Council on Pharmacy and Chemistry

REPORT OF THE COUNCIL

The Council has authorized publication of the following report by Dr. Harry E. Morton and Mr. Frank B. Engley Jr.

AUSTIN SMITH, M.D., Secretary.

DYSENTERY BACTERIOPHAGE

REVIEW OF THE LITERATURE ON ITS PROPHYLACTIC AND THERAPEUTIC USES IN MAN AND IN EXPERIMENTAL INFECTIONS IN ANIMALS

HARRY E. MORTON, Sc.D.
AND
FRANK B. ENGLE JR., M.S.
PHILADELPHIA

Within the last ten years two reports on the nature and use of bacteriophage have appeared in the Journal. The first review was by Eaton and Bayne-Jones and the second by Krueger and Scribner. Both of these reports covered all bacteriophages which had been used therapeutically. There may be a fallacy in drawing conclusions from the action of one bacteriophage and attempting to predict the behavior of bacteriophages in general. Some of the conclusions arrived at by Krueger and Scribner did not appear to apply to dysentery phages and so we have found it necessary to review the literature on the use of dysentery phage. This review, along with work reported since the previous reviews, presents dysentery phage in an aspect different from the impression gained from the two previous reviews.

REVIEW OF THE LITERATURE ON THE THERAPEUTIC USE OF DYSENTERY BACTERIOPHAGE

In order to evaluate the effectiveness of dysentery bacteriophage, or any other agent, in the treatment of bacillary dysentery it is necessary to demonstrate dysentery bacilli, the causative micro-organisms, in every patient studied. Moreover, it is important to determine whether or not the preparation of dysentery bacteriophage to be used therapeutically is active against the dysentery bacilli isolated from the patient. This can be done conveniently in vitro. There are differences between strains of bacteriophages as there are between different strains of bacteria. If one preparation or strain of dysentery bacteriophage does not act against a particular culture of dysentery bacilli, other strains or preparations from different sources may do so. An outstanding feature of bacillary dysentery in man is that the mortality is not very great, so, as Seidlmayer has pointed out, one cannot take the reduction in mortality as a criterion of bacteriophage therapy. He suggests that the criterion of cure should be the time when the dysentery bacilli disappear from the stool. That is desirable because in the treatment of bacterial infections one is interested not only in ridding the patient of his symptoms but also in ridding him of the pathogenic micro-organisms so as to prevent a recurrence of the disease or the transmission of the micro-organisms to susceptible individuals. Using as the criterion of cure the time when dysentery bacilli disappear from the stool involves the difficulties of growing dysentery bacilli from stools which also contain a dysentery phage. Micro-organisms often fail to grow in cultures when a specific bacteriophage is also present in the specimen. This is a recognized difficulty in the laboratory diagnosis of bacillary dysentery because many patients possess a natural dysentery phage in their stools, especially during the later stages of the infection. It would be, even more, a complicating feature if dysentery phage was administered to the patient.

Kligler, Oleinik and Czakzes have described a technique for culturing dysentery bacilli from specimens containing dysentery phage. It is based on the observation that dysentery phage is inactivated by high dilutions of solution of formaldehyde at a faster rate than are dysentery bacilli. Stools suspected of containing a mixture of dysentery bacilli and dysentery phage are mixed with equal volumes of dilute solution of formaldehyde so as to give a final concentration of solution of formaldehyde U.S. P. 1 to 10,000 and 1 to 7,500. Cultures of the stool suspensions are streaked on solid media immediately, and about six, eighteen and twenty-four hours after mixing with the solution of formaldehyde. An exposure of six, twelve or eighteen hours to the concentrations of solution of formaldehyde mentioned causes an inactivation of the dysentery phage and allows the dysentery bacilli to grow. After twenty-four hours' exposure the dysentery bacilli begin to be killed by the solution of formaldehyde. This improved technique should be valuable in a more accurate laboratory diagnosis in cases of dysentery and should be helpful if dysentery phage is being administered to the patient.

Since the issue of whether or not dysentery phage is of value as a therapeutic agent is still far from settled, the reports, pro and con, will be discussed separately.

(a) The following reports are frequently cited as evidence of the ineffectiveness of dysentery bacteriophage as a therapeutic agent. They are cited here with sufficient information to indicate that they are poor scientific tests or that the information is inadequate to justify the interpretation usually assigned to the results.

Davidson employed dysentery phage in the treatment of 12 children with bacillary dysentery of the Flexner type. However the stool cultures of 2 of the children were negative for dysentery bacilli and in only 7 cases was the dysentery phage tested and found active against the dysentery bacilli. Of these 7 patients 4 died (ages ranged from 3 to 14 months) and 3 recovered (ages ranged from 2 to 30 months). To the 4 patients who died the dysentery phage was given orally. Of the 3 patients who survived 2 received the dysentery phage rectally and 1 received it orally. Two of the 3 patients who survived received the dysentery phage early in the course of the disease.

The number of cases is too small to justify a statement as to the ineffectiveness of dysentery phage in the treatment of bacillary dysentery.

From the Department of Bacteriology, University of Pennsylvania School of Medicine.


Fletcher and Kanagarayy tried some dysentery phage, provided by d’Herelle himself, in the treatment of 1 case of bacillary dysentery of the Shiga type and 22 cases of the Flexner type in the Federated Malay States. One tube of bacteriophage containing about 2 cc. was given orally at twelve hour intervals for three doses; in some cases a second course of three doses was given, and in others a subsequent dose was given every morning. Daily bacteriologic examinations of the stools were made, but there was no apparent reduction in the number of dysentery bacilli in the stools. Good results appeared to be obtained in the 1 case caused by Shiga’s bacillus, and the dysentery phage produced complete lysis of Shiga bacilli in vitro. The dysentery phage was tested in vitro against the Flexner strains and it did not produce complete lysis of the organisms. The authors felt that an attempt should be made to find a bacteriophage which would produce a complete and permanent lysis of the Flexner strains, as was available for the Shiga strains.

Taylor, Greval and Thant employed bacteriophage therapy in the Rangoon General Hospital during 1928 and 1929 with 14 cases of bacillary dysentery of the Shiga type. A mixed bacteriophage was used at the beginning of the case before isolation and identification of the causative organism. Two cc. was given by mouth three times daily. Eight cases served as controls. There was one death among the 8 controls (reported as 12 per cent mortality) and two deaths among the 14 treated cases (reported as 14 per cent mortality). There was a natural bacteriophage present in all the controls. In another series of 6 cases of Shiga dysentery and 6 controls in 1929 there were no deaths in either group. Again there was a natural bacteriophage present in all the controls. In 1929 there was also a series of 6 cases of Flexner dysentery and 6 control cases. One death from uncomplicated dysentery occurred in each group. In the control group 1 patient also died of dysentery complicated with chronic interstitial nephritis. These series of cases contribute nothing to the evaluation of the therapeutic value of dysentery phage, as the authors state that all the control cases showed the presence of a natural bacteriophage at some period. These results were also reported in part II of the report by Asheshov, Taylor and Morison.

Riding studied 60 cases of acute bacillary dysentery. Of sixty strains of dysentery bacilli isolated, twelve were not lysed by the standard dysentery phage. Of forty-two strains of dysentery bacilli confirmed serologically, two showed no evidence of bacteriophagy. The clinical course of bacillary dysentery was not altered by dysentery phage given orally. It is possible that the strain of dysentery phage employed was destroyed by the pH of the fluids in the gastrointestinal tract, because 6 normal volunteers took the dysentery phage orally, and the dysentery phage could not be detected in their stools. Riding adjusted some of the dysentery phage with 0.1 normal hydrochloric acid to pH 3 and found that there was no loss in activity of the bacteriophage. This, of course, is not comparable to the maximum acidity of the stomach.

Nabarro and Signy observing the effect of dysentery phage in the treatment of dysentery in children, concluded that the results were disappointing and did not feel justified in recommending it. They used d’Herelle’s dysentery phage and stated that it did not contain any Sonne phage. During the four year period of their observations they encountered 87 cases of Sonne infection, 18 cases of Flexner Z and 1 case of Flexner W infection. From the information given, one would not expect beneficial effects of the phage therapy.

In a study of 33 infants less than 2 years old, Johnston, Ebbs and Kaake used a polyvalent phage active against Sonne (R and S types), Hiss-Russell and Flexner strains of dysentery bacilli, typhoid-like organisms and some strains of chol bacilli. There were 37 children of the same age distribution in the control group. From 51 of 70 cases, organisms of pathogenic character were isolated. These included ten strains of Sonne, five Hiss-Russell or Flexner, twenty-six Schimtz, six Asiaticus and two paratyphoid B. The dysentery phage was given orally with dextrin-maltose solution. Clinically, no difference was observed in the two groups.

Vaill and Morton administered dysentery phage to 21 patients. Only 1 control patient without phage is mentioned. Detailed observations are presented for only 5 cases. Quite justifiably the authors make no statement as to whether dysentery phage is or is not of value in the treatment of bacillary dysentery.

A closer approach to a scientific evaluation of dysentery phage therapy is the report by Kessel and Rose. Although only 68 cases are included in their data, Shigella paratyphosicaeFlexner was isolated from each case, verified by biochemical and serologic reactions and shown to be completely lysed by the dysentery phage used for treatment. Three to 5 cc. of dysentery phage was given orally at twelve day intervals with a minimum of three doses. Alternate cases were selected for “phage treatment” and for “controls,” care being taken that comparable cases exhibited nearly uniform symptoms. In the 1930 series of cases the lytic property of the phage was tested against each organism isolated before the phage was administered. In 12 of the treated cases the dysentery phage was given on the average of four days after admission to the hospital. No deaths occurred in either the group of 12 treated cases or the control group of 10 cases. The average number of days in the hospital was 11.9 for the patients receiving dysentery phage as compared with an average of 10.1 days for the control group. In the 1931 series of cases the dysentery phage was administered within a few hours after admission to the hospital because it was found that about 90 per cent of all Flexner strains were lysed by the particular strain of dysentery phage in use. The phage later was tested against the organisms for its activity. The average number of days in the hospital was eleven for the phage treated patients as compared with 12.1 days for the controls. There were four deaths in the phage treated cases and three deaths in the control cases. Two of the four deaths in the

---

treated group and one of the three deaths in the control group occurred within twenty-four hours after admission to the hospital, and it was thought that these cases should be considered as reaching the hospital too late for any treatment to be of value. This leaves two deaths in each of the treated and the control groups.

A recent report by Boyd and Portnoy describes the use in a prisoner of war camp of some dysentery phage which was captured during the retreat of the Axis from El Alamein. The highest titer of the dysentery phage against Shigella strains was 1:1,000 by the patch test. Either the test, as employed, was not very sensitive or the titer of the dysentery phage was not very high, because 1:1,000 is not a high titer for a bacteriophage. The authors stated that therapeutically the dysentery phage did not abort dysentery. Of 4,590 controls 6.16 per cent, or 283, developed symptoms of dysentery and 2.96 per cent, or 136 of these, had to be admitted to the hospital. Among the phage treated group of 4,070 prisoners 8.52 per cent, or 347, developed symptoms of dysentery and 3.1 per cent, or 126 of these, had to be admitted to the hospital. On the average it required 9.03 days for the blood and mucus to disappear from the stools of 126 control cases as compared with 9.08 days for 124 phage treated cases. The average stay in the hospital was 19.83 days for the 124 control cases as compared with 16.97 days for the phage treated cases. Based on the figures supplied by Boyd and Portnoy, dysentery phage did not abort dysentery sufficiently to prevent hospitalization or shorten the period when blood and mucus were present in the stools, but it did shorten the average stay in the hospital by 2.86 days. It must be remembered that the titer of the dysentery phage was only 1:1,000 by the method of testing.

Comment.—There are nine reports which are cited at various times as evidence of the ineffectiveness of dysentery phage in the treatment of bacillary dysentery. When these reports are examined few, if any, are free of criticism and should not be considered as contributing any direct evidence as to the ineffectiveness of bacteriophage therapy for the following reasons: Davidson’s report really concerns only 7 cases. There was no close comparison in age of the patients, time of administration of the dysentery phage during the course of the infection or the route of administration. Fletcher and Kanagarayer thought they obtained beneficial results in the treatment of 1 case of Shiga dysentery, and there was complete lysis in vitro of the Shiga bacilli by the dysentery phage. They suggested that an active Flexner phage ought to be developed because they did not get beneficial results in the 22 cases of Flexner dysentery, nor were the Flexner bacilli completely lysed in vitro. The report by Taylor, Greval and Thant should not be considered, because the control groups in two studies were found to have a natural phage, and in another study there were only 6 treated and 6 control cases, with one death in each group. Only 40 of 60 cases in Riding’s study should be considered, but he was unable to find the phage in the stools after administering it to normal volunteers. He attempted to determine whether an acid reaction inactivated the dysentery phage and found that it was not inactivated by a reaction of pH 3. This is not comparable to the maximum acidity to which the dysentery phage might be exposed in the stomach and suggests that the technic employed for detecting the dysentery phage was inadequate. Boyd and Portnoy detected dysentery phage in the blood serum and stools of 2 normal volunteers who swallowed dysentery phage as well as in 5 patients with bacillary dysentery to whom dysentery phage was given orally. Nabarro and Signy employed a dysentery phage which did not contain any Sonne phage, and yet 87 of 106 cases of dysentery in their study were caused by the Sonne bacillus. It would be expected that they would obtain unsatisfactory results. Johnston, Ebbs and Kake employed bacteriophage in the treatment of the condition known as “summer diarrhea” in which the causative agent is too obscure to attempt to evaluate a substance like dysentery phage, which may be specific for only dysentery bacilli or even for certain strains of dysentery bacilli. The report of Vaill and Morton contained detailed observations on 5 patients and only 1 control, and even they made no attempt to evaluate dysentery phage as a therapeutic agent.

The reports by Kessel and Rose are the only reports of the nine cited in this section which are worthy of serious consideration. The conclusion which can be drawn from the report by Kessel and Rose is not that dysentery phage is no good in the treatment of bacillary dysentery but that a much larger series than 68 cases must be studied. In one study by these authors there were no deaths and in another study there were two deaths in each of the control and treated groups which could be attributed to bacillary dysentery. They recognized that the mortality in bacillary dysentery was very low and that some criterion other than a reduction in mortality needed to be used. They attempted to use as a criterion of cure the length of time spent in the hospital, but this is not as good for a criterion as the time of the disappearance of the organisms from the stools, as suggested by Seidmayer. The results of Boyd and Portnoy are important in that they demonstrate that dysentery phage, after being swallowed, can be detected in the blood serum, feces and urine of the patients. Although phage therapy did not lessen the percentage of individuals requiring hospitalization or shorten the period during which blood and mucus were present in the feces, in the phage treated group the average number of days in the hospital was less by 2.86. The dysentery phage had a titer of only 1:1,000 by the method of testing and its use was entrusted to the prisoners of war.

(b) The following reports are frequently cited as evidence of the effectiveness of dysentery bacteriophage as a therapeutic agent. They are cited here with sufficient information to indicate that they are poor scientific tests or that the information is inadequate to justify the interpretation usually assigned to the reports.

da Costa Cruz employed dysentery phage in the treatment of 24 cases of dysentery. However, a bacteriologic study was made in only 12 of the cases. One case was caused by the Shiga strain, 6 cases by the Hiss strain, 4 cases by the Flexner strain and in 1 case no dysentery bacilli were demonstrated. In 1 of the cases caused by Flexner bacilli the organisms were resistant to the action of the bacteriophage in vitro and the bacteriophage was without effect in vivo. Two cc. doses of dysentery phage were given by mouth. Some cases showed pronounced benefit in four or five hours, some recovering after one or two doses. In 2 cases the symptoms returned on suspension of treatment but disappeared permanently on resumption of the treatment. Actually only 11 cases of bacillary dysentery are described in the study, and although the phage

treatment appeared to be beneficial there were no controls. In 1924 he reported seemingly equally good results with a larger number of cases.

Spence and McKinley obtained good results with dysentery bacillus therapy in a comparative study on children. Of the 20 cases in which dysentery bacilli were demonstrated in the stools (9 Shiga and 11 Flexner) 19 of the cases were treated within the first week of the disease with only two deaths ("10 per cent mortality"). There were five deaths in the control group of 12 cases ("40 per cent mortality"). The ages of the children in the treated group ranged from 4 months to 6½ years, whereas in the control group the ages ranged only from 1 to 2 years. The average time of recovery was 12.8 days in the control group as compared with 5.8 days in the treated group.

Choudhury and Morison reported satisfactory results in preventing the spread of an epidemic of dysentery. Nearly all the inhabitants in a neighboring village, Nongsiar, had died of dysentery and the people in Solsjarang were becoming ill. Shiga and Flexner strains were isolated from some of the patients. Two cc. of bacitrophage in 4 ounces (120 cc.) of water was given each sick person three times during the first day and subsequently twice daily. There were 18 severe cases with three deaths, no deaths among the 19 moderately severe or 43 mild cases.

Compton reported on dysentery phage therapy in 66 cases, but a bacteriologic study was included for only 6 patients. Although he reported beneficial results, there was during this period of phage treatment a general lowering of the death rate for dysentery which he concluded may have been a coincidence or may have been due to the dissemination of the bacitrophage. In 1924 he reported that during the period of 1928-1940 the number of cases of bacillary dysentery in Alexandria was not appreciably reduced but that the case mortality dropped from 20 per cent to 6.5 per cent. The case mortality for typhoid in Alexandria for the period of 1928-1938 remained fairly constant at about 18 per cent.

In Cairo, where bacitrophage therapy was not used, the case mortality for bacillary dysentery averaged about 29 per cent for the years 1936-1938.

London reported that dysentery phage compared favorably with the results obtained by emetine and salines. One of the noteworthy features of the therapy appeared to be the early loss of toxicity. This is in variance with the observation of de Costa Cruz, who stated that dysentery phage did not act on the toxins of the dysentery bacilli and for that reason it was often desirable to combine dysentery phage therapy with immune serum therapy. It was impossible for London to do bacteriologic studies, but no amebas were observed microscopically and the symptoms were those of bacillary dysentery. There were 129 cured out of 141 patients treated with bacitrophage, a mortality of 8.5 per cent. In other tea gardens in the district there were 72 cases of dysentery among the population, with a mortality rate of 12.5 per cent. The conditions under which the tests were made prevent any scientific deduction from the results as to the value of dysentery phage as a therapeutic agent. Sen treated 36 cases of bacillary dysentery in children and reported the results no less striking than d'Herelle's results.

Burnet, McKie and Wood observed that the presence of a highly active phage in some cases appears to determine a rapid recovery. Of 21 patients studied, the stools of 7 who recovered and of 3 who died showed an active phage in the feces, whereas 4 who recovered and 7 who died did not have an active phage in the feces.

McCay reported that dysentery phage was successful in effecting a speedy cure in 30 per cent of the cases in which it was used, and in the remaining 70 per cent it appeared to have very little effect clinically. In 111 treated cases there were six deaths (5.4 per cent mortality). In the 120 untreated cases there were thirteen deaths (10.8 per cent mortality). The causative agent was isolated in nearly 50 per cent of the total number of cases.

Davenport and Johnsen successfully treated 1 case of accidental infection with Flexner bacilli. The Flexner bacilli were present in the stools at the onset and phage was absent. Five cc. amounts of the Flexner phage were given by mouth for three doses. Flexner bacilli soon disappeared from the stools, and phage appeared. After about one week the stools contained neither Flexner bacilli nor the phage.

Querangaud des Essarts treated 190 cases of bacillary dysentery occurring during twenty-nine days on board two ships in Brest; however, the causative organism was identified in only 59 cases. There were 16 caused by Shiga, 38 by Flexner and 5 by parabacillary bacilli. A polyvalent dysentery phage was employed in the treatment of 185 cases, 5 cc. being given in alkaline water the first day, 10 cc. on the second and third days and 5 cc. on the fourth day. Blood and mucus disappeared from the stools after the second or third day, and after four days the stools appeared normal macroscopically. None of the cases showed severe toxemia. There were no controls.

Melnik, Khastovitch and Nikhinich conducted several studies on the therapeutic use of dysentery phage. The preparation which they used was prepared against the Shiga-Kruse strain and was especially active against that strain. Treatment was a single dose of 5 to 15 cc. orally in saline solution repeated two or three times in some cases. Foods yielding acid products were withheld.

Charnock, in summarizing some general procedures for the administration of bacitrophage, suggested that the surroundings should be alkaline.

Melnik, Khastovitch and Nikhinich recommended no food for several hours after the administration of bacitrophage; then large amounts of fluid should be given.

were obtained in two to four days in the case of 32 adults and satisfactory results were reported in 3 children with severe infections. No controls were included.

Kliewe and Helmreich 44 reported that therapeutically dysentery phage was effective in cases of mild or moderately severe Flexner Y and it effectively eliminated the carrier state in 16 men. In severe illness there was frequently an exacerbation and only occasionally improvement. Melnick, Khastovitch and Nikolson 47 also reported that frequently the diarrhea was more intense following the administration of dysentery phage but soon disappeared.

Comment.—There are 19 reports which are cited at various times as evidence of the effectiveness of dysentery phage in the treatment of bacillary dysentery. When these reports are examined, few if any should be considered as contributing direct evidence to the effectiveness of bacteriophage therapy for the following reasons:

The cases of dysentery reported by de Costa Cruz in which bacteriologic studies were made was too small and there were no controls. The series reported by Soesman and Mikeladze, Nemseza, Alexidze and Assanichvil were likewise small. Inadequate bacteriologic studies and/or lack of controls form the basis of the criticism of the reports by Choudhyry and Morison, Compton, London, Sen. McCay, Murray, Querangul des Essarts, Guthof and Halser. In the report by Spence and McKinley the ages of the children in the control group varied from 1 to 2 years, whereas the ages of those in the treated group varied from 4 months to 6.5 years. In view of the difference in severity of bacillary dysentery in different age groups and the small number of cases, this report cannot be given much weight in evaluating bacteriophage therapy. Burnet, McKie and Wood did not commit themselves on the effectiveness of bacteriophage therapy but merely reported the observation that those individuals who recovered from bacillary dysentery had an active bacteriophage in their feces more often than the patients who did not recover. The single case reported by Davenport and Johnson cannot, in itself, contribute much in evaluating the effectiveness of bacteriophage therapy in bacillary dysentery, which has such a low mortality in adults.

In the largest number of cases reported in any one study, Melnik, Khastovitch and Nikolson observed only 2.5 per cent mortality in 1,059 controls as compared with 1.4 per cent mortality in 282 phage treated patients. Of greater significance was that 55.3 per cent of the phage treated patients were out of the hospital four days after treatment, whereas only 18.8 per cent of the controls were out in four days.

Mikeladze, Nemseza, Alexidze and Assanichvil treated 47 cases of dysentery with dysentery phage and experienced three deaths (6.4 per cent mortality), which was about one half the mortality usually observed by ordinary methods of treatment.

Murray 46 stated that he successfully treated 146 cases of bacillary dysentery between October 1931 and February 1937 in Shanghai by giving the phage one hour before meals or three hours after a previous meal, preferably in an alkaline medium three times daily.

Halser 43 employed dysentery phage in controlling dysentery due to the Sonne bacillus in a home for 32 blind children. All those who had been sick, all those unaffected and all the staff received dysentery phage three times daily for a fortnight and thereafter one dose daily. There were no deaths and no bacteriologic study as to whether the phage was active against the organisms for each patient. The sudden cessation of the epidemic is not beyond the realm of a coincidence and, like many of the other reports cited in this section, provides no unequivocal scientific evidence which can be used in evaluating the therapeutic value of dysentery phage.

Soesman 42 used a polyvalent dysentery phage in 50 cases (17 adults and 33 children). He reported full recovery and concluded that it was a valuable agent in general practice. Guthof, 48 a battalion medical officer in a German infantry regiment, treated bacillary dysentery with Dysentery Polyphenol (Behring). Good results

the effect of the association of other organisms with the
dysentery bacilli has been overlooked, for the most
part. The report of Kliwes and Helmreich did not
give sufficient details on the therapeutic trials to warrant
any more serious consideration than other reports which
have been cited. The curing of the 16 carriers is not a
definite accomplishment unless one knows that the
dysentery bacilli were actually eliminated from the intestines
and not that the workers failed to culture the bacilli
in the presence of their specific bacteriophage. Some
precautions such as those described by Kliger, Olenik
and Czazkes would have to be employed in order to
obtain unequivocal results.

**REVIEW OF THE LITERATURE ON THE PROPHYLACTIC USE OF DYSENTERY BACTERIOPHAGE**

Persons of all ages are susceptible to bacillary dysentery. It often is endemic in certain areas and frequently becomes epidemic. Except in infants and debilitated persons the mortality rate is not very great, whereas the morbidity rate may be high. An agent which would prevent infection of individuals with dysentery bacilli would be highly desirable. It becomes even more important when the normally hygienic living conditions throughout the world are disrupted by the conditions brought about by war. Only a few reports deal with the prophylactic value of dysentery phage.

Morison appeared to have been the first to try dysentery phage prophylactically. Two doses of phage were given orally to prisoners during each week of September. During that time 5 cases of dysentery appeared among the 192 prisoners receiving dysentery phage (2.6 per cent morbidity). Among 169 prisoners not receiving dysentery phage, who served as controls, there were 28 cases of dysentery (16.5 per cent morbidity).

Melnik, Nikhinson and Khustovich undertook field trials. The control group, comprising 1,126 children ranging in age from 1 to 15 years and living in the same districts as the other children, experienced 72 infections, or a morbidity of 6.38 per cent. Those treated were divided into two groups. The first group, comprising 692 children ranging in age from 1 to 15 years, received a dose of dysentery phage orally every two weeks, so that during June, July and August each child received a total of seven doses. There were 10 infections, or a morbidity of 1.44 per cent. The second group, comprising 662 children of the same age variation, received dysentery phage plus sterile ox bile under the same conditions. Only 1 child acquired bacillary dysentery, or a morbidity of 0.15 per cent.

Kliwes and Helmreich tested the prophylactic action of dysentery phage on German soldiers. One hundred and thirteen took 10 cc. of dysentery phage following a dose of sodium bicarbonate in ½ cup of tea or coffee on three successive mornings. In the same unit 250 men served as the controls. None of the treated soldiers developed dysentery, whereas there were 10 cases among the controls.

Boyd and Portnay tested prophylactically on prisoners of war some dysentery phage captured during the retreat of the Axis from El Alamein. The number of prisoners in the phage treated group varied from 672 to 811. The incidence of bacillary dysentery per thousand for the first four weeks prior to the administration of dysentery phage was 27.58, whereas the rate per thousand during the four weeks following the adminis-

**REVIEW OF THE LITERATURE ON THE ACTION OF DYSENTERY PHAGE IN EXPERIMENTAL ANIMALS**

It is logical that the in vivo action of dysentery phage should be tried in experimental animals following the observation of the lytic action in vitro. Such reports in the literature are not very numerous, and a fair proportion of them have not appeared until recently. It is obvious that early workers employed dysentery phage on human beings with no more indication for success than the fact that the phage lysed dysentery bacilli in the test tube. Indeed, many instances are reported in which dysentery phage was used indiscriminately. The improvement in our knowledge on animal experimentation during the last twenty-five years has better demonstrated protective action of dysentery phage in vivo. Perhaps it will lead to a more scientific trial of dysentery phage in human beings.

Studying the effect of Shiga phage on infections in rabbits produced by the Shiga bacillus, Kabesima found that after the intravenous injection of Shiga phage the bacteriophage entered the bile and was able to exercise its lytic action on the Shiga bacilli. Otto and Munter claimed that they confirmed the basic finding of d’Herelle. Bacteriophagia succeeded even in animal experiments. Appelmanns found that when bacteriophage was injected into guinea pigs it was present in the spleen for at least five days but not in the liver, kidneys, heart, blood, urine, testicles or lungs. When bacteriophage on bread was fed to mice and guinea pigs, it appeared in the stools but not in the organs of the animals killed. Injected bacteriophage passes into the blood stream in a few hours. It is eliminated through the kidney and intestine and thus disappears completely in twenty-four to forty-eight hours. The latter observations are at variance with more recent work in which mice were the experimental animals. Bacteriophagia is generally accepted as part of nature, and it is quite unlikely for a foreign particle to be completely eliminated from a normal animal twenty-four to forty-eight hours after injection. If the blood was tested in liquid medium for the presence of bacteriophage, the lytic action may have been inhibited or masked by the presence of the blood. If a solid medium was employed, the appearance of the lytic areas may have been so altered after exposure of the bacteriophage to the body fluids as to be not readily recognizable. Perez-Otero (personal observations, Morton and Perez-Otero) observed that when injecting the heart’s blood from mice for the presence of dysentery phage the phages sometimes were nearly microscopic in size. The minute plaques were detected with the unaided eye only by proper illumination. More important than detecting the presence of bacteriophage...
in the blood is the demonstration of a protective action of the bacteriophage. Arnold and Weiss, working with Shiga phage, demonstrated protective action in rabbits. They showed that the bacteriophage was present and active in the blood stream five minutes after injection.

Eliauva failed to find any protective action of dysentery phage when the dysentery phage was injected six hours later or simultaneously with the infecting dose of Shiga bacilli. If the dysentery phage was allowed to come in contact with the Shiga bacilli in vitro even for a few seconds prior to injection into rabbits the animals were protected. Normal rabbit serum or a 5 per cent suspension of rabbit erythrocytes had no effect on the bacteriophage.

After the subcutaneous injection of Shiga phage into guinea pigs, Smirnow and Goldin detected it in the spleen after seventeen days. In the lymph glands after thirteen days, in the liver after three days and in the blood after two days. In the case of immune guinea pigs the bacteriophage was present after twenty-four hours but not after forty-eight hours, which is to be expected. Specific antibodies combine with the foreign particle when introduced and bring about rapid elimination from the immune animal's body.

The Rakietens observed that developing chick embryos were killed with relatively small numbers of Flexner bacilli. Death of the embryos could be prevented by dropping a small amount of dysentery phage onto the choroid plexus, five hours after the inoculation of the embryos. There appeared to be a relationship between the survival of the embryos and demonstrable proliferation of the bacteriophage.

MacNeal, Blevins and Pacis studied the protective action of anti-Sonne phage in protecting chick embryos against infection with Shigella paradysenteriae, variety Sonne. The dysentery phage protected 60 per cent of the embryos for four days, compared with a survival of 19 per cent in the controls. Only 2 per cent of the five embryos hatched in the control group, as compared with 28 per cent in the group treated with Sonne phage, which is a significant difference in mortality rates.

By injecting Shiga bacilli intracerebrally, Dubos, Straus and Pierce were able to produce a meningitis in mice which was fatal in three to ten days. Shiga phage injected into the general circulation of the mice can multiply in the brain of infected mice. Under proper conditions the Shiga phage protects the mice against the fatal infections, and the protection appears to depend on the early establishment of a high bacteriophage level in the infected animal.

Employing white Swiss mice as experimental animals and Flexner strains of dysentery bacilli, Morton and Engley demonstrated prophylactic and therapeutic actions for dysentery phage. Separate groups of 3 mice each were injected intraperitoneally on successive days for seven days with 1 cc of dysentery phage containing approximately 1 billion phage particles per cubic centimeter. Groups of mice were injected in a similar manner with the same bacteriophage after its lytic activity had been destroyed by heat. On the seventh day all the mice received 10,000 lethal doses of dysentery bacilli (4 bacilli constituted 1 minimum lethal dose). The mice receiving the heat inactivated dysentery phage died, whereas those receiving the active dysentery phage remained well, thus demonstrating the prophylactic action of the dysentery phage. If 1 cc of dysentery phage was injected intraperitoneally practically simultaneously with 12,000 lethal doses of dysentery bacilli, the mice remained well. A majority of the mice could not be protected if more than three hours elapsed between the infecting dose of dysentery bacilli and the injection of dysentery phage. A strain of dysentery phage developed against the X type of Flexner bacilli protected mice against either the X or the Z type of Flexner bacilli. A strain of dysentery phage developed against the Y type of Flexner bacilli protected mice against virulent bacilli of the Z type. The X and Y dysentery phages lysed the X and Z types in vitro. Dysentery phage developed against the X type failed to lyse in vitro a virulent strain of the Newcastle type of Flexner bacilli, and it also failed to protect mice.

In the therapeutic tests when 2 out of 3 mice were protected, the ratio of phage particles to virulent dysentery bacilli was 1:8 in the case of X phage and the homologous X strain of bacilli, 1:7 in the case of X phage and a Z strain of bacilli and 1:5 in the case of a Y phage and a Z strain of bacilli. It is doubtful whether it would be possible to protect mice or other animals against so many virulent organisms with such a small amount of bacteriophage unless more of the specific bacteriophage was in some way produced within the body of the infected animals. The Rakietens observed a proliferation of Flexner phage in chick embryos which survived the experimental infections. Dubos, Straus and Pierce also demonstrated multiplication of Shiga phage in mice experimentally infected with Shiga bacilli. Morton and Perez-Otero demonstrated quantitatively the increase of dysentery phage in mice. They also demonstrated that the dysentery phage is not eliminated completely from the circulating blood of mice in a couple of days, as was reported by some of the earlier workers. One cc of dysentery phage containing 1,400,000,000 particles was injected intraperitoneally into mice. After twenty-four hours there were on the average 30,000,000 particles per cubic centimeter of blood as taken from the heart. There was a gradual decrease in the titer of the phage in the blood until seven days after the initial injection, when there were slightly less than 50 particles per cubic centimeter of blood. On the fourth day following the initial injection of dysentery phage, the titer of the phage in the blood of the mice was about 200 particles per cubic centimeter. If the mice at this time were injected with virulent dysentery bacilli not susceptible to the dysentery phage in the test tube, the mice died rapidly without any appreciable increase in the titer of the dysentery phage in their blood. However, if the mice were injected with virulent bacilli susceptible to the dysentery phage in the test tube, the mice remained well and there was an increase by as much as 80,000 fold in the amount of dysentery phage in the circulating blood. Within one hour after the intraperitoneal injection of dysentery bacilli into normal mice, it is possible
to demonstrate a bacteremia. In the case of mice possessing dysentery phage in their circulating blood it is also possible to demonstrate both dysentery phage and dysentery bacilli. The dysentery phage was inactivated with diluted solution of formaldehyde following the technic of Klglver, Oleinik and Czazkes\(^4\) in order to cultivate the dysentery bacilli successfully. This would imply that bacteriophagy may take place in the blood stream in addition to other places in the animal body.

Comment.—Within a year and a half five reports have appeared demonstrating, without any doubt, the fact that bacteriophagy can take place in vivo and protect developing chick embryos and white mice against fatal infections of dysentery bacilli. Three of the five reports demonstrated that the amount of bacteriophage increases when bacteriophagy takes place in vivo. There is only one report in the literature of the failure of dysentery phage to protect experimental animals. That is the report in the French literature of Eliava, and he was using the Shiga strain, the most toxic of the dysentery bacilli. Kabeshina, Arnold and Weiss and Dubos, Straus and Pierce used the Shiga strain and demonstrated protective action of Shiga phage in vivo, so perhaps the report of Eliava can be ruled out on the basis of personal technic. The earlier reports (Appelmans in 1921 and Smirnow and Goldin in 1931) on the rapid elimination of dysentery phage from the circulation are at variance with what we would expect following the injection of foreign particles into a normal animal. The quantitative work of Dubos, Straus and Pierce and of Morton and Perez-Otero offer proof that dysentery phage is not completely eliminated from the blood in twenty-four and forty-eight hours.

The quantitative work by Morton and Engley is important in that it demonstrates how little dysentery phage is really needed in protecting animals from experimentally induced infections with dysentery bacilli. Most investigators have worked with an amount of phage far in excess of that needed for protection. This may lead to erroneous assumptions of the amount of bacteriophage needed in treating animals larger than mice.

COMMENT AND SUMMARY

The history of dysentery phage may be divided into three phases. The first and most exciting period was the discovery by d’Herelle of the invisible agent which causes transmissible lysis of dysentery bacilli in the test tube. This phenomenon, called bacteriophagy by d’Herelle, was found to take place with micro-organisms other than Shigella, so that now it is regarded as commonplace. The second period in the history of dysentery phage was the attempt to use this microscopic parasite for bacteria in the treatment of bacillary dysentery in man. Many of the trials were not carefully planned scientific experiments, so the reports of such trials contributed little if anything toward evaluating dysentery phage as a therapeutic agent. Trials were made without suitable controls. Often a bacteriologic diagnosis of bacillary dysentery was not made; frequently no attempt was made to determine whether the dysentery phage was active against the patient’s strain of dysentery bacilli, and often the results were of no statistical importance because of the small number of patients employed. There are about twice as many favorable as unfavorable reports on the use of dysentery phage therapeutically, but the conditions under which the tests were carried out make all the reports, seemingly good or bad, unsuitable for scientific evaluation. Only a few investigators used the dysentery phage prophylactically, and these reports appear more encouraging than the therapeutic reports. The third phase of the history of dysentery phage has been the in vivo tests, attempts at preventing or curing experimentally induced infections in laboratory animals. The majority of these tests demonstrate that dysentery phage has a definite prophylactic and therapeutic action against experimentally induced infections with dysentery bacilli, both the Shiga and Flexner varieties. All of five recent reports are in agreement in this respect.

The next phase in the history of dysentery phage should be carefully planned prophylactic and therapeutic trials on human beings, taking advantage of the knowledge gained from in vitro tests and in vivo tests on experimental animals. Quite illogically, tests on man were made before the dysentery plaque was tried on experimental animals.

In summary it may be stated that:

1. The reports on the therapeutic trials of dysentery phage in man are inconclusive. This is in part due to the nature of the experiments, and the problem of the therapeutic action of dysentery phage must be considered as still unsettled.

2. In experimentally induced infections with dysentery bacilli in laboratory animals, dysentery phage has demonstrated an unmistakably therapeutic action.

3. In therapeutic trials it is necessary to perform a bacteriologic diagnosis and demonstrate dysentery bacilli in each case.

4. Susceptibility of the patients' organisms to the bacteriophage to be employed should be demonstrated in vitro in each case.

5. The criterion for cure should be the disappearance of the dysentery bacilli from the stools. This should be verified by repeated cultures, perhaps employing a technic such as that of Klglver, Oleinik and Czazkes,\(^4\) which makes possible the cultivation of dysentery bacilli even in the presence of a specific dysentery phage.

6. It is necessary to know that the dysentery phage reaches the dysentery bacilli in an active form.

7. Obviously, suitable controls are necessary. These should be of comparable age and duration of illness and should be free of a natural bacteriophage. Determining the presence of the same pathogenic organism in the controls and its susceptibility to the dysentery phage is not sufficient. Other organisms in the intestinal flora, in both the controls and the treated patients, should also be investigated in case symbiosis with other organisms interferes with the therapeutic effect of bacteriophage, as Melnick, Khaustovitch and Nikhinson thought it did.

8. Prophylactically, it has been shown that dysentery phage is capable of preventing bacillary dysentery in man and also capable of preventing lethal infections experimentally induced in laboratory animals.

9. It is necessary to know that the dysentery phage will be active against the strains of dysentery bacilli to be encountered.

10. No lytic action in vitro, no protective action in vivo.

11. A very striking feature of the in vivo action of dysentery phage has been the small amount of dysentery phage required for the protection of experimental animals. A ratio of 1 phage particle to 8 virulent dysentery bacilli has been shown to afford protection in animal experiments.

12. Bacteriophage is unique among antibacterial substances in that, as it is used to destroy bacteria in the animal body, more of the active agent is produced.