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Effect of Massive Doses of Bacteriophage on Excretion of Vibrios, Duration of Diarrhoea and Output of Stools in Acute Cases of Cholera*

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Each member of a group of 8 patients with acute cholera was treated with a mixture of four cholera bacteriophage preparations containing over 2 × 10^{12} phage particles/ml. These massive doses were intended to kill immediately all vibrios in the intestine by "lysis from without". The numbers of Vibrio cholerae *were drastically reduced rapidly. In 4 patients,* V. cholerae *was completely eliminated from the stools early in the treatment; the total stool volume and after-treatment duration of diarrhoea were reduced in comparison with a control group but were higher than in a group of patients treated with tetracycline. In the other 4 patients treated with phage, vibrios disappeared more slowly from the stools and there was no apparent clinical effect of the phage. In all the patients treated with phage, the duration of diarrhoea was longer than in patients in a control group who excreted vibrios for a similar length of time although the stool output was similar. This was interpreted as being due to the persistence of vibrios in foci of infection in the upper intestine.*

It is concluded that treatment of cholera with massive doses of bacteriophage is not as effective as treatment with tetracycline. However, phage can selectively eliminate the majority of vibrios without affecting the other intestinal flora and without any apparent toxic effect on the patient. Phage might therefore be useful as a research tool.

INTRODUCTION

Many studies of the therapeutic effect of bacteriophage in acute cases of cholera have been carried out since the discovery of bacteriophage. The daily doses of bacteriophage used in the majority of these studies have been of the order of 10 ml–100 ml with a probable concentration of 10^9 plaque-forming units (PFU) per ml (Pollitzer, 1959; Sayamov, 1963). This number is small in relation to the large numbers of vibrios excreted by an average cholera patient—namely, about 10^8 *Vibrio cholerae* per ml in the 5000 ml–20 000 ml of stools passed per day.

These early studies depended on the assumption that small numbers of phage particles would infect the vibrios, multiply, and infect other vibrios until

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all susceptible vibrios were eliminated from the intestine. The bursting time of a vibrio infected with phage has been estimated to be between 30 and 65 minutes (Mukerjee, 1961) while the gut transit time from duodenum to anus in an acute-cholera patient with severe diarrhoea may be 30 minutes or less (J. Taylor, J. L. Kinzie & R. Hare, unpublished data). Thus, an infected vibrio would normally be carried far down the intestine or perhaps be excreted before lysis occurred. For this reason, treatment based on the long-held assumption mentioned above seemed unlikely to succeed and it was felt that a new approach was needed.

If bacteriophage could be given in such a large dose that each vibrio would be infected by 100–300 phage particles, practically instantaneous lysis of the vibrios should take place by " lysis from without " (Delbruk, 1940). A large enough dose of bacteriophage would, in this manner, be expected to eliminate the vibrios from the whole intestine very rapidly. The problem of resistant mutants could be avoided by using a mixture of different phages with a wide host-range spectrum.

To this end, a method was devised by which cholera phage could be prepared in fairly large bulk at a concentration of over 2×10^{12} PFU/ml. Using a mixture of such high-titre cholera phages, a number of acute-cholera patients were treated by administering massive continuous doses during the acute illness until their diarrhoea ceased. The pattern of vibrio excretion, the output of stools and the duration of diarrhoea of these patients were then compared with these factors in patients treated with intravenous fluid alone and with tetracycline plus intravenous fluids.

MATERIALS AND METHODS

The indicator strain and phages

V. cholerae Ogawa strain 154 (received from Dr S. Mukerjee) was used for propagation of the phages and also for their bulk production. In the main series of 8 patients, the phage used was a mixture of approximately equal quantities of 4 phages, Mukerjee's group I and group IV phages, phage 326 and phage 268. The two latter phages were isolated by the senior author in the Pakistan–SEATO Cholera Research Laboratory. In the earlier preliminary experiments different phage mixtures were used.

Culture media

The fluid medium that was used contained 1.0% of peptone (Oxoid) and 1.0% of sodium chloride in distilled water. To increase the stability of the phage preparations, calcium chloride was added to give a final concentration of 10^{-2} M (Huq, 1968). For the preparation of normal agar medium, Bacto-agar (Difco) was added to give a concentration of 2.0%, or 0.7% for the preparation of soft agar. All media were autoclaved at 20 lbf/in^2 (1.4 kgf/cm^2) for 20 minutes. The pH was 6.5 and did not require any adjustment.

The gelatin–taurocholate–tellurite agar medium (Monsur, 1961), routinely used in the Institute of Public Health laboratory, Dacca, for the isolation of *V. cholerae*, was used for detecting the presence of vibrios in the stools.

Production of high-titre phage

V. cholerae Ogawa strain 154 was grown overnight in agar medium in several Kolle flasks and the growth from each flask was washed with 5.0 ml of broth and pooled. The viable vibrio count in such suspensions was of the order of 7.5×10^{10} per ml. Altogether, 6 2-litre filtration flasks, each containing 500 ml of broth, were inoculated with 35 ml of bacterial suspension prepared as described above. This gave a final viable vibrio count in the flask of about 5×10^9/ml. The flasks were placed in a water-bath maintained at 37°C. Filtered, sterile oxygen was bubbled through the medium under positive pressure through a glass tube which branched at the end into a number of short jets so that oxygen could reach all parts of the medium. Sterile air was sometimes used in place of oxygen but in that case the rate of flow had to be increased. After 1 hour of oxygenation, a sufficient quantity of high-titre phage was added to each of the flasks for each vibrio to be infected by about 4 particles. Oxygenation was continued for a further 6 hours and at the end of this time the flasks were allowed to stand overnight at room temperature (25°C–30°C). The following day, the phage harvests were freed from bacterial debris by centrifugation at 3000 rev/min. The clear supernatant, which normally had a phage titre of the order of 5×10^{11} PFU/ml, was preserved with chloroform. The supernatant was further concentrated by high-speed centrifugation at about 40 000 *g* for 1 hour. After centrifugation, the supernatant was poured off and the pellet of sediment was soaked in one-tenth of the original volume of broth and left overnight in the refrigerator. The

next day, the well-soaked pellets were gently, but thoroughly, resuspended in the broth. The concentrated phage suspensions from different tubes were pooled and any crude debris was separated by light centrifugation.

The final preparation was titrated for phage count, tested for bacterial sterility, and preserved with chloroform. The titre of such a concentrated phage preparation was approximately $2\text{–}4 \times 10^{12}$ PFU/ml. Titration of phage was carried out by the agar-layer method (Adams, 1959). For actual treatment of patients, approximately equal volumes of the four phage types, each with a titre higher than 10^{12} PFU/ml, were pooled. This pooled phage was then administered to the patients. About 200 ml of the concentrated phage could be prepared in the laboratory by the technique described above in an average working day.

Detection of V. cholerae *in stools in the presence of a high concentration of phage*

For the detection of *V. cholerae* in the presence of a high concentration of phage in the stools ($<10^{10}$ PFU/ml), the following two methods were used.

Polyvalent antiphage serum from which antibacterial antibodies had been removed by absorption was first added to the stool which was then centrifuged, while being maintained at 4°C, to sediment the bacteria. The supernatant was poured off, the bacterial sediment was resuspended in broth containing the antiphage serum, and the suspension was centrifuged again. This process of phage neutralization was repeated several times to reduce the phage count of the supernatant to a level low enough ($<10^5$ PFU/ml) not to affect vibrio counts (K. A. Monsur & M. A. Rahman, unpublished data). The sediment from the last washing was then inoculated on to gelatin–taurocholate–tellurite agar plates for the detection of *V. cholerae*.

Later on, it was found that results similar to, or better than, those obtained with the above method, could be achieved if several shreds of mucus from the stool were washed by gentle stirring in several changes of broth and then plated for the isolation of vibrios. Many of the colonies showed evidence of partial phage lysis, and their detection was made easier by incubating the plates for 48 hours.

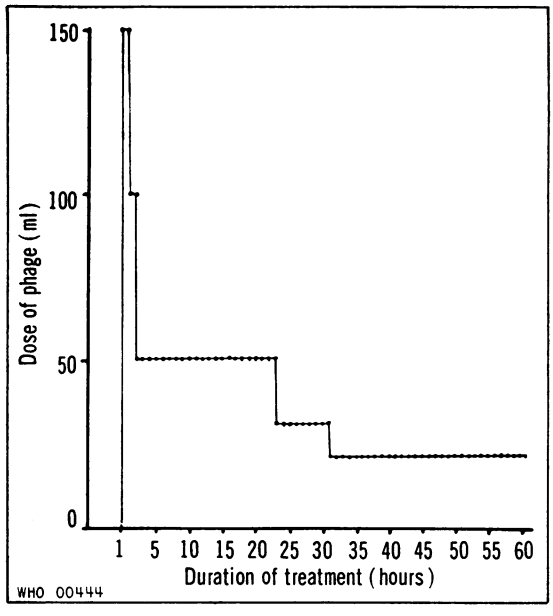
CLINICAL METHODS

The patients treated with phage were severely dehydrated on admission, had vibrios in their stools (as seen by dark-ground microscopy) and after rehydration passed over 400 ml of stool per hour for at least 8 hours; they had no complicating illness, were not pregnant and had received no treatment before coming to the Pakistan–SEATO Cholera Research Laboratory. From previous experience, it was known that patients with such a high initial stool output, if treated with intravenous fluid only, could be expected to purge for several days in a fairly predictable fashion (Hirschhorn, unpublished data). Such patients would therefore be most suitable for the assessment of any new treatment. Once a sufficient quantity of bacteriophage to treat a case had been prepared, the first available patient meeting the above-mentioned criteria was selected for study.

The guiding principle for fixing the dosage was as follows. Assuming a maximum gut volume of 2 litres in a 50-kg acute-cholera patient, the total number of vibrios present in the intestine at the beginning of treatment would be approximately 2×10^{11}. In order to achieve a level of 300 phage particles for each vibrio, at least 6×10^{13} phage particles must be administered. As the titre of the phage preparation was about 2×10^{12} PFU/ml, at least 30 ml would be required as the minimum initial dose. Allowing for variation of vibrio count in the stool, and also for some degree of non-specific inactivation of phage by gastric and intestinal contents, it was concluded that the initial dose should be at least 100 ml and preferably 200 ml. It was planned to maintain a concentration of phage in the intestine of over 10^{11} PFU/ml as long as the diarrhoea continued. Such a concentration should ensure a high probability of destruction of all the vibrios by "lysis from without" and help to eliminate those hidden in the crypts. This procedure would require a maintenance dose of up to 100 ml of phage preparation per hour but, depending on the stool output, the amount could be decreased. Even with the improved method of phage production, it was very difficult to maintain this huge dosage level in a severe case of cholera.

In practice, the patient was given an initial loading dose of 100 ml–200 ml followed by half-hourly or hourly doses at a gradually reduced level until the diarrhoea stopped. The phage was administered through an orogastric tube. With each dose of phage, 20 ml–50 ml of 10% peptone were also given to stabilize the phage and to prevent it from being inactivated by gastric acid. A typical dosage schedule is shown in Fig. 1.

FIG. 1
DOSAGE SCHEDULE FOR PHAGE TREATMENT OF PATIENT No. 8489



The patients were observed frequently for any indication of adverse effects. Urine output and intravenous fluid intake were carefully recorded and reviewed at 8-hourly intervals. Patients were maintained in water and electrolyte balance with the usual intravenous fluid therapy. Vital signs were recorded every 8 hours and the stool output was determined every 4 hours until no further liquid stool was passed.

Preliminary experiments in monkeys, rabbits and human volunteers established the fact that the phage preparation had no toxic or diarrhoea-inducing effects. The preparation of sufficient phage for treating a single patient required 4–6 weeks. Because of this, and also on account of the limited "seasonal" incidence of cholera in Dacca, the main study was spread out over 2 cholera "seasons"—namely, between April 1968 and March 1969.

RESULTS

The administration of phage to two patients treated prior to the main study with inadequate doses of phage was stopped because adequate quantities of the mixture were not available. The results suggest that in both cases the phage caused the output of stools to fall rapidly while the administration of the mixture continued but the output increased again after administration was stopped.

In the subsequent main study, 8 patients were treated. All of them had repeated bacteriological confirmation of the presence of *V. cholerae* Inaba serotype in their stools. The mean stool output prior to phage treatment was 662 ml per hour with a range of 454 ml–863 ml per hour (Table 1).

A sample of each patient's stool was cultured and examined with dark-field microscopy just before the phage therapy was started. In all cases, the count of *V. cholerae* Inaba was over 2.5×10^7 per ml. Organisms with the characteristic motility of *V. cholerae* were observed in each case under dark-field examination. In all the cases, addition of the bacteriophage mixture to the stools to give a final phage dilution of 1/20 caused immediate complete loss of motility of the vibrios in the stool. With a dilution of 1/100, this effect was produced within 10 minutes. These tests provided preliminary evidence that the phage mixture was effective *in vitro* against all strains of *V. cholerae* present in the patients.

In vivo results were similar. Between 30 and 90 minutes after phage administration was started vibrio activity, as observed by means of dark-field microscopy, disappeared from the stools. Bacteriophage and vibrio counts performed during this time showed that the appearance of large numbers of bacteriophage coincided with the disappearance of vibrios. Subsequently, the phage titre remained high in the stools, usually above 10^{11} PFU/ml, and the vibrios could not be detected by the usual cultural methods. Later, an occasional, vibrio could be isolated if mucus from the stool was repeatedly examined after washing or after antiserum treatment, as described earlier. The complete disappearance of vibrios, as judged by all attempts at detection, occurred between 8 and 81 hours following administration of the bacteriophage.

The response to the phage is shown in Table 1 In this table, the cases have been arranged in order of increasing time between the beginning of phage treatment and the last positive vibrio isolation. If, on this basis, the cases are divided arbitrarily into two groups (group A and group B), one with a shorter and the other with a longer duration of vibrio isolation, it will be seen that the 4 patients with a shorter duration of vibrio isolation had a shorter after-treatment mean duration of diarrhoea and a smaller after-treatment total stool as compared with the

TABLE 1

COMPARISON OF STOOL OUTPUT AND DURATION OF DIARRHOEA WITH VIBRIO EXCRETION IN 8 PATIENTS TREATED WITH PHAGE

Case No.	Before treatment			After treatment			Total no. of PFU of phage given
	Duration of diarrhoea in hospital (hours)	Stool output for the entire period		Time of last vibrio isolation (hours)	Duration of diarrhoea (hours)	Total stool (l)	
		ml/hour	Total stool (l)				
Group A (vibrios isolated for shorter time):							
8224	18.5	558	10.3	<8	68	14.6	9.7×10^{15}
9075	18	767	13.8	9	69	21.9	1.2×10^{16}
7855	11.5	743	8.5	12	76	8.0	2.9×10^{15}
8489	9	454	4.1	18	76	9.1	5.2×10^{15}
Mean	14.25	631	9.2		72.25	13.4	
Group B (vibrios isolated for longer time):							
8535	8	863	6.9	22	122	32.2	1.1×10^{16}
8110	8.5	520	4.4	48.5	133	27.9	1.6×10^{16}
9244	8	621	5.0	62.5	101	16.2	9.5×10^{15}
7926	8.5	767	6.5	81	111	29.6	2.7×10^{15}
Mean	8.25	693	5.7		116.8	26.8	

other group. For the two groups, the mean values were 72 hours and 116 hours duration of diarrhoea and 13 litres and 27 litres of stools, respectively. The only overlapping of values in the two groups was in stool volumes and it occurred in only one pair of cases—No. 9075 and No. 9244.

The over-all clinical response to the bacteriophage treatment is shown in Table 2. For purposes of comparison, the results from 8 patients treated with bacteriophage are presented together with those from a control group of 50 adult cholera patients treated with intravenous fluid alone, and a comparable group of 18 patients treated with tetracycline. The control group was obtained by retrospective analysis of the Pakistan–SEATO Cholera Research Laboratory records of all cholera patients treated between 1962 and 1968 with intravenous fluid therapy only. Each patient in the control group was severely dehydrated on admission, had vibrios in their stools, passed at least 400 ml of stools per hour after rehydration for at least 8 hours, had no complicating illness and had received no treatment before coming to hospital. In order that the control group could provide the basic data for comparison with the phage group, the same criteria were employed for selecting both the phage and control groups.

Another group of patients with the same criteria as the control patients but treated with tetracycline in 1966 (R. S. Northrup, unpublished data), provided the data for the tetracycline group. Antibiotic treatment in the tetracycline group was commenced after observation in the hospital for a mean period of 12.4 hours. Capsules, each containing 250 mg of tetracycline, were administered every 6 hours until the diarrhoea ceased.

No antibiotic was given to the control group of patients. For purpose of comparison with the other two groups, the periods corresponding to post-treatment duration of diarrhoea, the excretion of vibrios and the stool output for the control group was calculated from 12 hours after the patient had been admitted to the hospital.

Rectal swabs from both groups were collected each morning and cultured for *V. cholerae*. Stool outputs were measured every 8 hours. Because

TABLE 2
COMPARISON OF THE MEAN STOOL OUTPUT AND MEAN DURATION OF DIARRHOEA IN THE CONTROL, PHAGE AND TETRACYCLINE GROUPS

Groups [a]	Before treatment			After treatment	
	Duration of diarrhoea in hospital (hours)	Rate of stool output (ml/hour)	Total stool (l)	Duration of diarrhoea (hours)	Total stool (l)
Control (50)	12.0	638	7.7	95.2	29.9
Phage					
Group A (4)	14.25	631	9.2	72.25	13.4
Group B (4)	8.25	693	5.7	116.8	26.5
Group A+group B (8)	11.25	662	7.5	94.5	19.9
Tetracycline (18)	12.4	500	6.2	39.3	10.4

[a] In parentheses, the number of cases in each group.

cultures were taken much less frequently in the control and tetracycline groups than in the phage group, a point midway between the last positive culture and the first negative culture was taken to represent the end of vibrio excretion.

The mean duration of vibrio excretion in the phage group was well below that for the control group. These results are not strictly comparable since cultures in the control group were taken only once daily and were performed in a routine manner, while the stools of the phage group were cultured every 2–4 hours and an intensive effort was made to detect vibrios. It seems likely, however, that had the methods been identical, the duration of vibrio excretion in the control group would have been greater rather than less.

Table 2 shows that the patients in the phage group with a shorter duration of vibrio excretion had a shorter after-treatment mean duration of diarrhoea and smaller after-treatment total stool volume than the control patients. With tetracycline treatment the results were still better. The other 4 patients treated with phage in whom *V. cholerae* persisted for a longer period showed virtually no clinical response. *V. cholerae* disappeared fairly quickly (22 hours) from the stools of 1 patient in this group (No. 8535) but the after-treatment duration of diarrhoea and the stool output remained high. It may be noted, however, that this patient was the most severely affected of the group.

Taking the phage-treated group as a whole, the after-treatment mean duration of diarrhoea (94.5 hours) was approximately the same as that of the control group (95.2 hours) but the after-treatment total stool volume (19.9 litres) was appreciably less than that of the control (29.9 litres). Fig. 2 shows that in the phage-treated patients the stool output decreased rapidly to low levels after the administration of bacteriophage but continued at that low level for a prolonged period in comparison with the tetracycline group.

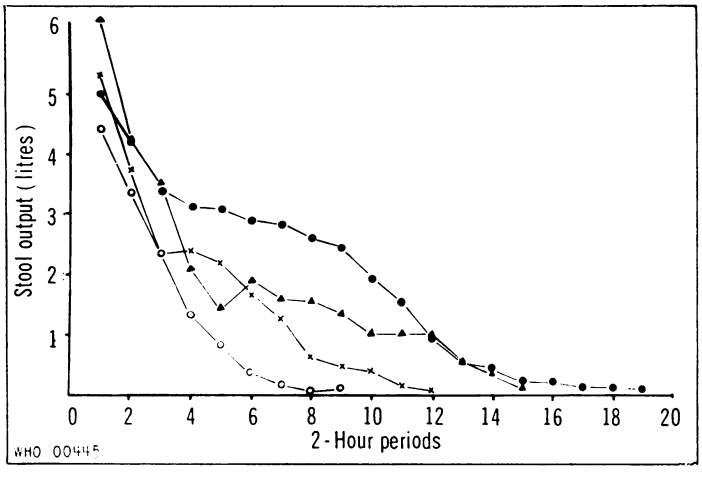
The massive doses of phage given to the patients seemed to be well tolerated and there were no indications of any adverse effects. During the course of the phage treatment the patient usually had a phage titre in the blood of about 10^2 PFU/ml.

In morphology, colony characteristics and routine biochemical behaviour, the vibrios isolated from the stools after treatment with phage did not differ from those isolated initially and were equally sensitive to the phage. The fact that the vibrios isolated after treatment showed no evidence of genetic change suggests that they had probably escaped infection by the phage.

DISCUSSION

In this study bacteriophage was administered to acute-cholera patients in amounts which were expected to cause the immediate death of all vibrios in the intestine by " lysis from without "; the phage titre in the stool was maintained at an extremely high level. In all 8 patients treated, *V. cholerae* were rapidly eliminated from the liquid portion of

FIG. 2
DURATION OF DIARRHOEA AND STOOL OUTPUT IN ACUTE CASES OF CHOLERA TREATED WITH PHAGE IN COMPARISON WITH CASES TREATED WITH TETRACYCLINE AND WITH CONTROLS



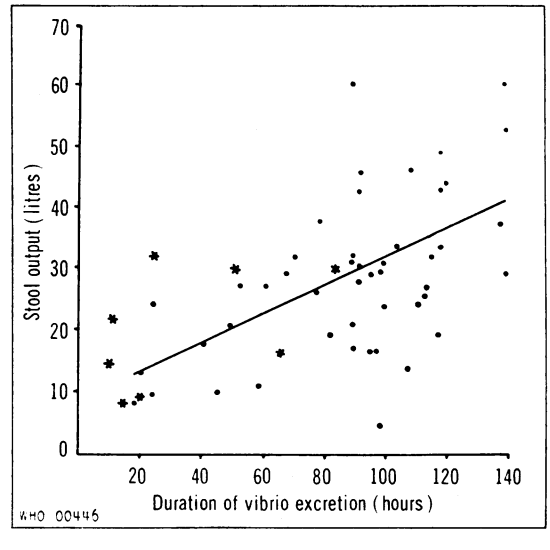
× Phage group A
▲ Phage group B
o Tetracycline group
● Control group

the stool but could be recovered from mucus shreds for varying periods. Despite this, an apparent clinical response, indicated by reduction of the stool output and a shorter duration of diarrhoea, was observed in only 4 out of the 8 patients. Even in those patients, the reduction in stool volume and duration of diarrhoea was less than that produced by tetracycline. One patient in whom vibrios disappeared after a comparable period (22 hours) showed no clinical response.

In Fig. 3, the after-treatment duration of vibrio excretion is plotted against the after-treatment total stool output. It will be seen that the after-treatment total stool output of the phage-treated patients was about the same as in control patients with a similar duration of vibrio excretion. Thus, early elimination of the vibrios in both groups appeared to result in a similar decrease in total stool output.

In Fig. 4 the after-treatment duration of vibrio excretion is plotted against after-treatment duration of diarrhoea. It will be seen that for a given duration of vibrio excretion, after-treatment duration of diarrhoea in the phage group was in all cases higher than in control patients. Thus, early elimination of vibrios from the stool did not appear to reduce

FIG. 3
EXCRETION OF VIBRIOS PLOTTED AGAINST STOOL OUTPUT AFTER THE INITIATION OF PHAGE TREATMENT OF CASES OF ACUTE CHOLERA [a]

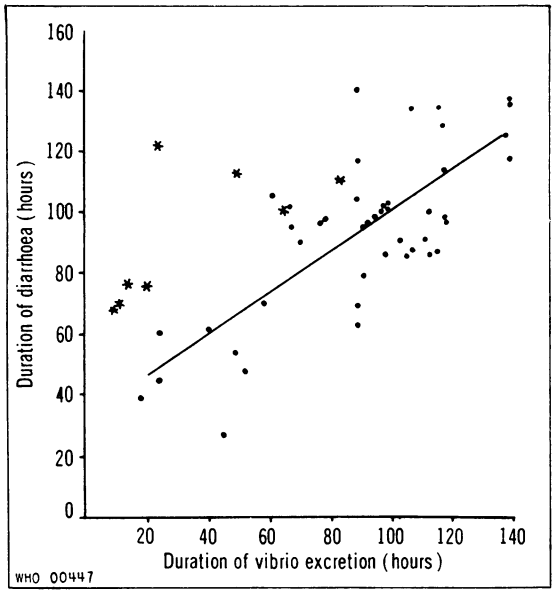


✱ Phage group
● Control group

[a] The straight line fitted to the data is a regression line for the control group.

FIG. 4

EXCRETION OF VIBRIOS PLOTTED AGAINST THE DURATION OF DIARRHOEA AFTER THE INITIATION OF PHAGE TREATMENT OF CASES OF ACUTE CHOLERA[a]



* Phage group

• Control group

[a] The straight line fitted to the data is a regression line for the control group.

markedly the duration of diarrhoea, despite a reduction in stool output.

It seems probable that the contrast between the control and phage groups noted above results from a difference between the presence of vibrio in the intestine and its presence in stool cultures. Elimination of the main bulk of the vibrios from the intestine appears to result in a reduction of total stool output. This occurred in both groups equally. The prolonged duration of diarrhoea at a low level in the phage group, however, was very likely due to the persistence of pockets of vibrios in certain parts of the intestine, such as the crypts, which were inaccessible to the phage. Vibrios coming from such pockets were probably attacked by phage immediately they entered the intestinal lumen and were thus not detectable in the stools. Thus, even in those phage-treated patients in whom vibrios appeared to be eliminated quickly, remaining foci of infection seem to have caused diarrhoea to persist, although at a reduced level, for some time after stool cultures had become negative.

The inability of bacteriophage to eliminate *V. cholerae* totally from the intestine may be related to the size of the particles. Compared with an antibiotic molecule, the particle is much larger; consequently, it is less able to diffuse into mucus, or into the depths of intestinal crypts, perhaps themselves plugged by mucus. Thus phage may be only poorly able to eliminate some vibrios which may, by virtue of their close proximity to epithelial cells, be instrumental in causing fluid loss, although phage rapidly eliminates vibrios from the intestinal fluid.

It is not known why the bacteriophage was able to eliminate vibrios from some of the patients and not from others. Only further study can determine whether host factors, such as inactivation of the phage by the stomach or intestinal contents, deficiency of co-factors, or others, may vary markedly from patient to patient, and whether correction of such factors could improve the clinical response.

The dosage of bacteriophage employed was massive compared with that used by other workers. The concentration of phage which other workers have used is about 10^9 PFU/ml. Had phage at that concentration been administered, about 4000 litres would have been required for each patient in order to equal the dose that was actually given. Despite the massive dosage, however, the over-all effect of bacteriophage was not as good as that obtained with tetracycline. It may be surmised from these studies that the use of a smaller dose of bacteriophage would be likely to have no effect at all.

A control series of experiments with administration of 10% peptone and medium without bacteriophage to patients in dosages similar to those used in the studies was not performed. In view of the known ability of glucose and glycine to enhance sodium and water absorption in cholera patients (Hirschhorn et al., 1968; Nalin & Cash, unpublished data), it may be argued that some of the observed reduction in stool output could be due to an absorption-enhancing effect of the peptone or medium. The relationship of clinical response to the apparent elimination of *V. cholerae*, however, makes it more likely that the effect of the bacteriophage therapy was due to the substantial reduction of vibrios in the intestine rather than to the peptone.

During the course of phage therapy, the patient received 2.3 litres–6.5 litres of fluid by mouth. This, at least initially, must have contributed substantially to the total stool output (Phillips, 1964). However,

this factor was not taken into account when the total stool output was calculated because an increasing amount of the orally administered fluid would be absorbed as the patient recovered (Phillips, 1964; R. A. Cash & D. R. Nalin, unpublished data).

In theory, a major advantage of using bacteriophage in cholera is its specificity. Bacteriophage attacks a single species of bacteria and leaves other organisms virtually unaffected; the present study has demonstrated the ability of bacteriophage to reduce markedly the number of vibrios present in the intestine of a cholera patient. This effect is in contrast to that of an antibiotic such as tetracycline which, by its activity against a wide range of bacteria, produces a change in bowel ecology as well as specifically eliminating the pathogenic organism. Despite its limited ability to destroy *V. cholerae* in the intestine, bacteriophage may serve as a research tool for investigating the pathophysiology of cholera without interference from vibrios but in the presence of the other intestinal flora.

The effort required to prepare sufficient bacteriophage to treat a single patient was very great and required the services of 3 technicians for 4–6 weeks. If the preparation could be reorganized on a large scale, using more flasks and constant-flow ultracentrifugation, the process could presumably produce large amounts of phage efficiently and cheaply. The limited effectiveness of the therapeutic regimen, however, makes it unlikely that bacteriophage will be more than a subject of research interest in the treatment of cholera.

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RÉSUMÉ

EFFET DE DOSES MASSIVES DE BACTÉRIOPHAGE SUR L'EXCRÉTION DES VIBRIONS, LA DURÉE DE LA DIARRHÉE ET LA QUANTITÉ DES SELLES ÉMISES DANS DES CAS AIGUS DE CHOLÉRA

On a traité 8 malades atteints d'une forme aiguë de choléra par un mélange de quatre suspensions de choléraphage titrant chacune plus de 2×10^{12} unités formatrices de plage par millilitre. La préparation a été administrée à doses très élevées dans l'espoir d'obtenir une destruction très rapide, par « lyse de l'extérieur », de tous les vibrions présents dans le tractus intestinal.

On a enregistré dans tous les cas une très forte réduction du nombre des vibrions dès le début du traitement et leur élimination complète des selles après 8 à 81 heures. Chez 4 malades, la disparition de *Vibrio cholerae* est intervenue précocement; le volume total des selles émises et la durée de la diarrhée, après le traitement, ont été moindres que chez des malades témoins traités uniquement par réhydratation, mais ont pris des valeurs plus élevées que chez des cholériques traités par la tétracycline. Dans les 4 autres cas, l'élimination des vibrions s'est faite plus lentement et le bactériophage n'a eu aucune action clinique décelable. Pour une même durée d'excrétion des vibrions, le syndrome diarrhéique a été plus durable chez les malades traités par le bactériophage que chez des malades témoins, bien que la quantité totale des selles émises ait été identique dans les deux cas. Ce fait est attribué à la persistance chez les premiers de foyers d'infection dans les parties hautes de l'intestin en dépit de l'élimination de la majeure partie des vibrions.

Même s'il est administré à doses massives, le bactériophage a donc une efficacité inférieure à celle de la tétracycline. Il présente cependant l'avantage d'agir spécifiquement sur *V. cholerae*, de ne pas léser la flore intestinale et, apparemment, d'être dépourvu de toxicité pour les malades. En raison de son activité limitée, son intérêt le plus immédiat semble résider dans son utilisation éventuelle comme instrument de recherche et d'étude du choléra.

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