

Interaction of *Vibrio cholerae* and *Vibrio El Tor*

S. BASU, M.B.B.S.,¹ P. BHATTACHARYA, M.B.B.S.¹ &
S. MUKERJEE, M.B., D.Phil., D.Sc.²

Soon after its entry into the Indo-Pakistan subcontinent in 1964, cholera El Tor progressively replaced classical cholera. One of the probable reasons for this was found from laboratory studies of the interaction of the two choleraenic vibrios, V. cholerae and V. El Tor. It was observed, in both in vitro and in vivo experiments, that in mixed culture El Tor vibrios from cholera cases are capable of outgrowing and rapidly eliminating V. cholerae. The apathogenic El Tor strains from the Middle East countries differed significantly from the pathogenic type of El Tor strains isolated from cholera cases in that the former did not inhibit the growth of V. cholerae strains. The possible mechanism of interaction of the pathogenic El Tor vibrios and V. cholerae was studied in detail. Various possibilities, including higher rates of multiplication of El Tor vibrios, competition for nutrients, secretion of inhibitory substances and liberation of lethal bacteriophage or vibriocins by El Tor vibrios, were examined. Although it was not possible to establish the actual mechanism of this interaction, the possible biological effect on the epidemiology of cholera was evident.

In recent years classical cholera, caused by *Vibrio cholerae*, has remained limited in the Indo-Pakistan subcontinent, whereas cholera El Tor, caused by *Vibrio El Tor*, has been spreading widely in the Western Pacific and south and south-east Asia. Since 1963, cholera El Tor has entered areas in the Indo-Pakistan subcontinent infected by *V. cholerae*. It was observed (Mukerjee et al., 1965) that, in many of the areas where both types of infection had been present, cases due to *V. El Tor* soon outnumbered or even completely replaced those due to *V. cholerae*. The present studies were undertaken to examine the possible effects of interaction of the two types of vibrio in relation to the present trend of cholera epidemiology.

MATERIALS AND METHODS

Vibrio strains

In most of the experiments one Inaba strain of *V. cholerae*, number GS1/65, and one Ogawa El Tor strain, GS 9/65, were used. These strains were

isolated in January 1965 from patients found to be infected at the Gangasagar festival³ and were the most recently isolated among available cholera and El Tor cultures from one outbreak. They were of epidemiological interest as they were likely to have been carried by the pilgrims back to their homes on their return journey and to have caused epidemics in the respective areas. The other *V. cholerae* strains used were Asm 1/65, Shillong 1/65, H74/64, Bg 22/64, Bg 40/64, Kul 124/64, Visak 45/65, Com 15/65, Com 15/64, Dac 5/64, Dac 42/64, Madu 10/64, Poona 43/64 and Bombay 6/65, all of which had been recently isolated in India or Pakistan. The pathogenic strains of *V. El Tor* were Phil 1/65, Phil 5/65, Kerala 1/65, Pat 2/65, Pat 11/65, HK TS 2/65, Mala 1/65, Boro 1/65, H 5/65, and Pak 1/65. The non-pathogenic *V. El Tor* strains consisted of ME 3, ME 4, ME 5 and ME 7, isolated from the Middle East countries, and EW 1, EW 5 and EW 6, isolated from Calcutta water sources at a time when there was no cholera El Tor infection in Calcutta. The *V. cholerae* strains studied were of the Inaba subtype and the El Tor vibrios of the Ogawa subtype, the selection having been made to facilitate sero-

¹ Senior Fellow, Indian Institute of Experimental Medicine, Calcutta 32, India.

² Head, Division of Microbiology, Indian Institute of Experimental Medicine, Calcutta 32, India; Director, WHO International Reference Centre for Vibrio Phage-typing, Indian Institute of Experimental Medicine.

³ This festival is held every year in Sagar island, about 75 miles (120 km) south of Calcutta, and people from all parts of India congregate there.

logical identification of the colonies in mixed cultures.

Media

In order to study the effect of the interaction of vibrios under poor nutrient conditions, most of the observations were made after culture in 0.25% peptone water. In some experiments, however, 1% peptone water and nutrient broth were used. Nutrient agar plates were used for the isolation of colonies. Blood agar plates with 5% sheep erythrocytes were used to differentiate between haemolytic and non-haemolytic colonies of *V. El Tor* and *V. cholerae* strains.

Growth of cholera and El Tor vibrios

Turbidimetric measurements at 540 m μ , using the Klett-Summerson photoelectric colorimeter with a suitable filter, as well as the method of viable counts, were used to measure the growth of vibrio strains.

Study of mixed cultures

In the absence of continuous-culture techniques, the following tests were undertaken to examine the effects of interaction of the two types of vibrios.

In solid media. A 2-hour growth of strains of *V. El Tor* in nutrient broth was spotted on the lawn of *V. cholerae*, and *vice versa*. The vibrio lawns were made by plating from two-hour broth cultures. In the absence of inhibition, the more vigorously growing culture would show a denser growth over the spotted area than the surrounding lawn.

In liquid media. To 25 ml of liquid medium in a flask, 10³-10⁶ cells of both types of vibrios taken from 2-hour broth culture were added in different proportions. Platings were made from this medium after incubation at 37°C for 0.5, 1, 1.5, 2, 4 and 6 hours and 1, 2 and 4 days; in some experiments platings were also made after 1, 2 and 4 weeks.

Effect of size of inoculum. In order to determine the effect of using heavier inocula of *V. cholerae* than of *V. El Tor*, the former was added in proportions of two, 10 and 100 times that of the latter.

Alteration of pH of medium. Changes in the pH of the media after the growth of vibrios were measured at different time intervals by means of the Beckman Automatic Titrator.

Effect of aeration of cultures. In order to study the effect of aeration on the mixed cultures, in some experiments the flasks containing the cultures were rotated in a rotary shaker.

Repeated addition of nutrient. To examine whether suppression of the growth of one type of vibrio was due to exhaustion of the available nutrients in the media as a result of their consumption by the other type, 2-ml samples of fresh media were added at intervals of 10 minutes with thorough mixing.

Addition of monospecific serum and El Tor phage. In preliminary experiments it was observed that *V. El Tor* grew more rapidly than *V. cholerae*. It was also found that very few *V. cholerae* could be isolated after 4-6 hours' incubation in admixture with growing *El Tor* vibrios. To determine whether *V. cholerae* were actually killed or were simply outnumbered by the rapid growth of the *El Tor* vibrios, attempts were made to agglutinate the *El Tor* cells by the addition of a homologous monospecific O-serum, the *V. cholerae* strain used being of the heterologous subtype, or to cause their lysis by adding high-titre bacteriophages active against *El Tor* vibrios but not against *V. cholerae*. The serum was added to the flask 2 hours after the incubation of the mixed culture medium and the phage was introduced 30 minutes after the addition of vibrio cultures in quantities sufficient to cause multiple infections of the *El Tor* vibrios.

The direct effect of phages from lysogenic *V. El Tor* on *V. cholerae* was tested by adding phage filtrates from *El Tor* strains to young cultures of *V. cholerae* in quantities sufficient to produce multiple infections.

Addition of high-titre anti-phage serum. All the pathogenic *El Tor* strains used in these experiments were lysogenic and it was possible that the phages liberated by these strains might have proved lethal to viable *V. cholerae* cells. In experiments to study this possibility, high-titre antiphage serum was added to the media in the proportion of one part of serum to 10-25 parts of the medium in order rapidly to neutralize the free phage particles, which might be liberated in the media during the growth of lysogenic *El Tor* strains.

Identification of colonies of V. cholerae and V. El Tor

Subcultures were made in triplicate on agar plates from mixed growths in liquid media; usually three hundred colonies were picked up. Vibrio colonies were identified by a combination of the following tests.

Phage-typing. The sensitivity pattern to a group IV cholera bacteriophage (Mukerjee, 1963) was used to

differentiate colonies due to *V. cholerae* and *V. El Tor* strains. *V. cholerae* cultures are sensitive to this phage, whereas all *V. El Tor* strains are resistant to lysis by it.

Polymyxin sensitivity test. The test was carried out according to the modified method of Roy et al. (1965). On nutrient agar plates containing 15 µg/ml of polymyxin B, spot cultures were made with 2-mm loopfuls of 2-hour growth in a broth containing the test strains. *El Tor* vibrios produced confluent or semi-confluent growths, whereas *V. cholerae* showed no growth or only an occasional growth of fewer than 10-15 isolated colonies.

Haemagglutination test. This was carried out according to the method of Finkelstein & Mukerjee (1963).

Serological test. Serological identification of colonies was made by agglutination tests with known monospecific Inaba and Ogawa sera. In most of the tests of interaction, the serotypes of the strains employed were different.

Tests for V. cholerae-inhibitory substances in El Tor cultures

These tests were made by studying the inhibitory action of culture filtrates, cell lysates and killed cultures of *El Tor* strains on *V. cholerae* cultures in the following manner. In all cases growth was measured turbidimetrically.

Addition of *El Tor* culture filtrate. In order to examine whether *El Tor* vibrios could liberate in the media any substance inhibitory to the growth of *V. cholerae*, 2-, 4-, 6- and 24-hour broth-culture filtrates of *V. El Tor* strains were added to equal volumes of nutrient broth and then inoculated with *V. cholerae* cultures. Growth was measured at regular intervals.

Addition of lysates of *El Tor* cultures. Washed suspensions of agar culture of *V. El Tor* were lysed in the Nossel shaker and then filtered through sintered-glass filters, the experiment being carried out at 4°C. The filtrate was added to an equal quantity of peptone water, which was then inoculated with cultures of *V. cholerae*. Turbidimetric measurements were made after incubation at 37°C.

Addition of killed culture of *V. El Tor*. Two-hour and 24-hour broth cultures of an *El Tor* vibrio were killed by maintaining them in a water-bath at 60°C for one hour. The killed cultures were added to equal quantities of nutrient broth and then inoculated with *V. cholerae* cultures.

Vibriocin in El Tor cultures

Attempts were made to demonstrate the presence of a vibriocin liberated by *El Tor* vibrios by the streak-culture (Shannon, 1957) and stab-culture (Fredericq, 1957) methods.

In vivo experiments

Interaction in mixed growths of *V. cholerae* and *V. El Tor* in the intestine of laboratory animals was studied in the following models.

In ligated loops of the intestine of adult rabbits (De & Chatterjee, 1953). Mixed suspensions containing approximately 10⁸ cells of each type of vibrio were injected intra-intestinally into the ligated loops of the small intestine of adult rabbits. After 4, 6 and 24 hours the animals were sacrificed and the contents of the loop washed out in normal saline and centrifuged at low speed; the supernatant fluid was plated undiluted and also in suitable dilutions. After overnight incubation, about 500 colonies were picked up and the types of vibrio present identified.

In the intestine of infant rabbits (Dutta & Habbu, 1955). Saline suspensions containing approximately 10⁸ cells of *V. cholerae* and 10⁸ cells of *V. El Tor* were injected into the upper part of the small intestine of 12-day-old rabbits. After 24 hours the small and large intestines were washed out in normal saline. The washings were centrifuged and the supernatant fluids plated. After incubation, the colonies formed were identified in the usual way.

RESULTS

Differences in growth rates of V. cholerae and V. El Tor

The rates of growth of the two types of vibrios in 1% peptone water at pH 8.2 were measured turbidimetrically (Table 1) as well as by the viable-count method (Table 2). They are represented graphically in Fig. 1 and 2, from which it can be seen that *V. cholerae* strains grew more slowly than *V. El Tor* strains. The optical densities of both cholera and *El Tor* vibrio cultures showed a steady rise for the first six hours. However, the growth rates determined by the viable-count method were fairly constant initially, but the viable-count curves then flattened out; for some strains there was, in fact, a decrease in count after about four hours. The rise in optical densities continued up to and even after 24 hours, whereas there was a noticeable fall in viable-count at 24 hours. Some degree of variation in growth rate

TABLE 1
GROWTH RATES OF *V. CHOLERAE* AND *V. EL TOR* MEASURED TURBIDIMETRICALLY^a

Type of vibrio	Strain	Original optical density	Optical density after interval of					
			0.5 hour	1 hour	2 hours	4 hours	6 hours	24 hours
<i>V. cholerae</i>	GS 1/65	0.0	0.001	0.002	0.004	0.017	0.055	0.127
<i>V. El Tor</i>	GS 9/65	0.0	0.002	0.008	0.021	0.090	0.150	0.260
<i>V. cholerae</i>	Asm 1/65	0.002	0.003	0.006	0.013	0.053	0.092	0.236
<i>V. El Tor</i>	Phil 1/65	0.0	0.002	0.008	0.020	0.086	0.160	0.410

^a Values are averages of three experiments.

could be observed between strains of the same type of vibrio. The discrepancy between the colorimetric readings and viable counts was possibly due to the continuous autolysis of the vibrio cells, which, together with the dead cells, accounted for the rise in optical density. Therefore, in all tests the viable-count method was used to ascertain the effects of interaction of vibrios on the viability of cultures under different conditions.

Mixed culture in solid media

When *V. El Tor* strains were grown on spot cultures over lawns of *V. cholerae* strains on agar plates, overgrowth of *El Tor* strains was indicated by denser growths over the spots of *El Tor* cultures, as compared with the culture of *V. cholerae* in the lawn. On the other hand, spots of *V. cholerae* cultures on lawns of *El Tor* vibrios did not show any evidence of overgrowth.

Mixed culture in liquid media

The results are given in Table 3, which shows that, when *V. cholerae* strain GS 1/65 was grown in

mixed culture in a liquid medium with *V. El Tor* strain GS 9/65, at a starting inoculum of 10^3 cells of each, the former was rapidly outnumbered and eliminated from the culture medium by overgrowth of the latter. After two hours no viable *V. cholerae* cell could be isolated on plating the mixture.

Similar results were obtained when the starting inoculum contained twice as many viable cells of *V. cholerae* as of *V. El Tor*. Even when *V. cholerae* were added in considerable excess, their number was very quickly reduced. When another strain of *V. cholerae* (Am 1/65) and another of *V. El Tor* (Phil. 1/65) were used, the results were similar. However, in some of these experiments, a few *V. cholerae* colonies could be isolated up to six hours after the start. Although in most instances Inaba *V. cholerae* and Ogawa *V. El Tor* strains were used, identical results were obtained when the subtypes of the vibrios were reversed, indicating that the interaction is between *V. cholerae* and *V. El Tor* and not between the subtypes.

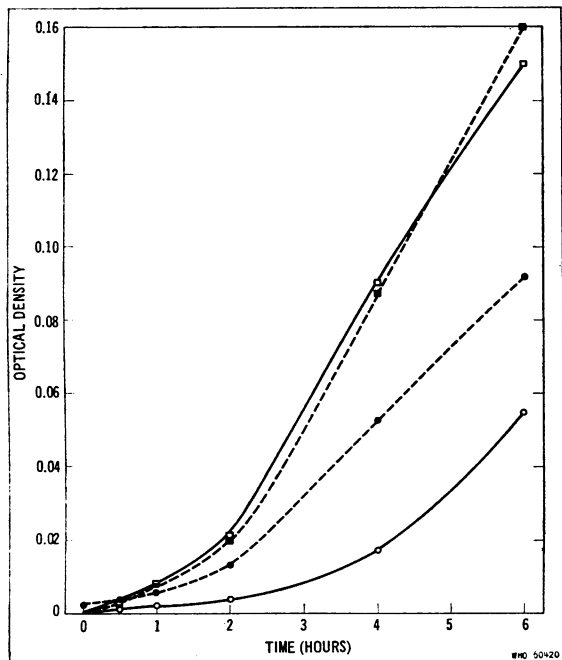
However, in mixed cultures of an apathogenic *V. El Tor* and a *V. cholerae* strain, there was no

TABLE 2
GROWTH RATES OF *V. CHOLERAE* AND *V. EL TOR* MEASURED BY VIABLE COUNTS^a

Type of vibrio	Strain	Original viable count	Viable counts after interval of					
			0.5 hour	1 hour	2 hours	4 hours	6 hours	24 hours
<i>V. cholerae</i>	GS 1/65	3.7×10^4	5.8×10^4	9.7×10^4	5.4×10^5	2.4×10^7	1.1×10^8	8.7×10^7
<i>V. El Tor</i>	GS 9/65	3.1×10^4	2.8×10^5	3.4×10^6	5.2×10^7	6.7×10^8	7.5×10^8	7.1×10^8
<i>V. cholerae</i>	Asm 1/65	2.5×10^5	4.4×10^5	6.3×10^5	8.0×10^6	4.6×10^8	3.4×10^8	8.1×10^7
<i>V. El Tor</i>	Phil 1/65	2.3×10^5	6.0×10^5	6.7×10^6	8.0×10^7	1.7×10^9	8.7×10^8	7.6×10^8

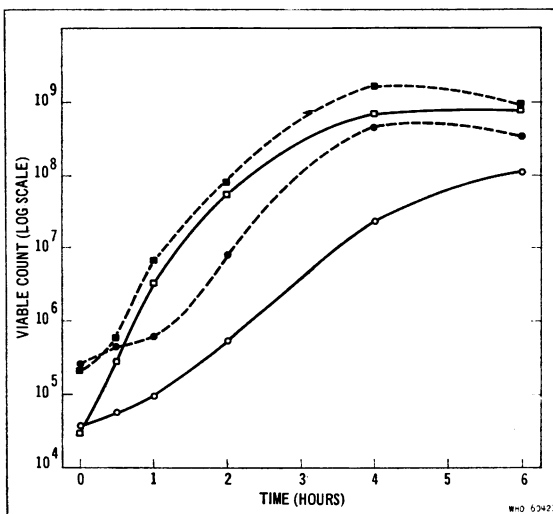
^a Values are averages of three experiments.

FIG. 1
GROWTH RATES OF VIBRIOS MEASURED BY OPTICAL DENSITY



Vibrio cholerae ○ GS 1/65 ● Asm 1/65
Vibrio El Tor □ GS 9/65 ■ Phil 1/65

FIG. 2
GROWTH RATES OF VIBRIOS MEASURED BY VIABLE COUNTS



Vibrio cholerae ○ GS 1/65 ● Asm 1/65
Vibrio El Tor □ GS 9/65 ■ Phil 1/65

inhibition of growth of *V. cholerae*. When a pathogenic (case) El Tor Ogawa and an apathogenic (water) El Tor Inaba strain were grown together, no elimination of the apathogenic type was observed in up to one week. The colonies of the apathogenic

TABLE 3
INTERACTION BETWEEN *V. CHOLERA* (GS1/65) AND *V. EL TOR* (GS9/65)^a

No. of cells added in 25 ml medium ^b		Original content of <i>V. cholerae</i> in medium (%)	Content (%) of <i>V. cholerae</i> recovered on plating at intervals of						
<i>V. cholerae</i>	<i>V. El Tor</i>		0.5 hour	1 hour	1.5 hours	2 hours	4 hours	6 hours	24 hours
10 ³	10 ³	50	25	12	3	0	0	0	0
10 ⁵	10 ⁵	50	40	32	25	11	3	0	0
2 × 10 ³	10 ³	67	31	16	5	0	0	0	0
10 ⁴	10 ³	91	68	50	42	36	8	0.4	0
10 ⁵	10 ³	99	82	72	66	32	28	7	0
10 ³ (and 2 ml media every 10 min)	10 ³	50	22	16	12	4	0	0	0
10 ³ (with aeration)	10 ³	50	24	14	8	0	0	0	0

^a Values are averages of three experiments.

^b In the first five experiments 0.25% peptone water was used, and in the last two nutrient broth.

strain were differentiated from the pathogenic strain on plating by the results of agglutination and haemolysis tests, as well as by the patterns of sensitivity to El Tor typing-phages.

Alteration in pH of medium

Measurement of the alteration in the hydrogen-ion concentration of the medium as a result of the growth of *V. cholerae* and *V. El Tor* showed that, in both instances, with an initial inoculum of vibrios of approximately 10^8 cells in 25 ml of nutrient broth, there was a progressive decrease in pH, which ranged, on average, from 8.2 at zero time to 7.8-8.0 after 2 hours, 7.4-7.6 after 4 hours, 7.0-7.2 after 6 hours and 6.4-6.8 after 24 hours. Thus variations in the pH of the media remained within the limits of survival and growth of both types of vibrio.

Aeration of media

V. El Tor, on growing in liquid media, forms a pellicle on the surface. Moreover, El Tor vibrios, being metabolically more active than *V. cholerae*, may utilize the available oxygen near the surface and deprive *V. cholerae* of the oxygen needed for their growth. Attempts were made to mix and aerate the cultures continually by the use of a rotary shaker. The growth appeared to be uniform throughout the medium, but the survival time of *V. cholerae* was not increased. After 2-4 hours no colonies of *V. cholerae* could be isolated.

Exhaustion of nutrient

Addition of 2 ml of the nutrient broth in 25 ml of the inoculated media at intervals of 10 minutes did not increase the survival time of *V. cholerae* in the presence of El Tor vibrios: *V. cholerae* colonies could not be isolated after 4-6 hours. On the other hand, overnight culture filtrates of *V. El Tor* were found to contain enough nutrient to permit visible growth of *V. cholerae* from a small inoculum. The elimination of *V. cholerae* by *V. El Tor* in a mixed culture was not therefore due to exhaustion of nutrients.

Addition of monospecific Ogawa serum and El Tor phages and El Tor antiphage serum

When a mixed culture consisted of El Tor vibrios of the Ogawa type and *V. cholerae* of the Inaba type in nutrient broth, addition of a high-titre monospecific Ogawa serum after growth for 2 hours resulted in agglutination of the El Tor vibrios, which quickly settled to the bottom of the tube. Viable cells of *V. cholerae*, even if present in small numbers,

could then be more easily observed in the supernatant liquor. Even after such treatment only a few colonies could be identified as being of *V. cholerae* when the supernatant medium was plated after 2, 4 or 6 hours; after 24 hours the few surviving *V. cholerae* cells multiplied as in a pure culture and the Ogawa El Tor cells almost completely disappeared from the mixture, having apparently been inactivated by the serum added. Addition of El Tor phages active against *V. El Tor* did not prove useful, as the initial phase of lysis of young cultures of El Tor vibrios was soon followed by overgrowth of their phage-resistant secondary growths. Addition of El Tor antiphage serum did not increase the survival rate of *V. cholerae*, colonies of which could not be isolated after 4-6 hours. Addition of lysogenic El Tor phage to young cultures of *V. cholerae* did not lower the viable count of *V. cholerae*, nor did the *V. cholerae* show any evidence of lysogenization. This indicated that the interaction of *V. El Tor* and *V. cholerae* was not due to lysogenization or lethal phage action.

Tests for inhibitory substances in El Tor vibrios

Addition of dead El Tor cells, lysates and culture filtrates after different periods of growth did not inhibit the growth of *V. cholerae* in nutrient broth. The rates of growth in presence of preparations from *V. El Tor* were identical with those obtained in their absence.

The *V. El Tor* strains were tested for the possible production of a bactericide active against *V. cholerae* strains by means of streak- and stab-culture methods. In no instance could the presence of vibriocins be demonstrated.

In vivo experiments

After administration of a mixed culture of *V. cholerae* and *V. El Tor* in the ligated intestinal loops of adult rabbits and intra-intestinally in infant rabbits, no *V. cholerae* colony could be found, among 400-500 colonies tested in each case, 6 and 24 hours after inoculation. At the end of 4 hours, however, in only one set of experiments, 12% of the colonies isolated from a mixed growth in the intestinal loop proved to be *V. cholerae*.

DISCUSSION

It can be seen from the results of tests for the interaction of *V. cholerae* and *V. El Tor* that in mixed culture, both *in vitro* and *in vivo*, the viable

counts of *V. cholerae* were progressively reduced and, within a short time, *V. cholerae* was eliminated from the mixture. The growth rates of El Tor vibrios were higher than those of *V. cholerae*, particularly in the early stages of growth. But this alone would be insufficient to explain the rapid alteration in the type of vibrio population in the mixture. Such alteration was noticeable even when the initial inoculum contained *V. cholerae* in 100-fold excess. These results could not have been due to the loss of available nutrients or oxygen in the media resulting from the rapid growth of *V. El Tor* in the early stages, since filtrates of a 24-hour broth culture of a *V. El Tor* strain contained enough nutrient to support the growth of *V. cholerae*. When a mono-specific Ogawa O-serum was added to the mixed culture after two-hours' growth, to agglutinate El Tor vibrios of the Ogawa subtype, the viable count of Inaba *V. cholerae* cells in the supernatant liquor showed a marked decrease, indicating the lethal effect on *V. cholerae* of interaction in the mixed growth. On continued incubation, *V. cholerae* cells alone were found to multiply in the supernatant liquor, suggesting that inhibition of *V. cholerae* takes place during the active multiplication of *V. El Tor*.

The possibility that temperate bacteriophages from the lysogenic El Tor cultures are lethal to the *V. cholerae* strains in the mixture is ruled out by the result of experiments in which antiphage serum was added to neutralize all free phages on their liberation by lysogenic El Tor cultures before they could affect the *V. cholerae* cells, no effect on the rate of survival of the latter organisms being observed. In fact, the El Tor phages were completely inactive against *V. cholerae* strains that were not even lysogenized. In the present series of tests, attempts to demonstrate the presence in the media of any substance liberated by El Tor vibrios that was vibriocidal or vibriostatic towards *V. cholerae* cultures proved unsuccessful. Barua (1963) has reported similar findings. Most of the El Tor strains used for studying interaction with *V. cholerae* cultures were lysogenic, but the presence of vibriocin could not be demonstrated in any of the El Tor cultures.

The apathogenic El Tor strains differed from the case strains of El Tor vibrios in that they had no inhibitory effect on the survival of *V. cholerae*. This difference between the pathogenic and apathogenic strains in their interaction with *V. cholerae* cultures may be significant.

Although the precise mechanism of the interaction between the two types of pathogenic O group I vibrios could not be ascertained, the possible biological effects of such interaction on the epidemiology of cholera would seem to be far-reaching. This has been evidenced in Pakistan and India, where classical cholera has been active for centuries and El Tor infection made its first entry in 1963 and 1964, respectively. In many areas of the subcontinent, cholera El Tor cases now either outnumber or have even completely replaced cases of classical cholera (Mukerjee et al., 1965). These epidemiological observations are to a large extent supported by the laboratory findings of interaction between *V. cholerae* and *V. El Tor* in mixed cultures *in vitro* and *in vivo*, which is likely to be one of the important mechanisms responsible for the change in type of the causative vibrios. Other factors, such as the longer survival time of El Tor vibrios in natural environments and the higher carrier rates and the longer duration of carrier states in El Tor infections, are also likely to play significant roles.

POSTSCRIPT

After this paper had been submitted, Wahba (1965) reported a technique for the demonstration of the production by *V. cholerae* and *V. El Tor* strains of bacteriocine (vibriocine) active against either type of vibrio. By this technique he was able to detect vibriocine-like action with some strains of *V. cholerae* and *V. El Tor*. However, in the present work no vibriocines could be demonstrated in El Tor strains GS 9/65 and Phil 1/65 when tested against *V. cholerae* indicator strains GS 1/65 and Asm 1/65. Since these were the strains mostly used in our interaction experiments, the results reported in this paper cannot be explained as due to the production of vibriocine by the El Tor strains.

ACKNOWLEDGMENTS

Our thanks are due to Dr A. Narayanaswami for his helpful suggestions. The technical assistance of Mr M. L. Chowdhury, Mr B. Sanyamat, Mr I. Guha Thakurta and Mr K. N. Maitra is acknowledged.

RÉSUMÉ

Au cours des dernières années, le choléra classique, provoqué par *Vibrio cholerae*, est resté limité à la région de l'Inde et du Pakistan, tandis que le choléra à *Vibrio El Tor* se répandait largement dans le Pacifique occidental et l'Asie du Sud et du Sud-Est. Depuis 1963, le choléra El Tor est apparu dans la région infectée par *V. cholerae* et les cas provoqués par *V. El Tor* ont rapidement dépassé en nombre, ou même ont complètement remplacé, ceux dus à *V. cholerae*. Les auteurs ont cherché dans l'interaction des deux types de vibrions une explication à la tendance épidémiologique actuelle du choléra.

En culture mixte de *V. cholerae* et de *V. El Tor*, le premier est éliminé en 2 à 6 heures. La croissance de *V. El Tor* est plus rapide que celle de *V. cholerae*, particulièrement au début de la culture, mais ceci n'explique pas entièrement l'éviction de ce dernier dont la diminution en nombre a encore été notable lorsqu'il était inoculé à dose cent fois plus élevée que *V. El Tor*. Les filtrats de culture permettant encore de cultiver *V. cholerae*, le phénomène n'est dû ni à une insuffisance de substances nutritives, ni à un manque d'oxygène provoqués par la pousse rapide de *V. El Tor*. Si, après deux heures de

culture, on agglutine *V. El Tor* par adjonction d'un sérum spécifique, la croissance de *V. cholerae* reste inhibée de façon marquée. En poursuivant l'incubation, on a constaté que *V. cholerae* se multipliait dans le liquide surnageant, ce qui laisse supposer que l'inhibition de *V. cholerae* a lieu au cours de la phase de multiplication active de *V. El Tor*. Cet effet inhibiteur n'a pas été retrouvé en utilisant la souche non pathogène de *V. El Tor*.

Les auteurs ont éliminé expérimentalement l'action possible d'un bactériophage apporté par la souche El Tor et léthal pour les souches de *V. cholerae*. Ils n'ont pu mettre en évidence aucune substance libérée dans le milieu par *V. El Tor*, expliquant l'action subie par *V. cholerae*. Ils ont reproduit *in vivo*, sur anse isolée d'intestin grêle de lapin adulte ou par inoculation intestinale à de jeunes lapereaux, leurs résultats *in vitro*. Ils concluent que ces résultats de laboratoire laissent leur place à d'autres mécanismes tels que le temps de survie plus grand de *V. El Tor* dans le milieu extérieur, le plus grand nombre de porteurs sains de ce type et la plus longue durée de l'état de porteur dans les infections qu'il provoque, comparés à ceux de *V. cholerae*.

REFERENCES

- Barua, D. (1963) *Nature* (Lond.), **200**, 710
 De, S. N. & Chatterjee, D. N. (1953) *J. Path. Bact.*, **66**, 559
 Dutta, N. K. & Habbu, M. K. (1955) *Brit. J. Pharmacol.*, **10**, 153
 Finkelstein, R. A. & Mukerjee, S. (1963) *Proc. Soc. exp. Biol. (N.Y.)*, **112**, 355
 Fredericq, P. (1957) *Ann. Rev. Microbiol.*, **11**, 7
 Mukerjee, S. (1963) *Bull. Wld Hlth Org.*, **28**, 333
 Mukerjee, S., Basu, S. & Bhattacharya, P. (1965) *Brit. med. J.*, **2**, 837
 Roy, C., Mridha, K. & Mukerjee, S. (1965) *Proc. Soc. exp. Biol. (N.Y.)*, **119**, 893
 Shannon, R. (1957) *J. med. Lab. Technol.*, **14**, 199
 Wahba, A. H. (1965) *Bull. Wld Hlth Org.*, **33**, 661