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BACTERIOPHAGE, ITS NATURE AND THERAPUETIC USE IN STAPHYLOCOCCUS INFECTIONS.

Senior Thesis

University of Nebraska
College of Medicine

Kenneth D. Grace

Introduction

In the first part of this work I have attempted to present, in rather brief form, a summary of the general characteristics of bacteriophage, and a review of the current theories regarding its nature and action. In the latter part, I have tried to present it in its clinical manifestations, showing as nearly as possible its uses, and especially its possibilities, as a therapuetice agent in staphylococcic infections. For the first portion of the work I have selected the monograph of d'Herelle as being the outstanding and most complete volume on which to base this work. Very few of d'Herelle's experimental observations have been improved upon or disproven since his volume was published (1921). Of course, this does not apply to the theories, chiefly those as to the nature of the bacteriophage, which he advances. The arguments on this matter continue. In fact, d'Herelle stands almost alone in his theory as to the nature of bacteriophage with most of his contemporaries ranged against him.

Review of the literature regarding clinical use of bacteriophage is marked by a most intense variation in enthusiasm among different authors, and even the same author at different times. There is one point on which all agree, however; that further experimentation, carefully controlled and standardized, is needed before final acceptance of the product as a definitely valuable therapuetic agent.

As a closing paraguaph to this work I have

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added mention of the firms who produce the bacteriophage commercially, since on its commercial availability in effective form will depend its final acceptance by the general practitioner.

History

The first record of a phenomenon which probably was bacteriophagy, though d'Herelle vigorously disputes this, was described by Twort.[44] During a series of experiments he observed glassy-looking areas on an agar culture of calf vaccinia which could not be subcultured. When the culture, including the growing portion of the material and the transparent areas, were washed off the medium the entire culture became clear. He further observed that the clarified suspension could not be cultured again and concluded that the living organisms had been killed. The micrococci were replaced by fine granules on the clear colonies, action proceeded from the edge of the colonies, the lytic action tended to spread, and the action was much more rapid on young cultures. The active principle, which he called an enzyme, remained active for six months and was destroyed at 60 degrees Centigrade.

A few others observed a similar phenomenon but general interest was not aroused in its true nature until the works of d'Herelle, begun three years later. While d'Herelle has cited many reasons why the phenomenon recorded by Twort is not bacteriophagy, it is

quite generally accepted that they are the same as those later reported by d'Herelle. d'Herelle has been able to make a more complete study of the matter, however, and his works have been veryslightly altered or improved upon since publishing of his monograph in 1921.

THE GENERAL PROPERTIES OF BACTERIOPHAGE

The Bacteriophage Phenomenon

The phenomenon known as bacteriophagy is best described by an experiment first noted by d'Herelle. Several specimens of fowl or horse feces are emulsified in broth and incubated for a few hours at room temperature. The larger particles are then filtered off and the remainder passed through a bacteria-proof porcelain filter, such as the Berkfeld or the Chamberland. Add a drop or two of the filtrate to a series of young broth cultures of Bac. dysenteriae (Shiga) which are heavy enough to show turbidity. On continuing incubation it will be found that some of them will clear, though often not become entirely limpid, and it will be found that most of the organisms have disappeared. If such a lysed culture is filtered and a portion added to another young broth culture, a similar clearing will occur. The process can be carried on in series indefinitely, and occasionally, though not always there will be noted an increase in virulence in the lytic principle. The principle responsible for this clearing is known as the bacteriophage, and the name is

usually prefixed by the name of the organism for which it is active, as Shiga bacteriophage or Staphylococcus bacteriophage.

Occurrence in Nature

Providing a sufficiently extensive series of test organisms is used, almost any filtrate from the fresh intestinal contents of a vertebrate will show the presence of a bacteriophage. Therefore, it is widely distributed in soils, surface waters, and anything similarly subject to such contact. The activity and range of virulence of such bacteriophages are variable, but, d'Herelle beleives, there is a tendendy to uniformity, in a given species. That is, Shiga phages are most often secured from the horse and fowl, B. Coli phages from man. In general, phage lysing a given organism most often occurs where the organism or related forms have grown in largest numbers. Mixed city sewage is the most convenient source of a wide variety of bacteriophage (Hadley, 1927). Ionescu-Mihaesti[29] isolated an easily adaptable phage form caged laboratory rabbits, and Kuttner[28] isolated a principle resembling bacteriophage, though less active, from a glycerine extract of small intestine.

Undoubtedly phage is often present, in a very active form against the causative organism, in some infections of man, and d'Herelle beleives, usually appears as recovery is manifest. The fact that the bacteriophage which he first isolated was present in

in the feces if a patient convalescent from a Shiga bacillus dusentery, led d'Herelle to beleive that it was the cause of recovery. His later experiments which determined that in several cases the virulence of the lytic principle varied directly as the patient's general improvement and showed coincident remissions, served to strengthen his views that the appearance of the lytic principle was an important contribution toward recovery. d'Herelle observed a similar phenomenon in typhoid fever cases, though his observations have been disputed. Larkum[30] reports that in 25% of cases of urinary infection, bacteriophage lytic for the causative organism is present, while it is never present in a normal urine. It is possible to obtain bacteriophage from filtered exudates, and suspensions of old cultures washed from the surface of an agar growth (Arnold,[8] Gratia,[20] Kuttner[29]), though its activity may not be great and not positive for the organism present in the exudate (Kuttner[29]). Doubtless there is a vast variety of sources as yet undiscovered.

Properties of the Bacteriophage

Bacteriophage exists in particles, as first observed by d'Herelle and later confirmed by other workers, which, though they cannot be seen even with the ultramicroscope can be noted if a system of titrations is used which involves high dilution. For example, if a small amount of an extremely diluted phage is added to each of a series of cultures of susceptible organisms, complete clearing will be observed in some with

with no change in other tubes. The assumption is that the lysed culture contained at least one particle, or corpuscle, while the unaffected one did not receive any. The phenomenon of plaque formation on an agar culture to which a small amount of highly diluted phage has been added points to the same thing, that is its particulate nature. The size of the particle has been variously estimated to be about 30μμ by d'Herelle, and 4.4mμ by Hetler and Bronfenbrenner,[48] both using the same method of dialysis through collodion membrane. The size is variable in different strains of phage.

Bacteriophage is most active in a medium at a pH of between 7.6 and 8.5. In more acid media lytic action is decreased. In a protein-free medium it has been demonstrated that its action is entirely destroyed at a pH of 4.0 to 5.0 in two or three hours.[27] The temperature at which the bacteriophage is active varies with different types but generally there is inhibition at 60°C (the phenomenon described by Twort designated 60° as the end of activity), and complete destruction at 70°-75°. Antistaphylococcus phage is usually destroyed at about 80°C. Krueger[50] was able to show that the process of heat inactivation of antistaphylococcus phage between 51°C and 62°C proceeded in accordance with the equation for a monomolecular reaction. The optimum temperature for bacteriophage action is about 30°-40°C.

Bacteriophage keeps indefinitely in broth filtrate, sealed. d'Herelle noted only slight dimin-

ution in virulence in ten years. Marshall and Paine[35] preserved specimens secured from d'Herelle for eight years, and noted a weakened but readily revived virulence.

Antiseptics generally inhibit phage action in the same concentration in which they inhibit bacterial growth (d'Herelle), though some ability to adaptation to antiseptic media has been shown. Merthiolate destroys bacteriophage action in 1:1,000,000 dilution (Rakietan). Ultra-violet radiation destroys phage action in about the same dosage required to kill bacteria under similar conditions.

Bacteriophage is antiseptic. Walker,[45] in a series of tests in which he mixed bacteria with various antiseptics and with bacteriophage, proved that the bacteriophage had a wider range of dilution at which tissue necrosis due to the infecting organism could be prevented. He concluded that antistaphylococcous phage should be a better dressing than any of the chemicals tried.

Bacteriophage is most active on young cultures, a fact first noted by Twort (1915), and since verified by every other experimenter. Arnold[6] attrubutes this to a characteristic of the bacteria, noting that at the beginning of the logarithmic growth period, approximately a three hour culture, and at its end, varying from two to eight hours later, the lysis was most rapid. During the subsequent stages when reproduction stops

and finally when the number of bacteria begin to decrease, the bacteriophage action is decreased or absent. This action is not influenced by solid or liquid media, or by by-products of bacterial reproduction.

A phenomenon of secondary growth ofter occurs in a bacteriophage filtrate previously clear. When cultured, the resulting colonies are atipical, but the change of media usually renders them susceptible to phage action, and addition of the active principle will again be followed by apparently complete lysis. After a period of a few hours to a few days the secondary culture will again appear.. This phenomenon is evidently due to development of a partial resistance to the phage activity by the bacteria.

Bacteriophage is not specific, but is adaptable: d'Herelle gives many instances of adaptation of a phage strongly lytic for one organism so that it exerts lysis on an entirely different one. Adaptation is easier to related organisms. McKinley[36] demonstrated the adaptation of a phage lytic to the Shiga bacillus so that it was lytic to meningococci. This was shown to be a group adaptation, as other cocci were also lysed. In another instance,[24] a filtrate lytic for both Shiga bacillus and the typhoid organism became lytic only for the Shiga bacillus after twenty serial passages through Shiga cultures. A similar series of paesages through typhoid cultures produced a specific typhoid phage. McKinley beleives that this adaptability suggests that there is only one race of bacteriophage which can

be adapted to any organism.

Bacteriophage is antigenic. Sera obtained from rabbits which had had a series of injections of active filtrates showed a definite antilytic power which retarded bacteriophage action. Arnold and Weiss[9] beleive that this antilytic production is specific for the phage used, though others beleive that it is specific only as to types of organisms, not a single one. It has also been shown that immunization of rabbits with a lytic principle results in disappearence of the similar phage from their intestines.[24] Phage is antigenic also in that it produces agglutinins for its respective bacteria. Larkum[31] used it a subetitute for typhoid vaccine, and found by titration that one innoculation of 2cc. of phage produced agglutination equal to that produced by three innoculations of regular typhoid vaccine. Moreover, the bactericidal power and opsonic index were greater in those produced by bacteriophage. Three injections of phage were less effective in agglutinin production than one, a fact which may be due to antilytic serum production. Arnold and Weiss[8,9] failed to confirm the latter observation (also made by d'Herelle), but they did confirm the other observation of definite antibody production to both typhoid and Shiga bacilli. An 'anaphylactic' phenomenon, probably due to antilytic serum production and observed by d'Herelle, occurs but rarely. There is apparently no reaction to prolonged staphylococcus bacteriophage use (Rice,[42] Larkum,[34] McKinley[37]) which would indicate a retarding antilytic action.

Production of Bacteriophage

Production of a bacteriophage lytic for a given organism proceeds, roughly, in this manner. Mixed sewage filtrate is passed through a porcelain filter, removing bacteria. The bacteria-free filtrate is then added to a series of young broth cultures of the organism to be lysed. Any tube which shows lysis, even in a small degree, is again filtered through the porcelain filter and a small amount of the filtrate added to another series of young broth cultures. By continuing in this way a bacteriophage which was only weakly active in the first instance can be made to increase in virulence until a very small amount, as little as a 1:10,000,000 dilution in many cases will produce a complete lysis. In the same way, autogenous bacteriophage can be increased in virulence by starting with a phage only weakly lytic to the invading organism.

Filtered exudates can often be made to yield an autogenous bacteriophage, by filtering the diluted exudate through a porcelain filter and adding a portion of the filtrate to young, actively growing, cultures of the organism causing the exudate (Arnold[8]). Kuttner[29] described production of a bacteriophage lytic for strains of staphylococcus by filtering a suspension of organisms from a normal agar culture and adding the filtrate to staphylococcus cultures. The filtrate was not active against its homologous strain,

however. Production of phage from old cultures in a manner resembling this has been described by other authors.

A method of production of bacteriophage in large amounts has been described by Keller.[25] This method requires a phage with a lytic titre of 10^{-6} or greater, and an 'innoculum' made as follows: A loop of homologous organisms is introduced into a 6cc. tube of broth (pH6.7) and incubated six hours at 37°. One part of this is added to one thousand parts of broth and incubated for one hour at 37°

To 100cc. of phage, 100cc. of innoculum is added and incubated at room temperature twenty-four hours. After twenty-four hours, andther 200cc. of innoculum is added, incubated twenty-four hours and 400cc. of innoculum are added, continuing in this way to double the amount until the desired proportion is reached. At this point, an amount of six-hour culture of the organism sufficient to produce opalescence in the mixture (ususlly about 100cc. per liter) is added, and the mixture allowed to remain for five days to a week. This step is based on the observation that if a secondary growth be allowed to develop and remain in the bacteriophage for a few days it would be more apt to remain clear after the final filtration. Opalescence having been allowed to remain, the material is filtered, checked for potency and contamination, and ready for use. The method can be varied as spec-

ial conditions arise, for example, insufficient clearing of innoculum when the turbid mixture is allowed to stand three or four days, filtered, and the usual process continued.

Based on their observation that a protein-free bacteriophage produces less systemic shock on intravenous administration, McNeal and Frisbee[47] used an almost protein-free asparagin medium prepared in this manner. 3 grams of asparagin, 2 grams of magnesium sulfate, 4.5 grams of sodium chloride, and 2 grams of dipotassium hydrogen phosphate are added to a liter of distilled water, the mixture autoclaved, and brought to a pH of 7.0 to 7.2. The organism is cultured in the medium, a small amount of known lytic bacteriophage added, and the material is ready to use. The only protein present is that produced by the bacterial disintegration.

Kliger and Clitzki[26] describe a method of elution with weak alkalies which, though it lowers the phage virulence somewhat, results in an almost protein-free principle. Arnold and Weiss[8,10] describe an agar layer method of phage production, which is considerably more involved and difficult.

The Nature of the Bacteriophage

Without attempting to support any of them as being the fact, I will merely mention the different views as to the nature of bacteriophage.

(A) d'Herelle's theory: The bacteriophage

is an autonomous ultramicrobe, a living parasite multiplying within the body of a sensitive organism. The main points designated by d'Herelle as proof of this theory are: (1) The individual characteristics of different races of the phage, maintained through successive generations; (2) The adaptability of the bacteriophage, both to developing virulence to organisms, and adapting itself to its surrounding medium; (3) The antigenic specificity, entirely apart from its related organism; (4) The fact that 'spontaneous' phages can be derived from only a few bacteria; (5) That phage multiplies only on actively growing bacteria, and is not, therefore, a result of bacterial disintegration; (6) That there are certain strains of bacteria which show no gross lytic abnormalities, yet that regularly produce a phage active against some other strain or species.

The theory of a living specific parasite has but few adherents. The theory assumes that phage action is entirely that of direct attack on living organisms, which, in view of the fact that it has been so often proven that stimulation of body defenses by increasing phagocytic invasion (Arnold and Weiss,[9] Smith[43]), makes the theory seem inadequate. The theory also assumes that direct contact with the invading organism must be established, which, though it is doubtless desirable, is not always possible and good results are obtained in spite of its impossibility (Larkum[34,33]).

(B) Theories of Bacterial Origin- which includes some of the theories of note besides d'Herelles. These include:

(1) Hadley's[22,23] theory of microbic dissociation, which concurrs, in most respects, with the theory held by Bordet.[14] It is, that the lytic areas arising in a colony and beleived by d'Herelle to be bacteriophage 'corpuscles' are in reality formed by the same organisms in a different cyclogenic state from others in the same substratum. Having multiplied to a slight extent, they disappear, leaving a bare area which develops later a sprinkling of colonies regarded by Bordet as secondary colonies but which are in reality tertiary. The phage is a filterable stage in the cyclogeny of the same species or a closely related one, and accomplishes its end by indirectly stimulating the rapid development of young, specialized cells already present in the substratum. These cells act, first by rapid multiplication until they reach the lytic threshold, then by lysis, accompanied by generation of a fresh brood of filterable forms. In other words, transmissable autolysis is a normal reaction carried to a pathological extreme, and microbic dissociation gives rise to a lytic virus, not the lytic virus giving rise to microbic dissociation as d'Herelle beleives.

(2) Bordet's theory that the phenomenon depends upon an 'hereditary nutritive vitiation', that is, a bacterium under certain stimuli undergoes

autophagy resulting from upset of equilibrium between assimilation and katabolism. This autolysis liberates substances which communicate to normal bacteria, in a receptive state, a tendency to produce descendants similarly liable to autolysis. This is the theory adopted by Kuttner,[29] that the phage is an autolytic secretion produced by the bacteria under certain conditions, acting as a catalyst to destroy the normal equilibrium of the actively growing cell. This explains, to a degree, the evident opsonizing power of the bacteriophage, remarked upon by Larkum[34] and others.

(C) The possibility that bacteriophage is a defense generated by the invaded organism against the invader (an hypothesis mentioned by d'Herelle) has received but little consideration because of its manifest inadequacies.

(D) The Besredka antivirus[13] has attracted some attention as a possible manifestation of the bacteriophage phenomenon, but fails to show the characteristic properties adopted as these of true bacteriophage.

There are, of course, many other theories as to the inherent nature of the bacteriophage, but most of them are variants of those mentioned above, and need not be quoted in detail.

THE THERAPUETIC USES OF BACTERIOPHAGE IN STAPHYLOCOCCUS INFECTIONS

In this section of my work I have attempted to bring out the clinical use of bacteriophage in staphylococcus infections, offering, as nearly as possible, a symposium of the different reports available. Case histories have been borrowed from these authors, when possible. In some types of cases they were not available. The most effective methods of use, the characteristic reactions, the end results, and the percentage of improvements in individual types of cases, have been recorded.

Abscesses

Excluding abscesses in osteomyelitic bone and superficial abscesses such as boils and carbuncles, this subject is a rather limited one. Rice[42] gives as his method of treatment the following: If the abscess is opened and draining, instill the bacteriophage directly into the crater, using a rubber catheter leading into the cavity, if deep enough to warrant it. A bacteriophage filtrate in 1% agar jelly is very easily handled and instilled. If the abscess is closed, inject the bacteriophage directly, anaesthetizing if necessary to perform the operation. Polyvalent staphylococcus phage is used if mixed infection is not present. If, as in an appendoceal abscess, the infection is apt to be multiple, a mixture of B. coli-staphylococcus phage is used. The amount is not especially important.

Theoretically, it is entirely unimportant since it is manufactured rapidly even though injected in very small amounts. Actually, all that could be injected without causing pain in the closed abscess, and a liberal application to the open cavities so that the material reaches the whole surface affected, should be used. Rice reports a total of forty-four cases (including seventeen appendoceal) of deep abscesses. Thirty-eight showed excellent results, three good results, one failure (in a moribund patient with appendoceal abscess), and two which did not report back. One of his patients with an appendoceal abscess and symptoms of delirium, high temperature, and with foul smelling fecal material draining from the wound, began to inprove in twenty-four hours and recovered completely. The reactions divided into excellent, good, and failure take somewhat this course: There is no effect in the failures, good or bad. Excellent results are those accompanied by marked reaction, usually increase in fever for one to three hours, occasionally pain for a few minutes to an hour after injection, and a slight general accentuation of symptoms. This is followed by complete releif from pain in a few hours, beginning temperature fall, and marked general improvement, progressing to complete recovery. The purulent discharge may be temporarily increased, followed by a change in its character to serous, watery discharge, and finally cessation. The temporary increase in symptoms as mentioned is generally regarded as favorable, the

more marked the initial reaction the more rapid the recovery. Good or medium results were so-called when the initial reaction was slight, but improvement manifested in two or three days, usually requiring a prolonged administration of the polyvalent phage. Rice and Harvey[48] report excellent results.

Alderson[4] used a method of direct injection of 2cc. of phage at twenty-four hour intervals until recovery is well under way. His reports are equally good.

Case Report--(Rice and Harvey,[48] case #8)

Bur. Male, age sixteen, who was thought to have an unknown focus of infection which caused him to develop numerous large abscesses in various parts of the body at intervals of about a week. When opened these customarily drained for weeks before healing. Staph. aureus was isolated from the pus. An active bacteriophage was then injected into two unopened abscesses present when first seen, one in the left breast, one in the left thigh. A similar lesion in the right breast had drained for three weeks. The one injected with bacteriophage was dry from the first, after being opened, and healed much more quickly than had been the habit of previous ones. The abscess in the thigh also healed promptly, and no other lesions developed.

-Acne-

Use of the bacteriophage in treatment of acne

has not been markedly successful.[11] Alderson[6] reports five cases as unimproved, with good temporary response but recurrence in one. Crutchfield and Stout[15] report that all but one of their cases recovered, even chronic cases, especially those characterized by deep and rather painful lesions. There was less effect on their cases of pustular acne than on those of the varioliformis type. Larkum[32] reports two cures, eight with marked improvement, and thirteen unimproved in twenty-three cases. Rice and Harvey[48] report much better success in bad cases of acne, that is, in the actively inflammatory, pustular cases, than in the mild.

The best method of treatment is as follows:[42,48] The face is scrubbed with soap and water, and then swabbed with alcohol. This is allowed to dry. The bacteriophage is then applied, using a cotton swab. It is absorbed by the skin at once, leaving no greasy or unpleasant effects. The performance is repeated night and morning, along with the usual general supportive measures. Pustules are evacuated before the treatment. In acute, severe acne of short standing the phage is most effective. In chronic, deep-seated, acne, it is much less effective.[42] Direct injection of individual pustules is effective, but tedious and impracticable. However, in very resistant cases the phage should be tried if other methods consistently fail.

Case Report-- (Crutchfield and Stout,[15] Case #7)

S.B., salesman, white, age forty, presented

a case of recurring small, hard, painful furuncles, of eight years duration, on the face neck, and scalp. The diagnosis was acne varioliformis. Aug. 13, 1929, 0.25cc. of bacteriophage was administered. On Aur. 17 the lesion was less painful and there was central liquefaction; 1cc. of bacteriophage was administered. On Aug. 21, the lesions were healing. Later observation showed no new lesions, and recovery was complete on Sept. 3. There were no recurrences.

-Bed Sores and Leg Ulcers-

Use of bacteriophage in treatment of bed sores has apparently not been extensive. Rice and Harvey[42,48] give the only reports which I have been able to find. Their results were very encouraging. The method of use was by direct application to the sore, and releiving pressure on the region as much as possible. Rice[42] reports twenty-one cases, with fifteen excellent results, two good, one poor, and three who died before the trial was satisfactory. Polyvalent commercial staphylococcus phage was used, except in those cases suspected of contamination by feces in which the mixed coli-staphylococcus phage was used. In those cases which Rice classifies as excellent results the discharge stopped in twelve to twenty-four hours, granulation tissue appeared at once, and healing began. The resultant scar was soft, pliable, and smaller than that ordinarily expected from such a lesion. He got some of his most spectacular results in paralytics with broken backs, and in chronic cases

of long standing.

In leg ulcer Rice[42] reports three cases, all of which were apparently improved by the local application of mixed B. coli-staphylococcus phage. Other supportive means were also used, so the actual benefit was hard to determine.

Case Report-- (Rice and Harvey,[48] Case #5)

A.C., male, age 69, had had hypertrophy of the prostate, residual urine, severe cystitis. Suprapubic cystotomy was followed by development of a periurethral abscess, a septic temperature, and general weakening of the patient. Two large bed sores developed, one over the sacrum, six inches in diameter, and deep, the other on the leg, about three by five inches. Death seemed imminent when bacteriophage dressing of the sores was begun and the same material instilled into the bladder. The temperature fell to normal the next day, and remained there. The bed sores began to clean themselves and were dry and odorless in three days. At the end of a week there were healthy granulations and new epithelium sprading from the edges. After a month the bed sores were practically healed, the cystitis much improved, and the surgeon ready to perform prostatectomy, which the patient refused. He left the hospital in good condition.

-Carbuncles-

Treatment of carbuncle represents one of the most universally successful of all treatments where bacteriophage is used to combat the staphylococcus. Reports of all who have used it are encouraging, with a high percentage of complete cures, rapid recovery, and freedom from further recurrence. Methods of treatment vary with different clinicians. d'Herelle[17] advises direct injection into the lesion, preceded by injection of novocaine if pain is too extreme to allow injection of phage without anaesthesia. d'Herelle goes so far as to state that the bacteriophage must be brought into direct contact with the invading organism in order to be effective. On the other hand, others state that injection at a distance is just as effective as it is directly into the lesion. The following seems to be the most generally accepted method of use: 2cc. of bacteriophage are injected into the carbuncle mass, preced by anaesthesia if pain is very severe. Preceding this, sufficient material may be removed for laboratory culture and testing for 'in vitro' lysis. Twenty-four hours later another injection is made into the carbuncle, if no reaction is manifest from the first. If there is a manifest reaction, direct injection may be stopped, and subcutaneous injection of 2cc. of the phage at a distance from the lesion may be used, repeating every twenty-four hours until inflammation has subsided and the lesion disappearing. Even a large number of injections

will not produce unfavorable reaction.[15,37] However, more than six injections are rarely required. Wet dressings of phage should be kept on the lesion at all times, whether there is an apparent opening to the surface or not. Polyvalent staphylococcus bacteriophage is used.

Reaction following injection is typical and occurs in nearly every case: Following injection there may be temporary discomfort of a stinging, burning nature or of a nature due to increase in subcutaneous tension at the site of the lesion. Within a few hours, often immediately,[48] pain almost entirely subsides, leaving the patient comfortable and with a feeling of well being. In a few cases, the temperature shows an initial rise, persisting for four to eight hours, often rising to 104°-105°F; with chills and symptoms of severe reaction in some cases. After the initial rise, it returns to the normal quickly, often within twelve hours to twenty-four hours and remains normal or only slightly elevated during the course of the healing. Some cases have been noted in which the first injection produced a much less marked reaction while the typical, more severe reaction followed the second or even the third or fourth injection of bacteriophage. In no case as yet reported has there been a permanently injurious effect from the reaction immediately following injection, or any injurious effect attributable to the bacteriophage (Larkum[34]). The possibility of foreign protein reaction or production of anti-bacteriophage antitoxin is mentioned

elsewhere.

The lesion itself shows an almost spectacular change. Following an initial redness or sign of reaction, the drainage, if any, changes from purulent to serous, almost a clear fluid. This includes drainage from any part of the mass, at either the central or peripheral portions. Fluctuation develops rapidly and artificial drainage through an almost pinpoint opening will be entirely sufficient, eliminating disfiguring and dangerous incisions.

Carbuncle treatment by bacteriophage must, of course, be accompanied by general supportive or systemic treatment, especially in the case of diabetics. Rice[42] reports that in diabetics with carbuncles treatment was more prolonged and results less spectacularly rapid but just as certain as in non-diabetics.

Because of the conveniently decreased amount of mutilation necessary to cure with bacteriophage, the resulting scar is small. Usually it is also pliable, soft, and consists of obviously healthy tissue. Rice and Harvey[48] report that the immediate and spectacular releif from pain is an almost constant and one of the most desirable of clinical reaction.

Because of its almost universal success it would almost seem that treatment of carbuncle by bacteriophage should become the standard, doing radical proceedures only in the rew cases which fail to respond. Alderson[3] reports 100% success in five cases.

Rice[42] reports four failures in sixty-six cases. Others report similar results. The fact that results are so rapid, that releif from pain and general toxemia so prompt, that radical operation with hospitalization and a long period of disability is often unnecessary, that the patient can continue his work almost at once and that disfiguration is almost entirely absent, would point to this as the ideal initial therapy in all cases.

Case Report--(McLean[38])-

A male baby, age ten months was severely ill with large carbuncles involving practically the entire buttocks and presenting multiple pinpoint openings. General condition was very poor. 1cc. of a 1:10 dilution of bacteriophage (in a protein-free asparagin medium) was injected into the subcutaneous tissue near the lesion. (Culture at this time revealed Staph. aureus, susceptible to 'in vitro' lysis). There was immediate improvement in general condition and behavior. Locally, induration seemed more extensive the following day. Local injections in increasing amount up to 2cc. were give daily for six days. Culture at this time gave a very slight growth of organism and ten day's after the first injection the lesion was healed except for a small furuncle which was drained two days later. Meanwhile, two other small furuncles had developed, one on the finger, one on the toe, and were quickly involuted by small local injections. There was almost no scarring of the involved area and recovery was complete.

-Furunculosis-

Bacteriophage as a therapuetic agent in furunculosis offers clinical results comparable to those of carbunculosis treatment. The method of use varies with different clinicians and brings out again the contention as to whether the phage must come into actual contact with the invading organism or is just as effective if injected at a distance. Larkum and Pratt[33] report 45% complete cures with 20% failure in a controlled group of cases in which they made only two injections subcutaneously of 2cc. of bacteriophage with a twenty-four hour interval between injections. In an earlier work, Larkum[32] reported 78% complete cures with 19% mild recurrences in 208 cases, while only 3% were unimproved. He used two subcutaneous injections as above, with local application. Alderson[6] reports no failures in forty-eight cases he treated, varying his treatment by direct injection, direct application, or subcutaneous injection at a distance from the lesion, as the lesion itself indicated. Rice[42] reports ten cases of generalized boils in children, in which fifty to three hundred fifty were present at once, all of which cleared up at once with local application or direct injection. Walker[46] found it to be of definite benefit only in cases where its direct application was possible.

The exact method of use is, therefore, a question. Certainly direct application on wet dressing to an open lesion is to be preferred because of its simplicity. This might be augumented by subcut-

aneous injection if it is thought desirable, using 1-2cc. of bacteriophage as often as every twenty-four hours in severe cases, until definite recovery is is evident. Where possible, 1-2cc. of phage should be injected directly into the lesion, using a small amount of novocaine if necessary to releive the pain of injection. In multiple boils this may prove too painful for injection of each lesion, of course. In deep-seated lesions it is probably the method of choice. Polyvalent phage should be used unless it is convenient to prepare the autogenous phage, which it so rarely is. If response is not rapid, that is, with three or four applications or injections, it probably will not occur. It may be that one commercial product will produce lysis where another will not, as the lytic power must certainly vary in the polyvalent products, and this fact may be of importance in case of apparent failure.

The reaction following bacteriophage injection of boils is similar to that of carbuncle injection. Temperature lowers at once, pain decreases or entirely disappears, and there is marked improvement in twenty-four hours, in the general condition. Locally, nearly every author reported liquefaction of pus occurring almost at once with quick resolution, healing being apparent in many cases within twenty-four hours. Larkum[32] divided the reactions in furunculosis treatment into mild, gemeral, and severe. 47% of his cases showed mild reaction of local erythema and soreness

at the site of the lesion. In most cases it was of very short duration, usually only a few hours. 10% showed a more general readtion with fever and malaise, lasting from four to twelve hours. 1% showed a severe toxic reaction, with nausea, vomitting, fever, and malaise, coming on about four hours after injection and persisting for several hours. In no case has there been a permanently harmful effect, however. The more severe the reaction, the more marked the clinical effect which followed, and the more rapid the cure.

The permanent effect on patients subject to recurring boils is questionable, as is the antigenic effect of staphylococcus bacterteriophage (which is discussed elsewhere). Larkum[33] reported that 19% of his cases showed recurrences within six months, thirty of whom cleared up without further recurrence, ten of whom had occasional mild recurrence, while, sixe cases persistently recurred. In Alderson's[6] series of cases recurrences occurred but were of a small rapidly resolving type of lesion. Rice[42] reported that in only one-half of his cases with 'crops' of boils was the condition entirely cleared up, but says that subsequent attacks were easier to control and less severe. Rice also reported that diabetics in his eries of cases showed slower but just as satisfactory results.

It is interesting to note that many of the cases reported had been under treatment for a long time with other therapuetic agents. Crutchfield and Stout[15] and Larkum cite instances of cure in patients prev-

iously under autogenous vaccine therapy. While this may be due in part to poor preparation of the vaccine, as suggested by Greenbaum and Harkins,[21] it is certain that vaccine therapy has never warranted so enthusiastic a reception on its clinical merits as has bacteriophage. The lessened tissue destruction and elimination of painful incision, as well as the probable effect of preventing recurrences in many cases indicate its use in cases of furunculosis. Despite the contention of Applebaum and McNeal[7] that bacteriophage will not cause disappearance of organisms in the presence of either pus or blood, as they show in their 'in vitro' experiments, and that the actual method of its working is still problematical, its clinical effects are so satisfactory and its deleterious effects so conspiciously absent it seems that it should become a valuable and commonly used therapuetic agent. Larkum's[34] warning that carefully controlled clinical experimentation is necessary before its universal acceptance, as well as his advice that its nature should be more thoroughly studied, should be heeded. An editorial[19] (1929) even seriously warned against its over-enthusiastic acceptance, comparing it with that accorded the coming of specific vaccine therapy. Nevertheless, results are very encouraging.

Case Report-- (Alderson[6]). Case #10.

L.S., male, age 16, with chronic furunculosis of neck, cheeks, and buttocks for one and a half years. Dietary regulation and autogenous vaccine begun

sixteen months before presentation produced partial releif but three acute recurrences had followed. When first seen there were three moderately large, fluctuating boils, already suppurating, on the back of the the neck , and one smaller non-fluctuant boil on the right cheek.

Culture from one boil showed Staph. aureus which was completely lysed by the bacteriophage.

Three subcutaneous injections of 3cc. each were made at twenty-four intervals, with only slight reaction at the site of injection. About twelve hours after the first injection, the unincised furuncles on the back of the neck became more turgid and painful and opened spontaneously. On the day after the second injection the cheek lesion underwent rapid regression, and the neck boils were practically healed. At the time of the third injection, healing was nearly complete in all lesions. There have been no recurrences in ten months.

-Impetigo-

Impetigo contagiosa treatment with bacteriophage offers quite favorable results in reported cases. Alderson[6] reported one cure in seven days by local application and subcutaneous injection of 1cc. of known lytic phage for six injections. Another[11] reports cure in seven days by direct application only. Rice[42] performed a comparative test using bacteriophage (stock, polyvalent) by direct application to one side of the face while he used ammoniated mercury on the other side. In the three cases reported, all of the

sides treated with bacteriophage cleared more quickly.

The method of choice for using phage in impetigo is direct application to the lesions, preceded by gentle cleansing with soap and water. It may be applied as often as desired, though twice or three times should be the minimum number each day. It may be dabbed onto the lesions with cotton pledgets, or smeared on if in the jelly form.

Effects are not spectacular in treatment of impetigo, but the possibilities are evident.

Case Report-- (Rice and Harvey,[48] case #24).

Infant female, aged fourteen days, which developed impetigo the tenth day after birth. Anti-staphylococcus bacteriophage was used on a part of the lesions, ammoniated mercury on the others. Two days later the lesions were all improved but those treated with phage were more improved. Ammoniated mercury was discontinued, bacteriophage used exclusively, and recovery was complete in two more days, much quicker than the average of such cases in that hospital.

-Infected Wounds-

Bacteriophage has not been widely used in treatment of infected wounds, but the few cases reported are encouraging. Alderson[6] reports one case of stitch infection in which a single application resulted in cure within twenty-four hours. Rice[42] reported that forty of his forty-four showed excellent results, three showed improvement, while one case, in which there was absolutely no apparent reason, failed to improve. The

lytic principle was active 'in vitro' in that case. McLean[38] reports favorable results.

The method of application is direct, in the form of dressings saturated with the lytic material.

The reaction reported by Rice were uniform. In nearly every case, pus production was increased for twenty-four to thirty-six hours, often to the point of saturating the dressings. After the initial increase in amount of pus, the wound cleared rapidly, and healthy granulation tissue appeared and developed rapidly. In some cases an initial burning sensation at the time of application was apparent. This was followed in one or two hours by a soothing sensation. In cases in which this reaction occurred, results were even quicher and more marked that those in which it did not occur.

-Osteomyelitis-

Direct application of bacteriophage, that is, artificially introduced bacteriophage, has not met with so marked success as has its use in some lesions. McKinley[36] reported good results in four cases. Larkum[32] reported six cases, three of which recovered rapidly while three were markedly improved. Bagley and Keller[12] reported ten cases of osteomyelitis in which they used bacteriophage therapy. Three showed marked improvement. One other case complicated by septicemia survived, though four blood transfusions were given in addition to intravenous bacteriophage therapy. Rice,[42] with eleven cases, had excellent results in four cases, fair

results in three, with no effect in four cases. He regards bacteriophage as of little value. McLean[38] reports use in osteomyelitis but gives no report as to its effectiveness.

The method of use varies, but it seems to be generally accepted that bacteriophage is of little value if the following are present: (1) Multiple infection, that is, mixed infection (Bagley and Keller[12]); (2) Dead bone.

Thus the primary treatment is that generally accepted in osteomyelitis. Saucerization, with removal of necrotic bone and sequestrae should be performed. The bacteriophage is then applied directly to the wound, which is immobilized. McKinley[38] used a combination of direct application and subcutaneous injection. His average dose was 2cc. of bacteriophage injected into the wound or tissue immediately surrounding it, every day for three doses. He usually accompanied this by injection of 2cc. subcutaneously for three or four doses. In most of his cases the remainder of the treatment was by direct application, continuous, with subcutaneous injection of 2cc. for two doses. Bagley and Keller[12] found the most effective method to consist of injection of an average of 2cc. every twenty-four to forty-eight hours subcutaneously with continuous direct application with wet dressing. McLean[38] used only direct application in acute osteomyelitis. In chronic osteomyelitis without definite localization or positive blood culture, he used it intravenously.

The reaction to bacteriophage therapy connects in an interesting manner to the results. In brief, if there is no reaction there are slower results or no results at all. McKinley[36] reports a case in which there was no improvement after three injections of 2½cc. each into the wound and surrounding tissue on successive days. On the fourth day, an injection of 4cc. was followed immediately by chills, a temperature of 104°, pulse of 120, and marked prostration. Within a few hours the temperature returned to normal, discharge decreased at once, and healing was evident without delay. In another case, 2cc. subcutaneously and 2cc. into the wound were used alternately every other day for four days. The wound began to improve, and discharge decreased greatly. Three days later another 2cc. were injected, and a temperature of 103°, cramping pains all over the body, chills, and emesis, resulted. This began to subside in five hours and the condition continued to improve. Three weeks later, without further therapy, the wound was healed except for a small area. Local application to this caused a similar reaction to the one described before, which persisted for seven days, after which healing progressed rapidly.

Bagley and Keller[12] report slightly different reactions. In their four cases of purely staphylococcic osteomyelitis they used a combination of injection at twenty-four to forty-eight hour intervals with direct application. In all their cases the discharge was not decreased, but continued to be copious. The

granulation tissue at the same time developed rapidly, was healthy, and pain was absent. Cure resulted in three of four cases.

The use of bacteriophage intravenously is probably indicated only in cases complicated by septicemia. This will be taken up later.

One of the most interesting viewpoints with regard to bacteriophage therapy in osteomyelitis is presented in a group of articles by Albee,[1,2,3,4,5] in connection with Patterson.[2] When Albee adopted the Orr treatment for osteomyelitis he became interested in just what caused the results which followed. In brief, the principles of the Orr treatment are as follows (Albee)[3]: (1) Primary asepsis or antisepsis to reduce the possibilities of focal infection; (2) Adequate drainage, that is, an opportunity for drainage to prevent pressure necrosis and prevention of cure by an accumulation of pus; (3) A post-operative dressing that protects against reinfection; (4) Complete immobilization. In his articles Albee summarized the stages of development of osteomyelitis treatment in which the Carrel-Daykin treatment, with its extensive and bothersome proceedures, its necessity for constant manipulation, and its attending dangers had still become the most favored and effective previous to the principles brought out by Orr. The Orr treatment, as modified and used by Albee is as follows: (1) In acute osteomyelitis the bone is opened freely. Saucerization is performed to remove all necrotic bone and the resulting cavity shaped as nearly

as possible into a V-shaped cross-section. Strong antiseptics are not used due to their destructive effect on normal tissue. Care is taken to prevent injury to periosteum, as it is from this as an osteogenic base that new bone must form. When the cavity is thoroughly cleaned out, a semifluid dressing of sterile vaseline is packed into it. The limb is then immobilized in a plaster case, without window opening or any further provision for drainage. The cast is not reopened or manipulated in any way for eight to twelve weeks depending on the extent of the original lesion. After this period, the sequestrum is detected by X-ray, the cast taken off, and the sequestrum removed. It will be found that the granulation tissue present will be red, healthy, and normal in spite of the presence of foul-smelling pus which may have completely displaced the gauze and saturated the whole leg and cast. After sequestrectomy, the lesion is redressed with vaseline pack and left for another six or eight weeks, redressed again, and continuing thus till bony replacement is complete. At the dressings, nothing is done to sterilize or in any way manipulate the healing region. (2) In chronic osteomyelitis the proceedure is essentially the same, except that necrotic or involved tissue removal usually has to be more extensive. The first immobilization need only be for six to eight weeks, with the same interval between subsequent manipulations. The results are the same. The wound will be absolutely saturated, steeped, in pus on removal of the cast. Yet, the patient

is almost invariably comfortable and practically symptom-free for the period of immobilization. The advantages to the patient, who needs to pay only a few days hospital bill at each redressing spending the intervals at home, are obvious. The physician's saving in time, trouble and work are incalculable.

The thing of interest here is that in 94% of the cases observed by Albee[2] in a group which he reports he was able to discover bacteriophage, lytic for the organism present, at some time during the course of treatment. In 3% of the remaining cases he was able to prepare an autogenous phage by laboratory methods, and use it in dressing the wound. If, as Albee beleives, the bacteriophage appearance is necessary to favorable healing, then the advantages of the Orr treatment is even more pronounced. By favoring pus retention and preventing the development of pressure which tends to retard circulation and healing, the best possible medium for bacteriophage development is provided. Besides this, there is the further indication that bacteriophage is a normal body defense mechanism which must have proper surroundings in which to develop, a fact which, if proven, might alter treatment of infections of many kinds. Albee himself likens the bacteriophage, as a microbic parasite, to other normal parasites in nature, and explain its presence as one of Nature's methods of balancing the scales of existence. Whatever its significance, it certainly sheds a new light on osteomyelitic treatment, and especially on bacteriophage therapy.

Following is one of his more or less typical cases in which the Orr treatment was used. The presence of native bacteriophage was not demonstrable throughout the entire course of the treatment.

Case Report--(Patterson and Albee, Case #2)

Woman, age 21. Two months previous to entrance had suffered a compound, comminuted fracture of the left radius and ulna. On admission, bone fragments projected through the wound, and there was sloughing on both dorsal and volar aspects of the forearm with osteomyelitis of both radius and ulna. Sequestrotomy was performed and the Orr treatment applied. The wound contained much pus, which on culture showed Bacillus coli predominating with a few Staph. aureus organisms, both susceptible to lysis. Broth filtrate of a bacteriophage originally present, was able to lyse the organisms present only after being exalted by adding stock-susceptible Staph. aureus organisms.

After seven weeks, at the first change of plaster dressings, the wounds were smaller, discharge moderate and not fetid, and healthy granulations apparent. Staph. aureus predominated in culture but no bacteriophage was demonstrable.

After another seven weeks Staph. aureus was the only organism present. No lytic principle could be isolated. The wounds were now practically healed and plaster casting discontinued. Healing was complete four weeks later.

In this case, phage was demonstrable only

once. It must be remembered, however, that 'in vitro' absence does not exclude its 'in vivo) presence.

-Otitis Media-

Rice[42] gives the only available reports of the use of bacteriophage in otitis media. He used it with success in eight cases out of nine, getting excellent results in six cases, and good results in two. His cases included mastoid infections also. Some of his best results were in cases where the ears had been draining for years.

The method of use was by direct application. The lytic principle was instilled directly into the ear, as well as into the mastoid wound. If there is dead bone in the mastoid, results are not good. He does not report the reaction in typical cases.

-Paronychia-

Alderson[6] gives the only available report of bacteriophage use in paronychia. He applied it by subcutaneous injection and local application, the former by using 2cc. subcutaneously every twenty-four hours, the latter by constant application. Results were excellent in both cases.

-Peritonitis-

Rice[42] gives the only report of use of bacteriophage in peritonitis. In three cases which he treated with commercial B. Coli-staphylococcus bacteriophage, in conjunction with other measures, two are alive and well, one died of actue heart failure. Rice beleives

that the bacteriophage was a big factor in recovery.

His method of use was direct introduction of the mixed phage into the peritoneal cavity. He does not describe the reaction occurring.

-Septicemia-

Treatment of staphylococcus septicemia by bacteriophage is in the nature of a last resort in most of the cases reported. However, McLean[38] reports fair success in one case which recovered. In two other cases of his the blood cleared but staphylococci developed in the pericardial sack and caused death. Bagley[12] reported three cases of septicemia complicating osteomyelitis, two of which died while one recovered. The one who recovered had four blood transfusions in addition to bacteriophage. Two cases treated by Rice,[42] using only local application and subcutaneous injection, expired. The life of one, he beleives, was prolonged by bacteriophage therapy.

Both McLean and Bagley used the concentrated bacteriophage in their cases, with severe reaction which I will describe later. d'Herelle[17] gives a more logical method of treatment. He recommends diluting the bacteriophage with normal saline, diluting 5cc. to 500cc., and prolonging the time of injection. d'Herelle states that 25cc. of bacteriophage can successfully be injected at one time, providing the injection is sufficiently prolonged, without shock to the patient. Without dilution, 2cc. of concentrated broth suspension of bacteriophage has, in nearly every instance, produced

severe reaction with fever, chills, cyanosis, and in two of Bagley's cases, delirium and coma.

In a later publication, McLean and Frisbee[47] report fifteen cases of staphylococcus bacteremia whom they treated, or directed the treatment, by bacteriophage. For intravenous therapy they used a nearly protein-free asparagin medium containing phage, made up by a method described earlier. Seven of their fifteen cases lived. They accompanied the intravenous administration with local and subcutaneous application of phage suspended in broth. They approve the following method of use: As soon as the organism is discovered in the blood intravenous therapy in instituted. Multiple small doses at intervals from thirty to ninety minutes apart are given the first few days, after which a longer interval of twelve to twenty-four hours may be allowed. They recommend starting treatment with 5cc. of a 1:10 dilution, doubling the dose and using undiluted phage until a definite reaction occurs with fever, chills, and symptoms of shock. This dose is continued even though clinical signs abate, for at least two weeks after symptoms subside and the organisms disappear from the blood. After this, for at least two weeks, the bacteriophage (as broth filtrate) is administered by subcutaneous injection of 1cc. every day. Biweekly injections are kept up for another month. By this method, latent or undestroyed organisms seem to be more completely removed, lowering the chance of remote lesions developing later after intravenous

therapy is stopped. This method of using phage was developed on clinical experience which they derived from their series of cases. Besides lowering the apparent shock on administration, this method allows a more constant application of the lytic principle directly to the involved tissue. Since no therapy has as yet been devised which offers much hope in staphylococcic bacteremia, bacteriophage therapy offers an improvement, at least. The experiments of Applebaum and McNeal[7] which demonstrated an inhibition of phage action in the presence of blood evidently are not altogether born out by clinical results.

Case Report--(McLean and Frisbee[47])

J.W., male, age 32, admitted to the hospital Dec. 3, 1931, with a septic temperature following varicose vein injection. The veins were incised and drained, and the patient was making satisfactory progress when he was allowed out of bed, Dec. 26, Previous blood cultures had been negative. Dec. 31 he had a relapse, with fever of 103°. Blood cultures at this time were positive. Bacteriophage therapy was begun Jan. 2, 1932, and its use was continued, as described before, on Jan. 3, 5, 7, etc., to January 27. A period of improvement was followed, on Jan. 19, by two chills ten hours apart and a fever of 103°. The next day the temperature was normal and continued so. Intravenous therapy was discontinued Feb. 1, though clinical signs had been absent since Jan. 19. Subcutaneous injection

of 1cc., every other day, was continued until Feb. 13, the date of discharge from the hospital. Biweekly injections were continued until March 18, when the patient was allowed to return to work.

-Staphylococcus Cellulitis-

Rice,[42] and Crutchfield and Stout,[15] report the only use of phage in staphylococcic cellulitis. In all five of Rice's cases the infection had previously refused to localize and liquefy. His method of application was by direct injection of .5cc. directly into the involved tissue. Releif from pain was immediate. Suppuration followed promptly, with good localization, and healing was evident within twenty-four hours. In some of the cases, pus production increased the day following injection, and in these cases, recovery was more prompt than in the others. Rice notes that this temporary increase in pus denotes phage activity and is a favorable sign. Using a bacteriophage previously tested for lytic potencey, Crutchfidd and Stout report approximately the same results, with complete releif from pain within forty-eight hours and early liquefaction and localization.

-Staphylococcic Purulent Arthritis-

Rice[42] reports four cases of staphylococcic purulent arthritis which involved nine different joints. He treated these, following aspiration and culture to determine the specific organism, with direct injection

of 1 to 2cc. of bacteriophage into the joint cavity. This was repeated until a therapuetic effect was noted. In every case there was marked improvement. In most joints, the resulting function was perfect. Drainage by incision of the joint was not used in any case.

-Sinusitis-

Rice[42] reports fifteen cases of sinusitis treated with bacteriophage, some with polyvalent bacteriophage, others with autogenous. He secured excellent results in seven cases with some improvement in four others. A few chronic cases presented marked releif, and in some cases, X-ray showed some evident decrease in involvement. The application was by direct instillation nasally.

Results are, of course, difficult to evaluate in such a small series of unselected and non-specific cases.

-Styes-

Larkum[32] reports the only cases of treatment of styes by bacteriophage. He used his standard method of treatment consisting of two, 2cc. subcutaneous injections on successive days with continuous local application. In five of his cases the styes disappeared without recurrence in six months. In one case there was a mild recurrence in two months. Three cases had mild and transient recurrences, while one case showed no improvement.

-Commercial Production of Bacteriophage-

Bacteriophage is available commercially from the Abbott Laboratories, Swan-Meyers division. It is marketed in liquid form, available in 5cc. ampules and 30cc. vials.

The Eli Lilly Company market the phage in a water-soluble jelly base (Staphylo-Jel, Strepto-Jel, and Colo-Jel), and in broth solution (Staphylo-Lysate, Strepto-Lysate, Colo-Lysate, and Ento-Lysate). The latter is prepared for market in 20cc. rubber-stoppered vials.

The Anglo-French Drug Company, New York, markets a bacteriophage produced under the scientific direction of Dr. d'Herelle. They market Bacte'-Pyo-Phage and Bacte'-Staphy-Phage, with others designed for use in intestinal infections.

E. R. Squibb and Son produce commercial phage.

A recent article (J. A. M. A. Jan. 14, 1933) by Straub and Applebaum comments on the efficiency of the different commercial bacteriophages, as demonstrated by their action in the laboratory. The phage marketed by Lilly and Company contains Merthiolate as a preservative, which in their opinion renders it ineffective and unsuitable. The bacteriophage of Squibb and Son showed a variable potency, different samples being more effective than others. The Staphylococcus phage marketed by the Swan-Meyers Company was apparently potent against most laboratory organism.

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