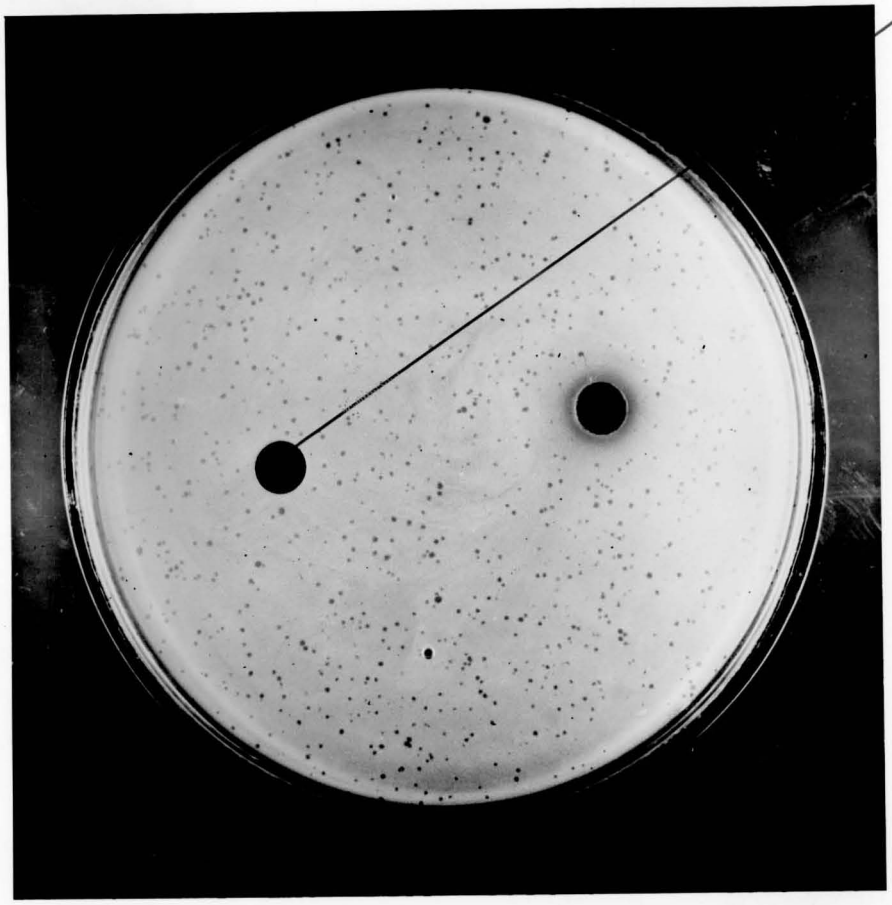
Figure No. III



COMBINED ANTIBACTERIAL AND ANTIPHAGE ACTIVITY

Pentamidine 15mg/ml

Figure No. IV



CONTROL NO EFFECT
AND
ANTIBACTERIAL ACTIVITY

CHAPTER VI

SUMMARY

1). One hundred purine, pyrimidine, amino acid analogues and other antimetabolite compounds were screened for their effect on E. coli W1485 cells and lambda bacteriophage combination by the paper disc method.

2). More than two dozens of the above chemical compounds showed antibacterial, antiphage activity or both by the method used.

3). A dozen of the most active compounds were tested further for their effect on free lambda phage, on cells of E. coli W1485, on the generation time of the same rganism, on the intracellular development of lambda phage by the one step growth experiment, and on their ability to induce lambda phage maturation from the prophage state in the lysogenic E. coli K12.

4). DL-Beta-Phenyllactic Acid, Benzimidazole, Bisulfan, and Dichloracetyl-L-Serine inhibit free lambda when used in high concentration.

5). Most of the drugs inhibit E. coli W1485 cells in the high concentration of 50, 25, 15, and 5mg/ml, but a different effect can be seen when 1mg/ml for each drug is used. Thus the drugs DL-Beta-Phenyllactic Acid, DL-Para-Fluorophenylalanine, N-CBZ-DL-Serine and DL-Beta-3-Thienylalanine stimulate growth of E. coli

W1485 by reducing the generation time, while Bisulfan, L-Arterenol-Bitartrate Hydrate, Dichloracetyl-L-Serine, Stilbamidine, Propamidine and Pentamidine inhibit the growth of E. coli W1485 to an extent by increasing the length of the average generation time.

6). In the intracellular development of lambda phage we have a varied effect. Thus the drugs Dichloracetyl-DL-Serine Na, DL-Para-Fluorophenylalanine, and N-CBZ-DL-Serine increase the burst size; Propamidine, Pentamidine, Stilbamidine, Bisulfan, Benzimidazole, DL-Beta-Phenyllactic Acid and L-Arterenol Bitartrate Hydrate decrease the burst size. Finally Dichloracetyl-L-Serine, and DL-Beta-3-Thienylalanine do not show any effect.

7). The drugs: DL-Para-Fluorophenylalanine, N-CBZ-DL-Serine, 3,4-Dihydroxynorephedrine HCL, DL-Beta-Phenyllactic Acid and Benzimidazole colud be used as inducing agents for the maturation of lambda phage from the prophage state in E. coli K12.

8). Possible mechanisms of action of most of the drugs have been discussed.

9). Infection of E. coli W1485 spheroplasts with lambda phage was achieved.

CHAPTER VII

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THE EFFECT OF CERTAIN DRUGS ON THE LYSOGENIC SYSTEM

This investigation is primarily the study of the lysogenic system in Escherichia coli, strain K12 and the effect of certain drugs on the lysogenic system. Another aspect of the investigation is the conversion of Escherichia coli W1485 cells into spheroplasts and the infection of these spheroplasts with the temperate bacteriophage lambda.

Escherichia coli, strain K12, E. coli W1485, E. coli C and the temperate bacteriophage lambda were used in the investigation. Standard methods and approved techniques were followed.

One hundred purine, pyrimidine, amino acid analogues and other antimetabolite compounds were screened for their effect on Escherichia coli W1485 cells and lambda bacteriophage combination by the filter paper disc method. Thirty-one of the above drugs showed antibacterial activity, antiphage activity or both by the method used.

A number of the above drugs that showed activity were tested for their effect on free lambda bacteriophage, on cells of E. coli W1485, on the generation time of the same microorganism, on the intracellular development of lambda bacteriophage and on their ability to induce lambda phage maturation from the prophage state in the lysogenic E. coli K12.

It was found that DL-Beta-Phenyllactic Acid, Benzimidazole,

Bisulfan and Dichloracetyl-L-Serine inhibited free lambda phage completely or to an extent at the concentrations tested.

It was also found that most of these drugs inhibited E. coli W1485 cells at the high concentration of 50mg, 25mg, 15mg and 5mg/ml while at the low concentration of 1mg/ml their effect varied.

DL-Beta-Phenyllactic Acid, DL-Para-Fluorophenylalanine, N-CBZ-DL-Serine and DL-Beta-3-Thienylalanine reduced the generation time of E. coli W1485 and thus stimulated its growth. Bisulfan, L-Arterenol Bitartrate Hydrate, Dichloracetyl-L-Serine, Stilbamidine, Propamidine and Pentamidine increased the generation time of E. coli W1485 and thus suppressed its growth.

The intracellular development of lambda phage was affected by a number of the drugs tested as was determined by the one step growth experiment. Dichloracetyl-DL-Serine Na, DL-Para-Fluorophenylalanine and N-CBZ-DL-Serine increased the burst size of lambda phage and had no effect on the latent period. Pentamidine, Propamidine, Stilbamidine, Bisulfan, Benzimidazole, DL-Beta-Phenyllactic Acid and L-Arterenol Bitartrate Hydrate decreased the burst size and increased the latent period. Dichloracetyl-L-Serine and DL-Beta-3-Thienylalanine did not effect the burst size or the latent period during the intracellular development of lambda phage.

A number of the drugs were tested for their ability to induce lambda phage maturation in E. coli K12. DL-Para-Fluorophenylalanine, 3,4-Dihydroxynorephedrine HCL, N-CBZ-DL-Serine,

DL-Beta-Phenyllactic Acid and Benzimidazole act as inducing agents.

Our attempt to produce spheroplasts from cells of E. coli W1485 was successful. These spheroplasts were easily maintained and were infected with lambda bacteriophage as was determined by the method followed. Adsorption of lambda bacteriophage on these spheroplasts took place at the same rate as for the whole cells of E. coli W1485.

APPROVAL SHEET

The dissertation submitted by Demetrios George Halkias has been read and approved by three members of the Department of Microbiology, one member of the Department of Biochemistry and one member of the Department of Pharmacology.

The final copies have been examine by the director of the dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the dissertation is now given final approval with reference to content, form, and mechanical accuracy.

The dissertation is therefore accepted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

January 15 1964
Date

Thomas J Bird
Signature of Advisor