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Upon the request of the Committee on Graduate Study the under-signed have examined a thesis entitled

Bacteriophagy in Intestinal Infections

presented by George M. Cameron candidate for the degree of Master of Science in Agriculture, and hereby certify that it is worthy of acceptance.

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BACTERIOPHAGY IN INTESTINAL INFECTIONS

A THESIS

Submitted to the Graduate Committee

of the

University of Tennessee

in

Partial Fulfillment of the requirements

for the degree of

Master of Science in Agriculture

George M. Cameron

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INTRODUCTION

Dr. d'Herelle[24] has given the name bacteriophage to a substance which he describes as a dissolving principle. The phenomenon, through which the bacterial solution is brought about, he has termed bacteriophagy.

Dr. d'Herelle describes this phenomenon as resulting in the absolute dissolution of the bacteria, brought about by the bacteriophage. He further states that by dissolution he means "passage of a solid body into a soluble state, without residue, macroscopically or microscopically visible".

The first observation interesting him in bacteriophagy was in 1909, while working with Coccobacillus acridiorum[24]. This was in connection with a disease of locusts in Mexico. He noticed irregularities in some cultures of the organism on laboratory media. They appeared as indented edges of colonies, and occasional clear areas in an otherwise normal, confluent growth of the organism.

In accordance with the work of Schweinitz and Dorset on hog cholera, d'Herelle developed an hypothesis. He believed the clear areas, hindering the normal development of the cultures, to be the growth of an ultramicroscopic virus. This virus, he believed to be either the direct cause of the disease, or the cause in conjunction with Coccobacillus acridiorum.

With a desire to know the truth about such a virus, d'Herelle endeavored to reconcile the results with facts observed in typhoid fever, and dysentery cases. He secured irregularities, such as described above, in some of the cultures made from stools of patients. However, his first results did not satisfy his expectations. He found that the irregularities always appeared during convalescence, and not at the beginning of the disease.

With this in view, in 1916 he made a systematic study of the occurrence of this dissolving principle in the stools of a dysentery patient. He found that when the principle appeared, it not only inhibited the growth of the dysentery bacillus in broth culture, but within 10 hours cleared a cloudy broth culture of the organism.

D'Herelle then conceived the idea that this principle was a parasite upon the organism. He transferred a drop of dissolved culture to an undissolved culture of the Shiga bacillus. This latter culture then dissolved. With repeated transfers, similar to this, d'Herelle reports a more pronounced activity of the virus, and more rapid dissolution of the organism.

D'Herelle then inoculated a young culture of Shigella dysenteriae with 1/10,000 cc. of a dissolved culture, and subcultured the same on agar slants. The first subculture was made immediately, and others after 1, 2, and 3

hours respectively. The first showed two plaques, or clear areas, the second six, the third one hundred and the fourth showed no growth of the organism. D'Herelle[24] says, "This gave a new proof that the dissolving principle actually regenerated in the course of action. It demonstrated that the principle was condensed in the form of active particles."

At this point it may be interesting to note a phenomenon reported by Twort in 1915[24]. D'Herelle has not accepted Twort's phenomenon as identical with the bacteriophage, though there are undoubtedly points of similarity. According to d'Herelle, Twort observed the phenomenon known as Twort's phenomenon, on agar cultures made from glycerinated calf vaccine. He noticed watery areas on some of these cultures. Some cultures failed to grow. When normal cultures were touched with small portions of the watery areas, similar areas developed and gradually spread over the entire growth. Such action was complete on young growing cultures; little effect being evidenced on dead cultures. He further reports fine stainable granules in these areas, which he believes to be the remains of bacterial life.

D'Herelle emphasizes the following points wherein his phenomenon differs from Twort's. He says that if a normal culture be touched with a portion of a clear area there is no dissolution of the culture except at the point of contact. He also states that in bacteriophagy there is a complete

dissolution of the organism in which the microscope reveals no remains, whereas Twort describes the presence of a residue of fine stainable granules in the clear areas, and complete clearing of normal cultures when touched at one point with a portion from a watery area.

The observations of these men have given rise to widespread investigation, and considerable controversy, among later workers in this field. The true nature of this lytic principle has come to be a factor of greatest interest to investigators.

There is an analogy between some of the possible theories of the nature of bacteriophage, and those which were held concerning the cause of fermentations, when Pasteur proved by unique experiments that fermentation of grain and fruit was brought about through the growth and development of microorganisms. Scientists recognized ferments as organized (living), and unorganized (chemical). Today, we have d'Herelle's corpuscular parasitic theory[24] concerning the bacteriophage as opposed to the possibility of its being a chemical substance foreign to the bacterium, or a principle derived from the bacterium. Concerning the last theory d'Herelle allows four possible solutions:

A. "It is an abnormal inert principle derived from the bacterium."

B. "It is an abnormal living principle derived from

the bacterium."

C. "It is an enzyme, normally elaborated by the bacterium."

D. "It is a living principle, existing normally in the bacterium."

The presentation of such divergent theories of bacteriophagy makes evident the great necessity for the further development and compilation of data bearing upon the nature of this phenomenon.

IMPORTANCE OF THE SUBJECT

Though few authors have endeavored to incorporate a concrete opinion concerning the nature of the bacteriophage in text-books, the following statement of Hiss and Zinsser[28] shows their interest in the phenomenon: "During the last few years a phenomenon has been observed with bacteria, which is likely to be of fundamental importance in the biological, and immunological study of bacteriology."

Sinclair Lewis[31] has introduced to the public the necessity for concentrated scientific experiment on the tremenduous possibilities of the adaptation of the phenomenon to therapeutic purposes. In "Arrowsmith" he has depicted the possible value of such a phenomenon used as therapeutic treatment in plague epidemics. In a popular way he has brought

this great principle, just recently disclosed to science, to the door of the lay reader, and made it a thing of general interest. He has presented to the average individual the tremenduous worth of such a phenomenon in one of the world's most terrible diseases. D'Herelle, not as a romancer but as a scientist, has given us a basis for hope in the prophylactic and therapeutic use of bacteriophage.

D'Herelle[24] has made use of cultures, lysed with bacteriophage, in immunizing against fowl typhoid, barbone (a septicemic disease of buffaloes and cattle), and against the toxic infection of rabbits with cultures of Shigella dysenteriae. He describes an initial immunity which he terms "exogenous immunity" and attributes it to the action of the bacteriophage corpuscle. This immunity, as described for fowl typhoid, continues to regenerate itself in an environment contaminated with the infecting agent.

In an environment free from contamination with the infecting agent, he has described the initial immunity as temporary, after which an "endogenous immunity" (or one which results due to the reaction of the body to substances introduced into it, by way of the lysed culture) is acquired by the treated animal, following an incubation period. The stimulus for this reaction, he attributes to the products of bacterial dissolution contained in the lysed culture. He says "These bacterial products, produced through

bacteriophagy, appear to be in a distinctive and peculiar physical state, one particularly suited to causing reaction of immunity by the body of the animal." In the case of barbone of buffaloes, he describes this "endogenous immunity" as approaching absolute immunity.

For prophylactic purposes, the bacteriophage is being distributed in Brazil, in 2 cc. ampules, for use by the public. D'Herelle says that prophylactic vaccination against bacillary dysentery by means of suspensions of the anti-dysentery bacteriophage is applicable to man. He has shown that animals can withstand 2000 times the fatal dose of a potent culture. He describes the immunity as absolute after the incubation period, but negative during this period.

D'Herelle[24] cites experiments which show the duration of the period of incubation during the development of "endogenous immunity" to be lengthened by large injections of lysed cultures. In the case of barbone, he found that 0.04 cc. of a lysed culture produced "solid" immunity by the fourth day, 0.25 cc. produced immunity by about the twentieth day, and 20 cc. required forty to sixty days. He attributes this to two reactions produced in the body when lysed cultures are introduced; one a sensitization, and the other an immunity. He suggests that the sensitization is increased with the size of the dose, whereas it disappears quickly in smaller doses. These factors may prove to be of utmost

importance in the study of the possible uses of cultures lysed by bacteriophage, in the production of immunity.

Another factor stressed by d'Herelle is the toxicity of fresh filtrates containing lysed dysentery organisms of the Shiga strain. He advises that these should be at least a month old.

In regard to man more data concerning the therapeutic than the prophylactic use of the bacteriophage has been accumulated.

Results obtained at the Institute Oswaldo Cruz, by da Costa Cruz, contain the following statements(24). "Up to the present, we have prepared about 10,000 ampoules of the bacteriophage." "The dysentery bacteriophage is by far the best therapeutic agent in the bacillary dysenteries known up to the present time." "Its action incomparably surpasses that of all other agents, including serum, for in the majority of cases the symptoms materially diminish in intensity within 4 to 8 hours after treatment, and the patient enters upon convalescence within 24 to 48 hours."

In a report on 23 cases of bacillary dysentery, Pereira says, "The anti-dysentery bacteriophage constitutes a specific treatment for the bacillary dysenteries, whether they be caused by Shiga bacillus or by paradysentery organisms. Without causing general reactions, the ingestion of this principle modifies completely within a very short space of time, the evolution of the dysenteric syndrome.... By the use of the bacteriophage the development of carriers is

avoided, for dysentery bacilli are never found in the excreta of patients who have been treated by the ingestion of the bacteriophage."

In cases of typhoid and para-typhoid fevers, the bacteriophage has according to d'Herelle, not invariably proven beneficial. However, where it has been of value, its value appears to be absolute. Beckerick and Hauduroy, Philibert, Arloing, Dufour, Bonnier, Sempe, Munter, Boenheim, and d'Herelle[24], all report successful treatment of coli infections of bladders and urinary tracts with use of lysed, filtered cultures, active for B. coli. A bacteriophage isolated by Gratia, polyvalent for staphylococci, has proven quite effective. Such a bacteriophage is now being distributed by the Michigan State Health Laboratories for treatment in cases of boils, carbuncles, and other staphylococci infections. It is furnished with the understanding that it may be used only when a control case is possible. The results are reported as favoring bacteriophage over other treatment because of greater curative and immunizing value. Possibilities are also held out for treatment of streptococci infections. Likewise in a few very successful treatments d'Herelle indicates the greatest possibilities for use of bacteriophage in plague epidemics. H. M. Winans[44] has reported the successful use of bacteriophage in several diseases. Thus from a therapeutical standpoint alone, bacteriophagy is of inestimable importance.

There are cases in which the action of bacteriophage differs from most prophylactic and therapeutic treatments of disease. There is the ineffectiveness of a virulent bacteriophage for Eb. typhi in some cases of typhoid fever, and there is the anti-toxic action, in vivo, of lysed cultures containing the bacteriophage. These are both problems of importance.

It will be necessary to study closely the nature of the elements in bacteriophagy which are responsible for the many inexplicable occurrences in order to develop its use.

It has been said that perhaps the action of the bacteriophage in its ability to bring about changes in the type of organisms is greater than its ability to lyse cultures. Hadley states that microbic dissociation and bacteriophagy are parts of the same problem. If it is true that in certain forms a pathogenic organism is decidedly virulent, and in other forms less so, then the ability to change the virulent to the less virulent type, if such is a characteristic of the action of bacteriophage, becomes a very important consideration. It becomes important to know whether secondary cultures appearing after lysis are more or less virulent than the original cultures. That the appearance of colony growth, in the case of secondary cultures after lysis is entirely different is set forth in reports by Fejgin[15] in work done with Proteus H X_{19}. Three yellow varieties, and Proteus O X_{19} are reported as variations

brought about in Proteus H X_{19} due to the action of bacteriophage. Proteus is associated with studies of typhus fever, and is frequent in outbreaks of the disease. Such facts make us reflect on the possibilities in relation to bacteriophagy.

It is a well-known fact that in pure-line cultures some individual organisms resist heat much longer than others. Likewise, some may be gentian-positive, and some gentian-negative. It is also known that there are strains of Eberthella typhi which are agglutinable and others which are non-agglutinable in the presence of specific immune serum. Yet both are Eberthella typhi. Hadley(20) says, "From the same pure-line strain may arise, depending on the manner of cultivation and on other environmental conditions, substrains possessing little resemblance either to each other or to the parent-strain. These different culture types may be spoken of conveniently as 'dissociated forms' or as 'dissociants'; and the phenomenon involved in their production has been termed 'microbic dissociation'".

The study of bacteriophage in prophylactic and therapeutic treatment of disease has opened a new and wide field of study, and a new era in bacteriology. The whole study of bacteriology is not old, and the progress in this science has been extremely rapid. Microbes were first studied by Pasteur as the cause of fermentation. He recognized certain types of organisms, morphologically.

Soon the use of gelatin as a medium for growth presented a means by which closer morphological studies could be made. In these studies Koch became a master. His idea of one form, one type, and one purpose for the progeny of a definite organism has until recently ruled the progress of the study of the morphology and the physiology of microorganisms.

Pasteur and Koch gave us the foundation whereby bacteriologists recognize a disease by isolating the causative organism. This organism is identified by its specific characteristics. Koch recognized the shape and size of organisms. Since then we have learned to study the specific by-products of organisms in different kinds of media. This was followed closely by the recognition of the resistance of individuals for specific diseases. It was found that the blood serum contained something which helped to protect an individual from a certain disease. Immunity was produced artificially by injecting attenuated organisms into the body, in response to which antibodies were produced. It was generally recognized that antibodies were specific in nature. Thus we secured the foundation of a greater study of prophylaxis, and therapeutics. Today this knowledge is one of the great things in both preventive and curative medicine. In spite of this great advance it is well known that cure and prevention are often due to poorly understood principles, no doubt related to physiology of microorganisms.

In discussing his belief that bacteriophage is a living parasite on bacteria, and that micellae which he calls "protobes", and not cells are the simplist forms of life, d'Herelle[26] makes the following statement: "Surely it is not through studying visible organisms, complex ones, that biologists will reach the solution to their fundamental problems, for with such the investigator finds himself surrounded by a multiplicity of diverse factors, which by their very number and by their complexity effectively prevent advance into the unknown." This statement is of great significance even should bacteriophage be shown to be an enzyme or auto-ferment of the bacterial cell. It is significant in that it points to the real basis for the study of such fields as physiology, adaptation to environment, and that other factor which no living thing evades, heredity. With the rapidity of regeneration among the simpler forms of life, we have not only the advantage of simplicity, but also that of numbers of individuals, as a factor in accuracy. The changes brought about by the action of bacteriophage, help to center our attention more closely on the individuality of microorganisms.

SURVEY OF LITERATURE

In regard to the source of the bacteriophage, d'Herelle[24] says that it is present everywhere, and that it has been found in the intestinal contents of healthy animals including man. It is especially prevalent in the feces of convalescents, in blood, pus, river water, and cultivated soil. He says, that as it is a constant inhabitant of the intestinal tract it may be encountered in everything which may be contaminated by fecal material. He suggests that certain bacterial strains may be found "contaminated", and that bacteriophage has been isolated from such; but he adds that such cultures are rare. To such sources Hadley has added: sea water, infected lesions, saliva, sterile abscesses (pus produced due to injected sterile agar), suppurative lesions, animal tissue, scrapings of mucosa, extracts of duodenum wall, pancreas, liver extracts, lymph, tonsils, prostate gland, and secretion of the latter. He also refers to its isolation by use of commercial trypsin and pepsin.

Hadley feels that rather than consider these places as the source of the bacteriophage it would be better to consider them as influences causing the bacteria to produce their own bacteriophage. He states that at the University of Michigan they have used the extract of the pancreas autoclaved at 15 pounds for 20 minutes, as a means for the isolation of the bacteriophage. They have secured similar

results using trypsin. He further states that in his laboratory the bacteriophage has been demonstrated by growing an organism for successive generations in its own filtrates, and that results show that bacteriophage must be generated the first time by a sensitive culture.

He lists the following three resistant cultures:

1. (a) Resistant to lysis

   (b) Non-lysogenic, or not possessing the power to transmit the lytic quality (its filtrate will not instigate lysis in a normal sensitive culture).

   (c) Non-lytic

2. (a) Resistant to lysis

   (b) Lysogenic (filtrate causes lysis)

   (c) Non-lytic

3. (a) Resistant to lysis

   (b) Lysogenic

   (c) Lytic

W. Borchardt[6] is inclined to attribute d'Herelle's phenomenon to the fermentative action of the pancreatic secretion activated by enterokinase. In introducing Shigella dysenteriae into the intestinal tract of rabbits and dogs, he found that a filtrate of the feces proved to be bacteriolytic. He reports the bacteriophage, or lytic principle as in greatest amount in the duodenum, and most active during the digestive period. He further found that a diltrate of duodenal fluid dissolved a great variety of organisms.

He also states that inasmuch as lytic filtrates cannot be produced by incubating mixtures of bacteria and immune serum, it would appear that preformed anti-bodies have nothing to do with the phenomenon.

K. Hajos[20] states that passage of bacteriophage through Hoen's membrane proves that the particles are no larger than an albumen molecule, and concludes that it cannot be a living thing. Smiyoshi[42] has introduced bacteriophage into the intestinal tract of guines pigs and later extracted the same from peritoneal exudates. Petrovanu[40] reports a lytic principle for Es. coli in filtrates from anginas, in scarlet fever, simple angina, Vincent's angina, and measles. He reports these filtrates as showing no lytic principle for Staphylococci, Streptococci, Micrococcus catarrhalis, Eb. typhosus, or Shigella dysenteriae. Kuttner[30] has secured bacteriophage from old cultures of typhoid and dysentery organisms, and in alkaline washings of Staphylococcus cultures, formerly resistant to lysis. She has concluded from her work, and other considerations that bacteriophage is a secretion of bacteria, produced under certain circumstances. She says that an autolysin is usually liberated in old cultures, from cell disintegration, and acts as a catalyst, destroying equilibrium in actively growing cells of constructive and and destructive forces in favor of the latter.

For the purpose of isolating bacteriophage d'Herelle says that the material in which it may be found should be filtered, first through filter paper through which water containing infusorial earth has passed. This makes a less permeable filter. Then it is filtered through a porcelain filter. If bacteriophage is present it will be found in the filtrate.

Hadley gives the following method:
For all work in bacteriophagy a culture less than sixteen, preferably twelve hours old should be used. The filtration of material (commonly sewage) to be used in the isolation of bacteriophage should be filtered according to d'Herelle's method followed by use of two parts of sterile broth to one part of the filtrate. To 30 cc. of this mixture add 15 drops of a young culture of the organism for which the bacteriophage is being isolated. Hadley emphasizes the condition of the culture. He holds that all broth cultures should be made from either a 12-hour agar culture or a 12-hour broth culture which has been made from a young agar culture. Younger but not older cultures may be used. The inoculated filtrate plus broth is incubated for 12 to 18 hours and then filtered. To the filtrate is added one-fourth the volume of fresh broth, and 15 drops of a young broth culture. This is repeated until the filtrate is clear at the proposed time to filter. When this occurs, 30 drops of the culture may be

used for inoculation. This amount may increase sometimes to 4 or 5 cc. in the fourth or fifth filtrate. Hadley states that unless bacteriophage is secured in this manner, by the sixth filtrate, one may as well begin again. One should seldom use over 400 mm. vacuum in filtering. Reserves may be kept from filtrates to test for the presence of bacteriophage.

Bacteriophage is recognized by its ability to bring about lysis in young clouded broth cultures, by its ability to inhibit growth of the organism on agar, and by its ability to produce plaques or clear areas. In the production of plaques a loopful of proper dilution is spread over an agar surface which has been inoculated with a young broth culture of the susceptible organism.

The method of isolating the bacteriophage takes into consideration the enhancement of virulence to susceptible cultures in successive transfers of the bacteriophage. It is also necessary to watch for the maximum concentration of the bacteriophage in the filtrates, for after securing an active and concentrated bacteriophage the concentration may be reduced by continued passage through a Berkefeld filter. In my own experience this has occurred with Proteus H X_{19}, an organism with which filtrable forms proved quite common.

Tomaselli[24] is credited with experimental work in which he has shown that after twenty days of contact of certain organisms with the bacteriophage "ultrabacteria" or

filtrable forms were no longer demonstrable, and the virulence of the bacteriophage was increased from weak (+) to maximum (++++) strength, by filtration at this time.

In July of 1927, using the method recommended by Hadley, I succeeded in isolating a bacteriophage active for Shigella dysenteriae (B. dysenteriae(Shiga)), and later one active for Proteus X_{19}.

After proper filtration the sewage was mixed with broth, two parts of broth to one of sewage filtrate. I followed through the filtrates according to the method advocated by Hadley. In the third filtrate I was able to demonstrate approximately 45 areas in one loop of a 10^{-4} dilution, and in the fourth filtrate this number had increased 22 times and could not be counted by use of a 10^{-4} dilution. This lytic principle, as contained in the fourth filtrate, had an inhibition titer of 10^{-7} and a lytic titer of 10^{-6}. In other words the growth in one loopful of a 12-hour culture of Shigella dysenteriae added to a tube containing 9 cc. of broth and 1 cc. of dilution of the fourth filtrate inhibited the growth of the dysentery bacillus, or rather caused entire clearing of the growth which appeared during the first twelve hours. In the case of the lytic titer, this represents the highest dilution from which 1 cc. added to a moderately clouded 9 cc. broth culture of the dysentery bacillus brings about complete clearing of the

culture. In each case these tubes were observed to remain clear over a period of ten days.

Using some of the sewage which had been secured 10 days before, I proceeded to isolate a lytic principle for Proteus X_{19} by the same method employed above. In this case I secured a lytic principle in the third filtrate which would produce 720,000 lytic areas per cc., and only 360,000 in the sixth, a circumstance previously referred to.

In November of 1927 for the purpose of attempting to isolate a lytic principle for Salmonella pullorum, I secured sewage from the end of a sewer pipe which carries sewage into the Tennessee River from West Knoxville. I followed the same isolation procedure outlined above, using Salmonella pullorum for growth in successive filtrates. In the third filtrate-reserve (about 5 cc. of the third filtrate kept to test for the presence of the lytic principle) I noticed growth. I had noticed similar passage of Proteus through a "W" Berkefeld filter.

I transferred a loopful of growth from the third filtrate-reserve of S. pullorum to a tube of sterile broth. From this tube I inoculated differential media, and found the organism to be physiologically identical with the pure culture of S. pullorum. However, it tended toward a slightly heavier growth on solid media, and the colony formation was less translucent than that of the pure culture.

Previous to the appearance of this growth in F_3-filtrate-reserve I had spread one loop of the filtrate over a freshly spread culture of S. pullorum, on an agar slant. After incubation this revealed about fifteen watery areas, each about 6 mm. in diameter, within a confluent growth of the organism. After three days these areas were still visible, but the center of each showed a growth heavier than normal, and almost opalescent in appearance. I carried the procedure for isolation of the bacteriophage through the fifth filtrate, and secured decidedly increased evidence of the presence of a transmissible principle inhibitory to the growth of S. pullorum, evidenced by scattered colonies on a spread agar slant in the presence of a loopful of the filtrate, whereas a control tube showed normal growth. However, I did not build up the principle to one of maximum activity.

Another attempt was made later to secure an active principle for S. pullorum. This time I used the sewage that I had secured one month before. It was filtered, and added to broth as previously described. Varying from previous practice, I used a 20-hour broth culture of S. pullorum and added 15 drops to the filtered sewage-broth mixture, at the same time making a transfer to a tube of sterile broth and one to an agar slant. Twenty-four hours later the sewage-broth mixture called hereafter F_0 filtrate, was entirely clear, whereas slant (b) and broth (b) both showed normal growth.

At this time I varied still more from previous practice and added to the clear F_0 filtrate 1 cc. more of the same broth culture used for the first inoculation, and in addition 10 cc. of broth. Transfers were also made from slant (b) to another agar slant and likewise to a tube of broth each labeled (c). Eight hours later the F_0 filtrate showed no marked growth. At this time, I transferred a loop from broth (b) to broth (b_1). Broth (b) had acted as a check on the F_0 filtrate inoculations, and was uniformly clouded.

Twenty-four hours later, making a total of thirty two hours after the last inoculation the F_0 filtrate was perfectly clear. Ten cc. were taken out for reserve. The remainder of the F_0 filtrate was fed 2 cc. of broth (b), a twenty-four hour culture, and 10 cc. of fresh broth. Thirty-six hours later the F_0 filtrate showed a definitely agglutinative growth.

With the F_0-reserve the following tests were made: one loopful was spread over a freshly prepared agar slant culture of S. pullorum, and this tube labeled (C_2). One drop was allowed to run down the center of a second spread culture, and this labeled (C_3) and a third spread culture was left as a control and labeled (C_1). These spread cultures were made from (C) which was about 30 hours old.

Slant (C_1) showed uniform growth, slant (C_2) showed no growth and slant (C_3) showed growth except where the drop

had run down over the spread culture.

By preparing dilutions of the reserve filtrate I found that the plaques secured would approximate a total of 1,500,000 per cc. Four serial transfers were made for inhibition tests, controls being run in all cases. In the last transfer one loopful of a 22-hour broth culture of S. pullorum was added to each of six tubes of broth, one was left for a control, and to the other five were added one loop of a cleared culture of S. pullorum. The results were typical of that which one might expect in the presence of an active lytic principle, the control tube being uniformly clouded.

After 56 days I noticed that one of the cultures just mentioned showed growth, while the others did not.

Following the above inhibition one loop from a cleared tube was added to a 28-hour broth culture of S. pullorum, having previously made a transfer from this broth culture to a second tube of broth. In this case lysis was brought about. In a similar case of lysis the cleared culture was used 103 days later in successful inhibition of the growth of pullorum in broth culture.

In January of 1928, I demonstrated an inhibitory principle for Es. coli, using sewage which had stood in the refrigerator for two and one-half months. In this case I prepared the mixture of filtered sewage and broth as formerly and inoculated it with 15 drops of a 12-hour broth culture

of Es. coli. After 12 hours I added 1 cc. more of a similar culture. This was filtered about sixteen hours after the second inoculation. The filtrate proved very effective in inhibiting growth of Es. coli, both in broth, and on solid media. I did no further work with it, at that time.

D'Herelle[24] says that there are races of bacteriophage virulent for only one strain, and races virulent for all strains of a given species and to different species. He says, "Ordinarily a bacteriophage is virulent, not only for bacteria of a given species, but also for bacteria of different species, sometimes to organisms very distantly related." He says that there are races of bacteriophage isolated from typhoid patients showing virulence in varying degrees for a number of species. In other words a bacteriophage isolated from the stools of a typhoid patient has been shown to be very active for the strain of Eb. typhi causing the disease, less active for another strain, and showing varying degrees of activity for Es. coli and Shigella dysenteriae. D'Herelle says that in view of the possible combinations of virulence in different races, no two races can be absolutely identical. Bail[6] has expressed his belief in a multiplicity of "phage", as particular strains of organisms show resistance to particular bacteriophages.

D'Herelle has applied a bacteriophage virulent for Es. coli and E. typhi to Shigella dysenteriae, through 1,200

passages, after which he claims it had not lost its ability to pyse the other two organisms. Inasmuch as the bacteriophage concentration would have been diminished within seven passages to the point of elimination of the original bacteriophage, and therefore all subsequent bacteriophage must have been developed during the lysis of Shigella dysenteriae, d'Herelle has taken the manifestation of undiminished multiple virulences to be an attribute of a single bacteriophage corpuscle.

He has shown that after 1500 passages with Shigella dysenteriae of a "phage" only slightly virulent for Eb. typhi the number of plaques formed with Eb. typhi is somewhat less than at the start. He believes it would be possible eventually to cause the complete loss of the ability to react with Eb. typhi. He has likewise demonstrated what he terms an adaptation of bacteriophage to an organism for which it formerly has shown no activity. He mentions work of Janzen and Wolff, da Costa Cruz and McKinley in the adaption of a "phage" virulent for one strain, to other strains and other species of organisms for which it formerly has shown no virulence. He describes the adaptation by McKinley(36) of a race of Shiga-bacteriophage to meningococcus by means of four passages in a bouillon containing glucose and blood. thereafter it proved active for Staphylococcus albus, aureus and citreus, for M. tetragenus, for Streptococcus hemolyticus

and for pneumococci of Group I, II, and III, for which it was formerly avirulent. He states that no race of bacteriophage is unchangeable, and that all vary continually some losing virulence very slowly, and others acquiring virulences by adaptation. He says further, "In a given suspension of the bacteriophage not all of the corpuscles are endowed with exactly the same properties. Virulence, in particular, is variable for each one of them." Matsumoto[35] decided that bacteriophage proven to be definitely specific is not adaptable to other organisms. He says that certain bacteriophages may act on a wide range of species; but this faculty is present from the time of isolation, and is not the adaptation of a specific bacteriophage to other organisms.

D'Herelle suggests the use of suspensions of young bacteria in broth for best results in lysis with bacteriophage. He says that in a 24-hour broth culture of Shigella dysenteriae about one-third of the organisms present are not capable of producing colonies on agar, whereas the organisms on a 24-hour agar slant culture are almost all living. He states that bacteriophage can develop at the expense of living bacteria only, and consequently all live organisms present the best conditions for lysis. He says that whereas a drop of a suspension of active bacteriophage corpuscle will completely dissolve a suspension of organisms from an 18- to 24-hour agar slant culture, it will leave a certain degree

of turbidity in a 15-day old broth culture of the same organism. He cites data to show that it is not the products of bacterial growth that prevent lysis in the old culture. Gratia[24] introduced a small portion of a potent Staphylo-bacteriophage with a suspension of Staphylococcus aureus. Bacterial dissolution began after one week, and continued to completion. In regard to this d'Herelle says, "The dissolution of modified bacteria, and dead cells as well, appears to be effected through the action of substances which become disseminated in the medium during the course of bacteriophagy".

In discussing conditions most favorable for plaque formation d'Herelle says, "The state of the bacteria is important. Young bacteria are most readily attacked, and the critical period is the moment of division." For application of bacteriophage, Hadley has advised the use of a 12-hour broth culture grown from an agar-slant culture not older than 24 hours. It is interesting to note that Hadley[20] has mentioned the aging of cultures as an influence in bacterial dissociation, or variance in type of organisms.

The above discussion leads us into the field of secondary cultures, or the growth of organisms in apparently lysed broth cultures, and within apparently lysed areas on agar. These organisms quite often show vigorous growth and in the case of secondary cultures of Shigella dysenteriae, I have found them resistant to the action of that "phage"

which by serial transfer had become quite active for the original culture from which these secondary colonies developed. It has been reported that organisms resistant to one strain of "phage" may be susceptible to other homologous strains. It is also reported that secondary cultures may regain their susceptibility to the "phage" to which they have shown resistance.

Bronfenbrenner has pointed out the decided differences between primary and secondary cultures, not only as concerns resistance to "phage", but also in morphology, size, and motility of organisms, as well as in morphological and physiological cultural characteristics. I have been decidedly impressed by the morphological differences in cultural characteristics in the presence of, and absence of "phage", under varying conditions. It is further reported by various workers that types of organisms which are resistant to "phage" may be secured from normal cultures, without the use of "phage" or other agents. Such types may also be secured in the presence of chemicals, heat, etc.

D'Herelle(24) says, "As a general rule it may be said that bacteriophagy may always take place at that temperature which is the most favorable for the development of the bacterium subjected to the action of the bacteriophage. This is not equivalent, however, to the saying that this temperature is that at which the phenomenon is effected at the

greatest rate. Moreover, the phenomenon may occur at temperatures very remote from the optimum growth temperature of the bacterium"..... "As regards the optimal temperature, that is to say, that at which the phenomenon manifests itself most quickly, and most completely, basing my statements upon the results of my experiments with B. dysenteriae and B. typhosus, I had concluded that the temperature most favorable for bacteriophagy was that which was likewise most favorable for the development of the bacteria. The results of many subsequent experiments, preformed with a variety of bacterial species, indicate that this conclusion may have been too restricted. As a general rule, the statement is certainly true for bacteriophagy of B. dysenteriae and B. typhosus, but it is not equally true for all other species of bacteria."

In cases of Shiga-bacteriophage a temperature range of from 8° to 41°C. has been given.

Eb. typhi and B. pestis have been lysed at 41°C, Staphylococcus at 43°C. and Es. coli at 45°C. Koser(29) has reported a bacteriophage for thermophilic organisms which acts best at 45° to 52°C., and Elder and Tanner report one active for a psychrophilic organism at temperatures from 0.05° to 4°C. This is in accord with d'Herelle's opinion that generally the best temperature for action of the bacteriophage is that at which the organism grows best.

Incidentally in explaining some works of Bail[5], Doerr and Berger[12], and Nakamura[38], d'Herelle has given results of the size of plaques on agar at different temperatures as follows:

I. Agar plates held at 37°C.

Suspension 4....About 50 plaques, having a diameter of about 5 mm.

II. Agar plates held at 30°C.

Suspension 4....About 50 plaques having a diameter of about 7 mm.

III. Agar plates held at 18°C.

Suspension 4....Confluent plaques (about 50), with a diameter of about 13 mm.

In regard to the effect of temperature on the size of the plaques, he says that it might be considered the result of a "race" between the multiplication rate of the bacteriophage and the bacteria. He says that the thickness of the film of bacterial growth normally prevents increase in the size of a plaque when such growth has hardly become visible. In his work with resistance of bacteria to bacteriophage d'Herelle used temperature variations. He found that at 25°C., using a Staphylo-bacteriophage he secured complete clearing of cultures which remained so two months. He secured similar results at 31°C. However, when after 6 days he incubated tubes cleared at this temperature using an incubator temperature of 41.5°C., clouding resulted in 24 hours. A temperature of 36°C. was less efficient in permanent lysis, one

tube not clearing at all. At 40°C. only 11 tubes cleared, and all were clouded after 72 hours.

For selection of a Staphlo-bacteriophage of great virulence from the race used in this work, d'Herelle recommends a temperature of 32°C. with a media more alkaline than pH 7.5.

Various experimental works have placed the temperature at which bacteriophage is completely destroyed at 75°C. Hauduroy[50] reported resistance of bacteriophage varying with the virulence, 102°C. being the temperature of actual destruction. Tchang Kono Ngen and Wgemans[51] also reported variation in resistance to heat according to the source of bacteriophage. A temperature of about - 180°C. has been found by d'Herelle to destroy bacteriophage from 47 days to 5 months of age, however, it did not destroy bacteriophage less than eighteen days old. He has presented only scant data as yet, and draws no conclusions concerning this.

According to d'Herelle, Marcuse[31] states that a filtrate mixed with a saturated solution of sodium chloride may be evaporated at a low temperature, and the active bacteriophage recovered from the crystals. In the case of other salts, mercuric chloride, sodium floride, potassium cyanide, and copper sulfate d'Herelle has reported destruction of bacteriophage in varying periods of time.

In regard to the presence of electrolytes in media various investigators[24] have reported that they are necessary for the activity of bacteriophage. D'Herelle claims that in acid media this is true, and may or may nor be true in neutral media. He believes that the phenomenon takes place in alkaline media with all races of bacteriophage in the absence of electrolytes. He reports active lysis in bouillon containing 25 per cent salt, and Brutsaert[10] reports lysis of Staphylococci in broth containing 14 per cent salt. Maitland's[33] experiments showing bacteriophagy in a medium such as 1 per cent buillon in physiological salt solution have been confirmed by d'Herelle. Although he has described complete clearing of a physiological salt solution suspension of an organism, he does not attribute it to a typical bacteriophage. D'Herelle attributes to Kabeshima the statement that lysis of Shigella dysenteriae occurs in the presence of antiseptic substances as sodium flouride or an excess of chloroform. All his other data indicates that lysis does not take place in the presence of sodium flouride although the bacteriophage is not destroyed by it. In commenting on work of Brutsaert he has presented the idea that the destructive concentration of sodium flouride varies between 0.25 per cent and 0.05 per cent for various bacterial species. He explains this as a result of the state of the bacterium, rather than

modification by the presence of the antiseptic.

Reports of adaptation of bacteriophage to phenol chloromine, immune serum, and mercuric chloride have been reported. Munter and Rasch[37] believe that such findings are to be explained by the lysogenic character of the organism used rather than by adaptation of the bacteriophage to the sustances. This adaptation is another of d'Herelle's points in proof of the living nature of bacteriophage. He also reflects upon adaptation of bacteriophage to organisms for which it has shown little or no virulence as proof of its living nature. However, here Hadley has questioned the ability of bacteriophage to adapt itself to organisms other than those within closely related groups.

As to the reaction of media and its effect on the action of the bacteriophage, d'Herelle says, "Unquestionably, alkalinity of the medium affords the most favorable reaction for accomplishing the phenomenon of bacteriophagy". "The optimal reaction is not a constant, varying not only with the bacterial species and the race of the bacteriophage concerned, but differing also with the nature of the medium." For routine purposes he advises ordinary peptone bouillon with a reaction of pH 7.8. He says that with an active bacteriophage lysis may or may not occur in media poor in electrolytes. It does not occur if the medium is acid, but always occurs in an alkaline medium.

Gratia[19] has set forth the effect of the reaction of media on the action of a bacteriophage for Es. coli. Using media of pH 6.8, 7.0, and 7.2 he demonstrated a fluctuation of clouding and clearing in broth, the growth becoming more decided with each successive fluctuation. In a media of pH 7.5 the lysis was complete and normal. Da Costa Cruz has been given credit for the isolation of bacteriophage active in a medium with a pH 5.1. Da Costa Cruz[11] has demonstrated flocculation of bacteriophage in the presence of acids. As concerns acids and bases in their effect on a Shiga-bacteriophage, Eliava and Pozerski[14] found that it was destroyed in 24 hours in media below pH 2.5 or above pH 8.4. According to Reichert[41] races of bacteriophage of different virulences are affected differently, some resisting acidity or alkalinity which may destroy others.

In regard to the inactivation of "phage" by antiserum, Bronfenbrenner says, "According to Bordet the active principle is destroyed by antiserum, if the "phage" is an organized corpuscular virus, one might expect by analogy with what is known about the mode of action of bactericidal sera that a virucidal action of antibacteriophage serum would require the cooperation of complement for its completion." Together with Korb, Bronfenbrenner has confirmed earlier findings of Bail that antibacteriophage serum is equally capable of neutralizing bacteriophage activity

before or after inactivation of complement. Arnold[2] concludes from his work with antibacteriophage serum that antigenicially the bacteriophage belongs to that group of substances that porduce neutralizing or antitoxic antibodies. That is to say it is of the group including true bacterial toxins, vegetable poisons, and enzymes produced by animals and plants. Arnold and Weiss[3] working with definite amounts of bacteria, and bacteriophage with antibacteriophage serum agree with Arnold's report that the antilysins belong to the group of antibodies which includes antitoxins, and antiferments. They demonstrated that susceptible bacteria grown in antibacteriophage serum develop no immunity to the lytic principle. They reduce agglutinins, opsonins, and amboceptors for homologous organisms; but do not reduce the antibacteriophage properties of the serum.

Osumi[39] prepared antisera in rabbits by injecting:

1. Lysate of culture of coli dissolved by bacteriophage
2. "Phage"-free emulsion - B. coli
3. Autolysate of colon bacilli made by solution of colon bacilli in distilled water ("phage"-free)

He reports only the first antiserum as inhibitory to the action of bacteriophage. He further reports that serum inactivated by heating was reactivated by adding complement, and concludes that here is a lack of analogy between antilysins and anti-toxins.

OBJECT OF INVESTIGATION

In general the object of this investigation is to attempt to add something to the rapidly accumulating data concerning bacteriophagy. The specific object of the work reported here is to compare eight organisms of the intestinal group, as concerns their reactions to methods of the isolation of bacteriophage, and as to their actions in the presence of a definite bacteriophage under controlled conditions.

PROCEDURE

In the following work media of a reaction between pH 6 and pH 8 has been used, except where otherwise indicated. This is in accordance with general practice in regard to media for the study of bacteriophage. It is generally considered that the best medium for the development of bacteriophage has a slight alkaline reaction. However, inasmuch as the bacteriophage feeds directly on the bacteria, and not on the media, he suggests that the exact pH value of the media is dependent upon the best growth of the bacteria. In the great majority of work with bacteriophage the phenomenon does not take place readily when the reaction of the medium is acid. Hadley, says that lysis will not occur under any conditions in which bacteria will not grow. He holds that bacteria are not lysed by bacteriophage in physiological salt solution but that upon transfer to broth lysis is immediate. Da Costa Cruz[21] gives a variation in the optimum reaction of media for bacteriophagy ranging from pH 6.5 to pH 8.3. He demonstrated the phenomena of the precipitation of bacteriophage from broth by addition of acid, and its solution by a reversion to alkalinity.

In using a portion of previously used sewage which had been in the refrigerator for 5 months, I began a series of inoculations and filtrations of broth. First I mixed the sewage and broth as described above, using eight organisms

taken from stock cultures and envigorated through successive transfers. For each organism used, 20 cc. of broth was mixed with 10 cc. of sewage, and filtered through a "W" Berkefeld filter. The eight tubes of broth-sewage mixture were inoculated with 15 drops each from respective 12-hour broth cultures of the eight organisms. In all work with this series no vacuum or pressure for filtrations was employed, and only the "W" Berkefeld filters were used. All organisms were treated in the same way. The treatment of them was governed by the results. When one filtrate was clear at the proposed time for filtration, and the other seven were cloudy, all were filtered irrespective of the possibility of developing a stronger lytic principle.

The eight organisms of the intestinal group used in this investigation were as follow: Salmonella pullorum, Alcaligenes abortus, Alcaligenes fecalis (B. Alkaligenes), Eb. typhi (B. typhosus), S. paratyphi (Para-A), Es. coli (B. coli), Shigella dysenteriae (B. dysenteriae (Shiga)), and S. schotermulleri (Para-B). At first in the attempt to isolate bacteriophage active for each of these organisms, a laboratory stock culture of Shigella dysenteriae was used. In applying a known bacteriophage of previously determined activity for Shigella dysenteriae, a culture was used which formerly had been used in isolating this bacteriophage at the University of Michigan. It was the practice in this work

to prepare broth cultures for use by transfers to broth from young agar slant cultures. These cultures were generally 12 hours old, and never over 24 hours old, unless otherwise specified. The broth cultures used were never over 16 hours old. After the first transfer of 15 drops of a 9-hour broth culture to broth-sewage mixture, these tubes were incubated at 37.5°C.

After growth for sixteen hours each of these was filtered, first asceptically through sterile paper filters, and then through Berkefeld "W" type filters. At this time all showed good growth except A. abortus and S. dysenteriae which were only slightly clouded. Inasmuch as the filtration was carried out in atmospheric pressure it covered a period of two to three hours. From each filtrate about 10 cc. was kept as a reserve to be tested for the presence of a lytic principle. The filtrates described above were labeled F_1 filtrates. For the organisms letters were used to designate each as follows: S. pullorum, A, A. abortus, B, Alcaligenes fecalis, C, S. schotermülleri, D, Eb. typhi, E, S. paratyphi, F, Es. coli, G, and Shigella, S. dysenteriae, H.

For each successive filtrate I used a sub-numeral with the letter F to designate the number of times filtration had been carried out. As to the reserve portions they were designated as A-F_1 reserve, A-F_2 reserve, B-F_1 reserve, B-F_2 reserve, etc., the first letter designating the organism which

has been grown in that particular broth-sewage mixture. After filtration for the F_1 filtrates, and after removing the F_1 reserves, 10 cc. of fresh broth was added to each of the eight filtrates, and then to each respective filtrate 15 drops of a 16-hour broth culture of the respective organisms. For B and H-F_1 filtrates it was necessary to use a 28-hour broth culture, because the 16-hour culture had not grown sufficiently for use. After eighteen hours of growth of these organisms in their respective F_1 filtrates A, C, and D were very cloudy and the others moderately so. All were filtered for the F_2 filtrates. Reserve portions were kept from each of these filtrates. To the remainder of the filtrates were added 10 cc. of fresh broth, and 15 drops of their respective 16½-hour broth cultures. After 21 hours all F_2 filtrates to which cultures had been added showed good growth. After 28 hours they were all filtered for the F_3 filtrates. As usual about 10 cc. of each filtrate was kept as the F_3 reserve. To the remainder of each of the filtrates was added 10 cc. of fresh broth and 15 drops of their respective broth cultures, 12 hours old. After 24½ hours duration in the incubator, at 37.5°C., all F_3 filtrates were filtered for F_4, although A and B-F_3 filtrates were absolutely clear. About 10 cc. of each F_4 filtrate was kept as a reserve, and to the remainder of each of the filtrates was added 15 drops of their respective 9-hour broth cultures,

and 10 cc. of fresh broth.

After 21 hours incubation the F_4 filtrates were filtered for F_5, except A. A was filtered 14 hours later, but was perfectly clear during this period. The reason for the delay in filtration of A was a broken filter. The F_5 filtrates were kept without adding either broth or culture. They were held at incubator temperature for several days to allow for growth of any filtrable forms. No further filtrations were carried out.

In order to test each of these filtrates from F_1 through F_4 for the presence of a lytic principle, plates of nutrient agar were poured and spread-cultures made of each of the organisms. One such culture of each organism was kept as a control plate. All plates were labeled according to the organism, and filtrate used, thus A-F_1, A-F_2, etc. To test the filtrates for a lytic principle 1/100 cc. of each of the filtrates was streaked across its respective spread-culture. Thus 1/100 cc. of A-F_1 filtrate was streaked across a spread-culture of A, and 1/100 cc. of B-F_1 filtrate was streaked across a spread-culture of B, etc. For the control plates 1/100 cc. of sterile broth was streaked across each. All of these plates were watched for inhibition of growth along the line over which the filtrate was streaked. In some cases there was proliferation of growth, rather than inhibition. The F_4 filtrates were tested later by preparing two spread-cultures

of each of the organisms on agar slants. One of each of these was kept as a control and over each of the others was spread 1/100 cc. of each of the respective F_4 filtrates.

During incubation and filtration the presence of growth due to the passage of organisms through the "W" Berkefeld filter was sought.

Five days after the last filtration, methylene blue stains were made from each filtrate in which growth had appeared. All observations were carefully tabulated.

After 21 days slant cultures were made from each of the filtrates showing growth, and in no case did they fail to grow on nutrient agar, although the growth in some cases varied somewhat in appearance from the usual laboratory stock culture of the organisms. From these slants, after about three weeks, broth cultures were prepared, and from these the organisms were transferred to some of the simpler kinds of media to determine the reactions that might occur, and to check reports that the organisms which pass porcelain filters often show physiological as well as morphological differences, as compared to standard characteristics. Hanging drop preparations were examined at 24 hours. Two days later methylene blue stains were made, and their microscopic appearance reproduced in blue printed drawings.

To check once more the presence of a lytic principle in any of the filtrates, transfers were made of

one loopful of a 15-hour broth culture of each organism to each of six tubes of broth. One broth culture of each organism was kept as a control, and to the other five were added a loopful of filtrates F_1, F_2, F_3, F_4, and F_5, respectively. Six hours later 1 cc. from each of these cultures, thus prepared, was spread over a Petri plate containing nutrient agar. The excess fluid was removed. These plates were incubated at 37.5°C. for 24 hours. In order to test filtrates from F_1 through F_5 for the presence of a lytic principle, plates of nutrient agar were poured and spread-cultures made of each of the organisms. One such culture of each organism was kept as a control plate. All plates were labelled according to the organism, and filtrate used, thus A-F_1, A-F_2, etc. To test the filtrates for a lytic principle 1/100 cc. of each of the filtrates was streaked across its respective spread-culture. Thus 1/100 cc. of A-F_1 filtrate was streaked across a spread-culture of A, and 1/100 cc. of B-F_1 filtrate was streaked across a spread-culture of B, etc. For the control plates 1/100 cc. of sterile broth was streaked across each. These plates were watched for inhibition of growth along the line over which the filtrate was streaked.

The F_4 filtrates were tested later by preparing two spread cultures of each of the organisms on agar slants. One of each of these was kept as a control, and over each of the others was spread 1/100 cc. of each of the respective F_4

filtrates.

The agar slant culture of Shigella dysenteriae which had been sealed at the University of Michigan on June 29, 1927, was opened on June 19, 1928. A direct transfer to a fresh agar slant was made and growth secured. This was repeated on June 25, 1928, using the same culture. Later this culture of Shigella dysenteriae was used because it was the culture on which the bacteriophage used in this work had been demonstrated.

A sealed tube was opened containing bacteriophage which eleven months before showed about one billion plaques per cc. This bacteriophage was tested in connection with the culture of Shigella dysenteriae mentioned above, and proved fit for use.

A second sealed tube was opened containing diluted bacteriophage, and of the age of that just mentioned. This diluted bacteriophage was used in all platings except where otherwise stated. It had, formerly, about 30 plaques in each loopful. By using one drop of this bacteriophage plated with Shigella dysenteriae a good distribution of plaques was secured under favorable conditions.

Knowing that the bacteriophage was sufficiently active to be used in contact with the culture of Shigella dysenteriae to be used, I proceeded to test its activity under varying conditions with the organisms under consideration.

Two broth cultures of each organism were prepared, using 9-hour broth cultures for seed. These in turn had been prepared from 16-hour agar slants. To one freshly prepared broth culture of each organism one loopful of dilute bacteriophage was added. All cultures were incubated for 12 hours. Using the 12-hour cultures, 1 cc. of each was spread over nutrient agar on Petri plates. One plate was prepared for each organism, using the cultures to which one loopful of bacteriophage had been added, and two plates for each organism using the cultures containing no bacteriophage. To one set of the last mentioned plates one drop of dilute bacteriophage was added before adding the bacterial cultures. The other set acted as a control for the growth of the organism. At this time I also centrifuged the broth cultures to which a loopful of dilute bacteriophage had been added. The centrifugal speed was approximately 4000 R.P.M., and the time was five minutes. After centrifugalization of one drop from the top, and one from the bottom of each culture were plated for a study of colony differences. These plates were all incubated at 37.5°C. for 24 hours.

Two weeks later 2 sets of eight plates each were prepared to test the action of concentrated bacteriophage on the eight organisms. To one set was added one drop of concentrated bacteriophage, the nutrient agar having been previously added, and hardened. Then to both sets were

added 1 cc. of a 12-hour broth culture, so there was one plate with bacteriophage, and one control plate for each organism. Even distribution over the agar surface was produced. The plates were incubated 24 hours.

To determine the effect of age and type of the organisms on the action of bacteriophage, 12-hour, 12-day and 28-day cultures of the organisms were used. This gave a variation in the age of the organism to which bacteriophage was applied, and theoretically a variation in type. Six sets of plates, eight in each set, were prepared by first adding nutrient agar and allowing it to solidify. Then to three sets one drop of dilute bacteriophage was added. Then 1 cc. of 12-hour broth cultures was added to each plate in one of these sets, and 1 cc. to each plate in one set containing no bacteriophage, so that there was one plate for a 12-hour culture of each organisms in each of the two sets. A similar procedure was followed, using 12-day cultures, and 28-day cultures respectively. In all cases the cultures were well distributed over the surface of the agar, and all plates were incubated at 37.5°C. for 24 hours.

For the purpose of demonstrating the effect of salt concentrations in solid media, on the action of bacteriophage, double peptone media was used with the following salt concentrations: 0.5%, 1%, 2%, 3%, 4%, and 5%. C. P. sodium

chloride was used. The reason for using double peptone was to avoid adding salt other than the exact amount desired. The action of bacteriophage on double peptone media was necessarily determined, using Shigella dysenteriae for which the bacteriophage had been isolated. Two sets of plates were used for each degree of salt concentration. First the media was poured and allowed to solidify. Then to one set for each concentration one drop of dilute bacteriophage was added. Then 1 cc. of 12-hour cultures of each of the various organisms was added and carefully spread over the plates. Thus, for each organism there was a control plate with the organism and no bacteriophage, and a plate with the organism and one drop of the bacteriophage, for each concentration of salt. Only Shigella dysenteriae with and without bacteriophage was plated on the double peptone media containing no salt.

In order to make observation of the effect of different temperatures on the action of the bacteriophage six sets of plates were prepared. As in previous work the nutrient agar was first poured into the plates. This was allowed to solidify, and a drop of dilute bacteriophage was added to each plate of three sets. Then 1 cc. of a 12-hour culture of each of the eight organisms was added to one plate of each set. The three different temperatures at which these plates were kept were 12°C., 26°C., and 37.5°C. These were all observed after 24 hours of incubation; but

the 12°C. plates were allowed to incubate 7 days for the final reading. The final readings of the 26°C. and 37.5°C. plates were taken after 24 hours.

Phenol and iodine were chosen in an attempt to demonstrate the effect of different concentrations on the action of bacteriophage applied to organisms under consideration.

A medium of pH 7.6 was used. To this the antiseptics were added, the iodine before sterilization, and the phenol after. The strength of iodine concentration may have been reduced slightly in sterilization of the media. Four concentrations of iodine in nutrient agar were used as follows: 0.006-2/3%, 0.01%, 0.02%, and 0.1%. Three concentrations of phenol were used as follows: 0.05%, 0.1%, and 0.2%. Two sets of eight plates were poured with each concentration of both antiseptic media used. In each case the control set had added to it 1 cc. from a 12-hour broth culture of the various organisms under observation. The other plates of each set had added one drop of dilute bacteriophage, and 1 cc. from a 12-hour culture of the various organisms. This gave seven sets of control plates, and seven sets of plates one of each set representing one of the organisms being used, and one drop of dilute bacteriophage. These plates were incubated at 37.5°C. for 24 hours.

In the work concerning the reaction of media, and its effect upon the action of the bacteriophage, a medium was used for routine work ranging between pH 7.6 and 7.8. For variation in reaction, media of pH value 6.8, 7.4, and 8.0 were used. Two sets of plates were prepared with each kind of medium, and this allowed to solidify. To one set, or eight plates of media of each pH value one drop of dilute bacteriophage was added. For this test the dilute bacteriophage previously used was further diluted by one part of sterile broth to one part of dilute bacteriophage. As a result one-half drop of the former dilute bacteriophage was used. This made the strength of the bacteriophage equal to about fifty plaques per drop, rather than 100 plaques per drop. Having added one drop of dilute bacteriophage to one set of plates of each plate, so that there was a control plate for each organism. There was also a plate with the organism together with one drop of bacteriophage for the medium of each pH value. These plates were incubated at 37.5°C. for 24 hours. Chicken blood serum was used in the work demonstrating the effect of blood serum on bacteriophagic action. First, two sets of plates were poured, using nutrient agar of pH 7.6 to 7.8. This was allowed to solidify. A large drop of serum was added to each of these sixteen plates, then one drop of dilute bacteriophage was added to each of eight plates. Then to each plate 1 cc. of a 12-hour

broth culture was added, thus making a control plate for the growth of each organism in the presence of serum, and one plate for the growth of each organism in the presence of serum and a drop of dilute bacteriophage.

RESULTS

In the course of the work covering incubation and filtration of cultures in a sewage broth mixture, it has been stated that a number of organisms passed through the "W" Berkefeld porcelain filter. Stains were made from these to determine the size and shape, and other microscopic features of the organisms to be found in these filtrates. The stained preparations revealed the following characteristics listed according to filtrates from which st ains were made:

F_1 S. pullorum--Innumerable minute coccoid forms, very few well stained, occasionally cells of abnormal swollen size. Organisms generally poorly stained. Few fibronous clusters, organisms mostly small. Very few rods larger than normal.

F_1 S. schotermülleri--Large rod-like appearances, swollen forms showing some normal shapes within them. Abundant minute forms to swollen short plump rods.

F_1 Eb. typhi--Very few stained organisms visible. A few clusters, hyaline appearances filled with very minute cells. None of normal size apparent.

F_1 S. paratyphi--Hyalin structures showing imbedded variations from stained threads to normal well stained organisms. Other organisms were poorly stained ranging from very small to normal size.

F_2 S. pullorum--No well stained organisms, many coccoid forms, few crowded clumps imbedded in hyaline substance. None normal size.

F_2 abortus--Very minute to normal sizes. Stained very poorly.

F_2 schotermülleri--Mostly poorly stained from very minute to normal size. Some well shaped organisms often evident in hyalin mass.

F_2 fecalis--Did not show growth when these stains were made.

F_2 paratyphi--From poorly stained, ill-shaped forms through coccoid and granular to normal well stained organisms.

F_3 fecalis--Many very plump almost round forms of variable sizes. Some well stained, many very poorly, and of granular appearance.

F_3 schotermülleri--Coccoid forms, rods with granular staining (bipolar), some club shapes. Hyalin branched, and tissue-like substance in which normal cells were clumped. Also masses of normal cells in brown clumps.

F_3 paratyphi--Granular clumps, showing innumerable minute coccoid forms to normal sized cells. Minute forms poorly stained, and normal cells well stained.

F_4 fecalis--Very few well stained organisms, very small coccoid to short rods, with a few rods bipolar staining.

F_4 schotermülleri--Minute coccoid forms, few long ill-defined rods, hyalin substance showing imbedded normal cells well stained, also clusters of imbedded minute to normal cells.

F_4 Eb. typhi--Many very small to normal organisms. Many small dark granular clumps, with occasional well stained hyalin masses, crowded with normal cells.

F_4 paratyphi--Some normal short rods, some clumps in brown mass. Many unstained and some very minute organisms.

F_4 dysenteriae--Many minute organisms, some granular clumps occasionally showing cell forms, coccoid to short rods. Staining quality variable.

F_5 fecalis--Many organisms clumped in masses. Some single, very small but mostly well shaped short rods, staining poorly. Some bipolar stained.

F_5 schotermülleri--Much substance of hyalin appearance, and some in which numerous organisms are clumped, varying in size from very minute to normal. Very few organisms stained well, many such tiny organisms hardly visible.

The growth on agar slants was in some cases more transluscent than the usual growth of these organisms, and showed a tendency in some respects toward individual colony formation. The F_2 Para-B growth was more proliferous than usual, and the growth of A. fecalis was less so, otherwise there were no marked differences. Rom the agar slant cultures

of the organisms just described together with alkaligenes of the F_2 filtrate, nutrient broth cultures were prepared. After 24 hours incubation these were examined in a hanging drop and the following observations made:

F_1 pullorum--Variable shape, with some short rods agglutinated. The coccoid forms were single. Non-motile.

F_1 schotermülleri--Some coccoid to actively motile rods. Some agglutinated.

F_1 Eb. typhi--Actively motile rods.

F_1 Paratyphi--Motile rods, few globular forms, and granular clumps.

F_2 pullorum--Non-motile rods, no agglutination.

F_2 abortus--Minute globular forms, ill shapes, and few rods. Clumps, hyalin, non-motile.

F_2 fecalis--Minute and larger globular forms. Non-motile.

F_2 schotermülleri--Granular and agglutinated clumps. Size variation from very small coccoid forms to very large rods in chains almost snake-like in appearance and motile.

F_2 paratyphi--Motile rods, and few flobules.

F_3 fecalis--Normal size, both motile and agglutinated forms.

F_3 schotermülleri--Motile rods and large non-motile strands. Few globules and granular masses.

F_3 paratyphi--Motile rods--some chains of several.

F_4 fecalis--Many free motile rods, few globular forms. Granular amalgamated clusters of organisms.

F_4 schotermülleri--Motile rods, agglutinated masses.

F_4 Eb. typhi--Actively motile rods.

F_4 paratyphi--Normal motile rods, non-motile small granular rods. Globules, gourd-like to ellipsoidel.

F_4 dysenteriae--Normal size, motile.

F_5 fecalis--Motile and agglutinated rods.

F_5 schotermülleri--Motile rods. Some globules.

F_5 Para-A showed growth. Not examined for the characteristics of the organisms. No visible growth during several days.

Transfers from the nutrient broth cultures to some of the simple sugars, and nitrate broth yielded results given in Table 1. Readings recorded represent four days growth in each broth.

TITLES OF FIGURES

Fig. 1	S. Pullorum after Passage of a "W" Berkefeld Filter
Fig. 2	Shigella Dysenteriae after Passage of a "W" Berkefeld Filter
Fig. 3	From Growth of S. Schotermülleri in Presence of F_3 Filtrate
Fig. 4	From Growth of S. Schotermülleri in Presence of F_5 Filtrate
Fig. 5 & 6	A. Fecalis after Passage of a "W" Berkefeld Filter with different Filtrates
Fig. 7 & 8	S. Schotermülleri after Passage of a "W" Berkefeld Filter with different Filtrates
Fig. 9 & 10	S. Schotermülleri after Passage of a "W" Berkefeld Filter with different Filtreates
Fig. 11	S. Schotermülleri after Passage of a "W" Berkefeld Filter
Fig. 12 & 13	Eb. Typhi after Passage of a "W" Berkefeld Filter with different Filtrates
Fig. 14 & 15	S. Paratyphi after Passage of a "W" Berkefeld Filter with different Filtrates

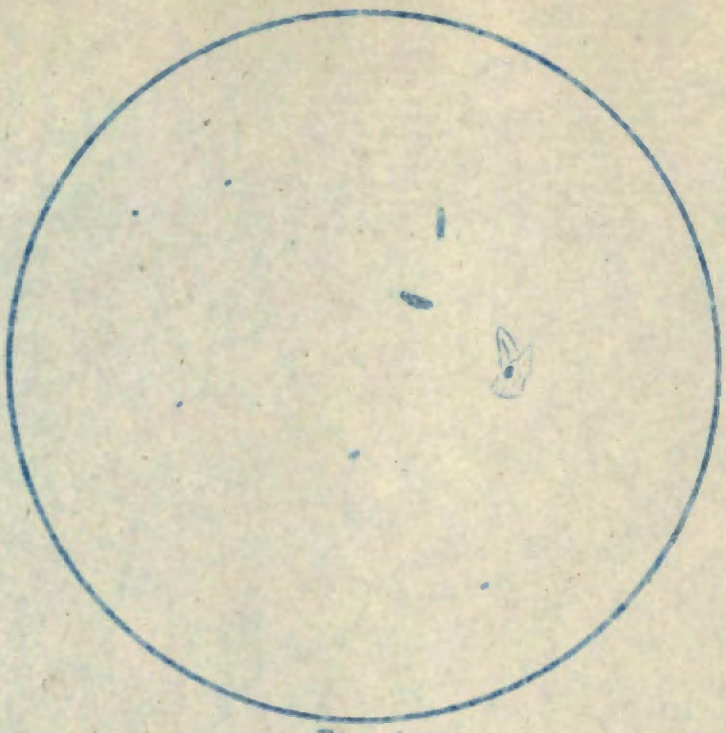


Fig. 1



Fig. 2

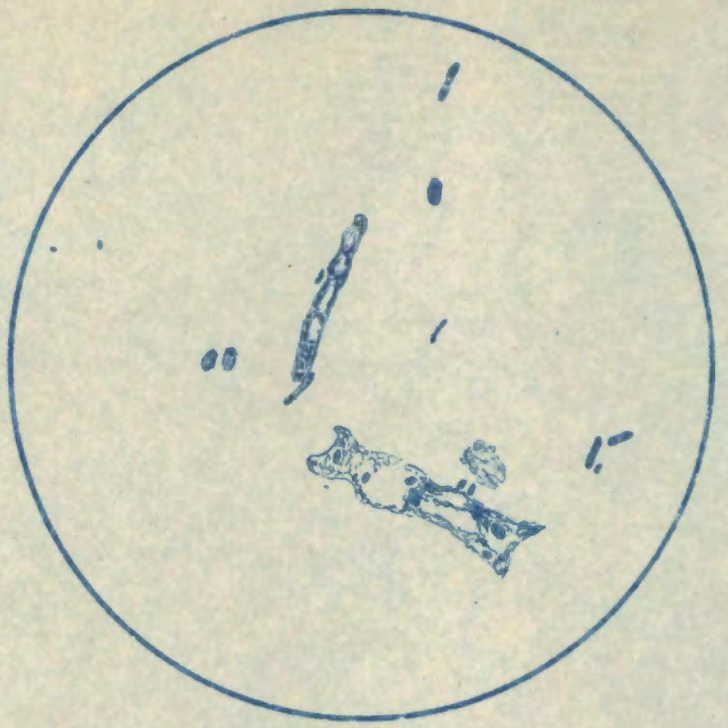


Fig. 3



Fig. 4



Fig. 5



Fig. 6



Fig. 7

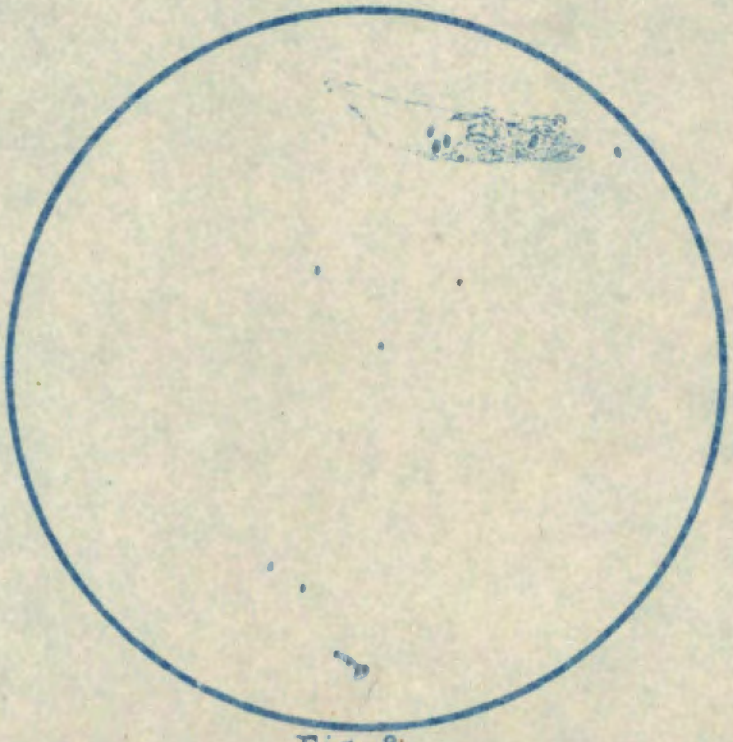


Fig. 8



Fig. 9



Fig. 10



Fig. 11

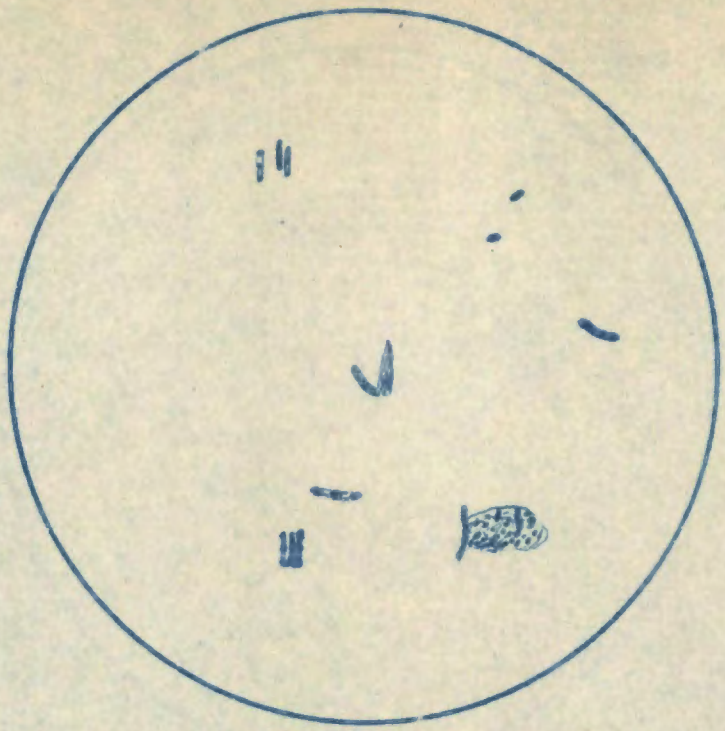


Fig. 12

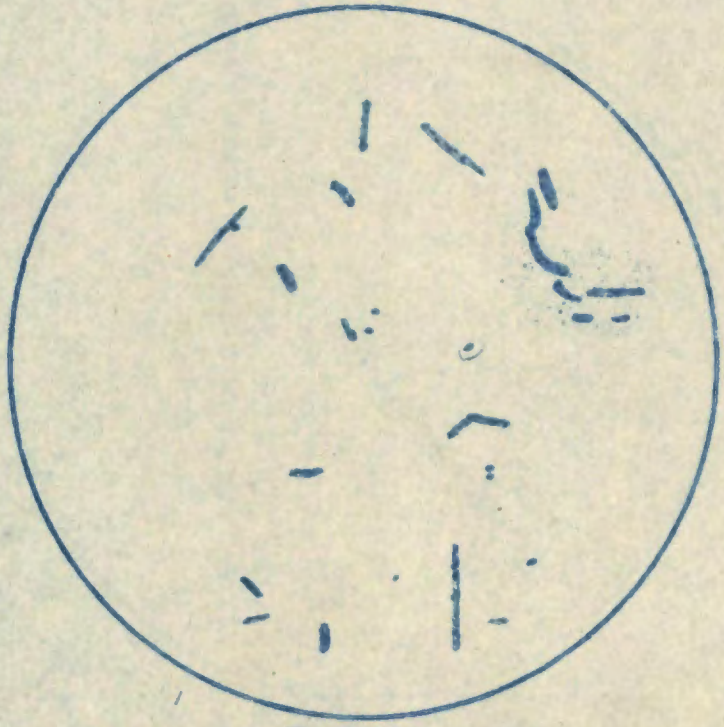


Fig. 13

TABLE 1

Reactions of filtrable Forms in Nitrate Broth and Sugar Broths

Filtrate	Nitrate reduction	Dextrose	Lactose	Sucrose	Levulose	Maltose
F_1 S. pullorum	—	Alk.	—	—	—	—
F_1 S. schotermülleri	—	⊕	⊕	—	⊕	⊕
F_1 Eb. typhi	±	⊕	⊖	—	⊕ V.S.	⊕ V.S.
F_1 S. paratyphi	—	⊕	⊕ V.S.	—	+	+
F_2 S. pullorum	+	Alk.	—	—	—	—
F_2 A. abortus	—	Alk.	—	—	..	Alk.
F_2 A. fecalis	—	—	Alk.	Alk.	..	Alk.
F_2 S. schotermülleri	—	⊕	⊕ V.S.	—	+	..
F_2 S. paratyphi	—	+	+	—	+	+
F_3 A. fecalis	—	Alk.	Alk.	Alk.	..	Alk.
F_3 S. schotermülleri	—	⊕	⊖	—	0	0 V.S.
F_3 S. paratyphi	—	+	0 V.S.	—		
F_4 A. fecalis	—	Alk.	Alk.	Alk.	—	Alk.
F_4 A. schotermülleri	±	⊕	⊖	—	+	⊕
F_4 Eb. typhi	±	+	+	—	0 V.S.	+
F_4 S. paratyphi	±	⊕	+	—	..	+
F_4 S. dysenteriae	±	⊕	⊕ V.S.	—	..	..
F_5 A. fecalis	—	Alk.	Alk.	Alk.	..	Alk.
F_5 S. schotermülleri	—	⊕	⊖	—	⊕	⊕

* In the above table symbols represent the following

V.S.= Very slight nitrate reduction or acid production as the case may be.

\+ = Acid production

— = No acid production

0 = Gas

.. = Indefinite

Alk. = Alkaline

V.S. = Very slight

Stained preparations were made from the nutrient broth cultures when they were three days old. Description of some of these, and a reference to drawings made from others follows:

F_1 S. pullorum--Mostly minute poorly stained cells.

F_1 S. schotermülleri--Fig. 8 represents this stain and in it may be seen the normal cells imbedded in hyalin substance.

F_1 Eb. typhi--Fig. 12 represents this stain, showing variation in sizes, hyalin substance, granular staining and a small projectile on a long slender rod.

F_1 S. paratyphi--Fig. 14 represents this stain.

F_2 S. pullorum--Fig. 1 represents this stain. Few well stained cells.

F_2 A. abortus--Cells mostly small and poorly stained.

F_2 A. fecalis--Large hyalin appearances some showing slight central staining. Stained and unstained large and normal forms in slightly stained hyalin substance. Represented in Fig. 5.

F_2 S. schotermülleri--Fig. 9 represents this stain.

F_2 S. paratyphi--Very minute forms and some short rods, stained very poorly.

F_3 A. alkaligenes--Forms as in Fig. 6 of F_5 alkaligenes.

F_3 S. schotermülleri--Poorly stained except such forms as appear in Fig. 11.

F_3 S. paratyphi--Hyalin forms as in Fig. 15, note the central amalgamated clusters of hyalin substances containing some poorly stained, and some well stained cells.

F_4 A. fecalis--Forms as in Fig. 6.

F_4 S. schotermülleri-- Mostly small cells, few stained as in Fig. 8.

F_4 Eb. typhi--Moderately well stained forms as in Fig. 13.

F_4 S. paratyphi--Mostly poorly stained minute coccid to normal rods.

F_4 S. dysenteriae--Fig. 2. Cells showing projections, and bulging and variable sizes. Also clumps containing normal as well as projecting rods.

F_5 A. fecalis--Represented by forms shown in Fig. 6.

F_5 S. schotermulleri--Variation in form, size and grouping, some showing swollen ends as in Fig. 10.

Tables have been prepared showing the effect of the application of 1/100 cc. of filtrates of the organisms under consideration to spread-cultures of the respective organisms. In all cases where growth of the organism is mentioned reference is made to the type of growth found on the control plate of that set.

The first filtrate had little effect on any cultures except in the case of S. pullorum. In this instance the growth was decidedly more cloudy and noticeable where the filtrate F_1 pullorum was applied, than on other portions of the spread culture. This is shown in Table #2.

Table #3., representing the application of 1/100 cc. of the F_2 filtrates to their respective spread cultures shows all growth to be normal except that along the line of the F_2 coli filtrate. This filtrate showed ability to inhibit the growth of Es. coli.

In Table #4., we find that the F_3 coli filtrate was not inhibitory to the normal growth of the organism. However, in the case of S. pullorum, A. abortus, and Eb. typhi there was definite inhibition of growth due to the presence of the F_3 filtrates of the respective organisms. Other F_3 filtrates showed neither stimulation nor inhibition of the normal growth.

Table #5. shows similar inhibition for F_4 S. pullorum, F_4 A. abortus, and F_4 A. fecalis filtrates. For F_4 S. schotermulleri filtrate it shows slight inhibition, and for F_4 Eb. typhi filtrate no inhibition whatsoever. The growth along the line of the F_4 dysenteriae filtrate was marked with a number of large colony formations, not present in the normal growth of Shigella dysenteriae.

TABLE No. 2.

Inhibition of Growth of Spread-Cultures on Line of 1/100 cc. F_1 Filtrate

Culture	Growth over line of filtrate	Growth on control plate
S. pullorum	Poliferous--Plate normal otherwise	Confluent
A. abortus	Normal	Confluent
A. fecalis	Normal	Confluent
S. schotermulleri	Normal	Confluent
Eb. typhi	Normal	Confluent
S. paratyphi	Normal	Confluent
Es. coli	Normal	Pitted, confluent
S. dysenteriae	Normal	Confluent

TABLE No. 5

Inhibition of Growth of Spread-Culture on

Line of 1/100 cc. F_3 Filtrate

Culture	Growth over line of filtrate	Growth on control plate
S. pullorum	Normal	Confluent
A. abortus	Normal	Confluent
A. fecalis	Normal	Confluent
S. schotermülleri	Normal	Confluent
Eb. typhi	Normal	Confluent
S. paratyphi	Normal	Confluent
Es. coli	No growth on line, otherwise normal	Confluent
Shigella dysenteriae	Normal	Confluent

TABLE No. 4

Inhibition of Growth of Spread-Cultures on Line of 1/100 cc. F_3 Filtrate

Culture	Growth over line of filtrate	Growth on control plate
S. pullorum	No growth on line, otherwise normal	Confluent
A. abortus	No growth on line, otherwise normal	Confluent
A. fecalis	Normal	Confluent
S. schotemuelleri	Normal	Confluent
Eb. typhi	No growth on line, otherwise normal	Confluent
S. paratyphi	Normal	Confluent
Es. coli	Normal	Pitted, confluent
Shigella dysenteriae	Normal	Confluent

TABLE No. 5

Inhibition of Growth of Spread-Cultures on Line of 1/100 cc. F_4 Filtrate

Culture	Growth over line of filtrate	Growth on control plate
S. pullorum	No growth on line, otherwise normal	Confluent
A. abortus	No growth on line, otherwise normal	Confluent
A. fecalis	No growth on line, otherwise normal	Confluent
S. schotermülleri	Slight growth on line, otherwise normal	Confluent
Eb. typhi	Normal	Confluent
S. paratyphi	Normal	Confluent
Es. coli	Normal	Pitted, confluent
Shigella dysenteriae	Large colonies on line, otherwise normal	Confluent

Plate I



Colony Types of Shigella Dysenteriae
in the Presence of F_3 Filtrate

Table No. 6. shows the effect on the normal growth of the organisms of spreading 1/100 cc. of the respective F_4 filtrates over a freshly spread culture of each of the organisms on an agar slant. In this case the F_4 A. fecalis filtrate failed to inhibit the growth of fecalis. The results on the growth of S. pullorum, A. abortus, S. schotermülleri, S. paratyphi, and Shigella dysenteriae of the presence of their respective F_4 filtrates indicate inhibition of growth of these organisms. The other F_4 filtrates showed no inhibitory factor for the normal growth of their respective organisms.

The next five tables were prepared to represent the inhibition or acceleration of normal growth of these organisms in contact with their respective filtrates in broth for a period of six hours. This was demonstrated by preparing spread-cultures, using 1 cc. of such cultures after 6-hours of incubation.

Table No. 7. indicates alteration of kind of growth by the F_1 filtrates for S. pullorum, A. fecalis, S. paratyphi, and Es. coli. It also indicates inhibition by the F_1 filtrates for A. abortus, S. schotermulleri, and S. paratyphi. The others showed no marked alteration or inhibition.

Table No. 8. shows a stimulative effect of F_2 filtrates of S. pullorum, A. fecalis, and S. schotermülleri

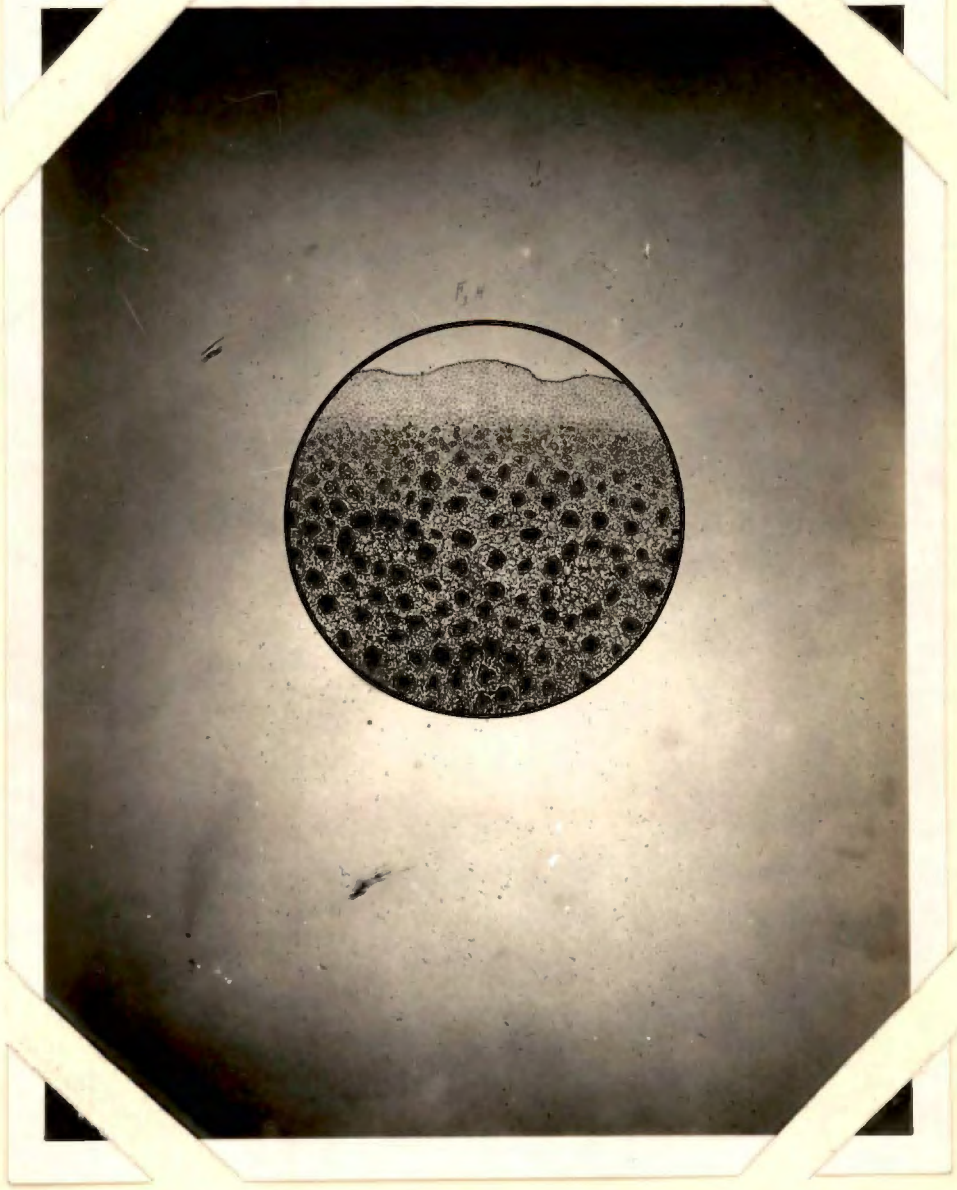
TABLE No. 6.

Inhibition of Growth of Spread-Cultures on Agar Slants in Presence of 1/100 cc. of F_4 Filtrates

Culture	Growth in presence of filtrate	Growth on control plate
S. pullorum	Marked inhibition F.S.C.*	Confluent
A. abortus	Marked inhibition F.S.C.	Confluent
A. fecalis	Normal	Confluent
S. schotermulleri	Irregular scattered colony growth	Confluent
Eb. typhi	Normal	Thin, confluent
S. paratyphi	Innumerable small plaques	Confluent
Es. coli	Normal	Confluent, pitted
Shigella dysenteriae	Only 6 colonies on slant	Individual colonies, innumerable

* F.S.C. = Few scattered colonies, as used in all future tables.

Plate II



Internal Structure of a Portion of large type Colony of Shigella Dysenteriae

TABLE No. 7.

Plates from 6-hour Broth Cultures with 1/100 cc. F_1 Filtrate

Culture	Growth of culture and filtrate	Growth on control plate
S. pullorum	Cloudy confluent	Thin confluent
A. abortus	One-half number of normal colonies	T.I.C.* colonies of two sizes
A. fecalis	Heavy and less T.I.C. than normal	T.I.C., confluent
S. schotemuelleri	Plaques, later showing cloudy growth	Confluent
Eb. typhi	Normal	Confluent
S. paratyphi	Slightly heavier than normal	Confluent
Es. coli	More confluency than normal	Confluent
Shigella dysenteriae	Very thin confluent	T.I.C.

* T.I.C. = Tendency toward individual colony formation, as used in all future tables.

TABLE No. 8

Plates from 6-hour Broth Cultures with 1/100 cc. F_2 Filtrate

Culture	Growth of culture and filtrate	Growth on control plate
S. pullorum	Cloudy confluent	Thin confluent
A. abortus	One-half number of colonies on control	T.I.C., colonies of two sizes
A. faecalis	T.I.C. and slightly more dense than control	T.I.C., confluent
S. schottmulleri	Abnormally heavy, confluent growth	Confluent
Eb. typhi	Normal	Confluent
S. paratyphi	D.C.*	Confluent
Es. coli	Less dense more translucent than normal	Pitted, confluent
Shigella dysenteriae	Normal with few S mm. colonies	T.I.C.

* D.C. = Dim or cloudy colonies in confluent growth as used in all future tubes.

Plate III

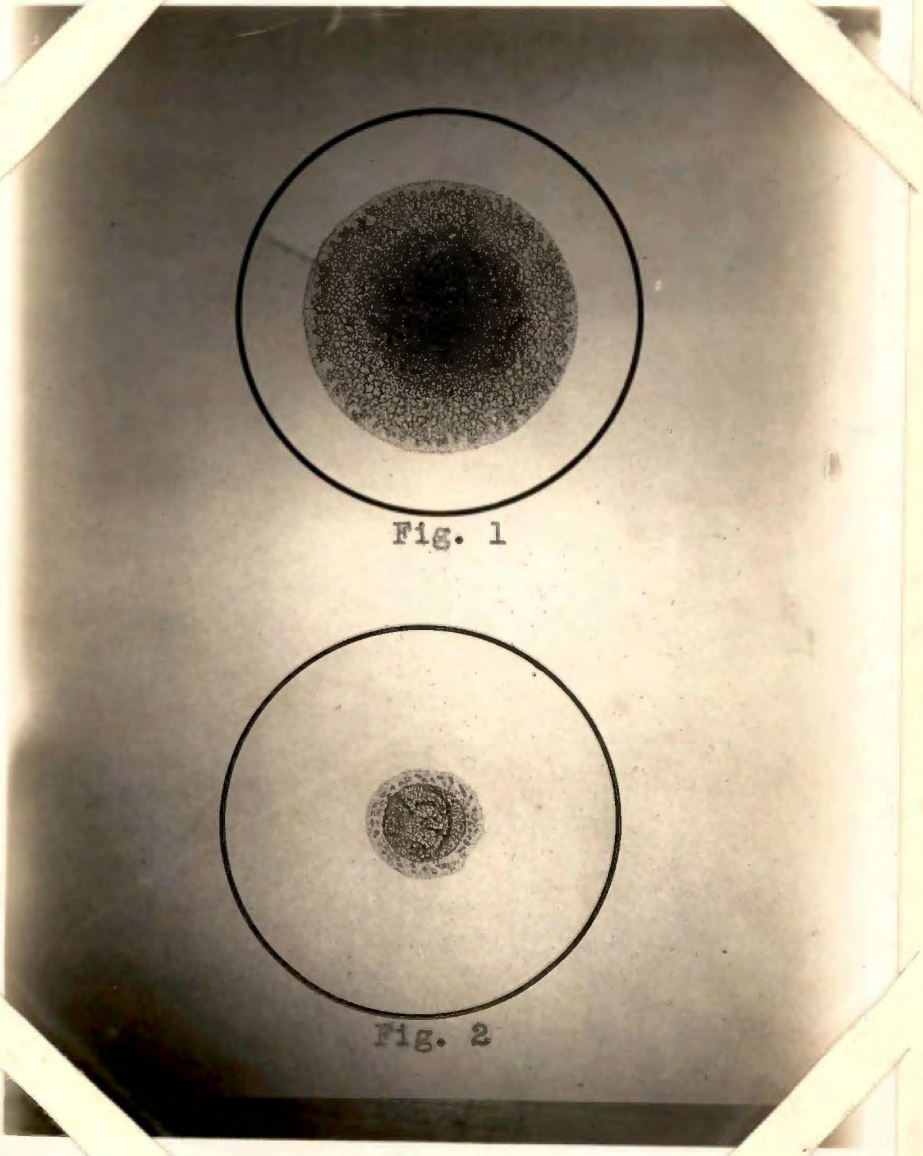


Fig. 1 Internal Structure of Medium sized Colony of Shigella Dysenteriae

Fig. 2 Internal Structure of small sized Colony of Shigella Dysenteriae

for the respective organisms. The F_2 filtrate of Shigella dysenteriae was also slightly stimulative. There was a difference in the type of growth of S. paratyphi, and Es. coli due to the presence of the F_2 filtrate. Inhibition occurred with A. abortus in contact with the F_2 filtrate and only in the case of Eb. typhi did the growth remain perfectly normal.

Table No. 9. shows a change of type of growth for S. pullorum, S. schotermulleri, A. fecalis, Eb. typhi, A. abortus, and Shigella dysenteriae in the presence of their respective F_3 filtrates. Growth of S. paratyphi and Es. coli appeared normal. Growth of A. abortus showed inhibition by F_3 filtrate of one type of growth. A fecalis growth was inhibited and the normal type of S. paratyphi growth was inhibited by the F_3 filtrate.

Plate I shows the types of colony growth formed by Shigella dysenteriae in the presence of the F_3 filtrate. Plate II shows the internal structure of the large colonies, from the edge to the center. Plate III, Figure 1 shows the internal structure of the medium sized, and Figure 2 of the small colonies respectively.

Table No.10. shows changes of type of growth for S. schotermulleri, Eb. typhi, and S. paratyphi due to contact with the respective F_4 filtrates. Inhibition is shown

in the growth of S. pullorum, A. abortus, S. paratyphi and Es. coli, in contact with their F_4 filtrates. For A. fecalis, and Shigella dysenteriae there was no noticeable effect brought about by the F_4 filtrates.

Table No. 11. shows changes of type in the growth of S. pullorum, A. fecalis, S. schotemulleri, Eb. typhi, S. paratyphi, and Shigella dysenteriae due to contact with the F_5 filtrates of these organisms. Inhibition was shown in the case of A. abortus, S. fecalis, Es. coli, with inhibition of the normal type of growth in the case of Shigella dysenteriae. In no case was the growth the same in the presence of the F_5 filtrate as on the control plate. Figure No. 4. shows the type of organisms found in the growth of S. schotemulleri.

Results are recorded in Table No. 12. showing the nature of growth on a spread agar plate culture prepared from the various filtrates, after inoculating each with its respective broth culture, adding 10 cc. of fresh broth and incubating for 12-15 hours. As recorded, pullorum grew as follows:

From F_1 filtrate - normal, from F_2, F_3, and F_4 plaques or lytic areas appeared as described, and from F_5 the growth was of a heavier, more opaque type.

TABLE No. 9.

Plates from 6-hour Broth Cultures with 1/100 cc. F_3 Filtrate

Culture	Growth of culture and filtrate	Growth on control
S. pullorum	T.I.C. (minute)	Thin, confluent
A. abortus	One-eight colonies on control. More large proportionally and up to three times the diameter of large colonies on control	T.I.C.--colonies of two sizes
A. fecalis	I*	T.I.C., confluent
S. schotemulleri	Heavy confluent control, no growth around edges	Confluent
Eb. typhi	D.C.	Confluent
S. paratyphi	Normal	Confluent
Es. coli	Normal	Pitted, confluent
Shigella dysenteriae	T.I.C., colonies of three sizes as in Plate No. 1	T.I.C.

*I = Irregular clearings in growth, with no clean cut round plaques; but shoreline effect, as used in all subsequent tables.

TABLE No. 10.

Plates from 6-hour Broth Culture with 1/100 cc. F_4 Filtrate

Culture	Growth of culture and filtrate	Growth on control
S. pullorum	F.S.C.	Thin confluent
A. abortus	One-twelfth total colonies on control, with no reduction in large colonies	T.I.C., colonies of two sizes
B. alkaligenes	Normal	T.I.C. confluent
Para B	G.V.S.* confluent	Confluent
Eb. typhi	Heavy confluent	Confluent
Para A	Cleared areas in which cloudy colonies later developed	Confluent
Es. coli	Pitted as with small plaques	Pitted, confluent
Shigella dysenteriae	T.I.C., no large colonies	T.I.C.

*G.V.S. = Growth very slight, as used in all subsequent tables.

TABLE No. 11.

Plates from 6-hour Broth Culture with 1/100

F_5 Filtrate

Culture	Growth of culture and filtrate	Growth on control
S. pullorum	Heavy type of confluent growth	Thin, confluent
A. abortus	One-half total colonies and 1/4 large ones on control	T.I.C., colonies of two sizes
A. fecalis	I	T.I.C., confluent
S. schottmulleri	D.C.	Confluent
Eb. typhi	Thin confluent with S.C.	Confluent
S. paratyphi	D.C.	Confluent
Es. coli	Pitted as with small plaques	Pitted, confluent
Shigella dysenteriae	Only few 3 mm. colonies and one large heavy type	T.I.C.

TABLE No. 12.

Plates from Filtrates after Inoculation with respective Organisms, and 12-15-hour Incubation*

Control	S. pullorum	A. abortus	A. fecalis	S. schoter-mulleri	Eb. typhi	S. para-typhi	Es. coli	Shigella dysen-teriae
Control	Thin con-fluent	P.C.	D.C.	Con-fluent	D.C.	Con-fluent	Con-fluent pitted	Thin confluent
Growth from F_1 filtrate	Normal	Heavy con-fluent	I	Normal	Normal	Normal	Normal	(1)
Growth from F_2 filtrate	One large plaque in normal growth	Thin con-fluentm	Con-fluent	Partly heavy type con-fluent Partly T.I.C.(1)	Thin con-fluent	Normal	Thin con-fluent	I
Groth from F_3 filtrate	0 In normal growth	I, and T.I.C.	Few (1)	I (2)	I Heavy type	Normal	Heavy pitted	T.I.C.
Growth from F_4 filtrate	(3) plaques	S.C. and P.C.	(1), (2) and (3)	D.C.	Thin con-fluent	Few (1)	Normal	(2)
Growth from F_5 filtrate	Heavy type	None	I	(1) and (2)	Thin con-fluent	Normal	Normal	Heavy pitted

*Abbreviations not previously used are explained below. Where I have designated plaques, plaques in confluent growth is understood.

P.C. = Prominent colonies in a confluent growth

⊖ = Colony surrounded by lytic area

(3) = Plaques approximately 5 mm. in diameter

(1) = Plaques, very small 1-2 mm. in diameter

(2) = Plaques between (1) and (3) in size.

A. abortus--From F_1 filtrate, heavy type of growth, from F_2 very light and from F_3 as F_2 light, but with a tendency toward colony formation. From the F_4 filtrate the growth was scattered, and from the F_5 no growth.

A. fecalis--From F_1 filtrate, irregular clearings in growth, from F_2 confluent, from F_3 and F_4 plaques, and from F_5 irregular growth.

S. schotermulleri--From F_1 normal, from F_2 partly heavy opaque type of growth and partly tendency toward individual opaque colonies, with some small plaques in the confluent growth. From the F_3 filtrate growth showed irregular clearings with some definite medium sized plaques. From F_4 dim milky colonies appeared in confluent growth, and from F_5 plaques of small and moderate size appeared in confluent growth.

Eb. typhi--From the F_1 filtrate normal growth, from the F_2 lighter confluent growth. From the F_3 the growth was of heavy opaque type with irregular clearings. From F_4 the growth was as from the F_2 and from the F_5 the growth was the same showing moderate sized plaques.

S. paratyphi--Normal growth from all filtrates, with a few small plaques from the F_4 filtrate.

Es. coli--Normal from the F_1, F_4, and F_5 filtrates, heavy from the F3 and a much more translusoent frowth from the F_2 filtrate. There were no plaques.

Plate IV



Growth of A. Fecalis and Es. Coli
with and without Bacteriophage

Shigella dysenteriae--With small plaques from the F_1 filtrate, irregular clearings in growth from F_2 filtrate, tendency toward individual colony formation from the F_3 and F_5 filtrates, the latter being heavier growth, and moderately sized plaques from the F_4 filtrate.

In all of the above plates Es. coli was the only organism showing no plaques or irregular clearing in the growth on any plate. S. pullorum showed lytic areas from F_2, F_3, and F_4, A. abortus from F_3, A. fecalis from F_1, F_3, F_4, and F_5, S. schotermulleri from F_2, F_3 and F_5, Eb. typhi from F_3 and F_5, S. paratyphi from F_4, and S. dysenteriae from F_1, F_2, and F_4.

The results of the interaction of a dilute bacteriophage active for Shigella dysenteriae on the eight intestinal organisms are given in Table No. 13. and No. 14. Table No. 13. shows that the Shigella growth was altered by contact with the bacteriophage, so that instead of a confluent growth individual colony formation showed on the plate. Pecularily enough Es. coli showed the same kind of alteration in growth, Plate IV, Figure No. 3. Growth of A. abortus was likewise altered. Growth on all other plates was normal regardless of previous contact with the dilute bacteriophage.

Table No. 14. shows an alteration in the type of growth of A. fecalis, and A. abortus in the presence of one

drop of dilute bacteriophage on an agar plate. Growth of Es. coli also shows a slight alteration being less pitted, whereas the dysenteriae plate shows a good distribution of plaques or clear areas over the whole surface of an otherwise confluent growth.

Plate IV, Petri plate No. 1 is the control plate of Es. coli. The pitted growth mentioned at various times in this thesis may be seen in the upper portion of the plate, to the right of the numeral. No. 2 is the confluent growth of Es. coli in the presence of one drop of dilute bacteriophage active for Shigella dysenteriae. Petri plate No. 3 shows the growth of prominent individual colonies, with no growth underlying these as a confluent substratum, due to six hours contact in broth with one loopful of dilute bacteriophage. Petri plate No. 4 represents the confluent growth on the control plate of A. fecalis. No. 5 shows the prominent colonies on a confluent growth of fecalis in the presence of one drop of dilute bacteriophage. No. 6 shows confluent growth after six hours contact with one loopful of the dilute bacteriophage active for Shigella dysenteriae.

Plate V, Figure No. 1 shows the individual fine colony growth developed by A. abortus in the presence of one drop of dilute bacteriophage. Plate V, Figure No. 2 shows the prominent colonies formed by alkaligenes in the presence of a similar amount of bacteriophage.

TABLE No. 13.

Spread-Cultures 1 cc. from 6-hour Cultures of Organisms and one Loopful of dilute Bacteriophage.

Cultures	Growth of 6-hour culture and phage	Growth on control
S. pullorum	Normal	Confluent
A. abortus	T.I.C.	Confluent, T.I.C.
A. fecalis	T.I.C.	Confluent
S. schotermulleri	T.I.C.	Confluent
Eb. typhi	T.I.C.	Confluent
S. paratyphi	T.I.C.	Confluent
Es. coli	P.C. Individual colonies	Confluent, somewhat pitted
Shigella dysenteriae	P.C. Individual colonies	Confluent

Plate V

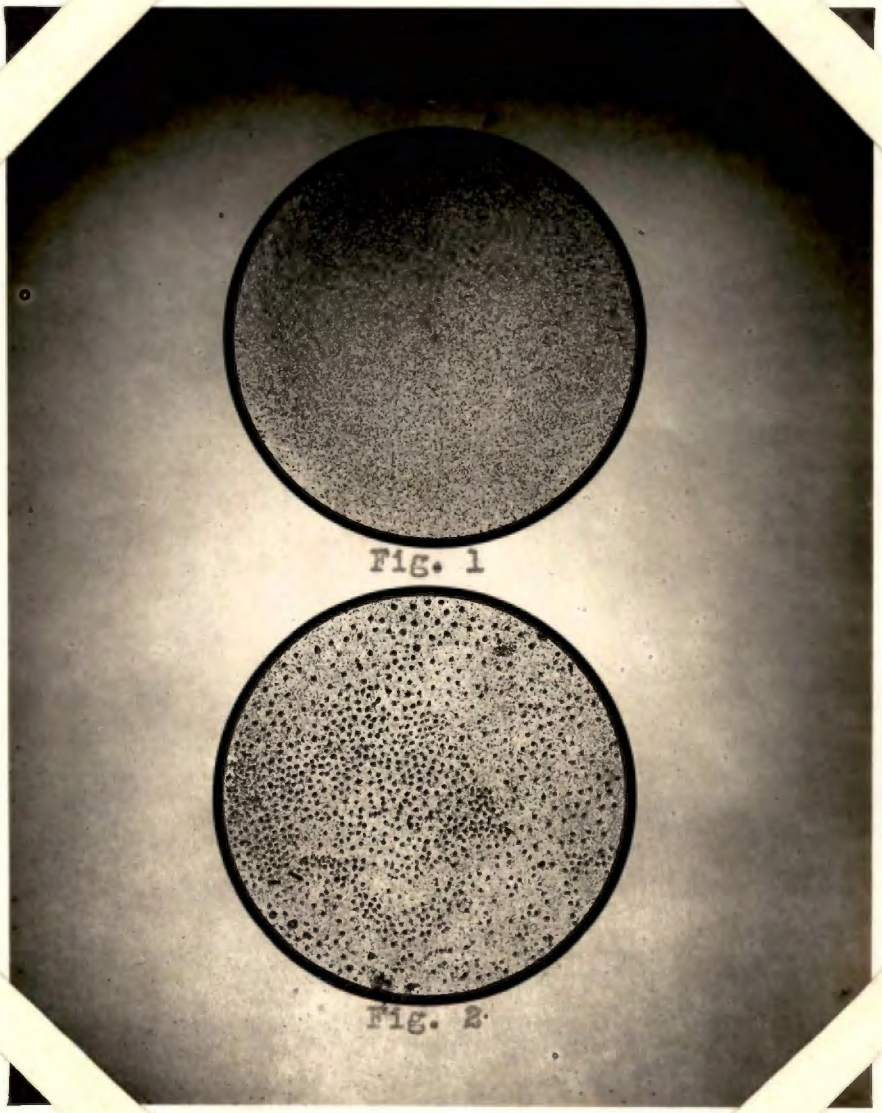


Fig. 1 Growth of A. Abortus in the Presence of Bacteriophage

Fig. 2 Growth of A. Fecalis in the Presence of Bacteriophage

TABLE No. 14.

Growth of Organisms on Agar Plates 1 cc. of Cultures with one drop of dilute Bacteriophage.

Cultures	Culture and one drop dilute phage	Control growth
S. pullorum	Normal	Confluent
A. abortus	T.I.C.	Confluent, T.I.C.
A. fecalis	P.C. in confluent growth	Confluent
S. schotermulleri	Normal	Confluent
Eb. typhi	Normal	Confluent
S. paratyphi	Normal	Confluent
Es. coli	Confluent	Confluent, somewhat pitted
Shigella dysenteriae	Plaques (2) and (3) over whole plate	Confluent, T.I.C.

As recorded in Table No. 14. all plates not already mentioned as showing variation grew normally confluent in the presence of one drop of dilute bacteriophage. This growth is represented in Plate VI, Figure No. 1. Plate VI, Figure No. 2 represents the plaques as they appear in the otherwise confluent growth of Shigella dysenteriae.

Plate VII, Figure No. 1 shows plaques as they first appeared in the confluent growth of dysenteriae, and Plate VII, Figure No. 2 represents the plaques at a later time, demonstrating the zone of diffusion, as well as the growth of secondary colonies.

In no case did the plates prepared for colony formation from the surface and bottom of the broth cultures, which had been centrifuged after six hours contact with dilute bacteriophage show differences in colony formation, for the same organism.

The effect of the age of the culture used in connection with bacteriophage is recorded in Tables Nos. 15, 16, and 17. It has been previously suggested that this also concerns the type of growth of the organism. It is to be noted, moreover, that Table No. 14. represents the effects of using a 9-hour culture under the same conditions as I have used the 12-hour, 12-day and 28-day cultures, although previous to a series of transfers which intervened.

Plate VI

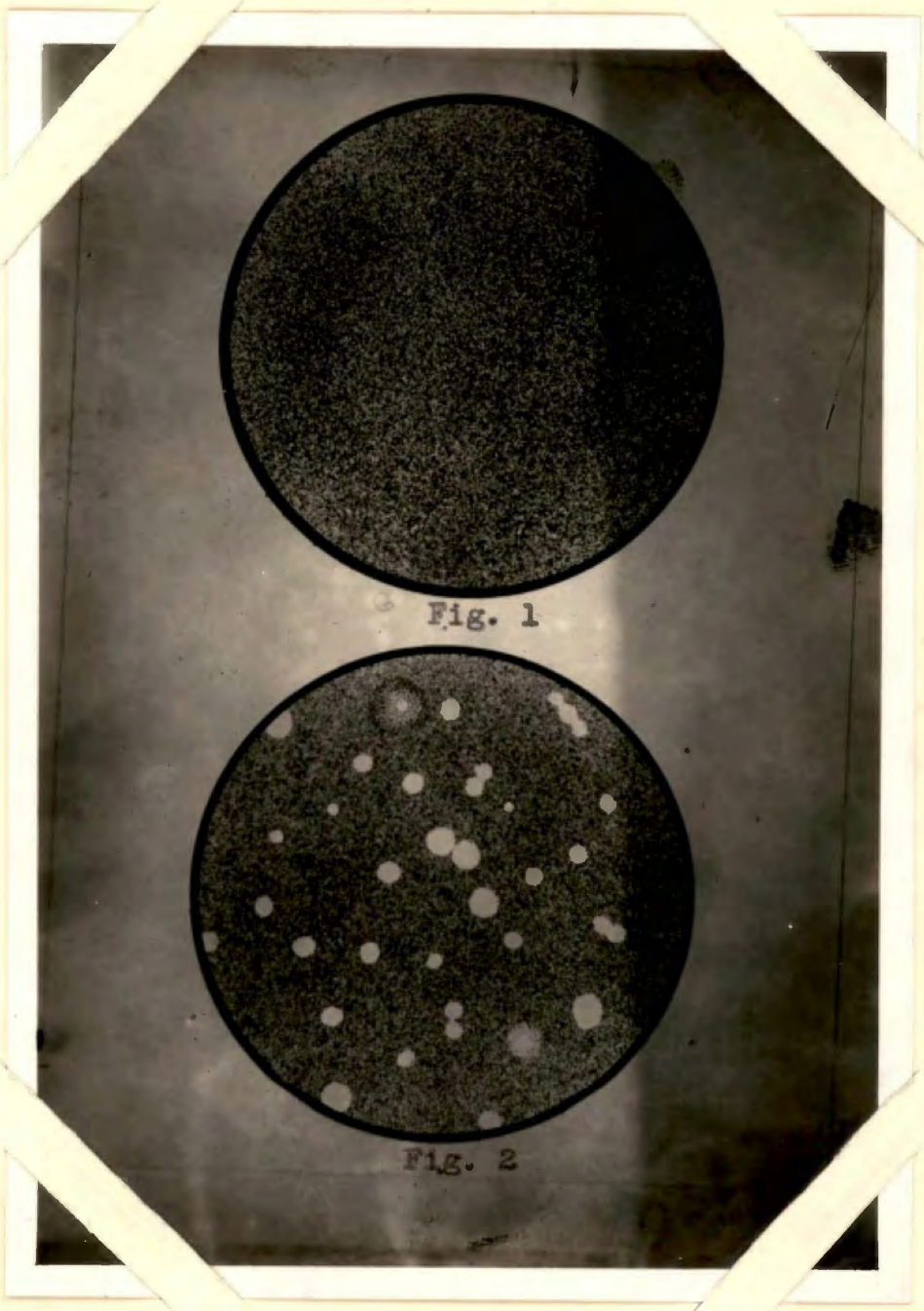


Fig. 1 Confluent Growth of an Organism in the Presence of Bacteriophage

Fig. 2 Plaques in Growth of Shigella Dysenteriae

In the case of the 9-hour culture, Table No. 14., A. abortus showed less confluency of growth, A. fecalis showed the appearance of individual colonies in a confluent growth, Es. coli showed a more decidedly confluent growth, and Shigella dysenteriae alone showed well defined plaques in the presence of dilute bacteriophage.

In using the 12-hour cultures, it is to be noted in Table No. 15. that as in the 9-hour cultures A. abortus showed a marked tendency toward colony formation in the presence of the bacteriophage. A. fecalis, and S. schotermülleri also showed irregular clearings in the growth. It also showed medium sized plaques and lytic areas surrounding minute colonies. Later this plate showed only a few streaks of minute colony growth, all other growth having disappeared. Paratyphi showed the presence of dim colonies in a confluent growth. Only S. pullorum and Es. coli grew normally. Shigella dysenteriae showed plaques of moderate size.

Action of the bacteriophage on 12-day cultures was less variable. In the case of S. schotermülleri there were some opaque colonies surrounded by less opaque areas, and other dim colonies in an irregular growth, though not more irregular than the control plate. Other organisms showed normal growth, which is to say no different in the presence of the bacteriophage than on the control plate, except

TABLE No. 15

Twelve-hour Spread-Culture with one Drop of dilute Bacteriophage

Cultures	Growth of culture with phage	Growth of control
S. pullorum	Normal	Confluent
A. abortus	T.I.C.	D.C.
A. fecalis	I	D.C.
S. schotermulleri 666	I	D.C.
Eb. typhi	(2) and D.C. @. Later only F.I.C.	Confluent
S. paratyphi	D.C.	Confluent
Es. coli	Normal	Pitted, confluent
Shigella dysenteriae	(1) and (2)	Confluent, G.V.S.

Plate VII

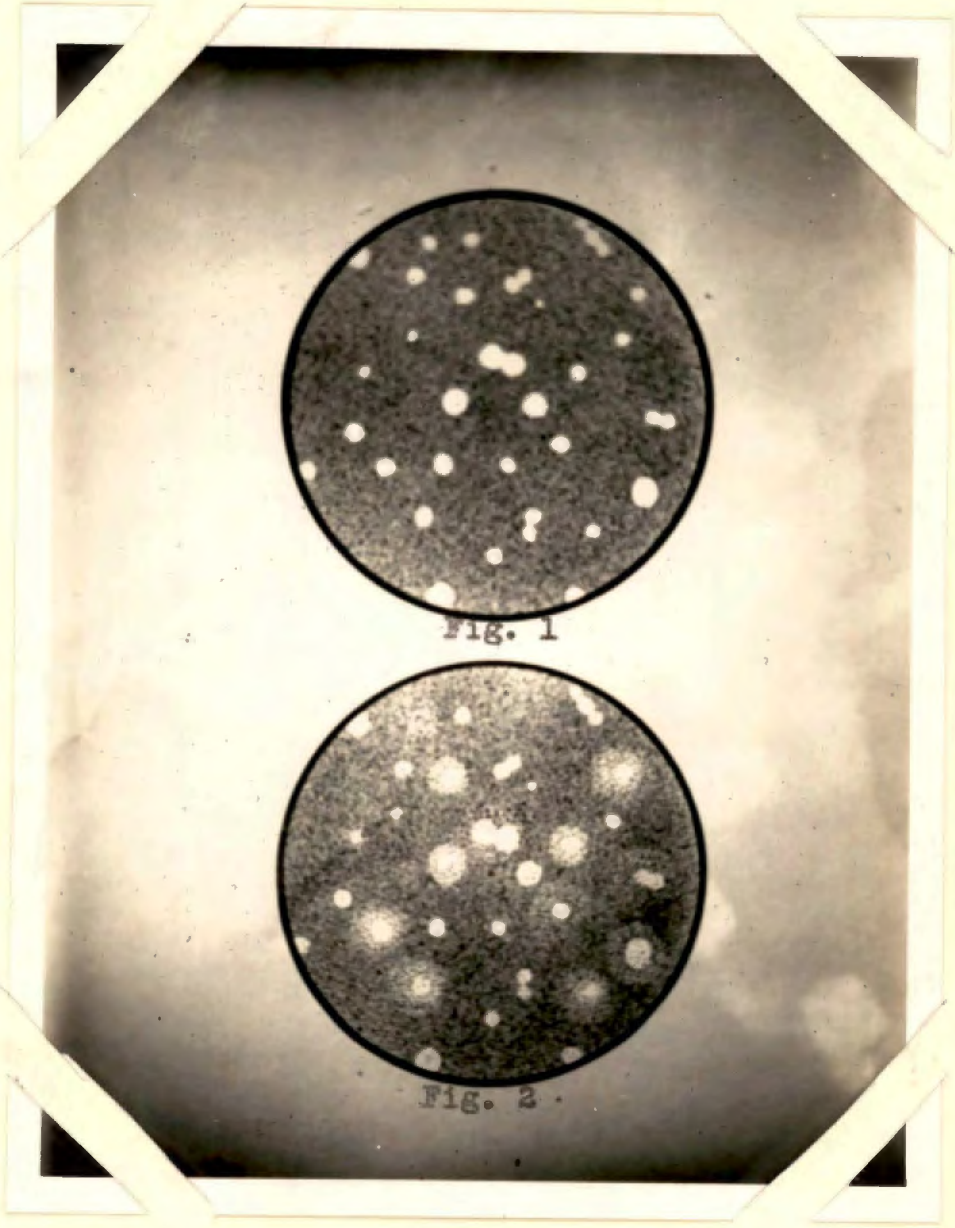


Fig. 1 Early Plaques in Growth of Shigella Dysenteriae

Fig. 2 Latent Zone of Diffusion of Plaques, and Secondary Colonies in Growth of Shigella Dysenteriae

TABLE No. 16

Twelve-day Spread-Culture with one Drop of dilute Bacteriophage.

Cultures	Growth of culture with phage	Growth of control
S. pullorum	Normal	Confluent
A. abortus	Normal	Confluent
A. fecalis	Normal	Confluent
S. schotermulleri	D.C. I	I
Eb. typhi	Normal	I
S. paratyphi	Normal	Confluent
Es. coli	Normal	Transluscent, finely pitted, confluent
Shigella dysenteriae	(1) and (2)	Confluent

dysenteriae, which showed small and medium sized plaques. However, the irregularity of growth for S. schotermulleri and Eb. typhi on control plates is to be noted, also the more translucent growth of Es. coli.

In the action of the dilute bacteriophage on 28-day cultures the results as given in Table No. 17. are somewhat unexpected in regard to pullorum and typhi. The growth of S. pullorum in this case was opaque in the presence of the bacteriophage, and showed innumerable small plaques, and colonies surrounded by lytic areas. These are to be seen reporduced in Plate VIII. In the case of Eb. typhi there were moderately sized plaques, as well as colonies surrounded by lytic areas. On Shigella dysenteriae as might be expected with an old culture only one plaque was visible, a fairly large plaque filled with secondary colonies. No other plates appeared to have growth altered due to the presence of bacteriophage, although several showed growth different than usual on control plates. Thus A. fecalis showed some irregular clearing, as did Eb. typhi, S. schotermülleri, Eb. typhi, and Es. coli showed dim cloudy colonies on a confluent growth.

The action of the bacteriophage applied to the organisms on a medium containing 0.5% sodium chloride is given in Table No. 18. A check of three plates on the action of the bacteriophage on the double peptone media showed a

TABLE No. 18.

Twelve-Hour Spread-Cultures with one drop of dilute Bacteriophage on a Medium Containing 0.5% NaCl

Cultures	Growth of culture with phage	Growth of control
S. pullorum	Normal and P.C.	Confluent
A. abortus	Normal	Confluent
A. fecalis	(1) and I	P.C. in confluent growth
S. schotemulleri	I	Confluent
Eb. typhi	Normal	Confluent
S. paratyphi	Normal	Confluent
Es. coli	Normal	Pitted, confluent
Shigella dysenteriae	G.V.S.	G.V.S. except one colony, large type
Shigella dysenteriae	Normal	Confluent
Shigella dysentariae No salt-- 3 plates	(2) and (3)	Confluent

TABLE No. 19.

Twelve-Hour Spread-Cultures with one drop of dilute Bacteriophage on a Medium Containing 1% NaCl

Cultures	Growth of culture with phage	Growth of culture
S. pullorum	T.I.C.	Confluent
A. abortus	Normal	Confluent
A. fecalis	Normal	Confluent
S. schotermulleri	Normal	I.
Eb. typhi	Normal	Confluent
S. paratyphi	Normal	Confluent
Es. coli	Normal	Pitted, confluent
Shigella dysenteriae	Dew-like colonies	P.C.--later yellow pigment
Shigella dysenteriae repeated	Normal	Confluent

reduction in the plaques produced in growth of Shigella dysenteriae. However, plaques of moderate and large size appeared. On double peptone medium containing 0.5% of salt the bacteriophage produced no plaques in growth of Shigella dysenteriae. In the growth of pullorum prominent colonies were formed on a confluent growth in the presence of the dilute bacteriophage. There were small plaques in an irregular growth of A. fecalis, and S. schotermülleri showed irregular clearing in the growth. Aside from the plates mentioned there was no difference in growth in the presence or absence of the bacteriophage.

As shown in Table No. 19. on a medium containing 1% sodium chloride, pullorum showed a tendency toward individual colony formation, in the presence of the bacteriophage. In the case of Shigella dysenteriae less marked colony formation in a confluent growth appeared in the presence of the bacteriophage. The control plate showed a more proliferous growth, and later a yellow pigmentation, which became deep yellow upon aging. This characteristic of pigmentation was easily lost in three transfers on nutrient agar. A. fecalis showed irregular clearings in the growth in the control plate, as well as in the presence of bacteriophage. Other plates showed no marked deviations from the usual growth on laboratory media.

On a medium containing 2% salt the growth of S. pullorum was inhibited somewhat showing irregularity. Shiga

Plate VIII

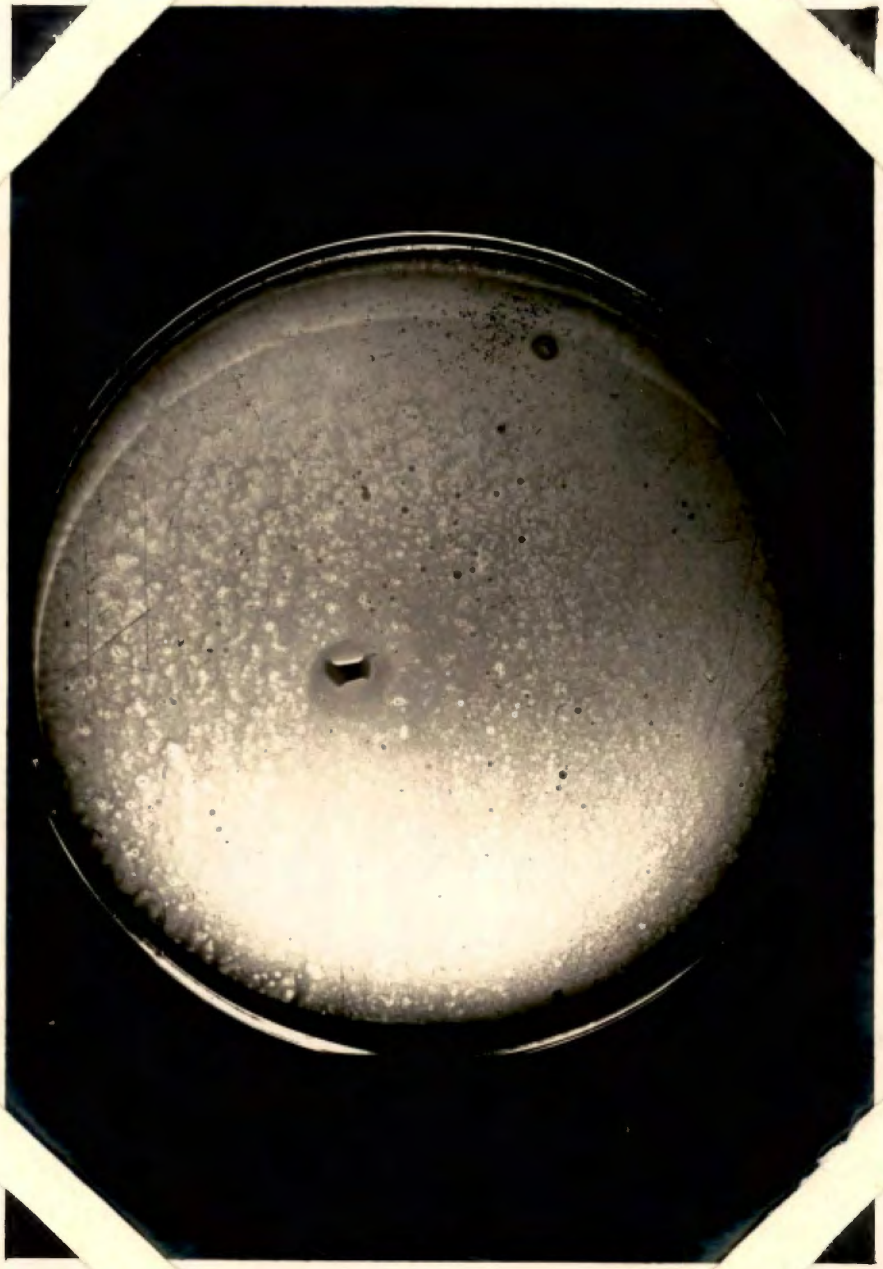


Plate VIII Small Plaques in Heavy Type of Growth from a 28-Day Culture of S. Pullorum in the Presence of Bacteriophage

TABLE No. 20.

Twelve-Hour Spread-Cultures with one Drop of dilute Bacteriophage on a Medium Containing 2% NaCl

Cultures	Growth of cultures with phage	Growth of control
S. pullorum	Normal	G.V.S. I
A. abortus	Normal	Confluent
A. fecalis	Normal	I
S. schotermulleri	Normal	D.C.
Eb. typhi	Normal	Confluent
S. paratyphi	Normal	Confluent
Es. coli	Normal	Confluent
Shigella dysenteriae	Dew-like colonies	Confluent
Shigella dysenteriae repeated	Normal	P.C.

TABLE No. 22

Twelve-Hour Spread-Culture with one Drop of dilute Bacteriophage on a Medium containing 4% NaCl

Culture	Growth of culture with phage	Growth of control
S. pullorum	Normal	Confluent cloudy
A. abortus	Normal	Few colonies
A. fecalis	I θ	Confluent, P.C.
S. schotermülleri	Normal	Pitted, confluent
Eb. typhi	Normal	Confluent
S. paratyphi	Normal	Confluent
Es. coli	Normal	Pitted, confluent
Shigella dysenteriae	Normal	N.G.*

*N.G. = No growth apparent

TABLE No. 23.

Twelve-Hour Spread-Culture with one Drop of dilute Bacteriophage on a Medium Containing 5% NaCl

Cultures	Growth of cultures with phage	Growth of control
S. pullorum	Normal	G.V.S.
A. abortus	Few scattered colonies	G.V.S.
A. fecalis	Normal	P.C.
S. schotermulleri	Normal	Pitted, confluent
Eb. typhi	Normal	G.V.S.
S. paratyphi	Normal	G.V.S., confluent
Es. coli	Normal	D.C.
Shigella dysenteriae	Normal	N.G.

dysenteriae showed no pigmentation, and the bacteriophage inhibited its growth somewhat.

The presence of 3% salt in the medium affected the growth of a few organisms as shown in Table No. 21. The growth of A. abortus was inhibited somewhat more than with the 2% salt concentration in the medium. Eb. typhi also showed a tendency toward colony formation, rather than confluent growth. Growth on the other plates was very similar to that on corresponding plates on media containing 2% salt. Only one plate of Shigella dysenteriae showed colony formation in the presence of bacteriophage as compared to a more confluent control plate.

On a medium containing 4% salt only A. fecalis showed variation in growth between the control plate and the plate to which bacteriophage was added as shown in Table No. 22. The growth in the presence of bacteriophage showed irregular clearings, and some colonies with lytic areas about them, as compared to confluent growth with definite colonies in it on the control plate. The control pullorum grew with more clouding than usual. The salt concentration seemed to limit the growth of A. abortus, schotermulleri, and dysenteriae.

The growth on control plates using medium containing 5% salt was generally decreased. There was no indication

that the bacteriophage produced any effect, as may be seen in Table No. 23.

In considering temperature as a factor in bacteriophagy some interesting results were secured, as evidenced in Tables Nos. 24, 25, and 26. At 26°C. Table No. 26. showed that Shigella dysenteriae was affected as might be expected, and plaques appeared. A. fecalis showed irregular clearings in the growth where bacteriophage was applied. The growth of S. pullorum in the presence of bacteriophage proved to be heavy, and opaque, as shown in Plate IX, a decidedly different growth than the usual type shown by S. pullorum, as seen in Plate X.

At 37.5°C. all of the organisms except Es.coli showed some difference in growth with and without bacteriophage as recorded in Table No. 25. S. pullorum showed a few moderately sized plaques, for the first time under these conditions. A. fecalis showed plaques and irregular clearings in the growth. A. abortus showed an inclination toward individual colony formation. S. schotermulleri showed irregular clearing in the growth. Eb. typhi showed medium sized plaques, and some slightly opaque colony formations. S. paratyphi showed similar colonies, and Shigella dysenteriae showed plaques. Thus, in this case four organisms showed clean-cut plaques, and only one no change in type of growth in the presence of bateriophage.

TABLE No. 24.

Twelve-Hour Spread-Culture of Organisms with one Drop of dilute Bacteriophage Incubated at 26°C.

Cultures	Growth of cultures with phage	Growth of control
S. pullorum	Opaque type	Confluent
A. abortus	Normal	Confluent, pitted
A. fecalis	I	Confluent
S. schotermulleri	Normal	Confluent
Eb. typhi	Normal	D.C.
S. paratyphi	Normal	Confluent
Es. coli	Normal	Pitted, confluent
Shigella dysenteriae	(2) and (3)	Confluent

TABLE No. 25.

Twelve-Hour Spread-Culture of Organisms with one Drop of dilute Bacteriophage Incubated at 37.5°C.

Cultures	Growth of culture with phage	Growth of control
S. pullorum	Growth light, few (2)	Confluent
A. abortus	Pitted	D.C.
A. fecalis	I Few (2)	D.C.
S. schotermulleri	I	Pitted, confluent
Eb. typhi	(2) D.C.	Pitted, confluent
S. paratyphi	D.C.	Finely pitted confluent
Es. coli	Normal	Pitted, confluent
Shigella dysenteriae	(1) and (2)	Confluent. Part of plate N.G.

TABLE No. 26.

Twelve-Hour Spread-Cultures of Organisms with one Drop dilute Bacteriophage Incubated 7 Days at 12°C.

Cultures	Growth of culture with phage	Growth of control
S. pullorum	(1) thin growth Heavy after @ 24 hours at 26°C.	G.V.S.
A. abortus	Normal	G.V.S.
A. fecalis	Normal	P.C.
S. schotermulleri	Normal	G. V.S.
Eb. typhi	(3) secondaries after 24 hours at 26°C.	T.I.C.
S. paratyphi	Normal	G.V.S., confluent
Es. coli	Normal	Pitted, confluent
Shigella dysenteriae	G.V.C. if any	(3), confluent
Shigella dysenteriae	24 hours at 26°C. Large plaques	(3), confluent

Plate IX



Heavy Type of Growth of S. Pullorum
at 26°C. in the Presence of
Bacteriophage

After seven days incubation at 12°C. S. pullorum showed small plaques and lytic areas about small colonies and Eb. typhi showed fairly large plaques in the presence of bacteriophage, as seen in Table No. 26. The growth on the dysenteriae plate in the presence of the bacteriophage was not visible when taken from the refrigerator. The control plate showed very definite large plaques, which later contained secondary colonies. The other plate of dysenteriae showed growth with large plaques after being at 26°C. for 24 hours.

The growth on most plates was slight but visible. After 24 hours at 26°C. S. pullorum in the presence of the bacteriophage had developed its characteristic opaque growth; but the plaques were unchanged. At this temperature A. fecalis showed no alterations in growth due to the presence of bacteriophage.

On a medium containing 0.05% phenol, S. pullorum showed a few small plaques on the control plate; but none in the presence of the bacteriophage. As recorded in Table No. 27. A. abortus, A. fecalis, and Eb. typhi showed irregular clearings in the growth on the plates to which bacteriophage had been added. On the corresponding plate for dysenteriae, plaques of moderate and large size appeared. Any other changes occuring were evident on the control plates. The growth of dysenteriae was noticeably heavy.

TABLE No. 27.

Twelve-Hour Spread-Cultures of Organisms with one Drop of dilute Bacteriophage on a Medium Containing 0.05% Phenol.

Cultures	Growth of organism with phage	Growth of control
S. pullorum	Confluent	Confluent
A. abortus	I	T.I.C.
A. fecalis	I	D.C.
S. schotermulleri	Normal	Confluent
Eb. typhi	I	Confluent
S. paratyphi	Normal	Confluent
Eb. coli	Normal	Pitted, transluscent confluent
Shigella dysenteriae	(2) and (3)	Heavy confluent

TABLE No. 28.

Twelve-Hour Spread-Cultures of Organisms with one Drop diluted Bacteriophage on a Medium 0.10% Phenol

Cultures	Growth of organism with phage	Growth of control
S. pullorum	Normal	Confluent
A. abortus	Normal	T.I.C.
A. fecalis	Normal	D.C.
S. schotermulleri	Normal	Pitted, confluent
Eb. typhi	Normal	Confluent
S. paratyphi	Normal	T.I.C.
Es. coli	Normal	Pitted, transparent
Shigella dysenteriae	(2) and (3)	Heavy, confluent

TABLE No. 29.

Twelve-Hour Spread-Cultures of Organisms with one Drop of dilute Bacteriophage on a Medium Containing 0.2% Phenol.

Cultures	Growth of Organism with phage	Growth of control
S. pullorum	Normal	N.G.
A. abortus	Normal	N.G.
A. fecalis	Normal	Confluent
S. schotermulleri	Normal	Confluent
Eb. typhi	Normal	Confluent
S. paratyphi	Normal	G.V.S.
Es. coli	Normal	S.C.
Shigella dysenteriae	N.G.	N.G.

TABLE No. 30.

Twelve-Hour Spread-Cultures of Organisms with one Drop of dilute Bacteriophage on a Medium Containing 0.006-2/3% Iodine.

Cultures	Growth of organism with phage	Growth of control
S. pullorum	Normal	Confluent
A. abortus	I	T.I.C.
A. fecalis	Normal	D.C.
S. schotermulleri	Normal	Confluent
Eb. typhi	Normal	D.C.
S. paratyphi	Normal	Confluent
Es. coli	Normal	Pitted, confluent
Shigella dysenteriae	(2)	Confluent

On a medium containing 0.1% phenol Shigella dysenteriae showed the same heavy growth, as well as large sized plaques in the presence of bacteriophage. The other cultures showed on the control plates, as given in Table No. 28., similar to the control plates recorded for the medium containing 0.05% phenol. The other plates showed normal growth.

In the presence of 0.2% phenol in the medim growth of all of the organisms, showed no change due to the presence of bacteriophage, as may be seen in Table No. 29.

With 0.006-2/3% iodine in the medim, Table No. 30., these organisms showed growth very similar to that on the medim containing 0.5% phenol. In the presence of bacteriophage, however, only A. abortus showed irregular clearing in the growth, and dysenteriae showed plaques, only moderately sized.

In the presence of 0.01% iodine, only Eb. typhi varied in the control growth, as compared to that on media with only 0.006-2/3% iodine present. As will be seen in Table No. 31., Eb. typhi on the control as well as in the presence of bacteriophage showed irregular clearing in the growth. Except for this difference the results were practically the same in the presence of the bacteriophage as they were with the smaller amount of iodine.

TABLE No. 31.

Twelve-Hour Spread-Cultures of Organisms with one Drop of dilute Bacteriophage on a Medium Containing 0.01% Iodine.

Cultures	Growth of culture with phage	Growth of control
S. pullorum	Normal	Confluent
A. abortus	I	T.I.C.
A. fecalis	Normal	D.C.
S. schotermulleri	Normal	D.C.
Eb. typhi	Normal	I
S. paratyphi	Normal	Confluent
Es. coli	Normal	Pitted, confluent
Shigella dysenteriae	(2)	Confluent

TABLE No. 32.

Twelve-Hour Spread-Cultures of Organisms with one Drop of dilute Bacteriophage on a Medium Containing 0.02% Iodine.

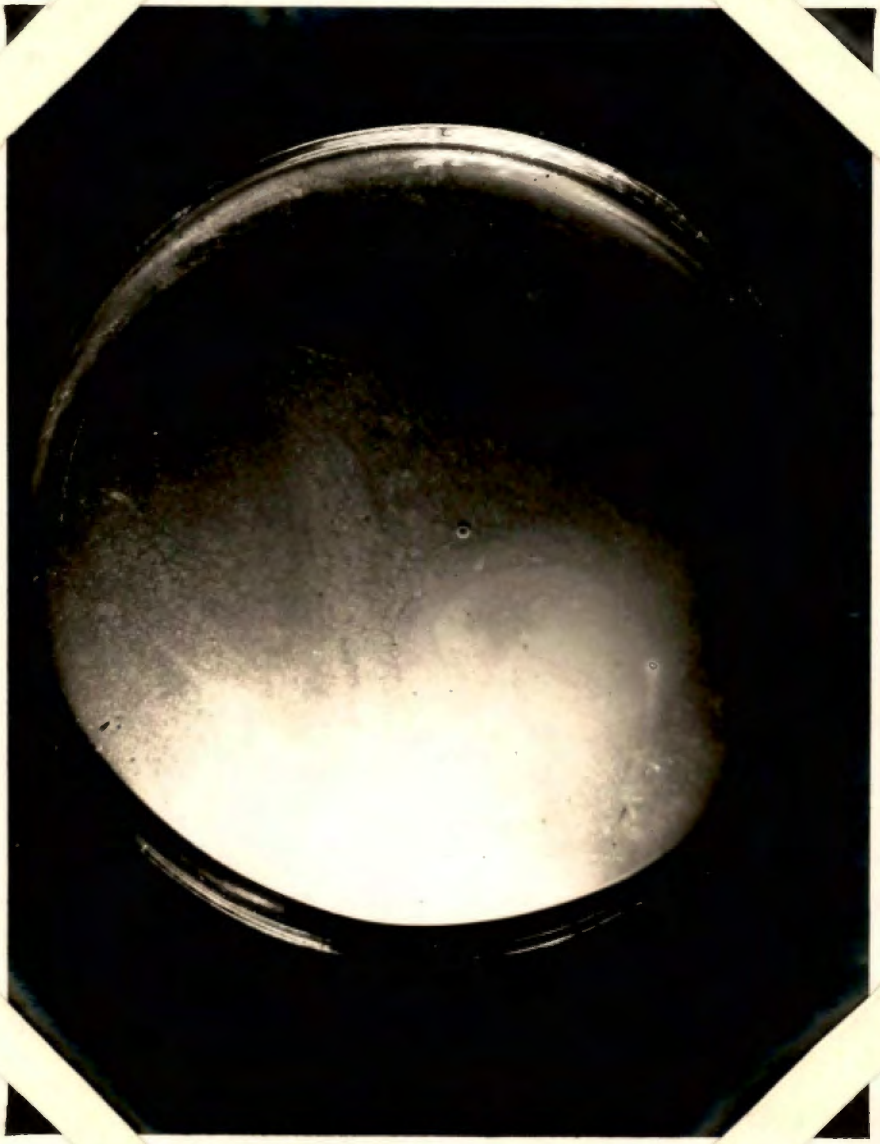
Cultures	Growth of culture with phage	Growth of control
S. pullorum	Normal	Confluent
A. abortus	I and T.I.C.	T.I.C.
A. fecalis	Normal	Confluent
S. schotermulleri	Normal	Confluent
Eb. typhi	Normal	D.C.
S. paratyphi	(2) many	T.I.C.
Es. coli	Normal	Pitted, confluent
Shigella dysenteriae	(2),(3) and two dozen large colonies	Confluent

TABLE No. 33.

Twelve-Hour Spread-Cultures of Organisms with one Drop of dilute Bacteriophage on a Medium Containing 0.1% Iodine.

Cultures	Growth of culture with phage	Growth of control
S. pullorum	Normal	Confluent
A. abortus	Large plaques 12 mm.	T.I.C.
A. fecalis	D.C.	D.C., I., (2) and (3)
S. schotermulleri	Normal	Pitted, confluent
Eb. typhi	Normal	Confluent
S. paratyphi	Normal	S.C.
Es. coli	Normal	Pitted, confluent
Shigella dysenteriae	Normal (2)	Confluent

Plate X



Normal Growth of S. Pullorum at 26°C.

Table No. 32. shows the growth on media containing 0.02% iodine to be the same as on media containing 0.006-2/3%, except in the case of paratyphi which showed a tendency toward individual colony formation. With this organism we find that medium sized plaques appeared in the presence of bacteriophage. A. abortus showed the same irregular clearing as in the previous two sets, and dysenteriae showed plaques of moderate and large size, and also some large colonies.

On a medium containing 0.1% iodine, it will be seen in Table No. 33. that the growth on the control plates of paratyphi, schotermulleri, and fecalis was altered by the greater iodine concentration. A. fecalis showed irregular clearing and plaques in the growth on the control plate. In the presence of bacteriophage these appearances did not occur. On the other hand, A. abortus showed very large plaques in the presence of bacteriophage, and not on the control plate. Shigella dysenteriae showed medium sized plaques in the presence of the bacteriophage.

As to the effect of a media with pH 6.8 on the action of the bacteriophage, no definite plaques were formed on Shigella dysenteriae, as may be seen in Table No. 34.

However, the growth was inhibited somewhat as compared to the control plate. Other organisms showed

TABLE No. 34.

Twelve-Hour Spread-Cultures of Organisms with one Drop of dilute Bacteriophage on a Medium of pH value 6.8

Cultures	Growth of culture with phage	Growth of control
S. pullorum	Normal	Confluent, heavy
A. abortus	Normal	D.C.
A. fecalis	Normal	D.C.
S. schotermulleri	Normal	D.C.
Eb. typhi	Normal	Confluent
S. paratyphi	Normal	Confluent
Es. coli	Normal	Pitted, confluent
Shigella dysenteria	No plaques, T.I.C. less in number	T.I.C.

TABLE No. 35.

Twelve-Hour Spread-Culture of Organisms with one Drop of dilute Bacteriophage on a Medium of pH value 7.4.

Cultures	Growth of cultures with phage	Growth of control
S. pullorum	Normal	Confluent
A. abortus	Normal	D.C.
A. fecalis	Normal	I
S. schotermulleri	P.C. opaque type of growth	I
Eb. typhi	Normal	Confluent
S. paratyphi	Normal	Confluent
Es. coli	Normal	Pitted, confluent
Shigella dysenteriae	T.I.C.	T.I.C.

TABLE No. 36.

Twelve-Hour Spread-Cultures of Organisms with one Drop of dilute Bacteriophage on a Medium with pH value of 8.

Cultures	Growth of culture with phage	Growth of control
S. pullorum	Normal	Confluent
A. abortus	Normal	P.C. confluent
A. fecalis	D.C.	(2) I
S. schotermulleri	Normal	D.C.
Eb. typhi	Normal	Confluent
S. paratyphi	Normal	Confluent
Es. coli	Normal	Pitted, confluent
Shigella dysenteriae	T.I.C.	Confluent

little effect due to the reaction of the medium, except that in no other case did the bacteriophage exhibit any action. S. pullorum showed a somewhat better growth than had been exhibited on more alkaline media.

Table No. 35. shows that in the case of A. fecalis and S. schotermulleri the growth showed irregular clear areas on media with a pH 7.4. The latter organism showed an opaque type of growth in the presence of the bacteriophage. All other plates showed no effects due to the presence of bacteriophage, except for a slight decrease in the growth for dysenteriae.

On media with a pH 8 A. fecalis showed irregular clearing and medium sized plaques, and as shown in Table No. 36. these were not present in growth with the bacteriophage, somewhat opaque colonies appearing in a confluent growth of the organism. The only other culture affected by the presence of the bacteriophage was dysenteriae, which showed a tendency toward individual colony formation rather than the confluent growth on the control plate.

In the presence of chicken blood serum pullorum appeared in a heavy opaque confluent growth. In the presence of the bacteriophage this growth was considerably less proliferous, and more cloudy than opaque. Table No. 37.

records a similar tendency toward less proliferous growth of A. abortus in the presence of bacteriophage than in its absence, as is also true in the case of schotermulleri. Es. coli shows a reverse of these conditions. A. fecalis shows more decided irregular clearing in the presence of the bacteriophage than in its absence. In the growth of Shigella dysenteriae only one medium sized plaque appeared in the presence of bacteriophage.

TABLE No. 37.

Twelve-Hour Spread-Cultures of Organisms on Agar with one Drop of dilute Bacteriophage and one Drop of Chicken Blood Serum

Cultures	Growth of culture with phage and serum	Growth of culture and serum
S. pullorum	Slightly cloudy, confluent	Opaque, confluent
A. abortus	Lighter, confluent	Confluent
A. fecalis	Decidedly I	Slightly I
S. schotermulleri	Less dense, normal	Confluent
Eb. typhi	Normal	D.C.
S. paratyphi	Normal	Confluent
Es. coli	Heavier, confluent	P.C., confluent
Shigella dysenteriae	One (2)	Confluent
S. pullorum repeated	P.C. cloudy confluent	Opaque, confluent

Plate XI



Growth of a pure Culture of S. Pullorum after Contact with a dilute Solution of $NaHSO_4$

DISCUSSION OF RESULTS

It is rapidly becoming a recognized fact that there are filtrable forms of a large number of known bacteria. D'Herelle describes such forms as occuring when in Shigella dysenteriae, also (Flexner) and (Hiss). He also mentions such forms of B. typhosus (Eb. typhi), B. pestis, B. gallinarum, and Es. coli. He attributes to the presence of the bacteriophage the occurrence of filtrable forms among non-spore forming bacteria, though he does not thereby preclude other causes. Laboratory stock cultures have passed through "W" Berkefeld filters in the University of Tennessee bacteriological laboratory. Consequently, I was not surprised to find growth in a number of filtrates. Though the eight intestinal organisms under condiseration were subjected to exactly the same treatment they did not all pass through the filters into any one set filtrate, and Es. coli did not pass through in any of my work. S. dysenteriae, and A. abortus/each passed through only into one filtrate, the fourth and second, respectively. On the other hand, S. paratyphi and S. schotermulleri passed through into all filtrates. Eb. typhi and S. pullorum passed through in two filtrates. The results showed that although at times organisms may have filtrable forms yet they do not always

give evidence of such forms, and such forms are not demonstrable in all organisms under identical conditions. The fact that with many organisms such forms may be demonstrated in normal 12-hour broth cultures, precludes the idea that adverse influence is absolutely essential to their development. It may be that growth in a sewage broth mixture has enhanced this quality in certain ones of the organisms under consideration, and this in turn may be due to the presence of a lytic principle, not strong enough to bring about complete lysis, but the creation of sufficiently advserse conditions to hasten bacterial alterations, in the struggle for existence.

The physiological characteristics of the organisms found in the filtrates showed some digression from the usual expectancy. In regard to such forms of Shigella dysenteriae d'Herelle quotes Hauduroy as saying, "the cultural characters and biochemical reactions of these elements are ordinarily different from those of the Shiga bacilli from which they are derived." He also describes masses without definite structure, and of variable size, having a mucus character, and in which granules can be detected. Minute granules are also described. Lohnis[32] speaks of Dunn's (1904) reference to the disappearance, and reappearance of motility in dysentery strains. Stamn (1914) is reported to have

demonstrated new forms secured from typical vibrio cholerae cultures which remained constant through 540 transfers. Bernhardt (1915) has reported similar results with meningococci, Eb. typhi, septicaemia, and diphtheria bacilli. Likewise Perisz (1904) is reported to have demonstrated so-called secondary cultures, in old cultures, producing rather constant strains, different both morphologically and physiologically.

The reports given in this paper are entirely in accord with these reports for both the morphological and physiological variations from the usually recognized characteristics of these organisms. The hyalin, or mucus masses described coincide closely with that stage in bacterial life, called by Löhnis the "symplastic" stage.

In growing the eight intestinal organisms in contact with their various respective filtrates it is a noticeable fact that none of them escaped variation in type of growth in the presence of one or another of the filtrates. In the case of the fourth or fifth filtrates plaques were demonstrated on growth of all the organisms except A. abortus, and for this organism there was decided inhibition of growth in the presence of the third, fourth, and fifth filtrates. Some inhibition was apparent for the normal growth of A. abortus in the first filtrate. This was also true in regard to Es. coli, and on one plate for A. fecalis, in

which case there had been a 12-hour contact previous to plating. F_3 filtrates for five of the organisms inhibited growth, and six F_4 filtrates.

The changes in type of growth brought about in all of the organisms in the presence of one or another of its filtrates interested me in that Hadley and others have mentioned the fact that bacteriophage is decidedly active in bringing about type variations in organisms. Had I been guided by the reactions of each filtrate in connection with the particular organism concerned, possibly an active transmissible lytic principle might readily have been secured for all organisms under consideration. In fact, with the same sewage I succeeded in isolating such a principle for these strains of S. pullorum, and Es. coli.

It is also of interest to note here that using the same sewage in connection with which the bacteriophage active for Shiga was isolated by me, bacteriophage was also isolated active for the following organisms: Eb. typhi, S. schotermulleri, S. paratyphi, Es. coli, S. suipestifer, and Proteus X 19.

D'Herelle says that he has never isolated two races of bacteriophage absolutely identical in their action. Thus considering the variation in characteristics between different species of organisms to say nothing of the well recognized variations of individuals within a pure culture

of a given species, it is not surprising that all species of a given group do not react exactly the same under identical treatment. To try to study the characteristic differences that occur in isolating the bacteriophage for a variety of organisms would be to attempt to lay down those absolute laws for bacteriophagy which d'Herelle says do not exist.

It is interesting to note again, in this connection the work of Kuttner(30) in which she concludes that the bacteriophage is a secretion of bacteria produced under certain circumstances. In this work she demonstrates a lytic principle in old resistant cultures of Eb. typhi and Shigella dysenteriae, and in alkaline washings of Staphylococcus cultures.

I have included Plates XII and XIII as demonstrations of lytic principles evident in some cultures. Plate XII shows lytic areas about certain colonies in a mixed culture. I have observed similar lytic areas about colonies in pure cultures. These lytic areas are round, and show marked similarity to those caused by bacteriophage, although they may be due merely to lytic action of the metabolic products of the organisms in the central colony.

Plate XIII shows lytic areas which appeared on a plate culture made from organisms previously isolated from teeth. The areas appeared only after one or two weeks

standing at 26°C. Here again we have a striking similarity to the lytic areas caused by bacteriophage. It may be that the organism carried the bacteriophage, and that it was inactive in vivo, allowing the teeth to decay in spite of its presence. These plates are not included as evidence of the source of bacteriophage; but merely as demonstrations of the frequency with which such lytic areas are to be found on ordinary plate cultures, and to attract attention to the clean-cut outline of such areas.

Results were quite different on those spread-cultures which were plated in the presence of a drop of concentrated bacteriophage. In that case Shigella dysenteriae showed no plaques; but a more decided growth with a tendency toward individual colonies. The growth of Es. coli was quite similar, being more transluscent than normally. Eb. typhi, S. pullorum, and S. paratyphi showed no change in growth due to the presence of the concentrated bacteriophage. However, A. abortus, A. fecalis and S. schotermülleri all showed plaques, of 5 mm. in diameter, with some on abortus and fecalis as large as 12 mm. in diameter.

Concerning results secured on interaction of bacteriophage on the eight intestinal organisms plated on nutrient agar of pH 7.6 incubated at 37.5°C. we have varying results. Tables Nos. 14., 15., and 25. represent plates under practically identical conditions. However, the results are not triplicated. In Table No. 25. it may be noted that

Plate XII



Lytic Areas about Colonies
in a mixed Culture

used. This, then appears to be another time when laws devised by our present knowledge can not be applied to govern the expectancy in results due to the action of bacteriophage.

It should be noted here that the bacteriophage in use has shown a reduced ability to lyse dysenteriae, as compared to its ability almost one year previously, when it was sealed up. However, I wish to add that the growth of the dysentery organism is also less active, and more translucent than it was when sealed up almost a year before. Consequently there are two possibilities as concerns the lessened activity of the bacteriophage.

These results considered with other works have a direct bearing upon the type of a culture in its effect upon the action of bacteriophage.

Certain authors have pointed to aging as a factor in causing variations in type of cultures. If the action of bacteriophage is dependent upon the type of the culture, this should be demonstrated in its application to cultures of various ages. The bacteriophage used showed a wider range of activity on 12-hour cultures than on 9-hour, though factors, other than age may have entered, as the result of frequent transfers. In the nine-hour cultures no action was evident for S. schotermülleri, nor for Eb. typhi. Both of these cultures showed inhibition, and Eb. typhi the results

of decided lysis when the 12-hour cultures were used. Here again we find decided action of dilute bacteriophage on an organism, Eb. typhi formerly not effected by the presence of a drop of concentrated bacteriophage.

In the case of 12-day cultures, any ability of the bacteriophage to inhibit the growth of abortus and fecalis had entirely disappeared. The growth of both schotermülleri and typhi showed irregular clear areas on the control plates as well as in the presence of bacteriophage. The action on the dysentery culture remained normal.

On the 28-day culture plaques appeared on S. pullorum, Plate VIII, and the growth was entirely different than that on the control plate, a demonstration not only of the action of bacteriophage in relation to aging cultures; but also of its ability to cause organisms to depart still further from their former type. S. schotermülleri showed more variation in growth due to aging, as did typhi; but only the latter showed plaques. However, S. schotermülleri showed colonies surrounded by lytic areas on both the control plate, and that to which bacteriophage was added. As to the action of the bacteriophage on a 28-day culture of dysenteriae, only one plaque appeared on the plate, and that was filled with secondary colonies. Thus whereas, what d'Herelle terms virulence of the bacteriophage was not as great for organisms from a 28-day culture of dysenteriae as for those

from a 12-hour or 12-day culture, the reverse was true for S. pullorum cultures.

The results are interesting as concerns growth on media containing varying amounts of sodium chloride. In the presence of bacteriophage A. fecalis showed plaques and irregular clear areas on that containing 0.5% salt and colonies surrounded by lytic areas on that containing 5% salt. Schotermülleri showed irregular clearing in the presence of bacteriophage on the medium containing 0.5% salt. In all other cases the bacteriophage showed no marked activity in connection with any of the organisms. On control plates of double peptone media, which was used to permit definite regulation of the salt concentration, Shigella dysenteriae showed plaques, though less numerous than on nutrient agar.

It is to be noted that on all media containing salt except on the control plates containing 0.5% and 4% and 5% A. fecalis showed irregular clear areas in the growth. S. pullorum showed such clear areas on the media containing 2% salt.

The salt per se can not account for the lack of activity of the bacteriophage when applied to Shigella dysenteriae, for Maitland has carried out experiments, confirmed by d'Herelle in which they claim that lysis was brought about by the bacteriophage in physiological salt

solution containing 1% bouillon. Brutsaert reports lysis of Staphylococci in broth containing 14% salt. It can be attributed only partly, if at all, to the double peptone media used; for in the absence of salt this media showed plaques when dysenteriae was grown in the presence of the same amount of bacteriophage used on the other plates. The tendency toward variations in the type of growth was noticeable with dysenteriae and other organisms, and may afford some explanation.

The action of the bacteriophage at varied temperatures is very interesting. In the first place, at 37.5°C. the activity was wide, plaques showing in the growth of four organisms, S. pullorum, A. fecalis, Eb. typhi, and Shigella dysenteriae, as well as irregular clear areas in the growth of S. schotermülleri. This is, as has been mentioned before, at variance with other results including the same kind of media, temperature, and time of incubation. At 26°C. the activity was less pronounced in plaque production, with organisms other than dysenteriae. In the growth of dysenteriae the plaques were generally larger than at 37.5°C. A. fecalis showed irregularly cleared areas; but no true plaques. The growth of S. pullorum was decidedly changed in type as shown on Plate IX. It has been known for some time that under stimulus of heat[1] organisms change certain characteristics, as their regeneration period. Such

Plate XIII



Lytic Areas on Old Plate Cultures
of dental Infection

characteristics as alcohol production of yeast have been changed by ultraviolet rays. Plate IX as compared to Plate X, shows what decided changes in morphological cultural development can take place in the presence of bacteriophage. For comparison with this note Plate XIX, which represents the growth of a pure culture of pullorum plated out by Allen, after the organism had been subjected to antiseptic action of $NaHSO_4$.

Returning to temperature variation, we find that after seven days at 12°C. S. pullorum growth showed innumerable small plaques in the presence of the bacteriophage. Eb. typhi also showed plaques, much larger in size. Shigella dysenteriae showed practically no growth in the presence of the bacteriophage, until after 24 hours at 26°C., when large plaques appeared. However, the control plate of S. dysenteriae to which no bacteriophage had been added showed large plaques which developed at 12°C. In the case of both Eb. typhi and the control plate of Shigella dysenteriae, typical secondary colonies developed within the plaques in 24 hours at 26°C. The pullorum culture showing plaques developed a growth similar to that shown in Plate IX; but within this the plaques remained distinctly visible. The plaques on the control plate of dysenteriae might be ascribed to a contaminated culture, or to the production of a lytic principle which produced plaques in which secondary

colonies occurred. These colonies are in all respects like those developed in the presence of bacteriophage. This would fit in well with Miss Kuttner's idea that under certain circumstances organisms produce an autolysin, which she believed to be the bacteriophage. If the culture carried the bacteriophage, I cannot understand why it appeared at no time under any other conditions. On the other hand, I have not proven that the lytic principle which developed plaques on the control plate was a true bacteriophage. Here as in other parts of my work I am lead to note the effect of the type of growth of organisms on the action of the bacteriophage, as well as the effect of the bacteriophage on the type of growth.

In the presence of 0.05% phenol the control plates showed a change in the type of growth of a number of the organisms evidently due to the effect of the phenol. S. pullorum showed some small plaques in the growth on the control plate. In the case of abortus, fecalis, and Eb. typhi irregular clearing occurred in the growth in the presence of bacteriophage. There were definite medium and large sized plaques on Shigella dysenteriae in the presence of bacteriophage. In the presence of 0.1% phenol in the medium plaques were formed only on dysenteriae in the presence of the bacteriophage. In the presence of 0.2% phenol the growth of the organisms was so slight that evidence of bacteriophage

action could not be detected.

Inasmuch as, according to d'Herelle, phenol requires approximately seven days in a 1% solution to destroy the bacteriophage, we would expect little impairment of its action in the presence of 0.05% or 0.1% phenol in the medium.

It has been reported that certain organisms are formed to change their type of growth in the presence of a small amount of phenol in the media, and such a tendency was evident on these plates. This may account for the lack of manifestation of inhibition in the growth of A. abortus, A. fecalis, and Eb. typhi in the presence of 0.1% phenol, in the presence of the bacteriophage.

In the presence of iodine the presence of the bacteriophage was decidedly evident throughout, in the growth of abortus. In the presence of 0.1% iodine in the medium, plaques 12 mm. in diameter were to be seen. Iodine did not prevent the formation of plaques on dysenteriae. On media containing 0.02% iodine many medium sized plaques were formed on S. paratyphi, and the control plate showed a tendency toward individual colony formation, not usually evident with S. paratyphi.

In the case of A. fecalis on the medium containing 0.1% iodine plaques were evident in the growth on the control plate; but in the presence of bacteriophage they were absent, and the growth merely showed the presence of cloudy colonies

in a confluent growth. All other cultures grown in the presence of iodine showed no change due to the influence of the bacteriophage, we find the type of growth of the organusms, S. paratyphi and A. fecalis, varied from the usual on the control plate in the presence of the amount of iodine present in each instance. In the preparation of the media used with iodine content, I wish to have it remembered that the iodine was added before sterilization.

On media of variable pH value we find no results conflicting with former observations. Definite plaques were not produced on Shigella dysenteria on media having a pH value of 6.8, 7.4 or 8. However, there was a marked inhibition of growth. This is in accord with considerable work done by various others on the effect of the pH value of media on the action of the bacteriophage. The bacteriophage in use in this work was isolated on a medium of pH 7.6 to 7.8, and it evidently gives the best results on such a medium.

In regard to the change in type of growth of S. schotermülleri on a medium of pH 7.4, in the presence of bacteriophage, it will be noted that the growth on the control plate was not duplicated in either of the other two media, and in each of these cases the bacteriophage caused no changes.

In the case of fecalis we have the occurrence of plaques on a medium of pH 8.0. The growth also showed

irregular clearing. Here as in an identical case on a medium containing iodine the growth reverted to a confluent growth containing cloudy colonies, in the presence of the bacteriophage. Thus it becomes evident that the pH concentrations used in this work did not destroy the action of the bacteriophage, although dysenteriae was effected differently than on a medium of pH 7.6 to 7.8. Likewise, we have once more a demonstration of type of growth as effected by the bacteriophage.

In the presence of chicken blood serum S. pullorum showed a digression in growth to the type shown in Plate IX. In the presence of the bacteriophage there was a reversion approximating the usual type of growth. This was repeated under the same conditions with approximately the same results. In the growth of dysenteriae there was only one plaque in the presence of the bacteriophage and chicken blood serum. In the case of A. abortus, A. fecalis, S. schotermülleri and coli there was some tendency toward change in type of growth in the presence of bacteriophage as compared to the control plate, where serum alone was added.

The ability of the bacteriophage to effect type of organisms is quite evident here as in other places in this work. The impairment of plaque production on Shigella dysenteriae may be explained by inhibition of bacteriophagic action by the serum, or change in type of Shigella

dysénteriae. Other workers have shown that normal rabbit serum does not inhibit the action of bacteriophage

CONCLUSIONS

1. In the isolation of bacteriophage for the eight intestinal organisms considered here, the use of sewage-broth mixture as a medium is effective when the incubation and filtration are carefully regulated.

2. Unalterable laws cannot be applied to the isolation and activation of bacteriophage, but individual regulation is necessary.

3. Concentrated bacteriophage may produce plaques in the growth of organisms which are apparently not affected with dilute bacteriophage.

4. In the growth of S. pullorum either in the 28-day culture at 26°C. or in the 12-hour culture at refrigerator temperature the occurrence of plaques seemed to indicate a definite phase of growth of the organisms.

5. In these experiments the inactivity of the bacteriophage for Shigella dysenteriae grown in the presence of various concentrations of salt appeared to be due to the type of growth.

6. Temperature does not seem to affect the bacteriophage separately from the organism.

7. The work here reported confirms d'Herelle as to the most effective pH value (7.6 to 7.8) for the action of bacteriophage.

8. It appears that the inhibition of the action of bacteriophage on Shigella dysenteriae in the presence of chicken blood serum is due to the effect of the serum on the organism rather than its effect on the bacteriophage.

9. The type of growth of an organism probably affects the action of bacteriophage to the same extent that the latter influences the type of growth of the organism.

10. The possibility of adaptation of bacteriophage to any particular organism may depend upon what might be termed the phase of growth of the organism.

11. It will be necessary to understand considerably more about the physiology of microorganisms before a true evaluation can be placed upon d'Herelle's parasitic theory.

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