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The Bacteriophage

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T H E   B A C T E R I O P H A G E

ITS VALUE IN THERAPY

With Reference to Osteomyelitis

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-- Charnock

T A B L E OF C O N T E N T S

I.

I N T R O D U C T I O N

The flames of the era of antiseptics are gradually becoming smoldering embers, though here and there an ember bursts into life anew, but with the accumulation of information regarding them they are more and more quickly trampled into temperance. Now we are more or less aware of their limitations and our enthusiasm, concerning their efficacy, is moderately controlled in spite of vigorous exploitations. Only a few years ago a new agent, destined to take its place among the combatants of infection, entered the field of therapy. After a period of critical analysis are we coming to look askance on this substance, called bacteriophage? Possibly not, for though our knowledge is as yet not satisfactorily complete, little enough is known about it to warrant an indifferent discard.

When the writer first became interested in bacteriophagy he was fired with enthusiasm over its possibilities in the field of therapy. As the subject matter unfolded its wealth he became discouraged and confused in the entanglements of controversy, and consequently became doubtful of its worth as a contribution to medical science. But slowly, by the process of elimination, an accumulation of materials became accessible for compilation worthy of some effort. This subject is presented, not in ignorance of the vast wealth of material on this topic, but in awareness of the limitations restricting such presentations to perusal form.

This paper is the culmination of factors born of aroused interest acquired while performing a bit of experimental work under Dr. H. Winnett Orr. The results obtained were of too little importance to include in this paper, but to observe, in the wards, the recovery of seriously infected patients under the Orr treatment is a spectacle one isn't likely to overlook. If, then, the striking results of this method can be shown to be a direct or indirect influence of bacteriophage, its value in therapy is worthy of every consideration.

II.

H I S T O R Y

Since the turn of the 20th century much literature has been presented in an attempt to fuse the phenomenon of bacteriophagy and the general syndrome of bacteriolysis. This confusion has led to a situation retarding the enlightenment regarding actual facts about the bacteriophage.

Twort (1), in 1915, advanced the phenomenon known as the Twort phenomenon, or bacterioclysis, and frequently referred to as the Twort-d'Herelle phenomenon because of the suggested similarity in nature and mechanism. d'Herelle (2) emphatically denies this relationship and maintains the phenomena are entirely different and separate in their character.

After years of almost repeated challenge by d'Herelle, Twort (3) published in 1930, his article, summing the characteristics of the lytic agent, and charging d'Herelle with misinterpretation of his (Twort's) original descriptions. Subsequent writers have really added little to the hypothesis set down by Twort. His work gained little recognition until revived by d'Herelle a few years later. During the Great War, Twort was forced to abandon his work and thus lost step with the rapidly progressive investigation of subsequent writers. d'Herelle's hypothesis gained recognition because of the simplicity of its conception and extensiveness of its development.

According to d'Herelle only three communications were found at the time of his publication in which the facts disclosed might be explained as due to the action of bacteriophage. These have been revealed through Hankin (4) who found that the waters of certain rivers in India possess bacteriocidal properties; Emmerich and Low (5) who advanced their "nuclease-immuno-proteidine" bacteriocidal properties in immunity; and Gildemeister (6) who described a phenomenon observed from colonies of typhoid and colon bacilli, which were believed by d'Herelle to be contaminated by bacteriophage.

There are many passages in the literature relating or hinting at such observations, but due to the difficulty which was experienced in reproducing the actions observed, no further mention was made of them.

It is certainly not from the lack of experimental endeavor that the story of bacteriophagy is not more complete, but rather that all avenues of investigation have not yet been explored.

d'Herelle expounded in exhaustive detail the story of bacteriophagy, but more recent investigators have vigorously attempted to strip his works of their original security. However, many of his basic principles remain intact.

Let it be assumed then, that bacteriophagy is a distinct entity, although much controversy has arisen over the nature and mechanism of the phenomenon.

It seems pertinent here to mention the incident which led to the investigation of bacteriophagy.

During the study of a bacterial disease affecting locusts in Mexico in 1909, it was found that, since the locust constituted an insect pest, the organisms could be enhanced in virulence and artificially implanted among the larval forms and thus destroy large numbers of the insects. In the laboratory work which ensued, unusual and peculiar observations were made. The colonies, when cultured, occasionally showed irregular and distorted configurations. In some cultures appeared plaques clear of growth. d'Herelle became curious and began a long series of experiments which resulted in the hypothesis which became bacteriophagy. (2)

III.

D E F I N I T I O N

A definition for bacteriophage is somewhat difficult to formulate, due to the various theories advanced as to its nature. d'Herelle's explanation seems general and sufficient for the present.

> "There is a principle, very widely distributed in nature, found in urine, blood, pus and normally occuring in the intestinal contents, which possesses the property of dissolving bacteria. This principle is present in a particularly active form in the intestinal canal and in the excreta during convalescence from a variety of infectious diseases." (2)

Kreugar (7) adds to this:

> "Bacteriophage is a generic term including a large group of agents which share in common the ability to produce dissolution of growing bacterial cultures (lysis). They all possess the curious property of regenerating themselves during contact with growing susceptible bacteria."

Note that here are two definitions essentially alike, but with emphasis placed on entirely varying aspects of the subject. One has neither added nor subtracted from the value of the other - though more than 20 years space the two writers. It is interesting to view the turmoil resulting from varying opinions regarding the nature of the phage and the diverse means contrived to explain it. Fundamentally the persistent question remains: '<u>What is the nature of bacteriophage?

IV.

THE NATURE OF THE BACTERIOPHAGE

It is not within the scope of this paper to detail the evidence derived to substantiate these theories, but a word concerning them seems obligatory.

d'Herelle is of the opinion that the phage is ultra microscopic particulate matter, for which he offers substantial evidence. (2). There is a definite ratio between concentration by titration and the number of colony plaques found on the culture plate. (8). Feemster (9) advances experimental evidence to show that the phage obeys closely the laws of chance distribution of particles in suspension. Charnock (10) is inclined to agree with d'Herelle and Feemster and others of this contention. d'Herelle likens the phage to a bacterial parasite, called Protobes, a substance foreign to the bacterial organism. It possesses the fundamental properties applicable to any living substance, namely assimilation and adaptability. d'Herelle became enthusiastic and implied that if the true identity of the phage be found, some light would be thrown on the source and origin of life. The primitive one cell is an accepted fact. But the persistent question as to the source of evolvement of the one cell is as yet theoretical and though many such theories have been advanced they lack the integrity of scientific facts. This will be discussed later as a matter of passing interest.

Bordet and Cuica (11) discovered bacteriophage in the peritoneal cavity of guinea pigs, which resulted in their theory of transmissible autolysin. They were of the opinion that the phage is an autolysin which originates from unfavorable bacterial metabolism when activated by leucocytes or leucocytic products. Arkwright (12) wrote of a substance with transmissible qualities.

Hadley (13) states that in the cyclogeny of the bacterial species, the organisms become 'fertilized' by a union of phage corpuscles, to produce changes observed in the bacteriophage phenomenon. Bacteria, as the result of this union, are empowered to develop into invisible forms. This has been advanced as the homogamic theory. Hadley, Delves and Klimek (14) believed that the original organism was found in filtrates of lysed cultures. d'Herelle and Rakieten (15) have shown these to be mutation variants, rather than cyclogenic transformations.

Kabishima (16) sponsored the Enzyme or Catalytic hypothesis. This theory embodies the presence of a proenzyme in the bacterial organism preactivated by internal gland secretion or leucocytic action in the body. Kreugar (7) is also of this opinion and would leave the problem settled by stating that in view of the evidence presented by him, in which he ran a series of experiments showing the catalytic influence of the magnesium ion on the phage, the burden of proof lay not with the exponents of the enzyme theory but with those aligned against it. Wollman (17), after presenting a review of the subject, states that the term 'bacteriophage' should be discarded in favor of the term 'lysogenic factors', or 'transmissible autolysis' or perhaps 'hereditary contagious autolysis'.

It is difficult to indifferently evaluate these theories. In scanning the literature one is inevitably drawn by the influence of d'Herelle. Nevertheless, in the presence of current knowledge, the work of the other investigators of bacteriophage cannot be ignored and this fact is fully emphasized in considering Herb's (18) paper 'Bacteriophage as the Missing Link between the One Celled Organ and the Inorganic World in Evolution'. If the imagination is not suppressed, all theories on phage may appear logical, and a component part in this intricate picture.

We are well acquainted with the one celled animal, the amoebia. It is a complex mass of protoplasm containing complex chemicals which may break down chemical compounds and synthesize even more complex compounds. By pulverizing the protoplasm it has been shown that though the mass as a cell ceases to function, the chemistry is still capable of activity. Thus it may be assumed that it is not the protoplasm that functions but the chemical bodies within, which are called enzymes, catalysts, and ferments. For the sake of simplicity these bodies are referred to as ferments. The catalysts represent the inorganic world, the enzymes the highly developed human ferments. To the category of ferments, ranging from inorganic to organic, belong the bacteriophage. The phage takes its place higher than the inorganic because it has been attributed the powers of assimilation and procreation. (18).

An editorial in the J.A.M.A. (19) aptly states that:

> "The subsequent extraction and preservation of the mosaic infectious agent by means of glycerin, the demonstration of its precipitation by alcohol, acetones and other protein precipitants, its absorption and subsequent elution from talc, and its partial isolation by electrolysis, have all been consistent with, but not proof of, its assumed enzyme character."

The phage classification can be made only by comparison with other known ferments. First consider its character.

1. Enzymes and ferments disintegrate and build

up chemical compounds for purposes of body building. The ability to dissolve microbes and propagate mark the phage as a ferment.

2. The ferment attaches itself to, or enters, the specific antigen before action begins, as is shown by the diptheria antitoxin to the diptheria bacillus. The organism becomes, in a word, sensitized. This action of the phage is well known.

3. Enzymes and bacteriophage are mostly specific in their actions.

4. Neither the enzyme or phage can be cultivated on artificial media.

5. Both work better in acute than in chronic cases.

6. Both are attracted to the site of infection regardless of the site of injection. (18).

Before going further, the role of hormones and vitamins as the compliment in these functions must be considered. On this basis was developed the compliment fixation reaction. In the absence of the compliment, lysis fails to occur. The bodies are present and are ready to act only when the compliment is present. Let us observe the rabbit to which is fed food lacking invitamins. It quickly dies. When the vitamins are supplied it lives. Or, when it is allowed to feed upon its excrement, it survives, - ample evidence that the essential chemicals are being supplied. Then it is found that the colon bacilli synthesize sufficient vitamins to maintain the proper balance essential to life. Similar results may be obtained when a gland such as the thyroid is removed. Any amount and quality of food may be provided but the patient expires because the hormone 'thyroxin' is lacking to influence the chemical balance of the organism. If this action can be demonstrated to affect the highly developed ferments down to the lowest organic ferment, then some such influence must prevail in the type of life, the simplest form, characterized by the bacteriophage. This statement is not without substantiation, providing, of course, the foregoing hypotheses have some basis, for which time is judge. (18).

Reference was made to the fact that both ferment and phage work better in acute than in chronic infections. In the acute infection, metabolism is increased considerably, the ferments are at work feverishly breaking down the protein molecules. The compliment, hormone, is abundantly present to stimulate action. Eventually the supply diminishes, due to the increase in disintegration of the materials, from which ferments procreate, over the reconstruction of such materials. Bacteriophage depends upon identical situations. This has been shown by clinical evidence. In the presence of bacteria, the phage rapidly and completely destroys its host, because of the presence of the compliment produced by the bacteria. When the bacteria are destroyed so is the source of compliment and the phage becomes inert. (18)

It is not preposterous to assume, then, that there exists such a relationship as that between enzyme, hormone and phage. It is not too fantastic then to imagine that the inorganic catalysts, constantly at work disintegrating and rebuilding metalic atoms and molecules, could add to its molecule a particle of atom to which it is in contact and gradually have other particles find an especially snug nook in its molecule until, with the passage of time, the catalyst becomes more complex and passes from the inorganic to the organic state. Then, with the complexity increased, the catalyst finds it more suitable to afford protection by cementing its components together by a mass of protoplasm, until there arises a unit composed of many chemical functions. (18)

Of course this picture is lacking abundantly in scientific proof, but it is no more fallible than the numerous and varied theories on the origin of life of the present day, and it certainly provides one more link, weak as it may appear, to the chain of evolution. Perhaps phage is neither living or inert, but whether it be particulate, enzyme or abnormal bacterial product, it has certain measurable properties.

V.

PHYSICAL AND CHEMICAL CHARACTERISTICS

The phage is filterable. d'Herelle found it would pass through ultra-fine filters. Twort (3) contributed much to prove its filterability. Upon this property was determined the size of the phage. By passage of the phage through collodion membranes of known mesh size, the phage was shown to vary from 10 milli-microns to 75 milli-microns in size. Considerable work by Elford and Andrews (20) substantiated this observation. Charnock (10) states that the phage is about 5 milli-microns in size. Kreugar's work determined the size varied from 10 milli-microns to 75 milli-microns and set the average at about 20 milli-microns. It is evident that the phage varies in proportion, or that definite evidence as to this property is as yet lacking in one or more respects. Frequent mention of this inconsistency is made in the literature.

Many writers give to phage the property of propagation. d'Herelle showed the phage increased at the expense of the bacteria undergoing lysis. Kreugar and Northrop (21) stated that lysis of cells occured in concentrated phage suspensions without occasioning an increase in phage concentration. Later Kreugar (7) writes that the rate of phage production is greater than bacterial reproduction. Thus the ratio of phage to bacteria is greater and is constantly increasing. That it increases in the presence of normal living bacteria is now generally accepted.

There are frequent references to the property of adaptability of the phage. d'Herelle and others demonstrated that phage resistant bacteria, by a series of transfers, could be made phage susceptible. He intimated that the protobes were capable of compensating for the environment surrounding them. Colvin's (22) work reveals that adaptation was not definitely shown, but does not deny the possibility that adaptation may occur. Schultz and Green (23) showed it became resistant to trypsin after a series of exposures. Asheslov (24) lamented that the adaptation to increased hydrogen ion concentration was shown with one phage only. Beard (25) was unable to demonstrate adaptability by any means. Kreugar (7) states that the bacteriophage is not capable of adaptability. Colvin (22) came to the same conclusion.

A property already referred to, transmissibility, has been shown by several investigators since d'Herelle. Recently Lemar (26) showed that the phage potency could be enhanced by serial transfer. This is an important factor in the preparation of bacteriophage for use in therapy.

Heat and cold are not without definite effects. d'Herelle stated that most bacteriophages are resistant to temperatures ranging up to 72-75° C., at which they are destroyed within thirty minutes. For example, the hemolytic Staphylococcus phage is destroyed when exposed at 60 ° C. for 30 minutes. In general, temperatures of 45° C. to the thermal death point inactivate, though not destroy, the phage. Lysis of organisms will occur within temperatures ranging from 8° C. to 45° C. Lack of lysis below 8° C. is believed to be due to the effect upon the bacteria, and above 45° C. due to the effect upon the lysin (phage). Charnock (10) states that the optimum temperature for activity varies with the optimum temperature for development of the bacteria being lysed. Nerb (27) believes that bacteriophage produced under conditions of heat has a higher titer and is more potent than that produced by cold. The culture is incubated for three hours at 32° C. - then placed in a water bath at 52° C. Lysis takes place within thirty minutes. If more time is consumed, young, more virulent organisms are added to the culture and returned to the same temperature. In this way more potent phage of higher titer may be produced in a shorter time.

Burnet and McKie (28) utilized a combination of heat and salt and found that heat inactivation which occurs readily in the presence of sodium, potassium and ammonium salts, is partially prevented by the addition of small amounts of calcium, magnesium and barium salts. This is an example of the complexity of the physical and chemical properties of bacteriophage. This reaction suggests that denaturation is an important phase of the phenomena and tallies a score in favor of its protein composition.

d'Herelle considered the composition of phage to consist of bacteria, proteins, fats, bacterial products, culture media and the lytic agent itself. Schlesinger (29) believed the phage to be a protein substance, and more specifically a nucleo protein. Hetler and Bronfenbrenner (30) were of the same opinion. Meyer, Thompson, Khorazo and Palmer (31) found that one preparation of phage contained 5.5% nitrogen and that in 1% Xanthoproteic, Greenburg-phenol reaction, and Molish test, the reaction was positive. Kreugar and Tamada (32) were able to extract nitrogen from the phage but no trace of protein. Clifton (33) was unable to produce a protein color reaction. Arnold and Weiss (34) were also unsuccessful in this respect. This failure to prove the presence of protein does not fully answer the question. It is possible that protein may be present in such minute amounts that our methods of detection do not discover them.(7)

Kabishima (16)* was of the opinion that the phage

* quoted from (7)

was related to the lipids. He reported that it was soluble in ether and chloroform. d'Herelle does not agree. Lemar (26) succeeded in extracting the phage from aqueous solutions with ether.

Whether it be colloid (20) or crystalloid (35) has been a paramount issue among recent writers. The effect this controversy has on many of the reactions claimed for the bacteriophage is evident. (7). There are suggestions in the literature to the fact that the phage is capable of transformation to crystalloid or colloid, as the situation demands. (35)

Among other physico-chemical properties which have been investigated is the effect of pH on the action of phage. d'Herelle's work set the range at 6.8 to 7.5. Clifton and Madison (36) in their work found a range from 2.1 to 7.6 in which the phenomenon was satisfactorily demonstrated. Charnock (10) states that the process of bacteriophage goes best in an alkaline medium of pH 7.2 to 8.2. Nerb (27) found that the phage on Savita medium flourished at a pH of 7.5.

The phage stimulates bacterial metabolism; respiration is increased during bacteriophagy, and free exposure to air accelerates lysis. (2) (37) (30). Eaton (38) found that oxygen was consumed and carbon dioxide produced during the action, and that even after death, oxygen consumption was maintained for 10 hours. Nerb (27) also mentions this finding. LeMar and Myers (26) found that there was stimulation of bacterial growth of the bacteria rather than stimulation of lysis. Kreugar and Northrop (21) denied any stimulation of bacterial growth.

VI.

E F F E C T OF A N T I S E P T I C S

Antiseptics inhibit bacteriophage. d'Herelle (2) has made a thorough study of this subject, experimenting with a number of antiseptics and their component chemicals, too numerous to mention. He found that the concentration limiting bacteriophage varies from 0.05% to 0.25% for each species studied.

> "This result might have been predicted for although the bacteriophage remains passive, it is certainly not because the antiseptic used had an effect upon it, but because it modifies the state of the bacterium. This conclusion is supported by the fact that in bouillon containing flouride the bacteria do not grow normally but in agglutinated clumps. These observations may be summarized very simply by stating that bacteriophagy is operative only with living and normal bacteria." (2)

Nerb (27) concludes that preservatives should be avoided. Kreugar (7) states that the production of phage is not dependent upon lysis alone, but upon bacterial reproduction, and anything which interfers with the latter, interfers with phage production. Bronfenbrenner and Muckenfuss (39) showed that the number of living bacteria in the culture controlled the speed and extent of lysis. Charnock (10) writes that dye stuffs alter bacteriophage. This statement is to be taken seriously if an understanding of the mechanism of phagy is shought. Ignorance of this fact and impatience are responsible for the many failures of the phage. Yet, in spite of these obstacles, successes have been recorded.

VII.

EFFECT OF BODY FLUIDS

"When added to a bacterium-bacteriophage mixture, substances devoid of action upon the bacteria have in general no effect upon the phenomenon. Thus the process is not modified, for example, by normal serum, acitic fluids or urine. Bile is unquestionably inhibitory." (2)

This statement has been repeatedly attacked by numerous investigators and abundant evidence brought out against its integrity. However, its stimulating effect has reaped much fruitful work along these lines.

Colvin (22) was apparently aware of the evasiveness of facts concerning the phage when he wrote:

"Since the characteristics of races of the bacteriophage are so variable, only a variety of experiments can lead to any general conclusions concerning the effect of tissues or body fluids."

Presumably the first reference to the inhibition of lysis by normal serum was made by Gratia and Jauman. (40). They found that there existed a nonspecific transient inhibition of lysis in a 1:500 solution of serum.

Colvin (22) studies the effect of many different serums on phage activity and found they all produced an inhibitory effect. Undiluted citrated blood yielded about the same results. He also found that urine, pus, acitic fluids and cerebro-spinal fluids exerted similar effects.

Appelbaum and McNeal (41) published studies on the effect of pus and blood on the phage phenomenon. These studies were suggested by the fact that reactions taking place in the test tube, in vitro, did not necessarily occur in vivo. The result was that purulent fluids inhibited phagy even after heating to 60° C., and led to an exhaustive investigation of the physico-chemical cause of this inhibitive action, which will be discussed later. Evans (42) found that the serum portion of whole blood was markedly inhibitory to lysis of streptococcus, staphylococcus, typhoid bacillus and colon bacillus. Riding (43) found that gastric secretions had no inhibitory effect, while autoclaved 25% intestinal mucous prevented complete lysis. He also substantiated Colvin's work on serums.

Caublot (44) stored bacteriophage in bile for 5 months after which the phage remained unchanged.

Dresel and Lewis (45) experimented with tissue cultures from the mouse and chicken. The hanging drop revealed no lysis though the phage remained in the tissues after eleven days and was still active. Conclusion was little of no effect. They state that the amount of inhibition depends upon the concentration of fluid medium. These experiments practically reverse the statement made by d'Herelle (2), but it must be remembered that the bacteriophage is delicate and identical results are not forthcoming unless investigation is carried on under exacting conditions.

VIII.

THE MECHANISM OF BACTERIOPHAGY

The mechanism of phage action is covered by abundant literature, though conclusions must be regarded with apprehension. A cross section of this work may be presented here.

d'Herelle (2) believed that the phage, when present with normal living bacteria, accosted the organism (fixation) and multiplied, causing the organism to swell and burst by explosive distention. Hetler and Bronfenbrenner (30) (37) showed by a series of moving pictures that swelling occured as evidenced by the appearance of large granules within the bacterial cell, followed by fragmentation with debris, visible throughout the field, which finally disappeared. Kreugar (7) presents literature describing the explosion as leaving a waste of cellular debris and a measurable increased viscosity of the medium. A study of lysis on bacillus coli and bacillus megatherum reveals that swelling occured in the coli organisms but was not present in bacillus megatherum. Bronfenbrenner, Muckenfuss and Hetler (37) were unable to demonstrate explosive lysis. Burnet (46) revealed that lysis occured without swelling.

Reference has been made to the compliment activating bacteriophagy. Herb (18) believes this to be a hormone. Colvin (22) infers that there is some influence exerted by protein.

> "Although there is a tendency to inhibit or modify phage lysis in serum, it is quite possible that each factor might so exist on rare occasions as to permit complete lysis, especially in fluids of low protein or no protein. Evidently lysis is prevented even though the lytic particle has become attached to the specific organism and as the organism grows the serum is altered to permit lysis."

Larkum (35) (47) opined that in serum the protein fraction is the main factor in inhibition while in urine the crystalloid fraction inhibits. A series of papers by Burnet (48) (49) (50) revealed that the susceptibility to lysis by phage was more or less directly related to the antigenic structure of the bacteria concerned. This structure is related to the polysaccharides. (51). Colvin (22) makes the statement that bacterial sensitization is due to the phage only. This question has run the guantlet of scientific investigation and at present remains indefinitely answered.

Kreugar (7) set down four stages by which the phage acted. The first consisted of fixation of the phage to the bacterial cell. The second provided increased metabolism in the organism - much as that occuring in the tissue cells in the presence of foreign bodies. The third revealed the swollen cells with the presence of granules, and the fourth stage was that of lysis. He believed that this phenomenon was more active during cell division, thereby rendering the phage more susceptible.

Kreugar and Northrop (21) state that there exists experimental evidence indicating that lysis is not a continuous process during the time of phage formation and bacterial reproduction but rather that it begins suddenly when the critical ratio of phage to bacteria has been built up in the system. Bronfenbrenner, Muckenfuss and Hetler (37) state that there is an increased activity in bacterial growth but lysis does not appear until absorption of the phage becomes complete. Schlesinger (52) states that during action about 95% of total phage is bound quickly and irreversibly by the bacteria, which are soon destroyed. A second portion is bound more slowly and reversibly, while a third fraction is not bound at all. Charnock (10) sums up the literature by stating that bacteriophagy takes place during the division of the organism. The addition of phage to a growing bacteria culture speeds up bacterial multiplication, accompanied by an influx of fluids, and bursts. This destruction of bacteria and regeneration of phage represents the mechanism of bacteriophagy.

This presentation of compiled materials by no means comprises the extent of literature on this phenomenon. The subject is much too broad to be covered in one paper, for the field of bacteriophagy is as far reaching as it is complex, and to date there are few definite conclusions to be extracted.

IX.

B A C T E R I O P H A G E IN T H E R A P Y

> "Therapy with bacteriophage is fast becoming important. As each new fact is added, the narrower becomes the gap between the theoretical and the practical. In the future it may become our cornerstone of defense against infection." (10)

The phage has been frequently applied in therapy promiscuously, and unjustly, too, for the protobes described by d'Herelle have been found to possess a marked degree of specificity. This property led Bail (53) to attempt a classification by employing the three criteria of plaque size, resistance, and serologic specificity. (54). This work was first done with the antidysentery phage, and confirmed by Burnet and McKie (55). d'Herelle and Rakieten (15) deplored the use of colony plaques because they found that a phage exhibiting three variants or mutants was capable of producing three sizes of plaques. These mutations are based on practically any influence which may occur during the stages of bacteriophagy. (54) (56) (55). Therapeutically we are only interested in classification as it applies to the virulence of the phage against an undesirable bacteria.

Twort (1) in his early works suggested the application of bacteriophage to disease, but consideration waned until d'Herelle (2) noticed the presence of phage in the stools of a patient convalescing from bacillary dysentary. This experience prompted him to the investigation of its use in infections. He made an exhaustive study of this problem and concluded that the absolute effectiveness of the phage was confined against the staphylococcus and colon bacillus with probable success in the future against plague.

According to experiences up to the present time, the affections most suited for bacteriophage treatment are staphylococci infections, infectious diseases of the digestive tract when evoked by the typhoid-paratyphoid-dysentary group, and coli infection of the urinary passages. (57). Other results were encouraging but as yet remain unproved. Since then contributions in the literature have been most abundant but little change has resulted.

Eaton and Bayne-Jones (58) present a very complete review of the literature dealing with the application of phage in urinary infections, (35) (59) (61) (60), typhoid and paratyphoid, (62) (63) (64) (65) (57), bacillus dysentery, (4) (14) (65) (57), cholera, (66) (58), plague (2) (results inconclusive), staphylococcus infections (67) (68) (69) (70) (71), and streptococcus infections (72) (73) (74) (75), all of which d'Herelle so thoroughly exploited.

These references from the literature do in no manner represent an exhaustive survey of the literature, but are merely examples selected at random. Naturally there are statements regarding failures and doubtful results. All in all, however, the scales tip considerably in favor of bacteriophage thereapy.

The inconsistency in therapeutic results is perhaps due to our limited knowledge of the nature and action of the phage, and certainly because of the lack of experimental check on laboratory animals. (40) (70)

> "It has been the general experience in medical research that therapeutic agents which are effective in human disease have previously been found effective in suitably conducted laboratory experiments on animals. This is not true of the bacteriophage." (58)

Albee (76), however, contends that bacteriophage is effective in experimental animals if injected away from the site of infection. In spite of this handicap great strides have been made in the treatment of infections with bacteriophage.

Like infection, it may respond in accordance with one of three reactions: the acute reaction in which lysis occurs promptly and healing is rapid; the chronic reaction whereby a symbiosis between bacteriophage and bacterial organism takes place and the disease becomes one of long duration; or the resistant reaction wherein the bacteria become immune to the lysis by phage. (7) McNeal and Frisbee (77) believe that the action of phage is not due to lysis but to sensitization of bacteria so that they become more susceptible to phagocytosis.

It may excite immunity within the body. This type of immunity is classified as temporary or permanent exogenous and endogenous immunity. It is temporary if, after the appearance of the phage, there is no reinfection to facilitate propagation or multiplication of the phage; and it is permanent if reinfection or contamination occurs creating a system of serial transfer in vivo. Endogenous immunity is that resistance or antibody response set up as the result of the antigenic lytic products. (7) (78). Arnold and Weiss (34) state that the only antibodies produced are antilysins. The above statements are hypothetical, though they do occasionally substantiate clinical observations.

Bear in mind that bacteriophage is not advocated as a specific in the treatment of any disease. It may be considered an adjunct or a contributing factor to healing. Albee and Patterson (79) showed that bacteriophage against the staphylococcus was an additional factor in promoting healing of wounds in general and particularly in those of osteomyelitis.

Soule (80) stated that there is no data to indicate that bacteriophage is on a sufficiently sound basis to warrant its unrestrained exploitation or promiscuous use. Hauduroy (65), after a series of therapeutic investigations, concludes that it is better, in the interest of the further development of this therapy, not to use it at all, rather than to use in the wrong place or to make it a panacea.

Though there is little evidence in the literature of a contraindication for the use of bacteriophage, the dangers of commercial exploitation constitute an ever-mindful hazard in the realm of medical thereapy. Many of the experimental failures and doubtful successes have been traced to commercial products, advertised as bacteriophage, containing preservatives inhibitory or lethal to the phage. (81)

To obtain satisfactory results the bacteriophage must satisfy these requirements: (a) specificity, (b) freshly prepared, (c) steril fluid medium.

X.

METHOD OF APPLICATION AND PREPARATION

The use of bacteriophage varies so widely in the hands of different therapists, that in lieu of these investigations, conclusions drawn from personal experience are deserving of more consideration. Suffice it to say here that all routes of administration have been utilized. Investigators have applied bacteriophage by all these routes, intravenously, subsutaneously, intramuscularly, and orally, and find that, with the exception of one, the bacteriophage is a valuable therapeutic agent. (58) Hauduroy (65) and Bagley and Keller (58) find that the intravenous administration is to be condemned. The generally accepted use is that of application nearest the site of infection.

There have been many techniques advanced for the preparation of the bacteriophage. A fair representation of such methods may be found in Feemster's article (82) 'Bacteriophage in Intestinal Infections'.

The simplest method of isolation is to place 5 to 10 cc. of plain broth in contact with not more than 1 gm. of feces and after 15 to 30 minutes decant into a Berkefeld filter and pass through an "N" candle. To a tube of broth seeded lightly with the organism causing the outbreak, or with a stock strain, add 0.5 cc. of the filtrate from the stool and incubate 18 to 24 hours.

A control tube containing only the organism should be set up. If a strong bacteriophage is present, the tube containing the filtrate may be clear while the control will be turbid. If the bacteriophage is weak, no clearing may appear in which case it is necessary to make sure no bacteriophage is present.. There are three methods by which this may be done.

1. The most accurate method of checking up on the doubtful tubes is to transfer 0.05 cc. to an 18 hour broth culture of the organism, thoroughly mix, and then smear 0.1 cc. evenly over the surface of an agar plate. This is best done with a bent glass rod sterilized by dipping in 95% alcohol two or three times, each time allowing the alcohol to burn. The inoculating culture should be heavy enough to produce a complete film of growth, with no separate colonies visible. If bacteriophage is present, plaques will show in the bacterial film on the surface of the plate. Sometimes, however, a very active bacteriophage may completely inhibit growth, and there will be practically no colonies on the plate.

2. Another method is to smear a loopful of broth from the doubtful tube and another loopful from the control, each on separate spots about 1 cm. in diameter or larger, on an agar plate. Lysis will be indicated by

(1) no growth; (2) a scanty growth; or (3) plaques in the bacterial film. The amount of reduction in growth can be determined by comparing with the control.

3. The third way to check up on the tubes showing no clearing is to seed thoroughly the surface of an agar plate with the organism. Then with a loopful of material from the tube make a circle on the surface of the agar. Make one also with the control for comparison. If bacteriophage is present, it will be indicated on the ring by more or less inhibition of growth of the organism or by a few well defined plaques. Usually the bacteriophage particles are much more numerous at 18 to 24 hours than the resistant organisms which have begun to grow out and the latter do not interfere materially in interpreting the results on the plates.

It is possible to isolate bacteriophage without filtering the material from the stool through a Berkefeld filter. The percentage of isolation will not be high however, since only the most active ones will be recovered in the presence of the rapidly growing and spreading bacteria. The stool specimen should be placed in contact with broth as outlined above, and 0.5 cc. of the broth placed in an 18 hour broth culture of the organism causing the outbreak. After 12 to 24 hours incubation, the tube is placed in a water bath and the temperature held at 60 degrees C. for a half hour. This will usually kill the vegetative bacteria and not harm the bacteriophage. A very small loopful of the material is then placed in an 18 hour broth culture of the organism and immediately plated out as described above. The presence of bacteriophage will be shown by plaques or by complete inhibition of growth.(82)

XI.

BACTERIOPHAGE IN OSTEOMYELITIS

Realizing, then, the circumstances under which phage exists, a survey of its relation to the treatment of osteomyelitis may be profitably considered. The use of bacteriophage in the treatment of osteomyelitis has been limited. (74). McKinley (83) reports four cases of osteomyelitis with rapid healings following the use of bacteriophage injected into and about the wound. Mocquot and Monard (84) treated two cases of osteitis with "vaccino-therapie-locale" bothered with persistent fistulae following operation for osteomyelitis of the tibia. Claeys and Peyre (85) reported two cases, that of a child who was cured in 40 days by the application of auto-bacteriophage to the wound following operation, and that of an adult with chronic osteomyelitis for 12 years, who had submitted to 14 operation, and was cured in five months by the use of bacteriophage.

To digress for a moment let us scan the formidable work performed by Orr. In a search for a more satisfactory treatment for osteomyelitis, he reclaimed the teachings of such historically prominent surgeons as Hunter and Lister, advocating asepsis, early drainage and prompt immobilization. (86) His plan began to take form during the Great War when the devastating mortality of gunshot fractures was noted. (87) In 1921 Orr (88) introduced the method which was to become widely known as the Orr Method in the Treatment of Osteomyelitis. A brief general outline of this treatment provides for immediate immobilization and traction on the operating table, secondary dressings requiring anesthesia if necessary; a thorough debridement or drainage operation if necessary; packing the entire wound open with sterile vaseline gauze, using no sutures, drainage tubes, or antiseptic dressings, to minimize infection; the application of an extensive well fitting plaster of paris cast; infrequent dressing except for certain definite complications such as continued sepsis and hemorrhage. Orr (87) maintains this constitutes a return to Listerian principles. These fundamentals which comprise the Orr treatment of today have gained widespread recognition because of the remarkably successful results dependent on them.

In the meantime Albee (89) became interested and reasoned that in spite of Orr's success, the apparent interference to free drainage by the use of vaseline gauze packing, the wide excision (trauma) of the wound, and the bathing of the limb in foul pus, while in the cast, were not consistent with the contention of success due to the fundamental requirements, immobilization and traction, debridement and drainage, minimizing reinfection, and infrequent dressings. Albee knew that bacteriophage accumulates as the conditions for bacteria become more favorable, and that any condition where, tension relieved, an allowance for the accumulation of the phage was ideal. This is exactly what Orr provides in his treatment. Albee (76) was inspired by d'Herelle's (2) experiment in which he planted .0001 cc. of bacterial culture in a young broth culture and transplanted to an agar slant. Luxuriant growth appeared, followed by minute opaque plaques. Then by inoculating a bacterial culture in a broth medium and transplanting an infinitesimal amount of material from one of the clear plaques into the broth and incubating for a few hours, a phenomenal clearing took place. Having learned of this spectacle in vitro, he wondered if such action could be going on in Orr's treatment. The analogy to the phage treated wound became obvious and Albee (73) (90) promptly began a series of investigations which revealed that phage spontaneously appeared in 94% of his cases. He then began to add autogenous phage to the wound at intervals, and noted that convalescence was shortened by several days and, in some cases, a matter of weeks, and when phage was absent recovery was slow. (91)

Orr (92), though not particularly concerned, admitted that bacteriophage may be an important factor in his treatment and stated that:—

> "maggots and bacteriophage may be of interest for what they can do in the wound but that the chief merit of their use lies in the fact that in the attempt to use them therapeutically, drainage tubes, chemical antiseptics and frequent dressing have been discontinued." (93)

However, in a recent communication, Orr revealed his rising enthusiasm for Albee's work and feels that enlightenment on this question may result from future investigations. Bagley and Keller (74) found that bacteriophage was successful in cases of staphylococcal osteomyelitis only when such cases were uncomplicated. When this occured they resorted to other treatment, though this practice is not consistent with Orr's teachings.

Of interest is the work of Livingston (94) who found that in the treatment of osteomyelitis with maggots the discharge consisted of serum, bacteria, pus, and a healing secretion believed to be bacteriophage.

Kirschenmann (95) treated eleven cases of osteomyelitis involving five different bones, the humerous, ulna, phalanges, tibia and toe, with a phage which he refers to as 'stock bacteriophage'. All recovered, and four cases would have previously been amputated.

Rankin (96), referring to Albee's (89) phage treatment, criticizes its use because it is eliminated from the body within 24 hours, repeated doses stimulate the production of antiphage, and the vaseline pack limits the necessary oxygen requirement for phage production. He also maintains that the dosage should not be increased because of its toxicity. Summing his statements he accepts the Orr treatment as ideal.

These statements, in effect, have more or less contradicted the literature presented. The first assertion is somewhat substantiated (35), but does not apply to the work of d'Herelle and others, including that of Albee, in which was shown that the presence of bacteriophage in convalescent patients was spontaneous. Jern (97) and her associates found that the potency of the phage in the wound decreased, and urged that it be repeatedly renewed. Repeated doses of phage do stimulate production of antiphage according to Arnold and Weiss (34) and in chronic resistant stages this appears plausible, but to make this statement a contraindication to its use is a fallacy as shown by Albee (90) and others that acute and subacute cases respond well to this treatment. The criticism to the vaseline pack would appear unfounded when considering the work of Dresel and Lewis (45) who found that there was nothing of inhibitory effect against the phage in tissues. It would seem then that the oxygen required in tissue metabolism is sufficient to permit the normal action of bacteriophage.

As for the toxic reactions resulting from the use of bacteriophage, the conclusion of Eaton and Bayne-Jones (58) may suffice to answer this statement:

> "The material called bacteriophage is usually a filtrate of dissolved organisms, containing, in addition to the lytic principle, antigenic bacterial substances, products of bacterial growth and constituents of the culture medium. The effects of all these components must be taken into consideration whenever therapeutic action is tested."

Jern (97) and associates have found that the administration of bacteriophage is never harmful. Frisbee and MacNeal (77) have used the fluid medium 'asparigin' in a series of cases, without noting serious reactions.

Rankin (96) concludes that the Orr method of treatment is ideal. In view of Albee's work on the relation of Orr's treatment to the phenomenon of bacteriophagy, Rankin's statements do not seem consistent.

XII.

C O M M E N T

Thus there is written in the literature of a man who advocated a treatment upon clinical observations and results and of another who partially substantiated the former's reasoning through the facilities of the laboratory. Through the years, the use of bacteriophage has been discarded and revived again, as investigation replaced the theoretical with the practical. It is believed that with progress in experimentation and improvement of the means of investigation, the bacteriophage will assume even more practical importance in the field of therapy. The failure of the laboratory to support the clinical and surgical results is not an indication for disapproval.

> "If the results are a step ahead of bacteriology, then one has a right to ask the bacteriologist to square his science with surgical practice." (89)

XIII.

C O N C L U S I O N

1. The Nature of the bacteriophage is as yet unknown although current information seems to point to the character of an enzyme. The work of Herb and Kreugar is strongly affirmative with this hypothesis.

2. The exact action of phage is that of lysis, though the intimate mechanism remains a problem of controversy. The writer is inclined to lean toward a "lytic-sensitization for phagocytosis" explanation, believing that the biological basis is firm enough to support this contention.

3. The success of the bacteriophage depends partly upon its purity and specificity. Obviously, a phage lytic for Staphylococci will not be so effective against the Streptococcus. It is better to use the agent in a medium as free as possible from materials which are apt to produce toxic reactions. Such reactions often obscure the clinical action of bacteriophage and necessitate an element of apprehension in eliciting definite conclusions.

4. The use of antiseptics with bacteriophage is a practice to be condemned if satisfactory results are to be expected. This has been no mean factor in many of the failures reported for the use of bacteriophage.

5. The phage works best in the early stages of disease. It is believed that in the chronic stage of

disease, the bacteria set up a condition approaching symbiosis, thus similarly influencing the action of bacteriophage.

6. In the hands of competent therapists, the true value of bacteriophage is revealed. Mention has been made concerning the inefficacy of the agent, and there is little doubt that the phage is of value only in certain selected cases. The promiscuous use of bacteriophage will condemn, more than anything else, its use in therapy.

7. The success of the Orr treatment is due in part to the phenomenon of bacteriophagy.

8. Emphasis must be placed on a thorough knowledge of bacteriophagy, as an essential requirement, to successfully and adequately evaluate the indications for, and the results of, the use of bacteriophage in the treatment of disease.

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