An Experimental Study on the Usefulness of Bacteriophage in the Prophylaxis and Treatment of Cholera

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Five different samples of bacteriophages were tried on weanling rabbits intra-intestinally infected with the Inaba 569 B strain of Vibrio cholerae. *Three of these prevented the onset of the disease and proved useful in the treatment of early cases of experimental cholera. The prophylactic and curative (if administered early) values of choleraphage observed during these studies appear to agree with the observations of several workers in clinical cases. One of the choleraphages used proved to be valuable both prophylactically and therapeutically in experimental cholera as well as in clinical cases. It was concluded that bacteriophage was most useful in the prevention of the disease, but that therapeutically its beneficial effect could be obtained only if treatment were initiated at the early stage.*

During the period between 1926 and 1945 several workers tried bacteriophage in clinical cases of cholera. The choleraphage was used either alone or in combination with routine saline treatment or with other remedies. Among the people who found bacteriophage to be useful were d'Herelle and colleagues (1930), Morison & Vardon (1929), Morison et al. (1930), Asheshov, Khan & Lahiri (1931), Morison and his co-workers (1934), Pandit et al. (1936), Boulnois (1936), Pasricha et al. (1936, 1939), Mitra (1939) and Misra (1944). There was an opposite school of workers who considered bacteriophage to be of no significant value in the treatment of the disease (Raja, 1934; Pandit & Rice, 1936). The Cholera Advisory Committee of the Indian Research Fund Association also reported in 1934 that choleraphage should not replace the orthodox measures of cholera control such as inoculation, sanitary measures, etc., and that its prophylactic and curative values are not well established.

Interest in the bacteriophage for both prophylactic and therapeutic use was revived when it was reported by Pastukhov (1959) that, out of all the patients (number not stated) treated with choleraphage prepared by Nikonov (1959), only two died, whereas the mortality in the other hospitals was very high. The choleraphage was tried in an epidemic at Dacca (Pakistan) in 1960. The report further stated that bacteriophage was also tried as a prophylactic measure in seven settlements around which cholera was raging in an epidemic form. The bacteriophage was administered to more than 30 000 persons. It was interesting to note that these localities remained free from cholera.

Bacteriophage treatment was not tried in a laboratory where experiments could be performed under controlled conditions. Recently Mukerjee & Ghosh (1961) have shown that cholera bacteriophage prevented the characteristic inflammatory reactions in ligated loops of the small intestine of the adult rabbit. These experiments have no resemblance to human cholera. On the other hand, Dutta & Habbu (1955) could reproduce the clinical disease in weanling rabbits, and it was considered to be of interest to evaluate the efficacy of choleraphage under controlled laboratory conditions. The choleraphage that was used in the Dacca epidemic, the clinical results of which are known, was fortunately made available through the local Russian consulate. The results of laboratory findings could be compared with those of clinical trials at Dacca.

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MATERIALS AND METHODS

The bacteriophage which was obtained from Russia is referred to here as " Phage R ". Four separate samples of choleraphage obtained from the Indian Institute of Biochemistry and Experimental Medicine were numbered 138, 145, 149 and 163. These phages were used for typing *Vibrio cholerae* strains at that Institute.

Test for lytic activity

A 16-hour growth of *Vibrio cholerae*, which included Inaba 569 B, Inaba 11, and Ogawa (Haffkine strains No. 41 and 5321, and freshly isolated strain No. 89), were inoculated into 2 ml of peptone broth separately and were incubated at 37°C for three hours. Culture spots were made on agar plates. After a lapse of 5-10 minutes, when the culture spots had dried, drops of the phage were added exactly on the spots. They were incubated for 18 hours at 37°C. All the phages had lytic activity on the strains of the vibrios mentioned above.

Experimental cholera

The method followed was that of Dutta & Habbu (1955). The strain of *Vibrio cholerae* (Inaba 569 B) preserved in a lyophilized state was regenerated, suitably cultured and finally administered to weanling rabbits intra-intestinally in a dose of 10^4 vibrios per 100 g body-weight.

The animals were divided into four groups for the purpose of treatment. With one group of animals, the treatment was started one hour before the infection; with the second group the treatment was begun eight hours after the infection; with the third, 16 hours after, and with the fourth, after the onset of diarrhoea. With each set of experiments, control experiments were carried out in which the infected animals were given no treatment. Bacteriophages were given in doses of 5 ml per 100 g of body-weight with 2% sodium bicarbonate. The total amount was divided into six doses which were administered at six-hourly intervals. The administration of sodium bicarbonate by itself had no effect on the progress of the disease.

RESULTS

It will be observed from Table 1 that when Phage R was administered one hour before infecting the animals, it prevented the appearance of the symptoms of the disease. Out of the six animals so treated none suffered from diarrhoea and no death resulted. When treatment began eight hours after infection, only two animals out of eight died of the disease. The untreated animals continued to pass watery stools until death and at autopsy showed typical signs of experimental cholera. When the treatment started 16 hours after the infection or after the onset of diarrhoea, none of the 12 animals survived.

Similarly, it will be seen from Table 2 that bacteriophage Nos. 138 and 163 were as potent as Phage R, whereas Nos. 145 and 149 were less effective. Nevertheless, the latter two phages prevented the disease from taking root.

DISCUSSION

The difficulty of clinical assessment of bacteriophage against cholera on the basis of field trials is too great. There are several variable factors which

TABLE 1
THE EFFECT OF ORALLY ADMINISTERED " PHAGE R " * ON CHOLERA-INFECTED RABBITS

	Treated infected rabbits			Untreated infected rabbits		
Start of treatment	Mean time of onset of diarrhoea (hours)	Mean survival time (hours) **	Mortality	Mean time of onset of diarrhoea (hours)	Mean survival time (hours)	Mortality
1 hour before infection	—	—	0/6	18	28	1/1
8 hours after infection	22	36 (2)	2/8	20	32	1/1
16 hours after infection	20.5	29.3 (6)	6/6	20	30	1/1
After onset of diarrhoea	19	30 (6)	6/6	22	32	1/1

* Also tried clinically.
** The figures between parentheses indicate the number of animals suffering from diarrhoea.

TABLE 2
THE EFFECT OF ORALLY ADMINISTERED BACTERIOPHAGES ON CHOLERA-INFECTED RABBITS

Phage No.	Start of treatment	Treated infected rabbits			Untreated infected rabbits		
		Mean time of onset of diarrhoea (hours) *	Mean survival time (hours)	Mortality	Mean time of onset of diarrhoea (hours)	Mean survival time (hours)	Mortality
138	1 hour before infection	—	—	0/4	20	32	1/1
	8 hours after infection	21.3 (2)	42	2/4	18	30	1/1
	16 hours after infection	20.5 (4)	29	4/4	22	32	1/1
	After onset of diarrhoea	20.0 (4)	31	4/4	19	24	1/1
145	1 hour before infection	18 (1)	24	1/4	22	34	1/1
	8 hours after infection	18.5 (2)	36.5	4/4	18	28	1/1
	16 hours after infection	22 (4)	30.0	4/4	20	28	1/1
	After onset of diarrhoea	19 (4)	24.0	4/4	26	32	1/1
149	1 hour before infection	20 (1)	34	1/4	22	30	1/1
	8 hours after infection	16 (2)	22	2/4	20	32	1/1
	16 hours after infection	22 (3)	34	4/4	18	24	1/1
	After onset of diarrhoea	21 (4)	30	4/4	16	28	1/1
163	1 hour before infection	nil	—	0/4	16	24	1/1
	8 hours after infection	18 (1)	—	0/4	16	24	1/1
	16 hours after infection	20 (3)	32	4/4	18	20	1/1
	After onset of diarrhoea	22 (4)	30	4/4	20	30	1/1

* The figures between parentheses indicate the number of animals suffering from diarrhoea.

restrict the value of the results. One epidemic cannot be compared with another even in the same locality because of possible differences in the nature and virulence of the organisms. There are other factors, such as the physical status of the health of the community, the type of bacteriophage used, the amount of phage given and its mode of administration, lack of adequate control experiments, inadequate number of patients taken for evaluation—all these inevitably affect the final assessment of the results. The results of field trials by different workers suffered from one or more of these defects, especially that of administration of inadequate amounts of choleraphage. It is, therefore, not unnatural to find contradictory statements on the usefulness or otherwise of choleraphage. The experiments carried out in hospitals under controlled conditions to some extent are more reliable than the field trials. Good results from phage treatments undertaken in hospitals have been reported by Ross, Bagchi & Roy (1928),

Asheshov, Khan & Lahiri (1930, 1931) and Pasricha (1936). The number of cases handled by Ross, Bagchi & Roy could not be called adequate for the purpose of evaluation. Cholera bacteriophage proved to be of no use in the hands of Taylor, Greval & U Thant (1939) and Souchard (1930). The number of cases studied by these workers was likewise too small for evaluation.

A notable contribution has been made by Pasricha, de Monte & O'Flynn (1936). In a well-planned and controlled investigation carried out in Calcutta, 1369 cases of cholera were studied in a local hospital. Adequate treatment with bacteriophage was given to 684 cases and the remainder served as control. The selection in the two series was made by taking alternate admissions into the wards, irrespective of age, severity of the infection, and general condition of the patients. Both series received the usual hospital routine treatment for cholera; the phage-treated cases received in addition choleraphage in

doses of 2 ml every four hours. It was found that bacteriophage caused a real reduction in cholera mortality among cases excreting vibrios.

If the results of the animal experiments are in any way an indication of clinical usefulness, it may be said that some choleraphages, when given in adequate doses, may avert an attack of cholera and may prove beneficial in the treatment of early cases.

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RÉSUMÉ

Bien qu'elles se soient manifestées à maintes occasions, en particulier lors de l'épidémie de choléra de Dacca, Pakistan, en 1960, les propriétés prophylactiques et thérapeutiques des choléraphages n'avaient pas été vérifiées en laboratoire, par des expériences contrôlées. Les auteurs ont entrepris de le faire, au moyen du phage utilisé par l'équipe russe à Dacca. Ils ont inoculé la souche Inaba 569 B de *V. cholerae* à des lapins de 10 jours. Les témoins moururent, dans les 32 heures, d'une infection rappelant le choléra humain. Le phage administré 1 heure avant l'infection a préservé les animaux de l'infection;

aucun d'entre eux ne fut malade ni ne mourut à la suite de l'infection cholérique. Lorsque le phage fut appliqué 8 heures après l'infection, 2 animaux sur 8 moururent. Traités par le phage 16 heures après avoir reçu le vibrion cholérique, tous les animaux moururent. Des phages d'origine locale donnèrent les mêmes résultats.

Les auteurs concluent que le choléraphage administré à un stade précoce peut empêcher le développement de la maladie. La clinique et le laboratoire sont en accord sur ce point.

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