

*Comment.*—All of the antimicrobial agents tried were effective in treatment of cholera El Tor cases, since duration of hydration and the period of excretion of vibrios could be shortened and the amount of intravenous fluid needed could be reduced. It is difficult, however, to conclude which of the drugs was most effective, though some differences were recognized in their effectiveness. As far as the period of excretion of vibrios is concerned, ery-

thromycin and tetracycline were superior to the other drugs; but as regards duration of hydration and amount of fluid required, chloramphenicol appeared to be better than the others.

It may be possible to work further on these problems during the Joint Study in order to find the most effective dosage schedule and the relative efficacy of the various antimicrobial drugs.

## Nonvibrio Cholera<sup>1</sup>

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In February and March 1964, during a seasonal period usually associated with a fall in the incidence of cholera in Dacca, a number of cases were admitted to the Pakistan-SEATO Cholera Research Laboratory ward in a state of circulatory collapse after an acute illness of less than 48 hours' duration, associated with the passage of copious rice-water stool. In most of these cases no vibrios were isolated from daily rectal swab cultures. During this period 15 such patients were admitted with profound dehydration in a completely collapsed state, without pulse or blood pressure, in whom no bacteriologic evidence of cholera infection was found. An additional 29 bacteriologically negative cases were admitted with moderately severe dehydration (plasma protein greater than 9 gm. percentage) associated with watery diarrhea. During the same period a total of 14 bacteriologically proven cases of cholera were admitted. It was apparent, therefore, that during this period a choleralike illness without bacteriologic evidence of cholera infection was more than twice as common on our wards as cholera itself. Accordingly, a prospective study was undertaken to obtain more complete clinical, physiologic, bacteriologic, and immunologic data on these cases and to compare them with established cases of cholera.

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### PLAN OF STUDY

The study was initiated on March 27, 1964 and continued until June 11, 1964. All patients with diarrheal disease admitted to the CRL ward in a state of circulatory collapse (absent pulse or pulse too rapid and faint to be counted), were admitted to the study. On each patient, admission plasma protein, electrolytes, white blood-cell count and differential, serum for agglutinin titers, and electrocardiogram were obtained before the initiation of any therapy. All patients were treated with intravenous fluids. Only two patients, both of whom had bacteriologically documented infection with *V. cholerae* Inaba, received antibiotics. All the determinations performed on admission were repeated on the seventh hospital day. Thirty to 100 ml. of liquid stool were obtained under oil from the sigmoid colon by means of a sterile catheter inserted through the rectum within a few hours of admission for determination of electrolytes, pH, sugar, gross and microscopic examination, and guaiac test for occult blood, as well as bacteriologic studies. The material was streaked on SS, MacConkey's, blood, gelatin (GA), and tellurite-taurocholate-gelatin (TTGA) agars and was placed in bile peptone enrichment fluid (Monsur, 1963). Seven ml. aliquots were distributed in 5 to 7 vials as soon as possible and stored at minus 45° C. In addition, dark-field examination was performed on fresh stool or rectal catheter specimens (Benenson, Islam, and Greenough, 1964). Throughout the hospital stay, daily rectal swab material was plated on GA and on TTGA and was placed in bile peptone enrichment fluid to be plated the following day. Strict intake and output measurements were made throughout the duration of illness. Stool was collected at 8-hour intervals for description and volume measurement. The end of the last 8-hour period before

the passage of nonliquid, semifformed-to-formed stool was considered to be "end of diarrhea."

**Immunologic studies.**—Paired acute and convalescent sera were tested for agglutinating antibodies against live *V. cholerae* Inaba and Ogawa (Goodner *et al.*, 1962). Three cases from whom noncholera vibrios were obtained on stool culture were also tested for agglutinating antibodies against the homologous organism.

**Bacteriophage studies.**—Frozen stool aliquots from 28 of the patients were examined for the presence of bacteriophage lytic for cholera vibrios as well as for bacteriophage which could lysogenize cholera vibrios. Eight classical cholera vibrios, four El Tor vibrios, and two noncholera vibrios were used as indicator organisms. Stool aliquots were treated with chloroform, heat, and ultraviolet light to liberate temperate phage. Samples were also enriched with the indicator organisms overnight and then plated to detect the presence of phage.

#### RESULTS

**Bacteriologic findings.**—Stool cultures from 14 patients were positive for *V. cholerae* (11 Inaba, 3 Ogawa) on 2 to 7 successive days. In four other patients cholera vibrios were not isolated, but noncholera vibrios were demonstrated for 2 to 5 consecutive days on TTGA and/or GA plates streaked directly with the rectal swab. No vibrios or other recognized enteric pathogens were isolated from the stools of 15 patients. In a single patient *V. cholerae* Inaba was reported on rectal swab culture from the second hospital day only. His admission bacteriologic cultures and dark-field examination were negative, as were cultures on days 3-8, and no serum agglutinating antibodies against *V. cholerae* were detected in either acute or convalescent sera. The positive second-day culture therefore appeared to be a sampling error. However, this patient will be excluded from subsequent analysis of the data.

Admission blood cultures were obtained from 27 patients. None showed any growth after 14 days' incubation.

Bacteriologically, therefore, the patients in the study fall into 3 groups: (1) cholera vibrios, 14 cases; (2) noncholera vibrios, 4 cases; and (3) no vibrios or pathogens, 15 cases.

**Dark-field microscopy.**—In all of the 14 patients repeatedly positive for *V. cholerae* by culture, the identical diagnosis, including Inaba or Ogawa sero-typing, was independently made on dark-field examination. In the 15 cases where no pathogen was isolated, no vibrios were noted on dark-field examination. In the four cases with noncholera vibrios on culture, noncholera vibrios were identified in the dark-field in two, no vibrios were seen in one, and *V. cholerae* Ogawa was reported in one. In the last case no cholera vibrios were isolated bacteriologically

TABLE 1.—Bacterial isolations and vibrio agglutinating antibody titer rises

Bacterial isolate	Significant antibody rise <sup>1</sup>	No antibody rise	Inadequate sera	Total
<i>V. cholerae</i> Inaba . . . . .	11	.....	.....	11
<i>V. cholerae</i> Ogawa . . . . .	3	.....	.....	3
Noncholera vibrios <sup>2</sup> . . . . .	2	1	1	4
No pathogens . . . . .	0	15	.....	15

<sup>1</sup> Fourfold or greater rise in titer.

<sup>2</sup> Rise in antibody against homologous organism; none against *V. cholerae* Inaba or Ogawa.

at any time during the patient's illness, and no antibodies against cholera vibrios were detectable in acute and convalescent sera; this case was considered to be a dark-field false-positive. In the 15 cases from whom no pathogen was isolated, no vibrios were noted on dark-field examination.

**Immunologic studies** (table 1).—Paired sera were available in 32 cases. In 14 of 14 cases from whom *V. cholerae* was isolated, a fourfold or greater rise in agglutinin titer occurred between specimens taken on admission and those taken after the sixth hospital day. In two of three cases with noncholera vibrios, a fourfold or greater rise in agglutinin titer against the homologous organism (but not against *V. cholerae*) was demonstrated. No rise in agglutinating antibody titer against *V. cholerae* was observed in any of the 15 cases from whom no pathogen was isolated.

**Bacteriophage observations.**—Lytic phage was not isolated from any of the 28 stools examined. From four stools temperate phage was isolated, two from stools with *V. cholerae* Ogawa and two from stools with noncholera vibrios (table 2). After overnight incubation of the stools with the indicator strains, temperate phage was detected in 4 of 14 stools that were bacteriologically negative.

**Clinical and biochemical observations.**—Various clinical and biochemical features of the cases are summarized

TABLE 2.—Bacteriophage isolations

Bacteriological findings	Temperate phage	No phage isolated	Total
<i>V. cholerae</i> Inaba . . . . .	0	9	9
<i>V. cholerae</i> Ogawa . . . . .	2	0	2
Noncholera vibrio . . . . .	2	1	3
No pathogen isolated . . . . .	4	10	14
Total . . . . .	8	20	28

TABLE 3.—Clinical features

	Cholera	No pathogens	Non-cholera vibrios
Watery diarrhea.....	14/14	15/15	4/4
Vomiting.....	14/14	15/15	4/4
Muscle cramps.....	13/14	15/15	4/4
Abdominal pain.....	5/14	11/15	3/4
Hoarseness.....	14/14	14/14	4/4
Unconsciousness.....	3/14	4/15	1/4
Oliguria.....	13/14	13/15	2/4
Documented fever.....	10/14	11/15	4/4
Duration of symptoms from onset to time of admission:			
Mean..... hours..	14	10	11
Range..... hours..	3-32	5-21	4-18

and compared in tables 3 through 6. It is apparent that the clinical symptomatology in the three groups (table 3) was identical, as was the degree of dehydration on admission as judged by a plasma protein level (table 4). Most of the patients were young adults, predominantly male. There were two 6-year-old children in the group with *V. cholerae*, while the youngest patient in the group

with no pathogens was 12. Leucocytosis was similar in degree and character (increased neutrophils, with a predominance of band forms) in the three groups. Admission blood electrolytes were similar (table 4). Similar electrocardiographic abnormalities (including giant "p" waves and an axis shift towards the right) were common in the 3 groups. Stool was alkaline and electrolyte-rich in all groups (table 5). The patients with *V. cholerae* tended to have a slightly higher mean pH and bicarbonate content than those with no pathogens. The so-called "typical rice-water stool" of cholera was seen in all groups, though none of the patients with *V. cholerae* had the reddish-brown stool frequent in the patients with non-cholera vibrios and with no pathogens. The most striking differences were in the mean duration and volume of diarrhea after admission (table 6). The average cholera patient purged for 4.6 days, in comparison with the 1.5 days for those with no pathogens, and passed more than eight times as much watery stool. Even in these respects, however, there was an overlap between the two groups. Thus, one patient with *V. cholerae* passed a total of 5.2 liters of stool during 2.7 days of diarrhea, while a patient from whom no pathogens were isolated had diarrhea for 4.3 days and purged 17.9 liters of stool. In general, however, the duration of illness in the group with

TABLE 4.—Clinical and biochemical findings<sup>1</sup>

	Number of cases	Sex ratio M : F	Age in years	Body weight in kg.	Plasma protein in gm. (percentage)	
					Admission	Convalescent
<i>V. cholerae</i> .....	14	11 : 3	28 (6-45)	39 (13-63)	11.7 (8.7-14.2)	7.1 (6.0-8.6)
No pathogens.....	15	10 : 5	35 (12-60)	38 (34-41)	11.8 (9.8-14.4)	7.2 (6.0-8.0)
Noncholera vibrios.....	4	2 : 2	26 (18-30)	39 (23-54)	11.3 (10-13.2)	6.9 (6.5-7.4)

  

	Total white blood count	Serum electrolytes mEq./l.			
		Na	K	Cl	CO <sub>2</sub>
<i>V. cholerae</i> .....	21,700 (4,000-36,000)	140 (139-144)	4.5 (3.5-5.0)	96 (86-119)	15.3 (9.1-22.0)
No pathogens.....	22,300 (6,450-38,000)	138 (126-142)	4.1 (3.2-4.7)	96 (82-110)	14.5 (9.5-20.3)
Noncholera vibrios.....	21,400 (14,800-32,200)	141 (138-144)	4.2 (4.0-4.6)	96 (90-100)	16.0 (14.5-18.9)

<sup>1</sup> Comparison of various parameters in the three groups of patients included in the study. Mean values are presented. The figures in parentheses following the mean values indicate the ranges of values recorded.

TABLE 5.—Stool characteristics

	pH <sup>1</sup>	Na <sup>1</sup>	K <sup>1</sup>	Cl <sup>1</sup>	CO <sub>2</sub> <sup>1</sup>
<i>V. cholerae</i> .....	7.54 (6.85-7.95)	139 (46-161)	24.3 (10.2-82.0)	106 (63-129)	48.5 (26.9-66.5)
No pathogens.....	7.11 (6.36-7.80)	123 (70-163)	31.1 (7.7-66.5)	99 (89-130)	41.5 (18.1-68.0)
Noncholera vibrios.....	7.52 (7.08-8.12)	111 (99-130)	46.4 (25.6-56.4)	91 (82-98)	45.3 (39.6-55.2)

  

	Glucose <sup>1</sup>	Appearance	Mucus	WBC	RBC	Guiaic positive
<i>V. cholerae</i> .....	0 (0-0)	9 "rice-water".....	+ - + + +	+ - + + +	0 - +	3/13
		5 yellow.....				
No pathogens.....	2.8 (0-17)	5 "rice-water".....	+ - + + +	+ - + + +	0 - + + +	5/11
		4 yellow.....				
		6 red-brown.....				
Noncholera vibrios.....	1.7 (0-5)	1 "rice-water".....	+ - + + +	+ - + + +	0 - +	0/3
		3 red-brown.....				

<sup>1</sup> Mean values (ranges in parentheses).

no pathogens was short, and most of these patients stopped purging by the second hospital day. All of the patients in the three groups responded rapidly to intravenous fluid and electrolyte replacement therapy, and there was no mortality in any of the groups.

TABLE 6.—Duration and volume of diarrhea

	Number of cases	Mean duration, days after admission	Range	Mean volume, liters	Range
<i>V. cholerae</i> .....	<sup>1</sup> 12	4.7	2.7-6.3	30.8	5.2-69.1
No pathogens.....	15	1.5	0-4.3	3.7	0-17.9
Noncholera vibrios.	4	2.1	1-3	3.0	2.3-3.4

<sup>1</sup> 2 cases of the 14 with *V. cholerae* that received Tetracycline therapy are not included.

DISCUSSION

These findings indicate that a syndrome occurs commonly in East Pakistan which often mimics classical cholera gravis in every respect, but which cannot be attributed to infection with *V. cholerae*. In none of the 15 patients with watery diarrhea and circulatory collapse who presented repeated negative bacteriologic cultures were vibrios found on dark-field examination; and in no case was there a rise in serum agglutinating antibody

titers against *V. cholerae*. The striking agreement between three independent techniques (bacteriologic culture, dark-field, and serologic) in failure to demonstrate evidence of vibrio infection in all 15 cases would appear to rule out *V. cholerae* as an etiologic agent. The bacteriologic studies undertaken did not reveal an agent responsible for the watery diarrhea. Virus studies have not yet been completed on the material collected from these cases. In the light of our present state of ignorance, and of the clinical similarity of the cases to classical cholera, the syndrome may be dubbed "nonvibrio cholera."

The findings further support a view held by many clinicians since the nineteenth century—that the cholera syndrome in all its manifestations, including circulatory collapse, muscle cramps, hoarseness, unconsciousness, and urinary suppression, is not specific for infection with *V. cholerae*, but is rather the physiologic result of extreme dehydration and electrolyte loss resulting from diarrhea and vomiting (Pollitzer, 1959).

Each of these 15 cases on admission presented the full-blown clinical cholera syndrome and could not be differentiated from classical cholera. Each responded dramatically to therapy with intravenous electrolyte solutions. Even on retrospective analysis only relative, rather than absolute, differences were noted between the patients with true cholera and those in whom no pathogens were found. In the latter group the diarrhea usually tended to be shorter in duration and less voluminous, and was sometimes associated with a pinkish or reddish-brown stool

color that is uncommon in patients with *V. cholerae* infection.

In view of the failure to demonstrate an etiologic agent, it may be asked whether "nonvibrio cholera" as seen in East Pakistan represents a single disease entity, or is rather a mixture of conditions of varying etiology having in common watery diarrhea, severe dehydration, and absence of infection with vibrios. Present information is inadequate to resolve this question. Though our experience with the syndrome is limited, certain features suggest that at least some of the cases may represent a specific disease entity. During the past 12 months the syndrome was encountered frequently in Dacca in February through May, but occurred as relatively rare sporadic cases in other months. Similiar observations were made in the course of a cholera vaccine field trial among 14,057 persons in a group of 23 villages near Matlab Bazar, 40 miles south of Dacca (Oseasohn, Benenson, and Fahimuddin, 1965). The incidence of severe, disabling true cholera (i.e., associated with inability to walk or respond) in a control group (TAB vaccinated) during the 1-year interval from December 1963 through November 1964 was 3.4 per 1,000. During the same period the incidence of severe, disabling diarrheal illnesses (using the same criteria of severity) from which *V. cholerae* was not isolated was 18.2 per 1,000. Nearly half (45 percent) of these illnesses unassociated with vibrios occurred during March, April, and May (table 7). While most of the severely disabling diarrhea

TABLE 7.—Frequency of severely disabling diarrheal illnesses not associated with *V. cholerae*, Matlab Bazar field trial area, December 1963 through November 1964

Time interval	Total cases	Rate/1,000
December-February.....	43	3.7
March-May.....	94	8.2
June-August.....	44	3.8
September-November.....	29	2.5
Total, December-November.....	210	18.2

due to *V. cholerae* occurred in persons below 15 years of age, nonvibrio diarrhea of similar severity was more frequent among those 15 and over.

Information collected both from the Matlab Bazar field trial area and from other areas of East Pakistan indicates that untreated or inadequately treated nonvibrio cholera may, like true cholera gravis, result in a fatal outcome. It must also be emphasized that the present study selected only the severest cases, those with the most profound de-

hydration. In the absence of a known etiologic agent certain identification of milder cases of the syndrome is difficult, and description of its full clinical spectrum is impossible.

Noncholera vibrios were demonstrated on repeated stool culture from four of the patients included in this study. Like those in whom no known pathogens were recovered, these cases mimicked classical acute cholera (tables 3 through 5), though, as in the group with no pathogens, average duration and volume of diarrhea were less than in the patients with *V. cholerae* infection (table 6). In two of the cases with noncholera vibrios, rises in antibody titer occurred against homologous noncholera vibrios, but not against *V. cholerae*. That infection with noncholera vibrios may occasionally give rise to the full-blown cholera syndrome has been observed repeatedly (Pollitzer, 1959; McIntyre *et al.*, 1964).

#### SUMMARY AND CONCLUSIONS

The syndrome in which the passage of copious quantities of rice-watery stool results in extreme dehydration and circulatory collapse is not specific for infection with *V. cholerae*. Such a syndrome, in which no evidence for *V. cholerae* infection was demonstrable by repeated bacteriologic culture, dark-field examination, and immunologic methods, occurred more frequently in areas of East Pakistan in the spring of 1964 than did true cholera itself. The duration and volume of watery diarrhea were usually less than in cases of true cholera. In a few cases infection with noncholera vibrios was associated with the syndrome, but in most cases no known pathogen was recovered.

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