USES OF BACTERIOPHAGE IN STAPHYLOCOCCIC INFECTIONS

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Bacteriology is, comparatively speaking, a new science. During the short course of its existence, however, it has yielded an abundance of useful information and is now one of the most important of the basic medical sciences. Through bacteriology, the specific factors in the etiology of many previously obscure conditions have been disclosed; effective therapy, active and prophylactic, has been made possible in many instances; prognosis has been made more intelligent and rational. Every branch of clinical medicine has been perceptibly and favorably affected by the knowledge supplied through the study of this new science.

Immunology, the study of ways and means of protecting the body against bacterial invasion, is undoubtedly one of the most important of the contributions of bacteriology to clinical medicine. Bacteriology has afforded the basis for theoretical conceptions and actual accomplishments in immunology which have resulted in the frequent concept of immunology as a special science although it is essentially a component of the broader sciences of bacteriology. The study of immunity has opened a field so vast that it offers practically unlimited opportunities for research into problems but little understood as yet.

It is the writer's purpose to discuss but one phase of immunology and to submit impressions and experiences in the making and use of bacteriophages.

It is of interest to note that certain immunologic principles were utilized by primitive peoples, thousands of years ago. Some are known to have treated the bites of poisonous snakes by the application of a paste containing the specific poison of the offending reptile. It is said to have been a common practice among ancient Chinese people to remove the crusts from smallpox lesions and to apply these crusts to the nasal mucous membranes of unaffected persons, thereby effecting immunization through a rudimentary method of innoculation. Other immunologic practices have been recorded, procedures which were effective although empiric. The step between ancient empiricism and

modern rationalism and specificity in immunologic methods was a long one. The dawn of modern immunology begins with the time of Louis Pasteur.

Pasteur it was who conceived the idea that invading bacteria might be led to bring about their own destruction. His early work was done with the anthrax bacillus which was at that time a menace to the French sheep breeders and a serious economic problem to the nation. Through experimentation and practical application, he demonstrated that anthrax could be eradicated by vaccination. Thus were the basic principles of immunology established. Metchnikoff, a countryman of Pasteur elaborated on Pasteur's work and gave it a broader significance. Under the microscope he observed that leukocytes possessed the property of destroying and absorbing debris and bacteria and he it was that applied to them the name "phagocytes" from the Greek term meaning "eating cells."

Scientific experimentation requires a certain understanding of the underlying factors or principles of the problems under investigation. Although Pasteur and his followers had unfolded the basic principles of immunity, it remained for the German scholar, Ehrlich, to evolve a workable and understandable theory of their mode of action. He projected his famous "side-chain theory" which, while it may have been incomplete or imperfect, was plausible and understandable and stimulated experimentation which has resulted in many invaluable contributions to the advancement of clinical medicine in general and bacteriology in particular. Without such a working-rule as the Ehrlich theory, many valuable discoveries (such as the Wassermann reaction, based on the principle of complement fixation) would probably not have been possible.

One year before the death of Ehrlich in 1916, Twort reported his remarkable discovery, known as "Twort's Phenomenon." This was that bacterial cultures contain ultramicroscopic particles, developed by the organisms in the culture, which are capable of destroying the bacterial growth. Two years later, in 1917, the French scientist d'Herelle corroborated Twort's findings and, by improving upon his procedures, succeeded in elaborating a practically available substance which was capable of counteracting the activity of the bacteria in the culture from which it was isolated. These particles were regarded as ultramicroscopic bacteria by d'Herelle and he called his preparation a "bacteriophage"—something which eats or destroys bacteria.

Since d'Herelle made his first report on bacteriophage in 1917, more than two thousand papers have been published on the subject. Notable among these contributions is the extensive work of Besredka

whose procedures varied somewhat from those of d'Herelle. Besredka used old cultures to obtain the substance which he termed "antivirus" but d'Herelle and his followers used very young, actively growing bacteria. Arnold, Bordet, and others, with whom we are inclined to agree, believed that the ultramicroscopic substance known as bacteriophage or antivirus is not a living substance but a ferment. Other valuable reports are those of Rice, Larkum, and Bozy.

Early investigators in bacteriology observed certain cultural irregularities in bacterial growth and noted that these irregularities were especially evident with bacteria from the intestinal tract. With the development of knowledge concerning bacteriophages, it was found possible in many cases, to isolate a phage from the feces of patients convalescing from infection, and also from sewage. This probably explains these cultural irregularities. Arnold, of the Illinois Public Health Department, has isolated a phage from Chicago River. Such discoveries are of clinical importance and open wide fields for the future of bacteriophage research.

When the use of a bacteriophage is contemplated in the treatment of an infection, it is of the utmost importance to first ascertain whether or not the available phage is active against the specific infecting bacteria. By obtaining a culture from the infected area, the efficacy of the phage may be tested in vitro before being used in vivo. The following method, a simple and feasible one, is used:

Make two cultures, one in 50 cc. of broth and one on plain agar. Incubate the agar culture for 12 to 18 hours.

Incubate the broth culture for 4 hours, then divide it among a number of sterile tubes, depending on how many tubes are to be tested.

Label one of these tubes "Control" and add nothing to it.

To each of the other tubes, add one-tenth volume of the phage to be tested. Label them properly for identification.

Incubate all the tubes for 12 to 18 hours or longer.

If any of the phages used are effective against the organism being tested, the cultures to which these tubes have been added will be clear. The control tube should be cloudy, indicating unimpeded growth.

Varying degrees of clearness may be observed in the different tubes. In such a case, the phage which has produced the clearest solution should be chosen for clinical use.

If none of the phages used will clear up the culture, it is an indication that none of them could be expected to be clinically effective. An autogenous bacteriophage should then be prepared. This may be accomplished through the "stepping-up process" as follows:

Make two cultures, one in broth and one in an agar tube.

Incubate both for 18 hours.

Filter the broth culture through a Berkfeld filter.

Innoculate the surface of an agar plate with a loop of filtrate and a loop from the agar tube.

Incubate the agar plate for 18 to 24 hours.

Pick out the abnormal colonies, those with the 'chewed' edges, and transfer them to another broth culture.

Incubate the new broth culture for 4 to 6 hours.

Add one-tenth volume of the first filtrate to the broth.

Incubate again for 24 to 72 hours.

If the broth becomes clear, it contains a specific bacteriophage; if not, the process may be repeated until it becomes active.

Filter the clear broth through a Berkfeld filter.

The filtrate contains the bacteriophage in the form for clinical use.

While it is extremely desirable, as mentioned before, that the available phages be tested in vitro against a culture of the specific offending organism before being applied clinically, it is not always possible or practicable to do so. Many conditions may make the delay inadvisable. A polyvalent bacteriophage may then be tried. The use of such preparations will not be uniformly successful, but they can do no harm and will succeed in many cases, provided the diagnosis of the type of organism is correct and the polyvalent phage contains the antagonistic substance for the particular strain. We now have a polyvalent bacteriophage which is effective against more than thirty strains of staphylococci and which has proved effectual in a number of cases where cultures of the infection organisms were not available.

Clinical Application

Most of our experience with the use of bacteriophages has been limited to its application locally at the site of infection and principally in the presence of staphylococcic infections. In some cases it has also been intramuscularly injected for a systemic effect. Local physicians have used our stock preparation and autogenous phages in the treatment of such varied conditions as infections in the external auditory canal and the nose, simple furuncles, multiple and persistent furunculosis, carbuncles on the neck and buttocks, secondary abscesses, etc. Their usual method of procedure is as follows:

About 1 cc. of the broth containing the phage is injected directly into the infected area.

A gauze or cotton dressing, saturated with the solution, is placed over the infected area, covered with oiled silk and held in position by a bandage or adhesive strips.

The dressing is kept moist by periodically saturating it with more of the solution.

When the infection is in the ear canal or the nose, pledgets of cotton saturated with the solution are left in the cavities.

Intramuscular or intradermal injections of the solution may be used in addition to the local treatment when indicated.

Results

The results from this treatment have been uniformily satisfactory. No failure has been encountered where the phage has been properly selected by typing in vitro previous to clinical application. Pain is promptly relieved, usually within a few hours, and a marked improvement is usually seen within twenty-four hours. When the infection is not too far advanced, abscesses are frequently absorbed without drainage. If the infection is advancing, localization takes place rapidly, drainage is established and often completed within forty-eight hours.

Discussion

Bacteriophage is made up of ultramicroscopic bodies which, whether they be bacteria or enzymes, are capable of reproduction as demonstrated through their growth in a culture. They reproduce at the expense of the bacteria in the culture which are dissolved and destroyed. Microscopic examination of a culture which has been exposed to an active bacteriophage shows no tract of bacteria or cell debris.

Repeated freezing of a solution of bacteriophage does not destroy its activity but heating to a temperature above 75° Centigrade does. Injection of a bacteriophage into an animal stimulates the production of an antibody that neutralizes the lytic action of the phage. The fact that there is a large amount of protein material in the bouillon together with the by-products of metabolism of the young culture of bacteria may be of significance.

Case Records

Case 1. Because of an unusual circumstance attending the successful management of this case a rather full report is warranted. The patient was a woman, 60 years of age, a diabetic who had for a long period shown a constant blood-sugar level of 200 to 250 milligrams, although glysosuria was absent. For four years she had suffered from furunculosis constantly, most of the lesions appearing about the face and neck. The causative organisms were found to be staphylococcus albus epidermitis. We had made two autogeneous vaccines which had been tried without giving any relief. Her physician then determined to try a bacteriophage. Our polyvalent phage failed to destroy the organisms obtained from the infected area, in vitro. We used it locally regardlessly but it failed also. An autogeneous bacteriophage was then prepared which, in spite of being repeatedly stepped up, failed to cause lysis of the infecting organisms in the test tube. Finally, on purely empiric grounds, we prepared another autogeneous bacteriophage, using 2 per cent dextrose broth in place of plain broth. This phage proved effective in vitro.

At this time, the patient was under the care of a surgeon, in addition to her regular physician, because of a carbuncle on the cheek. Because of her diabetes, the surgeon hesitated to incise it. Hot boric compresses irritated her and her condition was becoming serious.

When the dextrose phage was completed, the physician injected it directly into the carbuncle and applied a dressing saturated with the solution. Within two hours, the pain had subsided and, within 24 hours, free drainage had been established without the aid of surgery. The infected area was kept constantly covered with a dressing saturated with the bacteriophage and daily intramuscular injections were given.

The carbuncle healed promptly, without leaving a scar, and she has been entirely free from similar infections since that time, a period of nine months.

Case 2. A woman, 43 years of age, came to her doctor after suffering for three weeks from a carbuncle in the gluteal region. During this time, it had opened spontaneously and drained and closed again several times.

A culture showed a strain of staphylococcus aureus which was lysed in the test tube by one of our stock bacteriophages of a similar strain. The abscess was infiltrated with 1 cc. of the phage and the dressings kept saturated with the phage all night. Within 12 hours, the pain was relieved and free drainage established. After 24 hours more, the abscess stopped draining and healed rapidly, granulating from the base outward.

Case 3. A man, aged 52, presented himself with a furuncle within the nose. A culture revealed the staphylococcus aureus which succumbed to our polyvalent bacteriophage in the test tube. The nostril was packed with cotton saturated with the bacteriophage. This was left in place over night. The abscess was promptly absorbed without draining and no further treatment was necessary.

Case 4. A man, 28 years of age, was referred for typing of infection. He had a traumatic abscess on his leg which was causing him great pain. The culture showed the causative organism to be the staphylococcus albus epidermidis and the broth culture cleared after incubation with our phage No. 7 made from a similar strain.

The infection was spreading at this time. One cc. of the phage was injected into the infected area and the dressings were kept wet with the solution. Within 24 hours, the infection had localized but was still very painful. The area was re-injected with 1 cc. of the phage and the dressings continued as before. Drainage was established spontanoeusly and within 72 hours of the first injection, drainage was complete, pain was completely relieved and granulation was beginning.

Case 5. A woman, aged 55, had had a series of styes over a period of several months. The same condition had occurred periodically for the past several years. When seen by us, she had a row of draining styes which were causing great discomfort.

The culture showed the staphylococcus albus epidermidis as the offending organism and it responded, in vitro, to our polyvalent bacteriophage. Local applications of this phage for 24 hours caused complete disappearance of the styes and there has been no recurrence in the past two months.

Case 6. This case illustrates the necessity of using a specific bacteriophage for a specific organism. A man, aged 70, had a carbuncle on his back. He also gave a history of having lung abscess four years previously. It was

impossible to obtain constant cultures. It was a polybacterial infection showing various types of [illegible] and bacilli at different times. Our polyvalent bacteriophage was tried without success.

Case 7. An exceedingly painful condition was seen in a woman, aged 32, whose external auditory canal was literally filled with small furuncles. The canal was closed so tightly that she could hear nothing in that ear. She had been using a commercial preparation of bacteriophage in a jelly form for two weeks without effect. There was no drainage at the time we saw her and no culture was obtained.

A pledget of cotton saturated with our polyvalent staphylococcus bacteriophage was applied over the external ear and kept moist. Within 24 hours, the pain was notably decreased and drainage established. A culture was then made which revealed a staphylococcus which was sensitive to the phage used. Within 72 hours, the pain was entirely gone, drainage had ceased and granulation had begun.

Case 8. A woman, aged 37, suffered from a traumatic infection in the distal phalanx of one of her index fingers. When first seen, it was a spreading cellulitis, without localization, and streaks of lymphagitis were seen running up the hand and well above the wrist. No culture was possible at the time so the area of greatest infection was injected with one-half cc. of polyvalent bacteriophage and constant wet dressings, saturated with the phage, were begun. In 12 hours, the infection had localized and the lymphangitis had disappeared. Healing did not take place at once because considerable sloughing eventually took place but no more difficulty was encountered as far as the infection was concerned.

A culture taken after drainage had been established showed the staphylococcus aureus which was susceptible, in the test tube, to our polyvalent bacteriophage.

Case 9. A boy, aged 4, had an abscess of the lower eyelid. It was not draining and no culture was obtained. Local application of the polyvalent phage caused such rapid disappearance of the lesion that it was impossible to have the child returned for a culture or further observation.

Case 10. A surgeon, past 70 years of age, had been taking a series of vaccine treatments for arthritis for the past year. At this time, every injection was followed by an abscess and when seen by us he had about five abscesses on each arm. They were large and contained great quantities of pus although they did not cause much pain. Repeated cultures were obtained from a number of different abscesses but none of them showed any growth. The abscesses were sterile.

In spite of this each abscess was injected with 1 cc. of our polyvalent bacteriophage and dressings saturated with the phage were applied. The same procedure was repeated the following day. In two days all the abscesses had disappeared and there have been no recurrences, although the vaccine injections have not been discontinued.

Observations

Organisms with like cultural characteristics do not necessarily respond to the same bacteriophage. A culture of staphylococcus aureus may not be lysed by a phage prepared from another culture of staphylo-

coccus aureus. The same is true of staphylococcus albus. There are, apparently, numerous strains of similar organisms with similar cultural characteristics and the phage must be specific in order to be active. On the other hand, a staphylococcus aureus bacteriophage will, at times, destroy the staphylococcus albus and the reverse is also true.

This observation emphasized the fallacy of relying too greatly on stock preparations. Unless such phages are prepared from a large number of strains of the specific organism, failure will be frequent. It also substantiates our contention that, whenever possible, cultures of the infection organism should be obtained and tested, in vitro, for its reaction to a number of specific bacteriaphages or a polyvalent one. When a culture cannot be obtained, a polyvalent preparation should be used. The greater the number of strains represented in such a preparation, the greater will be the probabilities for success. When the available phages fail to destroy the culture in the test tube, an autogenous one should be prepared. Our own experience indicates that where a phage is inactive under laboratory tests, failure will attend its clinical use; and that in nearly every case where activity has been demonstrated in the laboratory, its clinical use will be attended by success.

Surface infections, especially in the region of the face, are usually caused by the staphylococcus albus whereas deeper infections such as furuncles and carbuncles, are usually caused by the staphylococcus aureus.

The local application of bacteriophage is now also being accomplished through the application of a salve or jelly with the phage incorporated into it. Our experience with this method is as yet too limited to discuss but, theoretically, it should have some advantages. Intramuscular and intradermal administration is also being largely used in the treatment of chronic infections, especially of the streptococcic types, with considerable success. Many cases of chronic arthritis, where the streptococcus is recovered from the nose, throat, rectum, or feces, have been successfully treated in this way.

Conclusion

Bacteriophage therapy offers a simple and unusually effective method of combatting many types of bacterial infection and opens a vast field for research. Its present accomplishments are limited but real; its future possibilities are unlimited and can only be conjectured.