1937

The effect of bacteriophage on reactions of certain members of the coli-aerogenes group of bacteria

Amedeo Bondi
University of Massachusetts Amherst

THE EFFECT OF BACTERIOPHAGE ON REACTIONS OF CERTAIN MEMBERS OF THE COLI-AEROGENES GROUP OF BACTERIA

BONDI, Jr. - 1937

THE EFFECT OF BACTERIOPHAGE ON REACTIONS OF CERTAIN MEMBERS OF THE COLI-AEROGENES GROUP OF BACTERIA

Amedeo Bondi, Jr.

Thesis submitted for the degree of

Master of Science

Massachusetts State College

May, 1937

TABLE OF CONTENTS.

INTRODUCTION.

Various bacteriological methods for testing the sanitary quality of drinking water have been devised. The test commonly accepted in this country is that which detects as evidence of pollution the presence of organisms of the coli-aerogenes group. This group includes those Gram-negative, non-spore-forming rods which ferment lactose with gas production.

Many workers have maintained that only one member of this group, Escherichia coli, is of intestinal origin, and that, therefore, the presence in water of other members of the group should not be considered as indicative of fecal pollution. For this reason various biochemical tests have been devised to permit the differentiation of Escherichia coli, believed to be of intestinal origin, from Aerobacter aerogenes, commonly considered to be of soil origin. These tests include a wide variety of qualitative and quantitative physiological reactions characteristic of these organisms.

Trouble arises, however, with other members of this group. These organisms have some characteristics in common with Escherichia coli, and still other characteristics in common with Aerobacter aerogenes. These so-called intermediates also vary among themselves. Because of these intermediate strains bacteriologists have experienced considerable difficulty in attempting to successfully classify the members of this group.

Bacteriologists often attribute the irregularity of bact-

erial activity to dissociation of microorganisms. Dissociation of organisms is not a new phenomenon, but one for which many varying and confusing theories have been advanced. Various morphological, cultural, and serological changes in bacteria have been observed. Some workers have attributed the irregularity in the behavior of members of the coli-aerogenes group to dissociation, although sufficient proof has not been advanced to completely substantiate this belief.

Dissociation is influenced by various factors, such as temperature, food, reaction, physical agents, and bacteriophage. This last named agent, whose nature is still disputed, is commonly considered to be parasitic to bacteria. It lyses bacterial cells in much the same fashion as do some disinfectants, causing complete disintegration of the cells. The action of most bacteriophages, as of many sera, is of a specific nature. In very weak concentrations a particular bacteriophage may lyse a specific organism, but still have no lytic effect, even in high concentrations, on related organisms. This specificity is not confined to genera, but may be restricted to species of the same genus or even to strains of the same species. Various strains of Escherichia coli sometimes show an amazing resistance to a coli-specific phage.

D'Herelle[28] makes this statement: "In summary, then, the most important fact to be derived is that, exposed to the action of the bacteriophage, bacteria undergo mutations, usually unstable ones, but that these may become fixed under conditions as

yet undetermined. These mutations are associated with a state of resistance acquired by the bacteria."

The purpose of the present investigation was to determine the effect of a bacteriophage, specific for a strain of Escherichia coli, on certain biochemical and serological reactions of several strains of this and other related species of bacteria.

LITERATURE REVIEW.

Bacteriophage, or the transmissible lytic agent as it was first named, was discovered independently by Twort[38] in 1915 and by d'Herelle[22] in 1917. It was soon after this time that reports were made concerning the influence of this lytic principle on dissociation. Although they differed considerably as to the nature of bacteriophage, both Bordet[3] and d'Herelle[23, 27] were among the first to report the generation of mutants from the substratum of the normal culture under the lytic stimulus. Bordet[3] stated that the lytic principle was an agent which, through its power of effecting mutations, acted as a "director of evolutional progress, and in this way controlled the destiny of the species". This was a daring inference to be made by this leader of the school which believed that the lytic principle was a substance derived from the bacterial cell as a result of some "vitiated" process, and this deduction consequently lead to his naming the phenomenon "transmissible bacterial autolysis". D'Herelle[23], on the other hand, maintained that bacteriophage was of a living virus-like nature, and gave the phenomenon its present name "bacteriophage". He recorded in his early work the origin of mutating culture types under the lytic stimulus.

The recognition of the influence of bacteriophage on bacterial dissociation was first recognized by these early workers in their studies on secondary, or resistant, cultures. When a bact-

eriophage is added to a young susceptible broth culture of homologous bacteria, lysis of the culture soon begins and a clearing of the broth results. Many times, however, this clearing of the medium lasts only for a time and is followed by a clouding from regrowth of the organisms. This growth constitutes the secondary, or resistant, culture. The same results may be obtained on a solid medium. If the culture to which phage has been added is streaked over the surface of an agar slant, a growth of the culture will appear after incubation, spotted with lytic areas of a size and number depending on the initial concentration of the lytic principle. These areas appear to contain no growth, but soon small colonies slowly appear. Many of these colonies are also the secondary, or resistant, cultures. These secondary cultures appearing after lysis differ in many respects from the original sensitive form, designated as the S form.

D'Herelle[25] reported that the cells of the resistant cultures differed microscopically from the original type. Commonly they approximated the coccobacillus, or even the coccus type. This, he stated, was true of Escherichia coli, Eberthella typhosa, Shigella dysenteriae, and many others. Such cultures often showed modified biochemical reactions, and according to d'Herelle often remained permanent in their new forms.

At this point, the question arose as to the significance of the relation of the lytic principle to the S and R bacterial types, the latter term being used at this time to designate the dissociated

type. Arkwright[1], contrary to the opinion of d'Herelle[28], believed that no significant relationship existed between the lytic principle and the S and R types. Gratia[18] was the first to report the action of bacteriophage on these two types. He found that lysis of the S gave the resistant SR, while lysis of the R gave the more resistant RR. The R form gave many more secondary colonies than did the S form. Gratia claimed that by using properly graded doses of bacteriophage he could produce types quite different from the normal. He reported three chief types of colonies: mucoid, non-mucoid and regular, and non-mucoid and irregular. The mucoid colonies were non-lysogenic and gave rise to mixed colonies, the non-mucoid regular colonies bred true, and the non-mucoid irregular colonies were lysogenic. By lysogenic is meant the ability to perpetuate the lytic action.

Just as the cultures arising from active dissociation are designated as primary dissociates, so Hadley[20] proposed to term the new cultures arising from bacteriophagic action (or passive dissociation) secondary dissociates. To make the subject clearer the following terms proposed by Hadley for the primary and secondary dissociates are presented:

Primary Dissociates:

Type X. The original culture from a natural source. Resembles the following type S with somewhat different serological reactions.

Type S. A normal culture such as an average laboratory stock culture. Contains, besides antigen S, some of the O antigen and, if an old culture, some R.

Type O. The intermediate or transitional group of cultures. Besides antigen O, may contain S and R antigens; culturally unstable and may be transformed to either S or R. Natural end product in further dissociation is the R.

Type R. A dissociate from S through the intermediate O. Found particularly in old laboratory cultures. Stable and often permanent, but commonly transforms to the normal S.

Type Ly. S type culture transforming rapidly to the intermediate O, with rapid dissolution of this culture form, leaving as its product the R. Plating yields S, R, and Ly forms. May be slightly lysogenic.

Secondary Dissociates:

Type SR. The secondary and somewhat resistant culture arising from the action of a lytic filtrate on its homologous substratum of the S type. Fairly stable. Plating gives mainly SR together with some S forms. Antigenic configuration unknown.

Type RR. The secondary and resistant culture arising from the reaction of lytic principle on R type culture. Usually more stable than SR. Plating gives mainly RR forms. Antigenic configuration unknown.

Type Lg (lysogenic). An SR or RR culture resistant to lysis but which carries the lytic agent. Filtrates may initiate serial lysis in the homologous S substratum. Plating gives SR or RR and Lg forms, but probably no S. Antigenic configuration unknown.

Types SR^2, SR^3, RR^2, RR^3, etc. SR and RR cultures of increasingly greater resistance to the lytic principle. Stability correspondingly increased.

Types SR^n, RR^n. SR and RR cultures possessing maximum resistance to the influence of the lytic principle. Hypothetical forms representing the most resistant and stable form of R, possessing "absolute" resistance to the lytic principle.

In regard to the origin of cultures resistant to lysis, d'Herelle(28) believed that selection operated, not on bacteria endowed with resistance, but on susceptible bacteria which are capable of acquiring resistance. He stated: "The secondary cultures, then, are the result of the adaptation undergone by the bacterium which acquires an immunity to its parasite". In his opinion bacteriophage always acted on a normal homogeneous culture. Gratia(18), however, suggested from his work that the resistance of cultures to bacteriophage results not so much from the gain of resistance by the bacteria, as from a selection of those organisms possessing natural resistance. This is in agreement with the experiments of Hadley(20), who found that cells resistant to a lytic agent of medium strength are identical with those of the R type, often mixed with the S forms. To him "their resistance was predetermined in the cyclogeny of the species". He did find that this state of resistance depended on the concentration of the phage, and that during the process the resistance might become enhanced. This, he believed, would account for the greater

number of secondary cultures from the R type than from the S type.

In his work on cultures secondary to lytic action, Fukeida[16] postulated a further theory involving the hereditary characters of the bacterial cell. He believed that there were two processes normal to bacteria: one, lysis, the function of the mother cell; the other, regeneration, the function of the daughter cell. With the latter, strains may be formed lacking genes with which bacteriophage can join, thus resulting in the formation of resistant strains. These will be produced faster and in a larger number if there are many young actively growing cells present. Studying the variants of "B. sanguinarum", Burnet[9, 10] believed that the formation of resistant cells was due to the inability of bacterial cells to absorb the phage concerned, although they may be lysed by other bacteriophages which they can "absorb".

At this time Blanc[2] reported the growth of mucoid colonies of "B. coli" after treatment with filtrates of sewage evidently containing a lytic principle. Many of these colonies resembled those of Friedlander's bacillus. Bordet and Ciuca[4] reported a similar transformation of "B. coli" into a mucoid aerogenes-like organism. Gory[17], using tap water presumably containing a lytic principle, made similar observations. Bronfenbrenner[5] noted changes in the viscosity of bacteria during lysis by bacteriophage.

Various workers have attributed to bacteriophage the ability to generate filtrable forms of bacteria. These dissociated

organisms, it has been observed, enter a stage of development in which they are filtrable through Berkefeld or Chamberland candles which ordinarily are capable of holding back all bacteria. D'Herelle[26] found such forms in the filtrates of lysed cultures of the Shiga bacillus. Haudurcy[21] reported filtrable stages of Esch. coli, E. typhosa, Shigella dysenteriae, and Micrococcus aureus, under the influence of weak concentrations of their specific bacteriophage. Tomasselli[37] reported the same observations with Escherichia coli. The presence of these forms was noted through the observation of faint, opalescent growth in broth. Haudurcy less frequently noted delicate growth on solid media. Fejgin[14] obtained a filtrable stage of the typhoid bacillus capable of producing a disease in guinea-pigs. Frobisher[15] disagreed with these workers. He was unable to show the presence of filtrable forms of similar organisms under the lytic stimulus. He concluded that such forms were contaminants resulting from faulty technique, or were the result of defective filters.

Dissociation of organisms under the influence of bacteriophage has been encountered when this agent has been used therapeutically. Much of the difficulty encountered in the treatment of infections with bacteriophage has resulted from the generation of resistant organisms. Dutton[12, 13] noted this in his use of the lytic agent in streptococcus infections. He found that some cells produced were more stable and resistant than the original forms. Following treatment of pyelitis with bacteriophage, Dimtza[11] isolated cultures of Escherichia coli of varying characteristics.

They were resistant to the lytic agent, showed a mucoid type of colony, and displayed other variant characteristics. Bronfenbrenner(6) noted that cultures of "B. pestis caviae", secondary to lysis, were avirulent when they were fed to, or were injected intraperitoneally into mice. These cultures remained resistant to lysis and were avirulent as long as they were cultured on solid agar, but when they were cultured in broth, they recovered their susceptibility to lysis and their virulence for mice. Kauffmann(29) discovered that the virulence of mucoid strains of members of the colon-typhoid-dysentery group of bacteria was altered under the lytic stimulus. These forms usually were more avirulent than the normal forms. Smith(34) found that bacteriophage stimulated phagocytosis of homologous susceptible bacteria by leucocytes. He did not find, however, that resistant bacteria were rendered susceptible to phagocytosis by exposure to the lytic principle. Sonnenschein(35) discovered that E. typhosa became hemolytic on blood agar under the influence of bacteriophage. He also reported that non-hemolytic strains acquired hemolytic power in bodies of mice, guinea-pigs, or rabbits, when bacteriophage was injected simultaneously into the animals.

Although but few studies have been made on the serological reactions of the various bacterial types produced under the influence of bacteriophage, these studies have been of considerable interest. Many workers have showed that the serological antigenicity of S and R forms produced by active dissociation may differ considerably.

D'Herelle[24] was among the first to note the effect of passive dissociation on serological reactions. He noticed the loss of agglutinability to specific antisera by cultures in a state of resistance to bacteriophage. McKinley[31] studied the serological relationships between the S and SR types. The serum of each type agglutinated its own antigen, but not the heterologous antigen. Hadley[19] compared the susceptibility to bacteriophage with the agglutination reactions of the various members of the colon-typhoid-dysentery group of bacteria. He found that cultures susceptible to agglutination in high dilutions were likewise susceptible to lysis by bacteriophage. Similarly, unagglutinable cultures showed a marked resistance to lysis by this agent. Shwartzman[33] found that when hemolytic streptococci were treated with bacteriophage to which they were susceptible, the property of specific agglutination was lost. These same cultures appeared also to show a complete loss of specific agglutinin-absorption and agglutinogenic properties. In some cases culture agglutinogens were transformed into antigens of entirely new specificity. Burnet[6] found that organisms possessing common heat-stable O agglutinogens were lysed by the same races of bacteriophage. Kauffmann[29] also studied the agglutination and agglutinin-absorption reactions of normal, and of bacteriophage-resistant, forms of Escherichia coli. The latter forms possessed a double receptor, whereas the normal form possessed only a single receptor. The agglutination tests reported by

Bruynoghe and Dubois[7] failed to demonstrate any acquired antigenic relationship with cultures of *E. typhosa* rendered resistant by exposure to a single race of bacteriophage. Reactions were not different from those manifested with sera of animals immunized with normal cultures.

EXPERIMENTAL.

Media Employed.

With the exception of the medium used for the isolation and cultivation of the specific bacteriophage employed in this problem, all media were prepared according to formulae of Standard Methods of Water Analysis(56). These media included lactose broth, Endo's medium, Clark and Lubs' medium, sodium malonate medium, Koser's sodium citrate medium, and tryptophane broth. These media are among those commonly recommended for the separation of members of the coli-aerogenes group of bacteria.

The ingredients of the savita broth(52) used for the isolation of the bacteriophage from sewage are as follows:

Savita (Battle Creek Food Co.)	10.0 gms.
Peptone, Difco	10.0 "
Salt (NaCl)	2.5 "
Dipotassium phosphate (K_2HPO_4)	8.0 "
Distilled Water	1000.0 ml.

The ingredients are dissolved with the aid of a little heat. The reaction is adjusted to pH 7.6. The medium is then heated in flowing steam for 15 minutes, and filtered through paper. The medium is sterilized in the autoclave at 15 pounds pressure for 15 minutes.

Deca-strength savita broth is prepared in the same manner, with the exception of the use of 100 ml. of water instead of 1000 ml. This medium is not heated after the reaction is checked, and is not filtered.

Cultures and Bacteriophage Employed.

The specific coli-phage employed in this problem was isolated from sewage obtained from the sewage disposal plant of the town of Amherst. Feces of man or experimental animals could likewise have been used for this purpose. Past experience had indicated, however, that sewage was a more certainly available source of bacteriophage, and for that reason it was used.

The homologous culture of the organism for which this phage was specific was also isolated from the sewage. This culture was designated Esch. coli IIA. It will be referred to hereafter as Coli IIA. This organism showed the typical fermentation of Esch. coli in lactose broth, and the characteristic colonies on Endo's medium. It was methyl-red positive, sodium citrate negative, Voges-Proskauer negative, sodium malonate negative, and indol negative. With the exception of the indol reaction, all of these reactions were typical of Esch. coli. However, as stated by Levine(30), the occurrence of indol-negative cultures of Esch. coli are not infrequently common in both water and soil.

The particular phage used in this problem, designated Phage IIA, was specific for the organism Coli IIA. It was unable to lyse other strains of Esch. coli, and was without lytic effect on Aero. aerogenes or any other member of the coli-aerogenes group tested. It was capable of producing a lysis of the cells of its homologous culture in a dilution 10^{-6}. Attempts to increase the virulence of the phage by

successive transplantations were unsuccessful. A phage capable of retaining its lytic power when diluted to 10^{-8} is considered to be moderately virulent. Stronger phages capable of lysis in dilutions of 10^{-10} and 10^{-11} are frequently encountered.

Other cultures employed in this experiment were all pure cultures of members of the coli-aerogenes group. These were all laboratory stock cultures originally isolated from water or from feces.

Procedure for Isolation of Bacteriophage.

The method used for the isolation and development of the bacteriophage was first recommended by d'Herelle[28], except that a different medium was employed: To 10 ml. of deca-strength savita broth in a 250 ml. Erlenmeyer flask was added 100 ml. of fresh raw sewage. A 24-hour broth culture of Coli IIA was then added to serve as the substratum. The flask was shaken thoroughly to mix the ingredients, and was then stoppered with cotton and incubated at 37° C.

After 24 hours' incubation, the flask was removed and to it was added a teaspoonful of Fuller's Earth. The flask was shaken well and then set aside to allow the heavier particles to settle. The supernatant fluid was first filtered through paper and then through a Chamberland candle, without disturbing the sediment in the flask. The filtrate was then tested for sterility.

The presence of a lytic principle was determined by adding varying quantities of the filtrate to tubes containing 10 ml.

quantities of sterile savita broth. To each tube was then added 0.5 ml. of a 24-hour broth culture of Coli IIA which served as the substratum. Filtrate and culture controls were also carried. The tubes were shaken well and placed in the incubator at 37° C. Lytic action was indicated by a clearing of the broth regardless of the presence later of a secondary bacterial growth. This required a reading of the tubes at intervals of one hour. Further proof of the presence of phage was obtained by inoculation on to agar slants in order to observe the appearance of any growth. A "moth eaten" growth on the slant indicated the presence of bacteriophage.

The susceptibility of other strains to this phage was determined by the addition of 0.5 ml. quantities of 24-hour broth cultures to tubes of savita broth containing varying amounts of the phage. Results with these strains were negative, indicating the specificity of Phage IIA, so named from its homologous culture which it was capable of lysing.

With a stock phage to start with, larger amounts of the phage were produced for use in the problem. This was accomplished by adding to flasks containing 100 ml. of savita broth two ml. of Phage IIA and one ml. of a 24-hour broth culture of Coli IIA. These were incubated over night at 37° C. As complete destruction of all the cells did not usually occur, the contents of each flask were filtered through Chamberland filters. The lytic filtrates were stored in the ice box until needed. Before the bacteriophage was

used it was retested for sterility and lytic activity. The latter was determined by finding the highest dilution of phage still capable of lysing the cells of its homologous culture. With but a slight variation this dilution was usually 10^{-6}.

Preparation of Test Bottles.

The experiment was set up in such a way as to simulate conditions naturally existing in bodies of standing water. Large laboratory reagent bottles were filled with two-liter quantities of brook water and sterilized in the autoclave at ten pounds pressure for thirty minutes. The contents of these bottles were tested for sterility. To these bottles were then added different quantities of cell suspensions of various organisms, and different quantities of Phage IIA. In order to determine whether the reaction of the water would influence the activity of the phage, the pH of the water in the bottles was varied. Table 1 contains a list of the bottles so prepared together with the agents added and the pH of the water in each.

The bottles were kept at room temperature and, over periods of time, reisolations were made from each. This was done by inoculating a series of lactose broth tubes from each bottle. The tubes were inoculated with one-ml. quantities of the contents of a bottle and incubated over night at 37° C. When these tubes showed the presence of gas, Endo plates were streaked from the growth in each. After incubation each characteristically different

Table 1. Method of setting up the test bottles.

Bottle No.	Ml. of Sterile Water	pH	Ml. of Phage IIA	Culture	Ml. of Cell Suspension
I *	2,000	7.2	None	Esch. coli IIA	100
II	2,000	7.2	100	Esch. coli IIA	100
III *	2,000	7.2	None	Esch. coli A1	100
IV	2,000	7.2	100	Esch. coli A1	100
V *	2,000	7.2	None	Aero. aerogenes A101	100
VI	2,000	7.2	100	Aero. aerogenes A101	100
VII	2,000	7.2	100	Esch. coli IIA	50
VIII	2,000	7.2	50	Esch. coli IIA	100
IX	2,000	6.2	100	Esch. coli IIA	100
X	2,000	8.0	100	Esch. coli IIA	100
XI	2,000	8.8	100	Esch. coli IIA	100

* Control bottles with no phage added.

colony was removed to an agar slant. Endo plates were again streaked from the agar slant cultures. Colonies were removed once more and transferred to agar slants. These subcultures, which were isolated in this manner from all the bottles, were saved for further study. Over a period of approximately five months a number of such reisolations were made from each bottle, including the control bottles. For convenience these subcultures will be referred to hereafter as cultures.

Effect of Bacteriophage on Biochemical Reactions.

In order to determine the effect of bacteriophage, if any, on the biochemical reactions of the organisms studied, various types of media were inoculated. These media, although mentioned previously, are repeated along with the test or reaction for which each was employed.

1. Lactose Broth. The fermentation of lactose with the formation of acid and gas.

2. Clark and Lubs' Medium. (a) Formation of acetyl-methyl-carbinol (Voges-Proskauer Test).
(b) Colorimetric determination of acidity (Methyl-Red Test).

3. Koser's Citrate Medium. The utilization of the citrate as the sole source of carbon.

4. Sodium Malonate Medium. The utilization of the malonate as the sole source of carbon.

5. Tryptophane Broth. The formation of indol as a decomposition product of tryptophane.

Each reisolated and purified culture was inoculated into these media in duplicate for the purpose of making the tests men-

tioned above. Any tests showing indeterminate results were repeated.

Table 2 shows the results obtained with the biochemical reactions of the cultures reisolated from the bottles listed in table 1. None of the biochemical reactions of the cultures appeared to be changed under the influence of phage. There was absolutely no change in the non-homologous cultures, namely, those reisolated from bottles IV and VI containing respectively Esch. coli A1 and Aero. aerogenes A101. The biochemical reactions of these cultures were identical with those of their respective control cultures reisolated from bottles III and V, neither of which contained any phage.

The phage also had no effect on the biochemical reactions of its homologous culture, Coli IIA. Variations in the quantities of phage and cell suspension added to the bottles, and in the pH of the water, did not appear to influence the bacterial activity in any way. As may be observed in table 2, these cultures gave reactions identical with those of the control cultures reisolated from bottle I.

Effect of Bacteriophage on Colony Characteristics.

In order to determine the effect of Phage IIA on colony characteristics, the cultures reisolated from all the bottles, including the control bottles, were streaked on Endo medium and in nutrient agar. After incubation for 24 hours at 37° C., these

Table 2. Summarized results of biochemical reactions and colony form of subcultures isolated from bottles listed in table 1.

Bottle No.	No. of Isolations	Endo Medium		Methyl Red		Voges-Proskauer		Indol		Sodium Citrate		Sodium Malonate	
		T	A	+	-	+	-	+	-	+	-	+	-
I	41	41	0	41	0	0	41	0	41	0	41	0	41
II	87	13	74	87	0	0	87	0	87	0	87	0	87
III	26	26	0	26	0	0	26	26	0	0	26	0	26
IV	27	27	0	27	0	0	27	27	0	0	27	0	27
V	21	21	0	0	21	21	0	0	21	21	0	21	0
VI	23	23	0	0	23	23	0	0	23	23	0	23	0
VII	20	4	20	20	0	0	20	0	20	0	20	0	20
VIII	15	2	13	15	0	0	15	0	15	0	15	0	15
IX	20	15	5	20	0	0	20	0	20	0	20	0	20
X	20	20	0	20	0	0	20	0	20	0	20	0	20
XI	20	20	0	20	0	0	20	0	20	0	20	0	20

T = typical colony form.
A = atypical colony form.
All cultures produced acid and gas in lactose broth.

media were observed for the presence of typical and atypical colonies.

Table 2 also contains the results obtained with these studies on colony characteristics. As the table indicates, the colonies of the non-homologous cultures of Esch. coli A1 and of Aero. aerogenes A101 reisolated from bottles IV and VI were unchanged. These cultures continued to produce typical colonies identical with those of the respective control cultures.(Fig. 1).

The homologous cultures of Coli IIA, which had been under the influence of Phage IIA, appeared in some cases to produce atypical colonies. The control cultures on Endo medium produced round, raised, convex colonies with glistening surfaces, having the characteristic metallic sheen. In contrast, many of the cultures of Coli IIA which had been in contact with phage produced irregularly oval colonies with somewhat radiate margins. These colonies were very flat, dry, and dull, with striated surfaces having metallic sheen. On nutrient agar the control culture colonies were those typical for Esch. coli on the medium, while the cultures that had been in contact with the homologous phage in bottle II produced colonies identical with those on Endo's medium, except that, of course, there was no color or sheen. Figure 2 shows some of the atypical colonies encountered.

Not all of the cultures of Coli IIA which had been under the influence of phage produced these atypical colonies on Endo's medium or on nutrient agar. As table 2 indicates, 74 of the 87 cultures

Figure 1. Typical colonies of Coli IIA produced by normal cultures.

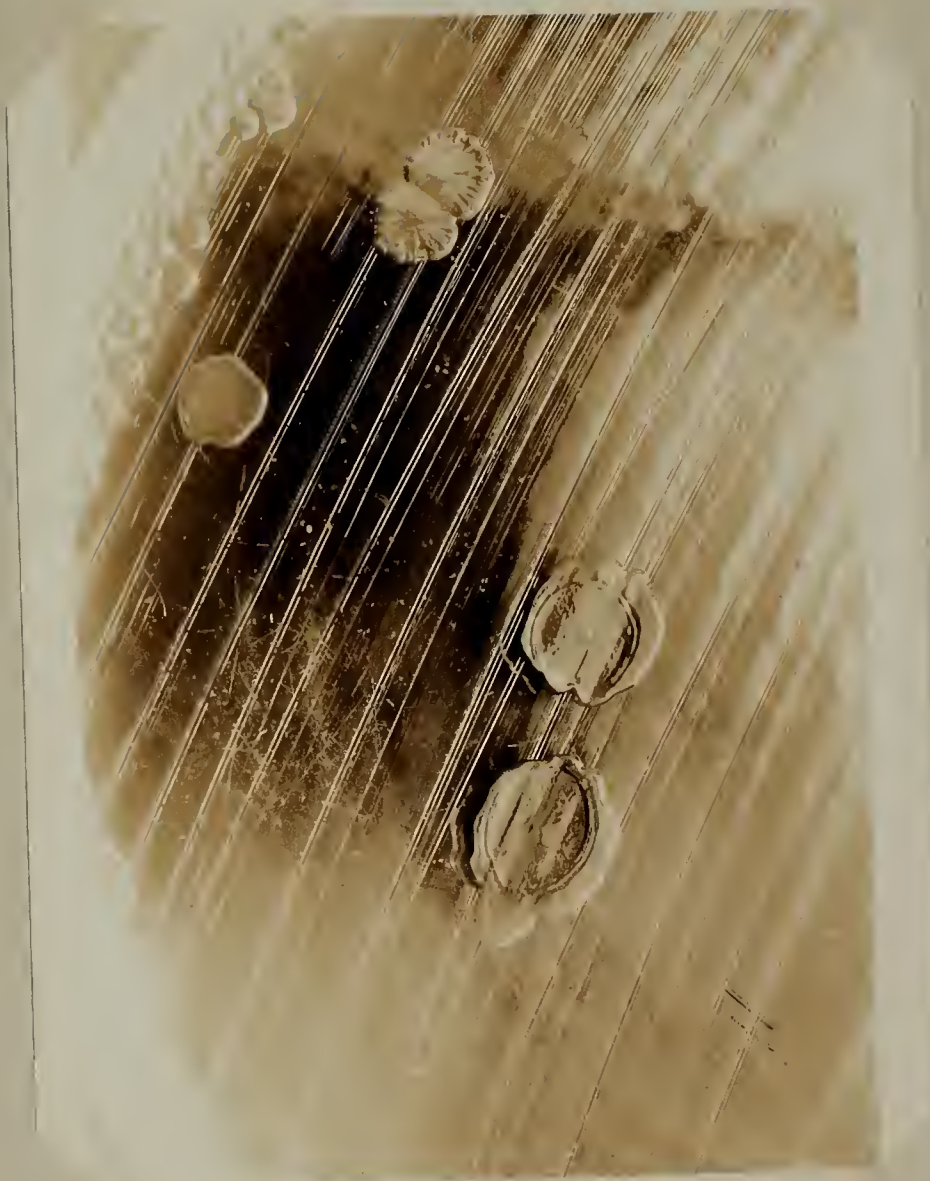

Figure 2. Atypical colonies of Coli IIA produced by culture under the influence of bacteriophage.

(85 per cent) reisolated from bottle II produced these atypical colonies. The remaining 13 cultures produced colonies identical with those of the control cultures. Table 5 shows the presence or absence of atypical colony produced with 20 of the cultures isolated from bottle II. These 20 cultures were not especially picked for this table, but represent the first 20 in the list of 87 cultures reisolated from bottle II. This table is included here because these cultures were used later in further tests to determine their resistance to the action of phage, and their serological reactions.

The cultures of Coli IIA reisolated from bottles VII, VIII, and IX showed a distribution of typical and atypical colony formation similar to those of bottle II. Regardless of the variation in the contents of these bottles, about the same proportionate number of cultures produced atypical colonies. None of the cultures reisolated from bottles X and XI produced atypical colonies. It seemed reasonable to believe that the cause of this was the alkaline reaction of the water in these bottles, which was unfavorable for the activity of the phage. The pH of the water in these bottles was 8.0 and 8.8 respectively. The optimum pH for phage activity ordinarily ranges between 7.2 and 7.8. However, it may be noted from table 2 that some of the cultures reisolated from bottle IX, in which the pH of the water was 6.2, produced atypical colonies. It would seem that such an acid reaction would have weakened the activity of the phage.

Table 3. Colony form of Coli IIA subcultures isolated from bottle II.

Control Culture	Colony Form	Phaged Culture	Colony Form
I1	T *	II1	A *
I2	T	II2	T
I3	T	II3	A
I4	T	II4	A
I5	T	II5	A
I6	T	II6	A
I7	T	II7	A
I8	T	II8	A
I9	T	II9	T
I10	T	II10	A
I11	T	II11	A
I12	T	II12	T
I13	T	II13	A
I14	T	II14	A
I15	T	II15	T
I16	T	II16	A
I17	T	II17	A
I18	T	II18	T
I19	T	II19	A
I20	T	II20	A

* T = typical.
A = atypical

** Colony form on Endo's medium agreed with that on nutrient agar.

Of considerable interest is the stability of the cultures which produced the atypical form of colonies. Over a period of five months following their initial isolation, these cultures were carried on nutrient agar and were transplanted from time to time. Throughout this period these cultures continued to produce this same atypical form of colony both on Endo's agar and on nutrient agar.

Resistance to Bacteriophage of Coli IIA (from bottle II).

A simple test was devised to determine the resistance to phage of cultures of Coli IIA isolated from bottle II. Different concentrations of phage were prepared in tubes containing sterile savita broth. These concentrations of phage ranged from 1:10 to 1:1,000. To the tubes were then added 0.5 ml. quantities of a 24-hour broth culture of the particular culture to be tested. These tubes were well shaken and placed in the incubator at 37° C. Readings were made at 24-hour intervals over a period of three days to observe the presence of any growth indicative of a culture's resistance to the phage.

The strains of Coli IIA from bottle II, as well as the control cultures reisolated from bottle I, were tested in this fashion. Although tests were run on 55 of the cultures from bottle II, only the results obtained with the first 20 of these cultures have been included. It was decided at this point to use only these cultures in future studies because it was felt that

the partial results would be representative of the whole, and the time and material required to test all of the cultures reisolated from all the bottles rendered the procedure impracticable. In addition to the results obtained in these tests with 20 of the Coli IIA cultures from bottle II, table 4 also includes the results obtained with 20 of the control cultures.

All but two of the twenty control cultures showed definite susceptibility to the lytic action of the phage. Lysis of eighteen of these cultures occurred in 24 hours in the presence of phage diluted to 10^{-5}, and the other two were lysed by 10^{-2} dilutions of the phage. It is certain from earlier work that even higher dilutions of the phage would have lysed most of these cultures. A few of the control cultures showed the presence of secondary growths after incubation for 48 or 72 hours.

On the other hand 14 of the 20 cultures of Coli IIA which had been reisolated from bottles containing phage displayed a marked resistance to its action, even when a 1:10 concentration of the phage was employed. The remaining 6 cultures (II2, II9, II12, II13, II15 and II18) were readily lysed in a fashion similar to the control cultures. It is not surprising that these 6 cultures were not resistant to the phage. It may be observed from table 3 that these were the same cultures of this group, culture II13 excepted, which did not produce the atypical type of colony. Apparently these susceptible cultures were unaffected by the phage in bottle II, and were identical with the normal unaffected con-

Table 4. Resistance to Phage IIA of Coli IIA subcultures isolated from bottle II.

Control Culture	24 hours			48 hours			72 hours			Phaged Culture	24 hours			48 hours			72 hours		
	1/10	1/100	1/1000	1/10	1/100	1/1000	1/10	1/100	1/1000		1/10	1/100	1/1000	1/10	1/100	1/1000	1/10	1/100	1/1000
I1	-	-	-	-	-	-	-	-	-	II1	+	+	+	+	+	+	+	+	+
I2	-	-	-	-	-	+	-	-	+	II2	-	-	-	-	-	-	-	-	+
I3	-	-	-	-	+	-	-	+	+	II3	+	+	+	+	+	+	+	+	+
I4	-	-	-	-	+_	+_	-	+_	+_	II4	+	+	+	+	+	+	+	+	+
I5	-	-	-	-	-	+_	-	+_	+_	II5	+	+	+	+	+	+	+	+	+
I6	-	-	-	-	-	-	-	-	-	II6	+	+	+	+	+	+	+	+	+
I7	-	-	-	-	-	+_	-	+	+	II7	+	+	+	+	+	+	+	+	+
I8	-	-	-	-	-	-	-	-	-	II8	+_	+	+	+	+	+	+	+	+
I9	-	-	-	-	-	-	-	-	-	II9	-	-	-	-	-	-	-	-	+
I10	-	-	-	-	-	-	-	+	+	II10	+	+	+	+	+	+	+	+	+
I11	-	-	-	-	-	-	-	-	-	II11	+	+	+	+	+	+	+	+	+
I12	-	-	-	-	-	-	-	-	-	II12	-	-	+	-	+	+	-	+	+
I13	-	-	-	-	-	-	-	-	-	II13	-	+_	+	-	+_	+	-	+_	+
I14	-	-	-	-	-	-	-	-	-	II14	+	+	+	+	+	+	+	+	+
I15	-	-	-	-	-	+	-	-	+	II15	-	-	+	-	-	+	-	-	+
I16	-	-	-	-	-	-	-	-	-	II16	+	+	+	+	+	+	+	+	+
I17	-	-	+	-	-	+	-	+	+	II17	+	+	+	+	+	+	+	+	+
I18	-	-	-	-	-	-	-	-	-	II18	-	-	-	-	-	-	-	-	-
I19	-	-	+	-	-	+	-	-	+	II19	+_	+	+	+	+	+	+_	+	+
I20	-	-	-	-	-	-	-	-	-	II20	+	+	+	+	+	+	+	+	+

+ indicates presence of growth.
+_ indicates faint growth.
- indicates no growth.

trol cultures. This point will be considered further in connection with a later phase of the study.

Effect of Bacteriophage on the Serological Reactions of Coli IIA (from bottle II).

In this part of the experimental work, an attempt was made to discover the effect of bacteriophage on the antigenicity of Coli IIA which had been reisolated from bottle II. Two rabbits were inoculated with antigens for the production of agglutinins. One rabbit was injected with a saline suspension of cells of a control culture of Coli IIA isolated from bottle I, and the other rabbit with a saline suspension of the cells of a phage-resistant culture of Coli IIA which had been in contact with phage in bottle II. At intervals of three days, over a period of approximately three weeks, these rabbits were injected with gradually increased doses of the respective antigens. At the end of this time, when sera obtained in trial bleedings showed suitable titers, the rabbits were bled and serum was obtained from each. The serum of the rabbit injected with the control culture was designated Serum I, and the serum of the rabbit injected with the culture which had been in contact with phage was designated Serum II. Each was named after the bottle from which the culture used in its production was isolated.

With these sera two sets of agglutination tests were made. Each serum was tested first against its homologous antigens, and then against the non-homologous antigens. For Serum I, the homolo-

gous antigens were the control cultures, while the non-homologous antigens were the cultures which had been in contact with phage. For Serum II, these latter antigens were homologous, while the control cultures were non-homologous.

Antigens both for inoculation and for agglutination tests were prepared by washing off the growth of 24-hour agar slant cultures with sterile physiological saline. These saline suspensions were adjusted to a turbidity corresponding to a 1.5 McFarland nephelometer standard. For the agglutination tests the antigens were added in 0.5 ml. quantities to agglutination tubes containing 0.5 ml. quantities of serum in varying concentrations. All tubes were shaken thoroughly and maintained in a water bath at 37° C. for 24 hours, at which time the tubes were read.

The results of the agglutination tests are summarized in tables 5 and 6. Both sera showed the same tendencies in their reactions on the two groups of cultures. With but few variations, Serum I completely agglutinated its homologous antigens at a dilution of 1:20, and partially agglutinated most of these antigens at a dilution of 1:40. It was necessary to use Serum II in a stronger concentration, namely 1:10, to effect even a partial agglutination of the same antigens. On the other hand, Serum I was capable of completely agglutinating the antigens of the phage-affected cultures at a dilution of 1:3,200. Similarly, Serum II was capable of agglutinating these antigens, which were its homologous antigens, at a dilution of almost 1:6,400.

Table 5. Agglutination reactions of Coli IIA control subcultures isolated from bottle I.

Culture	Serum I				Serum II			
	1:10	1:20	1:40	1:80	1:10	1:20	1:40	1:80
I1	++++	++++	++	-	+	-	-	-
I2	++++	++++	+	-	++	-	-	-
I3	++++	++++	++	-	-	-	-	-
I4	++++	+++	-	-	++	-	-	-
I5	++++	++++	+++	-	+	-	-	-
I6	++++	++++	+++	-	++	-	-	-
I7	++++	++++	+++	-	+	-	-	-
I8	++++	++++	+++	++	+	-	-	-
I9	++++	++++	+++	-	++	-	-	-
I10	++++	++++	+++	-	++	-	-	-
I11	++++	++++	++	-	+	-	-	-
I12	++++	++++	++	-	+	-	-	-
I13	++++	++++	+++	-	+	-	-	-
I14	++++	++++	++	-	+	-	-	-
I15	++++	++++	++++	++	+	-	-	-
I16	++++	++++	++	-	++	-	-	-
I17	++++	++++	-	-	-	-	-	-
I18	++++	++++	+	-	+	-	-	-
I19	++++	++++	-	-	-	-	-	-
I20	++++	++++	-	-	++	-	-	-

++++ = complete agglutination.
+++ = 75% agglutination.
++ = 50% agglutination.
+ = 25% agglutination.

Table 6. Agglutination reactions of Coli IIA subcultures isolated from bottle II.

Culture	Serum I				Serum II			
	1:1600	1:3200	1:6400	1:12800	1:1600	1:3200	1:6400	1:12800
II1	++++	+++	-	-	++++	++++	+++	-
II2	-	-	-	-	-	-	-	-
II3	++++	++++	+++	++	++++	++++	++++	+++
II4	++++	+++	++	-	++++	++++	++++	-
II5	++++	+++	-	-	++++	++++	+++	-
II6	++++	++++	++	-	++++	++++	++++	+++
II7	++++	++++	+++	++	++++	++++	+++	++
II8	++++	++++	+++	+++	++++	++++	++++	+++
II9	++++	++++	+++	-	++++	++++	++++	+++
II10	++++	++++	+++	-	++++	++++	+++	+++
II11	++++	++++	++++	-	++++	++++	++++	-
II12	-	-	-	-	-	-	-	-
II13	++++	++++	+++	-	++++	++++	+++	-
II14	++++	++++	+++	-	++++	++++	++++	++
II15	-	-	-	-	-	-	-	-
II16	++++	++++	++++	-	++++	++++	++++	++
II17	++++	++++	++++	-	++++	++++	++++	++
II18	-	-	-	-	-	-	-	-
II19	++++	++++	+++	-	++++	++++	++++	++
II20	++++	++++	++++	-	++++	++++	++++	++

++++ = complete agglutination.
+++ = 75% agglutination.
++ = 50% agglutination.
+ = 25% agglutination.

The effective dilutions of both sera were much higher when they were reacted with the antigens of the phage-affected cultures, than when they were reacted with the antigens of the control cultures. This difference in results of the agglutination tests of the two groups of cultures apparently was not due to a difference embodied in the two sera. The difference appeared to lie with the antigens. The cultures which had been in contact with phage evidently had undergone a change to the extent that they gained the property of being much more easily agglutinated. The control cultures evidently lacked this property and displayed a resistance to agglutination, regardless of the serum with which they were reacted.

Again it is noticed that cultures II2, II12, II15, and II18, displayed reactions similar to those of the control cultures. These were the cultures which had not given the atypical type of colony formation, nor the same resistance to phage as did the other cultures from bottle II. Of all the cultures, II9 was the only culture giving an agglutination reaction which did not correlate with its type of colony formation and its susceptibility to phage.

DISCUSSION.

D'Herelle(28) reported that bacteria sometimes undergo modifications in their biochemical behavior when subjected to the influence of bacteriophage. An attempt at production of such biochemical modifications, in the work reported in this paper, was unsuccessful. The study undertaken was confined to that of a single specific bacteriophage, and the conclusions drawn apply to only this bacteriophage and not to bacteriophages in general. When homologous and non-homologous cultures of members of the coli-aerogenes group of bacteria were subjected to the action of the specific Phage IIA, their physiological reactions were unchanged. The particular tests made on these cultures were not by any means the only tests which could have been made, but rather only those which are commonly recommended for the differentiation of members of this group of bacteria, and which consequently are the most important from the standpoint of water analysis. These biochemical tests were not quantitative estimations of the activity of these organisms. On the contrary they were very rough indications of certain reactions functional in the normal existence of these organisms. For this reason it is not surprising that changes in these physiological reactions, as indicated by these tests, were not produced. This was especially true of the non-homologous cultures for which the specific Phage IIA had no affinity. Apparatus and methods capable of detecting very small quantitative variations in the activity of bacteria might have disclosed changes in the bio-

chemical behavior of these organisms under the influence of phage. Even if such apparatus and methods were available, unless the physiological changes were such that an organism which was a positive reactor to one of these tests became negative, or the opposite, these changes would not be significant. Quantitative changes of considerable degree may be expected as a result of many factors other than phage.

As mentioned previously, many workers have reported the production of variant atypical colonies by various bacteria under the influence of bacteriophage, similar to those encountered in this study. Hadley(20) reported that the production of such variants was not wholly peculiar to bacteriophage action. He believed that such abnormal forms appear in many normal cultures when they are aged or grown under unfavorable conditions. If this were true, in this instance, one would expect that some of the control cultures reisolated from bottle I would show these modifications. As it was, however, every one of the 41 control cultures isolated was normal in all respects. Similar changes might have been noted with these control cultures if isolations were made from bottle I after it had stood for a longer period of time. The important point to be remembered, however, is that the marked speed with which bacteriophage brings about these so-called transmutations stamps the reaction as unique to the action of phage.

Of considerable interest is the possible connection between the production, as a result of phage influence, of these variant colonies on Endo's medium and difficulty which often confronts the

bacteriologist in his use of this medium in water analysis. Endo's medium is often used to confirm the presence of members of the coli-aerogenes group of organisms from water. Upon isolation, the organisms producing these atypical colonies were found to be morphologically and physiologically identical. It might be assumed that such abnormality in the colony formation by these organisms is the result of bacteriophage action in the water. If some such agent were a factor in the formation of these atypical colonies, colony formation would be less dependable in characterizing different members of this group.

The study of the serological reactions of the cultures resistant to bacteriophage definitely showed the need of further study on the antigenic relationships of these dissociated forms. As the results indicate, some definite change occurred in the serological reactions of cultures of Coli IIA which had been in contact with their specific phage. The change brought about is the exact opposite to that reported by d'Herelle[24], Hadley[20], Shwartzman[33], and others. These workers reported that cultures acquiring resistance to phage lose the property of agglutination. The cultures of Coli IIA resistant to phage, however, acquired the property of agglutination which it did not have originally, as typified by the failure of the control cultures to be agglutinated. It is not the intention of the author at this time to offer an explanation for this unusual serological change which was evidently the result of bacteriophage action. Further serological tests, particularly agglutinin-absorption tests, should throw some light on the anti-

genic configuration of the different forms produced. The agglutination reactions of these cultures employing H and O antigens might also furnish further information.

Table 7 gives a summary of all the reactions of the cultures of Coli IIA isolated from bottle II. These reactions, namely colony formation, resistance to phage, and agglutination by a specific serum, show a close correlation. One group of these cultures was resistant to phage, produced an atypical type of colony, and was agglutinated by its specific serum in high dilutions. The cultures of this group were evidently dissociated forms of Coli IIA produced by the action of its specific phage. On the other hand, another group of these cultures (II2, II12, II15, and II18) were susceptible to the lytic action of the phage, produced typical colonies, and were agglutinated by the specific Serum II only in very low dilutions. These cultures appeared to be identical with the control cultures and are probably of the same type. However, one must not be led to assume that these cultures escaped the action of the bacteriophage in bottle II. It is much more likely that the action of the phage in its production of these dissociated forms was the same on all the organisms present, and in the reproduction of these dissociated forms there was a regeneration of some of the original types. This seems more logical than the belief that these supposedly normal cultures, susceptible as they were to phage, could have escaped its action. Cultures II9 and II13 were the only cultures which gave reactions not correlating with those of either of the two groups of cultures. Culture II9

Table 7. Summary showing correlation of the reactions of Coli IIA subcultures isolated from bottle II.

Culture	* Colony Form	Resistance to Phage **		Agglutination by 1:3200 dilution of Serum II
		1:10	1:100	
II1	A	+	+	+
II2	T	-	-	-
II3	A	+	+	+
II4	A	+	+	+
II5	A	+	+	+
II6	A	+	+	+
II7	A	+	+	+
II8	A	+-	+	+
II9	T	-	-	+
II10	A	+	+	+
II11	A	+	+	+
II12	T	-	-	-
II13	A	-	+-	+
II14	A	+	+	+
II15	T	-	-	-
II16	A	+	+	+
II17	A	+	+	+
II18	T	-	-	-
II19	A	+-	+	+
II20	A	+	+	+
Control	T	-	-	-

* A = atypical.
T = typical.

** + = resistant.
- = non-resistant.
+- = partial resistance.

was similar to the control cultures, except for its failure to be agglutinated by both Sera I and II in high dilutions. Culture II13 was similar to the dissociated forms, except for its lack of resistance to the phage. These two strains were intermediate in their behavior between the undissociated cultures and those showing greater dissociation through their contact with phage.

It would be difficult indeed to place the chief variant type obtained in this study in Hadley's classification. It is commonly considered that R cultures are less readily agglutinated than S forms. On this basis, it would seem that possibly the original culture used in this study was an R and not an S type. Such an assumption would account for the exceedingly low titer of the specific antiserum. Ordinarily when bacterial antigens are used for the production of agglutinins, the resulting sera are capable of retaining this agglutinative power even in high dilutions. The fact that the original normal culture resisted phage dilutions higher than 10^{-5} adds further to the possibility of the original type being an R type. Most cultures of the S type are susceptible to much higher dilutions of their specific phages. Assuming that the original culture was an R type, it would be reasonable to assume that the dissociated forms produced were RR types. The stability of the dissociated forms, as shown by their own ability to continue to produce atypical colonies five months after their isolation, is certainly characteristic of RR types. Such reasoning, however, is purely hypothetical, and until further serological studies are made, it would be impossible to place these dissociated forms in Hadley's classification.

SUMMARY.

1. A study has been made of the effect of a specific bacteriophage on the biochemical reactions, colony formation, resistance to bacteriophage, and serological reactions of homologous, and non-homologous cultures of the coli-aerogenes group of bacteria.

2. Biochemical reactions of the homologous and non-homologous cultures, such as fermentation of lactose, production of indol, acid production, utilization of sodium citrate and sodium malonate as sole sources of carbon, and the production of acetyl-methyl-carbinol were not changed by the action of bacteriophage.

3. Under the influence of bacteriophage certain of the homologous cultures produced an atypical form of colony on Endo's medium and on nutrient agar.

4. Cultures producing atypical colonies also displayed the acquisition of resistance to bacteriophage.

5. A definite change was observed in the serological reactions of the phage-affected cultures as typified by agglutination tests. These cultures gained the property of being agglutinated by their specific serum in high dilutions, a property which the original normal cultures lacked.

6. Not all of the cultures isolated from under the influence of bacteriophage displayed these mutant characteristics.

Some cultures appeared to be similar to the normal cultures, but these were probably regeneration forms of the dissociated types.

7. The reactions of the phage-affected cultures correlated closely. Cultures which proved resistant to bacteriophage also produced atypical colonies and gave agglutination reactions foreign to those of the normal cultures.

LITERATURE CITED.

(1) Arkwright, J. A. 1924 The source and characteristics of certain cultures sensitive to the bacteriophage. Brit. Jour. Exper. Path., 5: 25-29.

(2) Blanc, J. 1923 Transformation muqueuse du Bacillus coli. Compt. Rend. Soc. Biol., 88: 49-51.

(3) Bordet, J. 1925 Le probleme de l'autolyse microbienne transmissible ou de bacteriophage. Ann. de l'Inst. Pasteur, 39: 717. Cited from Hadley(20).

(4) Bordet, J., and Ciuca, M. 1920 Exudats leucocytaires et autolyse microbienne transmissible. Ann. de l'Inst. Pasteur, 83: 1293. Cited from Hadley(20).

(5) Bronfenbrenner, J. J. 1926 Changes in viscosity during lysis of bacteria by bacteriophage. Proc. Soc. Exp. Biol. and Med., 23: 635-636.

(6) Bronfenbrenner, J. J. 1926 Studies on the bacteriophage of d'Herelle. VI. On the virulence of the overgrowth in the lysed cultures of B. pestis caviae. Jour. Exp. Med., 44: 607-622.

(7) Bruynoghe, R., and Dubois, A. 1927 La parente des microbes devenus resistants au bacteriophage. Compt. Rend. Soc. Biol., 96: 209-211.

(8) Burnet, F. M. 1927 The relationships between heat stable agglutinogens and sensitivity to bacteriophage in the Salmonella group. Brit. Jour. Exp. Path., 8: 121-129.

(9) Burnet, F. M. 1929 Further observations on the nature of bacterial resistance to bacteriophage. Jour. Path. and Bact., 32: 349-354.

(10) Burnet, F. M. 1929 Smooth-rough variation in bacteria in its relation to bacteriophage. Jour. Path. and Bact., 32: 15-42.

(11) Dimtza, A. 1927 Über Veränderungen von Coli-Stämmen durch Bakteriophagewirkung "in vivo and in vitro". Centbl. Bakt., I Orig., 101: 171-177.

(12) Dutton, L. O. 1926 The probable role of the bacteriophage in streptococcus infections. Jour. Lab. and Clin. Med., 11: 763-769.

(13) Dutton, L. G. 1927 The occurrence of the bacteriophage and variation in streptococcus colony formation. Jour. Bact., 13: 43-44.

(14) Fejgin, B. 1925 Sur la forme filtrante de Bacilli d'Eberth. Compt. Rend. Soc. Biol., 92: 1528-1530.

(15) Frobisher, M. 1928 On the action of bacteriophage in producing filterable forms and mutations of bacteria. Jour. Inf. Dis., 42: 461-472.

(16) Fukeida, Y. 1927 Über die Ausbildung bakteriophagenfester Bakterien. Ztschr. f. Immunitätsforsch. u. exp. Therap., 53: 252-262.

(17) Gory, M. 1923 Transformation muqueuse de Bacillus coli. Compt. Rend. Soc. Biol., 88: 49.

(18) Gratia, A. 1921 Studies on the d'Herelle phenomenon. Jour. Exper. Med., 34: 115-116.

(19) Hadley, P. 1926 Parallelism between serological and bacteriophagic response in B. typhosus and certain avian paratyphoids. Proc. Soc. Exp. Biol. and Med., 23: 445-446.

(20) Hadley, P. 1927 Microbic dissociation. The instability of bacterial species with special reference to active and transmissible autolysis. Jour. Inf. Dis., 40: 1-512.

(21) Hauduroy, P. 1926 Les formes invisibles et filtrants des bacteries visibles. Jour. Physiol. and Path. Gen., 26: 523-533.

(22) d'Herelle, F. 1917 Sur un microbes invisible antagoniste des bacilles dysenterique. Compt. Rend. Acad. Sci., 165: 373. Cited from Hadley(20).

(23) d'Herelle, F. 1919 Sur le microbe bacteriophage. Compt. Rend. Soc. Biol., 82: 1237-1239.

(24) d'Herelle, F. 1920 Sur la resistance de bacteries a l'action du microbe bacteriophage. Compt. Rend. Soc. Biol., 83: 57-59.

(25) d'Herelle, F. 1921 Phenomenes coincidant aver l'aquisition de la resistance des bacteries a l'action du bacteriophage. Compt. Rend. Soc. Biol., 84: 384-386.

(26) d'Herelle, F. 1922 The bacteriophage: It's role in immunity. (English translation, Smith). Williams & Wilkins, Baltimore.

(27) d'Herelle, F. 1924 Immunity in natural infectious disease. (English translation, Smith). Williams and Wilkins, Baltimore.

(28) d'Herelle, F. 1926 The bacteriophage and its behavior. (English translation, Smith). Williams and Wilkins, Baltimore.

(29) Kauffman, F. 1926 Keimenwandlung und lysinwirkung. Ztschr. Hyg. u. Infektionskr., 106: 520-531.

(30) Levine, Max. 1921 Bacteria fermenting lactose and their significance in water analysis. Iowa State College Engr. Exp. Sta. Bul. 62.

(31) McKinley, E. B. 1925 Transformation sous l'influence du principe lytique faible de la specificite antigenique d'une culture. Compt. Rend. Soc. Biol., 93: 1052-1054.

(32) Rakieten, M. 1932 Studies with staphylococcus bacteriophage. I. Preparation of polyvalent staphylococcus bacteriophage. Yale Jour. Biol. and Med., 4: 807-818.

(33) Schwartzman, G. 1927 Effect of bacteriophage upon the agglutination of hemolytic streptococci. Proc. Soc. Exp. Biol. and Med. 25: 227-228.

(34) Smith, C. H. 1928 Bacteriophage and phagocytosis. I. Effect on resistant and dead bacteria. Jour. Immunol., 15: 125-140.

(35) Sonnenschein, C. 1929 Wirkung von Bakteriophagen auf Typhusbakterien. Hämolysierende, schleimig wachsende, unbewegliche Formen von Typhus-bakterien. Münch. Med. Wochenschr., 76: 355-356.

(36) Standard Methods of Water Analysis, 7th edition. 1933 American Public Health Assn., New York.

(37) Tomaselli, C. 1925 Contributo allo studio del batteriofargo. Cited from Hadley(20).

(38) Twort, F. W. 1915 An investigation into the nature of the ultramicroscopic viruses. Lancet, 2: 124. Cited from Hadley(20).

ACKNOWLEDGEMENTS.

The author wishes to express his appreciation to Dr. James E. Fuller for his advice in the conduction of the research in this thesis and for his aid in the preparation of the manuscript.

The author also desires to express gratitude to Dr. Leon A. Bradley and to Miss Mary E. Garvey for their kindness in furnishing equipment and to the members of the committee, Dr. Paul Serex and Mr. John G. Archibald, for their kind cooperation.

We, the undersigned, members of Mr. Bondi's thesis committee, approve this thesis.

James E. Fuller
Chairman

Paul Lorez

J. E. Archibald

Date May 19-1937