CRL TECHNICAL COMMITTEE

22 - 24 October 1963

AGENDA

MORNING, TUESDAY, 22 OCTOBER

Dr. James A. Shannon, Chairman

0900 - 0915	Welcoming remarks - Dr. Benenson.
0915 - 0925	Report on CRL Ward - Miss Torrance.
0925 - 1015	Clinical & Therapeutic Aspects of Cholera - Dr. Greenough Discussion by Drs. Phillips, Carpenter, Wahed, Woodward.
1045 - 1115	Dedication of Plaque in Memory of Dr. Joseph E. Smadel.
1115 - 1135	Discussion of World Cholera Situation - Drs. Pease, Luang Binbakya Bidyabhed, Dizon, Phillips.
1135 - 1150	Bacteriologic Observations - Mr. Imdadul Huq.
1150 - 1210	Cholera Phage Studies - Dr. Monsur Discussion by Drs. Goodner and Cruickshank.
1230 - 1330	L U N C H
1330 - 1700	Field Trips

MORNING, WEDNESDAY, 23 OCTOBER

Dr. A.K.M. Abdul Wahed, Chairman

0845 - 0915	Household & Family Studies - Dr. Stockard.
0915 - 0930	Relation of Water to Cholera - Mr. Zafar Discussion by Drs. Black, Francis, Flynn, Yen.
1015 - 1100	Visit to laboratories; Coffee
1100 - 1130	Host Factors in Cholera Susceptibility - Dr. Gordon. Discussion by Drs. Kamaluddin, Yen, Berliner, Khuda.
1200 - 1230	A Cholera Focus in Dacca City - Dr. Fahimuddin & Mr. Zafar. Discussion by Drs. Dizon and Flynn.
1245 - 1330	L U N C H

AFTERNOON, WEDNESDAY, 23 OCTOBER

Dr. Robert Cruickshank, Chairman

1330 - 1400	Immunological Observations on Patients and Exp. Animals - Dr. Benenson. Discussion by Drs. Goodner and Phillips.

Continued....

1420 - 1440	Vaccine Studies of March - April, 1963 - Dr. Q. Khan.
1440 - 1450	Responses and reactions to Cholera Vaccine - Dr. Benenson.
1450 - 1505	Distribution of Cholera Patients in Dacca and Keraniganj - Dr. Stockard.
1505 - 1535	Matlab Bazar - Dr. Oseasohn and Dr. Majumdar. Discussion by Drs. Francis, Black, Luang Binbakya Bidyabhed, Lewthwaite, and Shannon.

THURSDAY, 24 OCTOBER

0900 -	Executive Session - Technical Committee and Selected Participants.
	Others - Laboratory and Field Discussions.

PAKISTAN-SEATO CHOLERA RESEARCH WARD

In November, 1962, the ward attached to the Cholera Research Laboratory was opened. The staff consisted of Dr. Gordon and Dr. Greenough with three Pakistani trained nurses and two nursing aids. Two male nurses worked in the pharmacy and central supply. Domestic staff assisted in the ward, kitchen, and laundry. During this first month, the patients were male convalescent cholera cases transferred from Mitford Hospital. In December and January, male and female patients, including children, were admitted directly. More trained nurses and aids were employed. Dr. Gordon and Dr. Greenough kept records, and measured intakes and outputs, as the nurseswere not familiar with the system of weights and measures used here. However, the nurses quickly learned the new techniques and relieved the doctors of this work.

During the peak period, December and January, 137 patients were treated; 5 deaths occurred - 2 male adults, 1 female adult, 1 boy and 1 baby girl. These 5 died of acute cholera (Inaba). In February and March, four Pakistani doctors joined the ward. The number of patients decreased to 40 direct admissions and 22 transfers. In April and May, more nursing staff was employed. The number of patients increased, direct admissions amounting to 61, and transfers to 22. Two deaths occurred in April, both acute cholera (Ogawa), 1 male child and 1 female adult.

A Land Rover ambulance arrived at this time, and helped considerably. Previously, patients had been brought to the CRL either by autorickshaw or jeep. In June, the x-ray machine was installed, and a technician employed. The technician is responsible for taking and developing all films. From 1 July until 16 October, 280 roentgengraphic examinations were done:

Small bowel series	50
Oesophagus	2
Total opaque medium	52
Chest and other	228

In July, August, andSeptember, the medical and nursing staff was increased. The total staff now employed in the ward is as follows:

Doctors	- 6	Staff Nurses for ward	- 7
Hospital Supervisor (Nursing)	- 1	Nursing Aids	- 6
Senior Staff Nurses (Clinical Research)	- 2	Male Nurses (Pharmacy & ECG)	- 2
Senior Staff Nurse (Field & Follow-up)	- 1	Male Nursing Aids (Pharmacy & Field)	- 2

Domestic:

Housekeeper	- 1	Ward boys	- 2
Cooks	- 2	Cooks Asst.	- 1
Laundry	- 3	Sweepers	- 5

The ward consists of 19 beds plus 8 cholera cots. During the past ten months, 418 patients have been admitted to the ward: direct admissions,274; transfers from other hospitals, 144. An estimate ofthe total intravenous fluid administered is 1500 liters. New therapeutic techniques have been taught. The work done in the CRL ward over the past ten months shows that with prompt medical attention and good nursing care a low mortality can be achieved.

Clinical & Therapeutic Aspects of Cholera

Introduction:

From 28 November, 1962, when the first case of convalescent cholera entered the CRL, until 1 October, 1963, a total of 418 patients have been admitted. Among these were 225 cases from whom V. cholerae Inaba or Ogawa was isolated, 13 cases from whom only a non-cholera vibrio was isolated, and 180 that were not cholera. The cholera cases have included a complete spectrum of clinical severity ranging from asymptomatic carriers to patients who were pulseless on admission and subsequently put out more than 100% of their body weight in stool.

As the operation of the hospital improved through the year there have been changes in the case population admitted (Table I). In November and December, before the physical plant was completed, and when only a minimum staff of nurses were available, we gained experience by taking in transfer from Mitford Hospital convalescent male cholera cases. We started to receive acute patients and direct admissions from case number 20 onward. It was not until the end of December that we began to solicit admissions of women and children. Since then there has been a steady increase in the proportion of direct admissions to transfers. The duration of time between the onset of symptoms and admission to the hospital has steadily decreased. For this reason fewer patients are now receiving treatment before they are seen and there are fewer artifacts created by previous therapy. The proportion of males and females since February has been fairly equal. Children and babies have constituted a constant fraction. In February our Pakistani doctors began taking clinical responsibility. By May the ward was being entirely run by a group of four Pakistani doctors under our supervision.

Methods:

On each admission we expect to accomplish the following observations:

1. History and physical examination.
2. Routinely a rectal swab on admission was examined for vibrios and other pathogens and daily thereafter rectal swabs were studied only for vibrios.
3. Body weight on admission and daily thereafter.
4. Pulse, temperature, blood pressure, and respiratory rate as indicated by the severity of the patient's illness.
5. Intake and output at 6 hourly intervals on all cases with active diarrhea.
6. ECG and blood tests on admission, and on the 2nd, 4th, and 7th hospital days. The blood tests included are hemoglobin, microhematocrit, WBC and stained smear, plasma protein, plasma Na, K, Cl, CO_2, and red blood cell Na and K. Creatinine and blood sugar are determined when clinically indicated.
7. Since June our x-ray unit has been functioning and all patients are having chest x-rays during their hospital stay.
8. Urinalysis and stool examination for parasites during convalescence.

All clinical and laboratory data were coded on ICT cards and analyzed. A sample sheet of code 401 is included.

Basic fluid and electrolyte replacement is administered to all cases with clinical evidence of dehydration. In cholera cases an initial clinical appraisal of the degree of dehydration is made, a blood sample is drawn - usually from the femoral vein, and initial rehydration is begun with isotonic saline by the same route. The clinical estimate is checked by the level of the plasma protein. After rapid re-establishment of circulation the object of treatment has been to match the stool flow in quantity and composition by intravenous replacement. We make our solutions by adding reagent grade chemicals to bottles of sterile distilled water. Solutions of 5 gms. $NaHCO_3$, 4 gms NaCl, and 1 gm KCl or 4 gms $NaHCO_3$, 4 gms NaCl, and 2 gms KCl are used. If acidosis is severe as judged by CO_2 values solutions of isotonic $NaHCO_3$ with 1 or 2 gms of KCl are employed. Any special modes of therapy such as antibiotics are used in addition to the basic replacement of fluid and electrolyte losses.

Patients are generally kept for 7 days to allow observations of antibody response to their infection. Since February an effort to make at least one follow-up contact of every cholera cases has been made. At that time a sample of blood and urine is taken and ECG is done.

Results and discussion:

During the peak of admissions in December and January we saw many patients with prolonged heavy purging and continued heavy excretion of V. cholerae. The logistic problem of supplying fluids, and the tedium of maintaining adequate watch prompted us to reconsider using a potent vibriocidal antibiotic agent. Although no clinical effect of antibiotics has been reported it seemed reasonable to us that eradication of the organism should result in an effect on the clinical course of the disease. We used tetracycline as a relatively nontoxic potent vibriocidal agent that could be given parenterally as well as orally. We did not initially have either placebos or the staff to carry out a good double blind study. Cases were assigned to treatment groups according to the decision of the physician on duty. The details of the populations studied and the results of the study are contained in an appended paper which is being submitted to Lancet.

Despite poor experimental design, and bias in the populations compared, we feel the results indicate a decisive effect of tetracycline on the clinical course of cholera. The difference between the groups is in the direction of placing more severely ill cases on tetracycline treatment. There is also a discrepancy in vitamin treatment between the groups. In the case of the first bias - severity of initial syndrome- an analysis of our cases shows a strong positive correlation of initial severity with subsequent course ($p < .01$) as measured by initial 24 hour stool volume compared with subsequent stool output or with duration of diarrhea. Thus the bias would tend to obscure a therapeutic effect. Cases receiving vitamin therapy but not antibiotic have been compared with controls on fluid treatment alone and no difference is apparent. In all other important respects the tetracycline treated and control groups are nicely matched. The results of treatment as measured by stool flow after 24 hours, intravenous fluid requirement after 24 hours, and duration of diarrhea are all striking ($p < .001$). It is also apparent that the stool is rapidly cleared of vibrios. These statistical facts were also very apparent to us at

the bedside. There were no cases treated with tetracycline that continued purging to a significant degree after the third day while there were a number of cases not treated that went on for 5 - 7 days. In general we expected that we would be able to discontinue i.v. fluid treatment within 24 hours of starting tetracycline.

After the initial series of cases treated by tetracycline we instituted an alternate case double blind study using oxytetracycline and an inert placebo.* These were administered by the intravenous route in a dose of 0.5 gm in the first infusion and every 12 hours thereafter while the patient was receiving i.v. fluids. In addition, an oral dose of 250 mgm every 6 hours was given. We studied a total of 12 positive cases in one group and 14 in the other before running out of materials. The results were not suggestive of any clinical effect (Table II). Finally in another study this September we again employed oxytetracycline, but without placebo. This was done in hopes that the greater severity and duration of the cases entering during this month might permit us more easily to see a clinical effect. The results of this study are summarized in Table III. A definite effect seems to be present.

Finally a word should be said about therapeutic trials with vitamins. Since vitamin deficiencies had been seen in cholera patients last winter, and we were studying the possible relationship of various deficiencies to susceptibility to cholera, we wished to explore the therapeutic potential of vitamins. This was especially important because of the significant amount of vitamin C in the intravenous preparations of tetracycline. Therefore the 39 control cases in the tetracycline study were divided into vitamin treated and not treated, and analyzed. The vitamin treated group were somewhat more severe and remained so despite the therapy.

There is a discrepancy in demonstrability of clinical effects of antibiotic therapy both within our own studies and between our results and those of others. It seems unlikely that this is due to inherent superiority of tetracycline over oxytetracycline, although the former is probably somewhat more potent in the dosages used here. We believe that variations in the natural severity of cholera are more important. We grouped our cases by the period of the year in which they were admitted. The results of a comparison may be seen in Table IV. Although there is no highly significant difference in severity of disease between the different seasons, (number of cases in each period being too small), the trends are noticeable. We were seeing less severe cases from February (at the end of the winter epidemic) through June and July than in August and September. The differences become significant if we look at cases coming directly to the CRL within the first 24 hours of illness, omitting the many winter cases transferred from other hospitals. The initial study on tetracycline was done during the peak of the winter epidemic, while the oxytetracycline double blind study was done during the wane of the spring epidemic in June and July. The later oxytetracycline study (Table IV.) was done when clinical observations indicated increasing severity of disease. Since the observation of a strong clinical effect of antibiotics on cholera depends on a severe and prolonged syndrome, the demonstration of an effect during the double blind study could be expected to require a large series since it was done during a period of mild cholera. It is interesting to look

* Supplied through the courtesy of Pfizer Ltd., Karachi.

at the case population reported from Bangkok in 1959 (Morgan et al.) and see that the duration of positive culture in their untreated cases corresponds closely with duration of positive culture in our tetracycline treated cases. Our winter control series is strikingly different. That the situation in Dacca may not be unique is illustrated by a graph of the results from J. S. Peterson (Chin. Med. J. 64, 276 - 284, 1946) which shows that at that time in China cholera had a pattern similar to that seen at CRL. (Table V.)

Further observations on cholera not related to therapy have also been made. A comparison of character of disease between cases of cholera due to the serotypes Inaba and Ogawa was carried out and no differences were found. A detailed comparison of all cholera cases and non-cholera cases was carried out and is summarized in Table VI. The differences found were as expected with the exception of the increased incidence of T. trichura in cholera cases, and increase in mean corpuscular hemoglobin concentration (MCHC) between admission and the convalescent period, suggesting a decrease in the size of the cells. Of greatest interest in the non-cholera cases is the rarity of any etiologic diagnosis. This suggests that we are basically very ignorant of the causes of diarrheal disease in this area. The group from whom we could isolate no vibrios contained two cases of typhoid fever, two cases of shigella associated diarrhea, and the remainder are mainly acute diarrheas of unknown cause. Non-cholera vibrios have been isolated from 13 cases as the sole agent present on direct plate cultures. In 4 of these there has been a significant titre rise against the homologous organism or against NCV No. 696. We have observed two cases with unequivocal rise in antibody titre against V. cholerae in association with acute diarrhea from which no pathogen could be isolated. There remain a very interesting group of cases that resemble cholera but on which no etiologic diagnosis has been made by any method. Many of these cases enter with a very severe initial syndrome which resembles cholera with respect to history, signs of dehydration, hemoconcentration, acidosis, and ECG abnormalities, but recover very rapidly. One such case fortuitously deposited some vomitus in the front yard of the hospital, which had a strongly positive culture for V. cholerae Inaba, but rectal swabs taken at the time and thereafter were negative. She had ECG changes typical of those seen in acute cholera.

A variety of other clinical observations has been made during the past year, all of which I shall not discuss. We have had a total of 9 deaths, only six of which were hospital deaths because of cholera. One was a case of pulmonary edema (without cholera) transferred in a moribund state to us from another hospital. One was a fetus at term, which probably died before treatment of the mother's cholera was begun, and which was stillborn in our ward. Four cases had been transferred from elsewhere after periods of prolonged vascular collapse with anuria and profound acidosis. Two babies with acute cholera were lost, one probably due to inadequate initial fluid replacement. The cause of the other's death is obscure. One cholera patient died in convalescence, probably not as a consequence of cholera. Of these cases 5 were autopsied and a summary of findings is included in appendix 3.

We have seen varying degrees of urinary suppression, but no death in uremia. In one case a peritoneal dialysis was carried out with an exchange of 41 litres and excellent response. All others regained kidney function without need of dialysis. All cases in this category were transfers

who had experienced a variable duration of vascular collapse and inadequate replacement. All cases admitted directly to CRL within the first 24 hours of disease put out urine copiously within 24-48 hours. It is interesting, however, that many cases despite prompt and early rehydration fail to put out urine as promptly as would be expected after resoration of circulation.

Clinical tetany responding to intravenous calcium has been seen in five cases. None of these had elevations of CO_2 above normal. However, all these have occurred in the past 2-3 months since we have made a more vigorous effort to correct acidosis within the first 24 hours of hospitalization.

Severe hypoglycemic coma has been seen in 8 cholera cases and 2 non-cholera cases on the first to fourth day of diarrhea and fasting. There have also been many cases with low blood sugars at this time of disease with no symptoms other than pronounced hunger. Two diabetics have been seen in association with proven clinical cholera. Two cases of coma recovering spontaneously late in convalescence from cholera were seen in transferred cases. No cholera case has been observed to have elevated blood pressure on convalescence or at follow-up. The overall incidence of BP greater than 140/90 from the Nutrition Survey is about 10%.

A large number of electrocardiograms have been done on both cholera and non-cholera patients. There are several marked deviations from normal. In cholera cases with vascular collapse there is marked increase in amplitude of the P waves, depression of ST. segments and high T waves. With return of circulation these T waves become flattened and the P wave and ST segment abnormalities return toward normal. Without potassium replacement the T waves remain flattened for several weeks. Infusion of large amounts of potassium chloride will return the T waves to normal rapidly. There are in addition to the abnormalities of ECG cardiac findings by auscultation in a few very severe cases including diastolic gallop rhythm, accentuated pulmonic second sound and basal rales despite inadequate rehydration.

Summary:

A large and varied group of cholera cases have been observed and studied over the past ten months. Studies on clinical effects of tetracycline, oxytetracycline and vitamins B and C have been carried out. We feel the differences observed with tetracycline justify a conclusion that this agent shortens the duration of diarrhea and decreases intravenous fluid requirements in cases of cholera, and oxytetracycline is probably effective as well. We have observed differences in the clinical severity of cholera at different times of the year. These differences seem to have influenced our ability to demonstrate the therapeutic effect of antibiotics.

Table I.

	Nov. & Dec.	Jan.	Feb.	March	April	May	June & July	Aug. & Sept.	Total
Total adm.	65	72	34	28	43	40	69	68	418
Direct adm.	18	35	23	17	30	31	57	63	274
Transfers:	47	37	11	11	13	9	11	5	144
from Mitford	47	29	7	8	12	5	11	4	123
" Victoria	-	8	4	1	-	3	-	1	17
" Other	-	-	-	2	1	1	-	-	4
Males	60	56	19	20	22	24	35	35	271
Females	5	16	15	8	21	15	34	33	147
Children less than 5 years	4	15	7	3	12	10	22	17	86
Deaths	1	4	-	-	2	-	-	1	8
Total cholera:	51	52	17	7	26	7	30	35	225
Inaba	51	47	14	6	14	5	1	-	138
Ogawa	-	4	2	1	12	2	27	29	77
Ogawa & N.C.V.	-	-	-	-	-	-	1	4	5
Ogawa & other pathogen	-	1	-	-	-	-	1	2	4
Inaba & Ogawa	-	-	1	-	-	-	-	-	1
N.C.V.	-	-	1	2	2	6	2	-	13
Non-cholera*	14	20	17	21	15	26	37	30	180

Composition of CRL ward admissions according to month of onset.
November 1962 through September 1963.

* All cases from which no vibrios were isolated.

Table II.

Double Blind Oxytetracycline Study
On Cholera Patients May - July, 1963.

		Group A	Group B	P
Males		8	9	$\geq .05$
Females		4	5	
Body weight	< 10 Kg.	2	2	$> .05$
	> 10 Kg.	10	12	
Admission Plasma Protein	< 10 gm.%	7	8	$> .05$
	> 10 gm.%	5	6	
First 24 hr. stool volume	<200 ml/kg.	10	11	$> .05$
	>200 ml/kg.	2	3	
Duration positive culture	< 3 days	10	14	$> .05$
	> 3 days	2	0	
Duration diarrhea	< 3 days	10	11	$> .05$
	> 3 days	2	3	
Stool volume after first 24 hrs.	<250 ml/kg.	9	12	$> .05$
	>250 ml/kg.	3	2	

Table III.

Oxytetracycline Study On Cholera Patients August - October 1963.

		Treated	Untreated	P
Males		4	11	> .05
Females		5	9	
Body weight	< 10 Kg.	0	4	> .05
	> 10 Kg.	9	16	
Admission plasma protein	< 10 gm.%	3	12	> .05
	> 10 gm.%	6	7	
First 24 hr. stool volume	< 200 ml/kg.	4	14	> .05
	> 200 ml/kg.	5	6	
Duration positive culture	< 3 days	9	10	< .05
	> 3 days	0	10	
Duration diarrhea	< 3 days	9	8	< .001
	> 3 days	0	11	
Stool volume after first 24 hours	< 250 ml/kg.	8	8	< .05
	> 250 ml/kg.	1	12	

Table IV.

Severity of Cholera in Patients Admitted To CRL From February to August and August To October with Less than 24 Hours Disease Before Admission.

		Feb.-Aug.	Aug.-Oct.	P
Males		17	9	> .05
Females		15	6	
Body weight	< 10 Kg.	4	2	> .05
	> 10 Kg.	28	13	
Admission plasma protein	< 10 gm.%	21	8	> .05
	> 10 gm.%	7	8	
First 24 hr. stool volume	< 200 ml/kg.	25	10	> .05
	> 200 ml/kg.	7	5	
Duration positive culture	< 3 days	25	5	< .01
	> 3 days	7	10	
Duration diarrhea	< 3 days	25	4	< .01
	> 3 days	7	11	
Total stool volume	< 500 ml/kg.	28	5	< .001
	> 500 ml/kg.	4	10	

Table V.

Percent Patients Continuing to excrete Vibrio Cholerae by Day of Disease.

Day of Disease	0	1	2	3	4	5	6	7	Number of Cases
Dacca - Winter	100	79	75	59	42	21	8	4	39
Dacca - Feb. to Aug.	100	72	41	28	22	19	9	9	32
Dacca - Aug. to Oct.	100	87	73	67	67	40	27	7	15
Bangkok (1)	100	51	22	3	2	0	0	0	115
China (2)	100	96	84	71	55	30	23	19	1949

(1) Morgan, F.M. et al. J. Med. Ass. Thailand 42, 413 (1959).

(2) Peterson, J.S. Chin. Med. J. 64, 271 (1946).

Table VI

Comparison of Cholera, N.C.V. & Non-Cholera
Cases Admitted to CRL During 1962 - 1963

	Cholera 225 cases	N.C.V. 13 cases	Non-cholera 180 cases
Age	16 ± 1 *	26 ± 5	19 ± 1
Weight in Kg.	27 ± 2	31 ± 3	29 ± 1
Sex Ratio M/F	69%	69%	61%
Vaccination history	43%	58%	46%
Previous cholera history	5%	8%	8%
Plasma protein on admission	9.4 ± .3	7.3 ± .4	7.4 ± .1
Plasma protein convalescent	6.6 ± .1	6.4 ± .4	6.6 ± .1
Homatocrit convalescent	34 ± 1	38 ± 1	34 ± 1
Parasites	82%	85%	84%
Ascaris	41%	46%	41%
Hook	35%	46%	33%
Trichuris	37%	54%	13%
Fever>99 at admission	15%	39%	40%
Days of diarrhea	1.2 ± .1	.5 ± .2	.3 ± .1
Total stool volume ml/kg.	154 ± 17	23 ± 17	19 ± 7
Tetany	5	0	0
Hypoglycemia Blood Sugar <40 mg.%	8	3	2
Increase in MCHC	34%	38%	14%

* ± Standard Error of the Mean.

TETRACYCLINE IN THE TREATMENT OF CHOLERA

Antibiotics are well known to be vibriocidal *in vitro* and *in vivo*; but, surprisingly, no beneficial effect of antibiotic therapy on the clinical course of cholera has been reported. Pollitzer (1959) has reviewed the available literature. If the clinical syndrome of cholera is the result of the presence of *Vibrio cholerae* in the gastrointestinal tract, removal of the organism should result in observable clinical benefit. During December 1962 cholera cases entering the ward facilities of the Pakistan SEATO Cholera Research Laboratory included many instances of severe and prolonged diarrhoea, associated with continued excretion of large numbers of vibrios. With replacement therapy alone, the management of these cases required large quantities of fluids and prolonged professional care. In hopes of shortening the course of the illness, we elected to administer a potent vibriocidal agent. This report presents the results observed in 77 patients with bacteriologically proven cholera, all of whom received fluid and electrolyte replacement. In 38, tetracycline was also administered by the intravenous and oral routes, while the other 39 received no antibiotic or other vibriocidal agent. Treated and control cases were not strictly alternated, but the groups were sufficiently alike to make comparison appear valid.

MATERIAL AND METHODS:

Proven cholera cases admitted to the ward of Pakistan-SEATO Cholera Research Laboratory during the last week of December 1962 and the first four months of 1963 were analyzed. Any case requiring fluid replacement on admission or passing at least 1% of body weight in liquid stool during a 24-hour period was included. Only patients receiving both intravenous and oral tetracycline were considered in the antibiotic-treated group. 39 cases meeting these criteria fell into the tetracycline-treated group, and 40 fell into the control group. Two deaths occurred in this series. One, in the tetracycline-treated group, was that of a 40-year old man who had been in vascular collapse for approximately 72 hours prior to admission. One two-year old child in the control group died as a result of insufficiently rapid initial rehydration. These two cases have been excluded from analysis in the comparison of the two groups with respect to morbidity.

On admission and daily thereafter, a rectal swab was plated directly on gelatin agar (Smith & Goodner, 1958), and bile tellurite agar (Monsur, 1961), as well as being put through enrichment media for vibrios (alkaline peptone or bile tellurite broth). Isolates were verified by slide agglutination using both group and specific Ogawa and Inaba antisera. No other pathogens were identified in any of these cases.

Serum proteins were measured by refractometry in a Bausch and Lomb serum protein meter. Serum CO_2 content was determined by the van Slyke manometric technique on blood drawn under oil. White blood cell counts, differential counts, microhematocrits and photoelectric hemoglobin (cyan-methemoglobin technique) were done on all cases in the acute and convalescent periods. Urinanalysis and stool examinations for ova and parasites were carried out during convalescence.

Patients were treated on specially designed cholera cots which permitted total collection of stool and urine. Total intake and output were summarized at 6-hourly intervals. The accuracy of intake and output measurements was verified by weighing patients at least once daily.

All patients were treated by fluid replacement along the lines suggested by the balance studies of Phillips and his collaborators (Watten, et al. 1959, Phillips 1962). An initial infusion of non-pyrogenic isotonic saline was followed by a bicarbonate-saline-potassium mixture consisting of 5 gm sodium chloride, 4 gm sodium bicarbonate and 1 or 2 gm potassium chloride per litre. The goal was to eliminate hypovolemia as soon as possible, correct acidosis, and then to maintain a balance between intake and output of fluids and electrolytes. No glucose was given by any route. In the majority of cases it was necessary to use the femoral vein for initial fluid administration and drawing of blood samples. Some cases received vitamin C, thiamine, and/or B complex vitamins parenterally.

In those cases treated with tetracycline, a dose of 100 milligrams (stabilized with 250 or 300 mgms of ascorbic acid) was added to the first or second litre of saline infused, and was given rapidly over a period of 15-20 minutes. An oral schedule of 250 mgms every 6 hours was begun immediately on admission unless vomiting prohibited, in which case the schedule was delayed 6-12 hours. Intravenous doses were repeated approximately avery 8 hours as long as medication could not be given reliably by the oral route.

The treatment groups were not established in a pre-arranged manner; but over the course of the epidemic, approximately equal numbers of patients fell into the tetracycline-treated and control groups (fig. 1). After an impression had developed that tetracycline therapy was beneficial, cases deemed to have a poor prognosis because of extremes of age or severity of symptoms were usually included in the tetracycline treatment group. In the analysis of records, the various parameters (listed in tables I and II) were examined to test the comparability of the two groups. The only points of comparison showing statistically significant discrepancy were vitamin therapy and initial intravenous fluid requirement. However, by all criteria examined, the group treated with tetracycline contained the more seriously affected patients.

RESULTS:

After the first 24 hours, both stool volume and requirement for intravenous fluids were strikingly less in the tetracycline-treated group (fig. 2). Tests of significance indicate t values of 3.9 and 18, respectively, and $p < .001$ for each comparison. Fig. 3 shows that duration of diarrhoea (defined to include any 24-hour period in which more than 1% of the body weight in liquid stool was passed) was significantly shortened in the tetracycline-treated group as compared to the control group ($t = 3.65$; $p < .001$).

The mean duration of bacteriological positivity of the stool was markedly reduced in the treated group ($t = 6.6$; $p < .001$), with no case having a positive culture for more than three days (fig.4).

Since vitamin therapy was not the same in the two groups, the 17 individuals in the control group who did not receive vitamins B or C were compared with the 22 who had received these vitamins. The same variables used to evaluate tetracycline were examined. No influence of vitamin therapy was demonstrable.

Because of the difference in initial intravenous fluid requirements of the two groups, the relationship of this variable to the subsequent course was examined in those cases not treated with antibiotic. The coefficient of correlation was positive and highly significant ($p < .001$) for the comparison of intravenous fluid requirement during the first six hours with each of the following variables: stool volume after the first 24 hours, intravenous fluid requirement after the first 24 hours, and duration of diarrhoea after hospital admission. The coefficient of correlation for the comparison of initial intravenous fluid requirement with the duration of positive cultures was lower, but still significant ($p < .05$). These correlations indicate that those cases appearing more severely ill on admission to the hospital are those most likely to have a prolonged and severe course.

DISCUSSION:

The present study was not planned in advance as a controlled trial, and no placebo medication was available. Although the cases treated with tetracycline and the controls were not strictly alternated, the only evident resulting bias lies in the direction of including cases of higher risk and greater severity in the tetracycline-treated group. As these severe cases are the ones most prone to prolonged illness, the bias in case selections is one that tends to obscure the therapeutic effect of the antibiotic. The ability of antibiotic to eliminate the organism from the stool has been recognized in the past (Pollitzer, 1959), and is evident in the results of the present study. The favorable effect on the course of disease, however, has not previously been reported. A preliminary communication (Carpenter et al.,1963) indicates that confirmatory data will be forthcoming.

It is generally agreed that replacement of fluid and electrolytes is essential in the treatment of cholera patients. Such replacement is the only necessary therapy for patients in whom the natural course of the disease is short. However, for patients in whom prolonged purging occurs, management can be difficult and costly, and death a common outcome unless sophisticated medical attention is available, along with laboratory support and adequate sterile supplies. Unless those difficult cases can be recognized in advance, a potent antibacterial agent should be administered to each case from the beginning. This will not only reduce the number of cases with prolonged diarrhoea with resultant savings in sterile fluids and hospital bed space, but will obviate returning to the community patients who are still bacteriologically positive.

SUMMARY:

Tetracycline as an adjunct in the treatment of cholera was evaluated by comparing the clinical course of patients who had received replacement of fluids and electrolytes alone with those who had been given tetracycline as well. The antibiotic eliminated bacteria from the stool, shortened the duration of diarrhoea, and decreased the requirement for intravenous fluids.

TABLE I

CLINICAL COMPARABILITY OF TETRACYCLINE-TREATED AND CONTROL GROUPS

	Number in Treated Group	Number in Control Group
Total numbers	38	39
Males	26	28
Females	12	11
V. cholerae Inaba	32	35
V. cholerae Ogawa	6	4
Other disease present	4*	5*
Intestinal parasites present	24	28
Cholera immunization within two years**	14	14
Cholera immunization greater than two years ago**	2	3
No cholera immunization**	19	17
No immunization history**	3	5
History of previous attack of cholera**	3	3
Treatment prior to admission:		
None	17	18
Sulfaguanidine and i.v. fluids	19	21
Other	2	0
Radial pulse weak or absent	17	17
Stuporous or unconscious	18	20
Temperature greater than 99°F during hospital stay	8	10
Vitamin therapy after admission		
Vitamin C	38***	11
Vitamin B	25	11
None	0	17

* Other diseases: One case each of asthma, pneumonia, chronic bronchitis, and previous gastrectomy in the treated group; and non-toxic goitre, herpes labialis, emphysema, bronchitis, and pregnancy in the control group.

** Undocumented statement by patient or relative.

*** The preparation of tetracycline for i.v. use contained a therapeutically significant quantity of ascorbic acid.

TABLE II

CLINICAL COMPARABILITY OF TETRACYCLINE TREATED AND CONTROL GROUPS

	TREATED GROUP		UNTREATED GROUP		SIGNIFICANCE OF DIFFERENCE	
	MEAN	95% CONFIDENCE LIMITS	MEAN	95% CONFIDENCE LIMITS	t	p
Age (years)	17	13 - 21	18	14 - 21	.1	$>.1$
Body weight(in kg.)	27	21 - 33	33	29 - 36	1.6	$>.1$
Hours of diarrhea before admission	18	13 - 23	21	15 - 26	.7	$>.1$
Plasma protein on admission (gm.%)	10.1	9.5 - 10.6	9.7	9.1 - 10.4	.8	$>.1$
Plasma protein convalescent (gm.%)	7.0	6.6 - 7.3	6.9	6.5 - 7.3	.18	$>.1$
CO_2 on adm. (mEq/l.)	12.4	10.9 - 13.9	12.3	10.7 - 13.8	.14	$>.1$
Hct. on adm.	47	45 - 50	44	41 - 47	1.5	$>.1$
Hct. convalescent	35	34 - 37	34	32 - 36	.65	$>.1$
WBC on adm. x 10^{-3}	23	20 - 26	20	17 - 23	1.2	$>.1$
Stool volume first 24 hrs. after adm. (ml./kg.)	100	68 - 133	72	42 - 104	1.2	$>.1$
i.v. fluids required first 6 hours after admission (ml./kg.)	85.2	74 - 96	62.6	48 - 77	2.1	$<.05$

References:

Carpenter, C.C.J., Mitra, P., Sack, B., Wells, S. Jr., Dans, P., Saxena R.S., Mandal, A. (1963). Bull. Calcutta Sch. Trop. Med. 11, 89.

Monsur, K.A. (1961). Trans. Roy. Soc. Trop. Med. Hyg. 66, 440.

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Pollitzer, R. (1959). Cholera. World Health Organization, Geneva.

Smith, H.L., Jr., Goodner, K. (1958). J. Bact. 76, 662.

Watten, R.H., Morgan, F.M., Songkhla, Y., Vanikiati, B., Phillips, R.A. (1959). J. Clin. Invest. 38, 1879.

FIGURE I

NUMBER OF TETRACYCLINE - TREATED AND CONTROL CASES,
BY MONTH OF ADMISSION

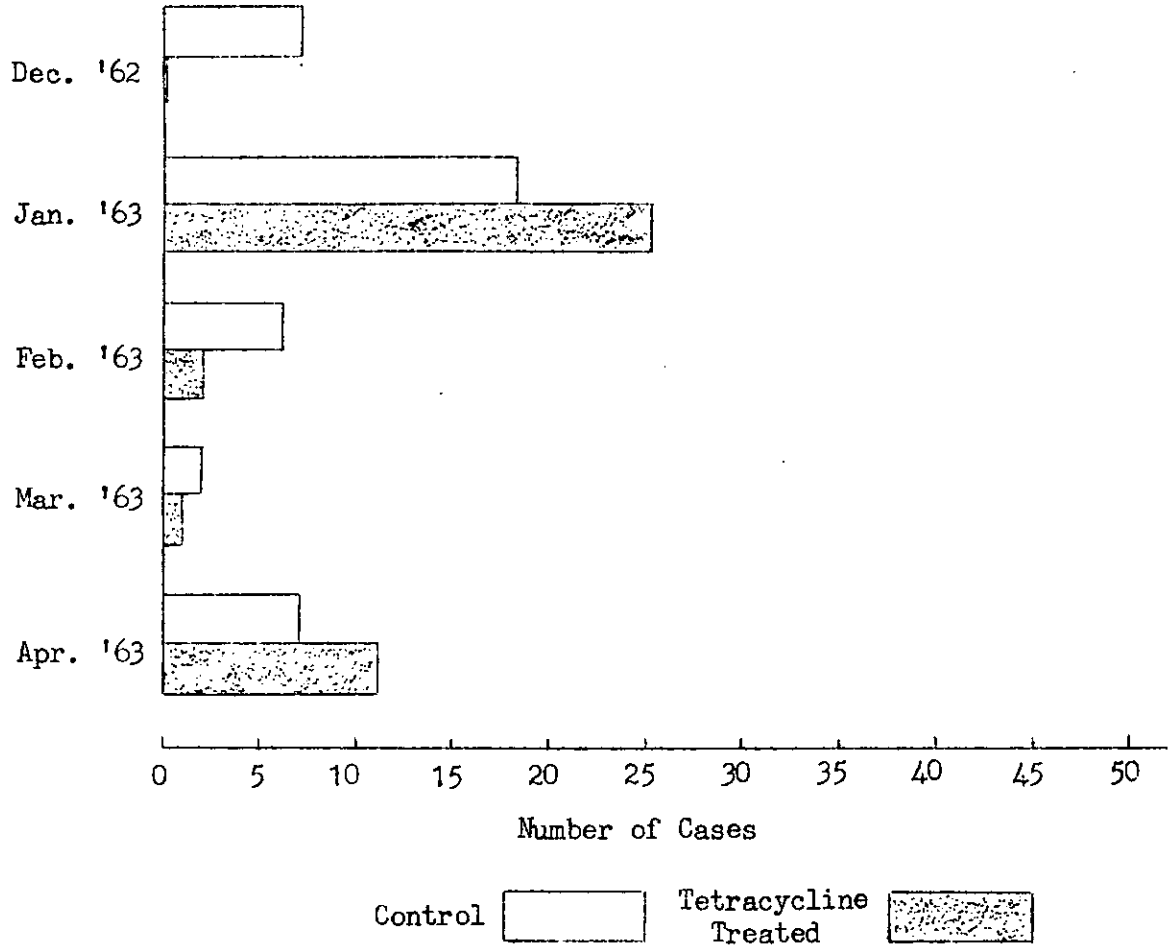


FIGURE II

MEAN STOOL VOLUMES AND INTRAVENOUS FLUID REQUIREMENTS AFTER FIRST 24 HOURS OF THERAPY

(95% confidence limits of mean indicated)

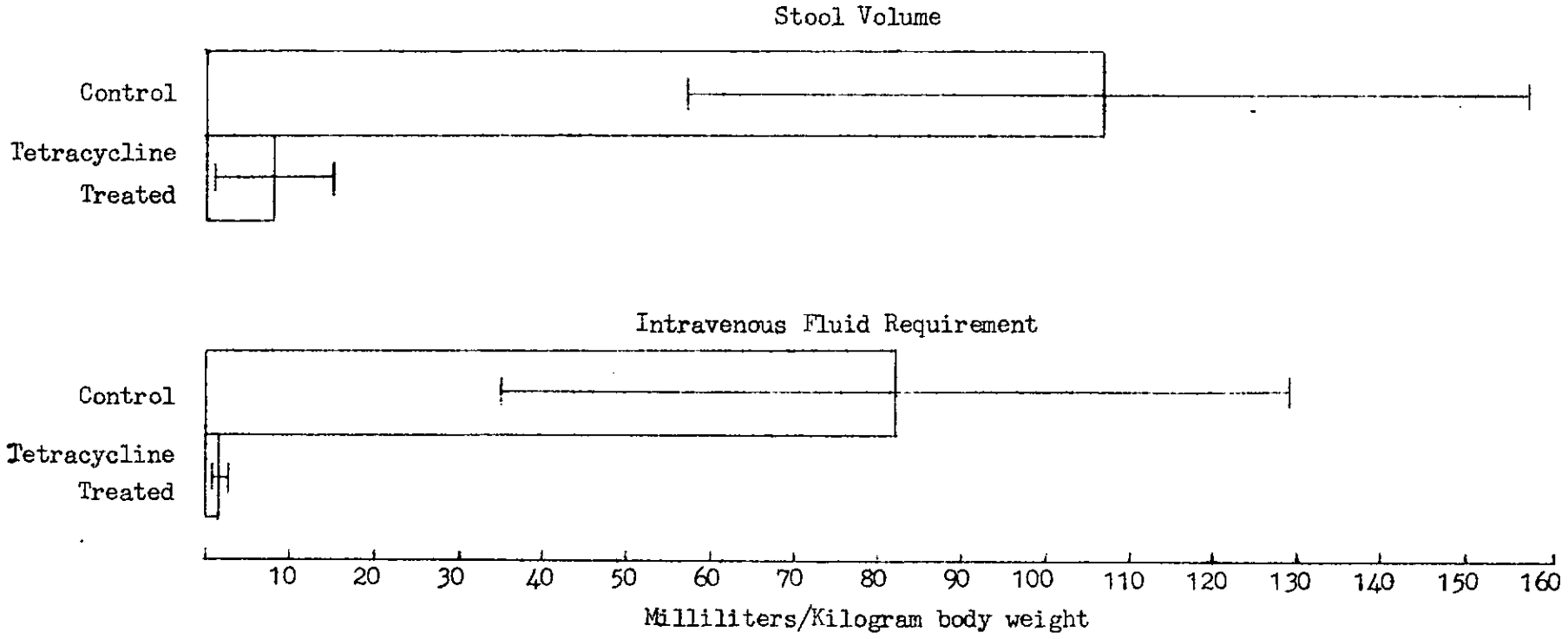


FIGURE III

DURATION OF DIARRHOEA AFTER START OF TREATMENT

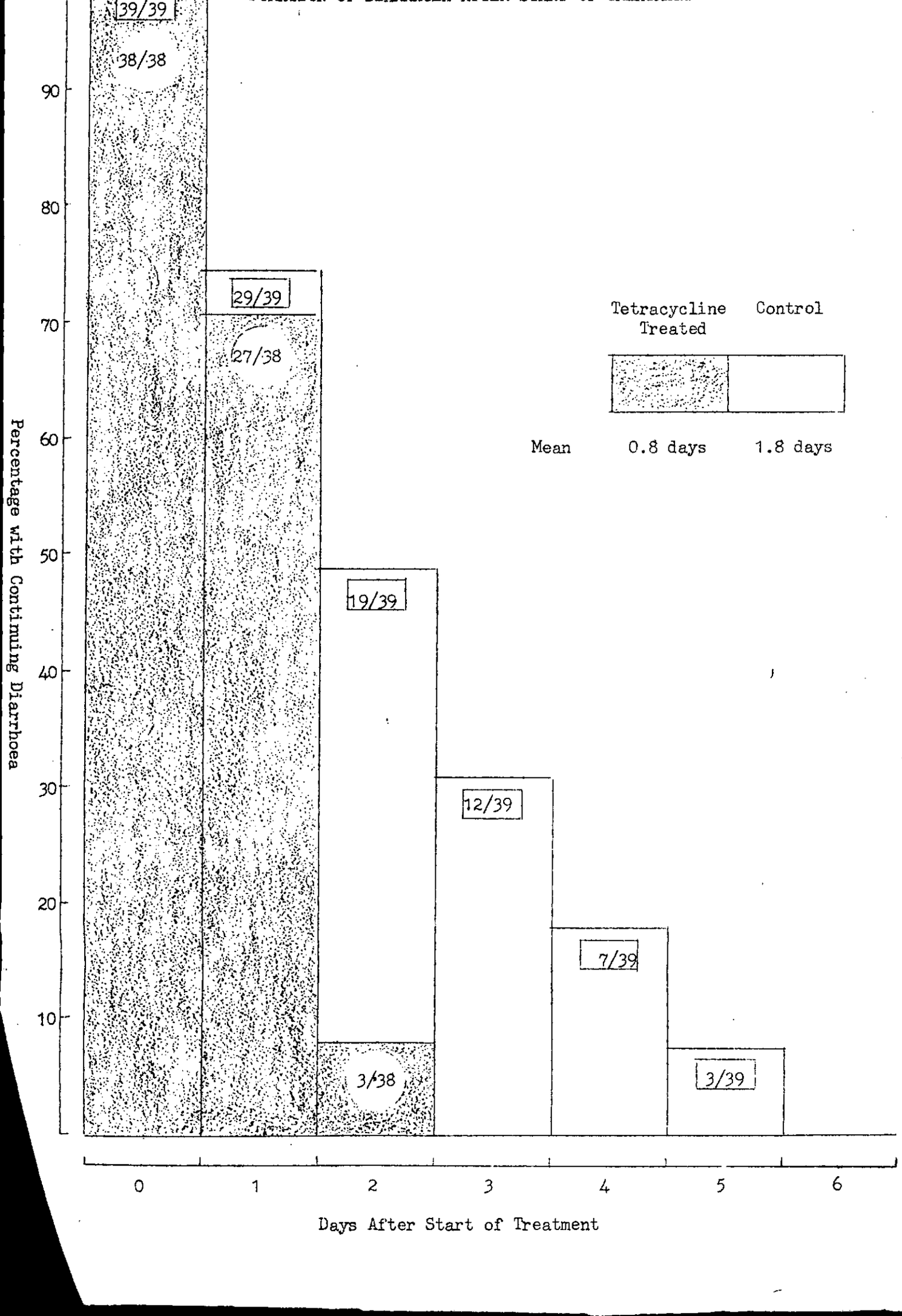


FIGURE IV

DURATION OF POSITIVE CULTURE AFTER START OF TREATMENT

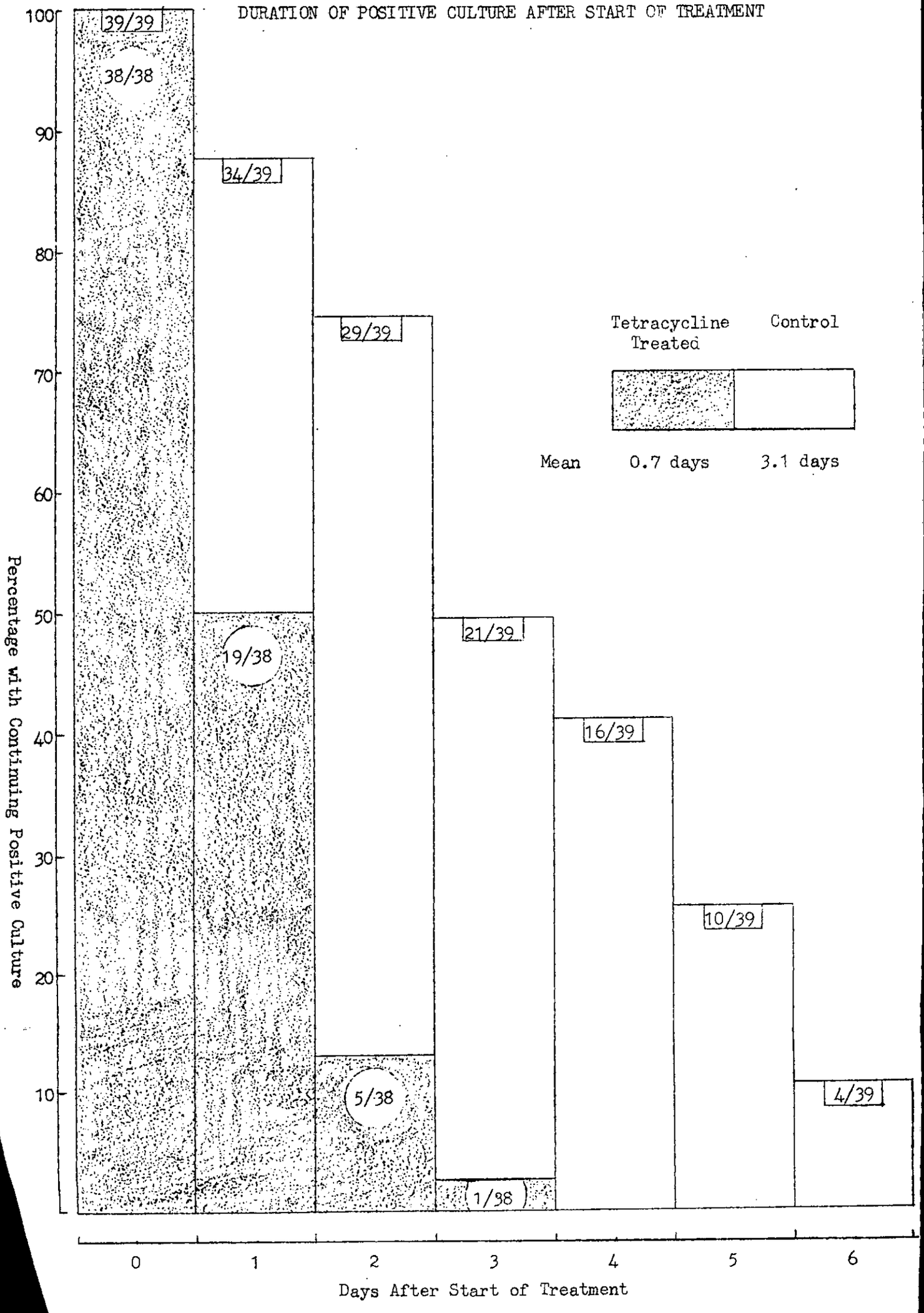
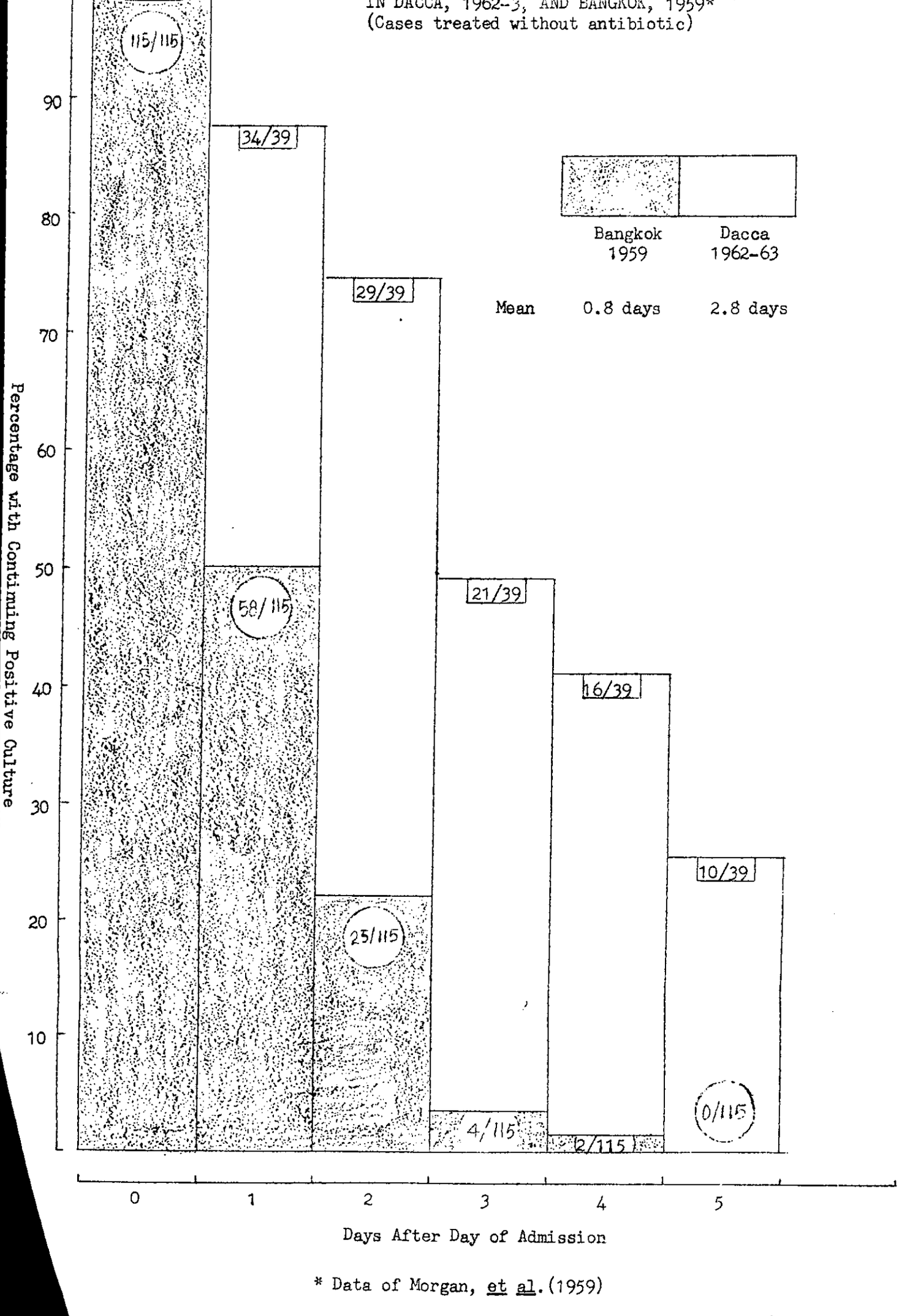


FIGURE V

PERSISTENCE OF V. CHOLERAE IN CASES IN DACCA, 1962-3, AND BANGKOK, 1959*
(Cases treated without antibiotic)



* Data of Morgan, et al. (1959)

Table I

Clinical Comparability of Vitamin Treated and Control Groups

	Number in Treated Group	Number in Control Group
Total numbers	22	17
Males	14	14
Females	8	3
V. Cholera Inaba	19	16
V. Cholera Ogawa	3	1
Other disease present	1	4
Intestinal parasites present	16	12
History of previous cholera*	2	1
Cholera immunization:		
Within 2 years*	7	7
Over 2 years*	2	1
None*	10	7
Unknown*	3	2
Treatment prior to admission:		
None	10	7
Sulfa drug and IV	2	0
Other	10	9
Pulse weak or absent (radial)	13	4
Temperature greater than 99°F during hospital stay.	5	5

* Statement of patient or relatives without documentation.

PROGRESS IN DIAGNOSTIC BACTERIOLOGY, 1962-1963

Isolation of Vibrio cholerae:

During the period from September 1962 to September 1963, 395 specimens were submitted to the bacteriology laboratory for examination. Of these, 59% were derived from field studies of the epidemiology section, 40% from clinical cholera suspects, and 1% from miscellaneous sources. The cholera suspects included patients in the CRL ward, in Mitford Hospital, Dacca, and in Victoria Hospital, Narayanganj. In addition, nine specimens were sent in by post from outlying hospitals. Table 1 shows the distribution of positive isolations of V. cholerae and other enteric pathogens in the material from the most important sources. Interestly, in 1962, only Inaba type cholera vibrios were encountered. Beginning in January, 1963, Ogawa was recognized with increasing frequency in Narayanganj, but did not appear in Dacca until April, 1963. Since then, isolations of the Inaba serotype have been rare. No El Tor organisms have been isolated from human sources. Of the non-cholera vibrios (N.C.V.) found in human material, 71% were of Heiberg group II, 17% of group I, and the remainder of groups III through V. No instance of a group VI N.C.V. has been encountered. Breakdown of the positive isolations is given in Table I.

El Tor vibrios:

In view of the increasing world wide interest in studies on El Tor, this laboratory obtained some El Tor vibrios from different sources. All these El Tor vibrios have been tested here by various techniques in common use for their characterization.

In Grieg's tube haemolysis method (1), 1cc and 0.1 cc of a 48-hour broth culture was mixed with 1cc of 5% goat cell. These tubes were incubated for 2 hours and then refrigerated. Readings were taken after 2 and 18 hours at $4^{\circ}C$. In Bhaskaran's plate haemolysis method (2), 1cc of 4% sheep cell suspension in normal saline was mixed with 9 cc of normal saline agar (normal saline with 0.6% agar*), and the mixture was poured on a 24-hour spot culture on a gelatine agar plate (6). In Finkelstein's chicken cell agglutination test (3), a 2.5% suspension of fresh chicken cells in normal saline was tested with a fresh culture of vibrios by the same technique applied to slide agglutination. The weak type agglutination may best be seen under a low power microscope. In Mukherjee's phage typing method (4), undiluted group IV phage (titer: 2.2×10^9 plaque forming units per ml.) was dropped on a lawn of the vibrio culture.

We have never isolated El Tor vibrios from patients; but among the strains isolated from water and sewage specimens from Dacca City,** we found 3 vibrios which are weakly haemolytic in both plate and tube haemolysis. These strains also agglutinate chicken cells and are not attacked by Mukherjee's phage group IV. In these characteristics the strains conform to the El Tor available from Malacca and elsewhere, as will be evident from Table 2.

* Bhaskaran used 0.4% agar in this experiment

** One (#107W/63) was isolated from a dug well in Lutfor Rahman Lane on July 9 and the other two (#152W/63 and 153W/63) from Narinda sewage pumping station, on the 28 and 29 August, respectively.

continued .....

Double isolates:

In 26 cases, cholera vibrios and non-cholera vibrios were found in the same clinical specimen. 22 of these were cultured both on direct plates and enrichment broth. Noteworthy is that in only 2 cases were cholera vibrios and non-cholera vibrios isolated from direct plating; in the other 20 cases, only cholera vibrios were isolated from the direct culture plates. It was not possible for technical reasons to plate out the enrichment broth after 6-8 hours incubation. After enrichment for 18 hours at 35°C, both cholera vibrios and non-cholera vibrios were isolated from 8 of these 20; in the other 12 cases, cholera vibrios were overgrown by the non-cholera vibrios and were not recognized.

4 specimens received only in enrichment broth yielded both cholera vibrio and non-cholera vibrios after 18 hours incubation at 35°C.

In three instances, Shigella and V. cholerae occurred together in clinical specimens; two Salmonella combined with N.C.V.

Special isolates:

One of the cholera vibrios isolated (# 6055/63) appeared to agglutinate in both Inaba and Ogawa typing sera, as well as in Group I antiserum. This isolate is provisionally considered to be of the intermediate "Hikojima" serotype. In tube agglutination, the vibrio titer with Ogawa antiserum is poor (1:20), although it agglutinates well with standard Inaba antiserum (1:80).

Shigellae isolated fell largely into Groups B and D (S. flexneri and S. sonnei), although 2 instances of Group A and 1 of Group C were found. Most of the Salmonellae isolated were derived from blood cultures taken from patients suspected of having typhoid fever in the medical service at Mitford Hospital. A detailed breakdown of these results is given in Table 3.

Enteropathogenic E. coli:

A pilot survey was initiated to investigate the amount of enteropathogenic E. coli present in Dacca area. Polyvalent sera and six specific types (026, 055, 0111, 0119, 0127 and 0128) were used in the survey. 180 rectal swab and stool specimens from out-patient diarrhea cases were examined during the period from 21 August 1963 to 23 September 1963. These were tested against the six types of antisera stated above. Out of the 180 specimens examined 16 pathogenic E. coli were isolated. The distribution of types is given in Table 4.

Comparison of Liquid Transport Media:

In order to learn which of the two liquid transport media commonly used at CRL was superior in the identification of cholera carriers, duplicate specimens were collected from household contacts by the epidemiology section in January and February, 1963. One was placed in T_1N_1 broth (trypticase 1%, NaCl 1%, pH 7.2), and the other (using a tellurite - impregnated swab) in bile peptone medium (Na taurocholate ½%, peptone 1%, pH 9.2) as described by Monsur (5). After being returned to the laboratory and incubated for 18 hours, specimens were plated identically on gelatin agar (6) and bile salt potassium tellurite agar (7) plates, and

continued .....

suspicious colonies identified by the usual procedures. The results are given in Table 5, which shows the apparent superiority of the bile-peptone tellurite fluid. It is pertinent that five of the specimens positive in T_1 and N_1 only were examined on a day that the bile peptone medium was incorrectly prepared and had a pH of 9.8.

TABLE 1

DISTRIBUTION OF POSITIVE ISOLATIONS BY MAJOR SOURCES, 1962 - 1963

Results	Sources			
	Epidemiology *	CRL Ward **	Mitford ***	Victoria ***
Total	10,225[1]	418[2]	1863[2]	361[2]
Positive	502	238	595	162
Ogawa	137	82	136	38
Inaba	178	139	374	106
NCV	182	18	58	18
Salmonella & Shigella	5	4	25	0

* Epidemiology submitted 10,225 specimens from 3,500 subjects. The results are tabulated on the basis of number of specimens.

** CRL ward submitted 4059 specimens from 418 patients. As there were repeated samples from each patient, many of whom were treated with antibiotics, the results are presented in terms of number of patients, not number of specimens.

*** In these hospitals, only one specimen per patient was submitted.

1. Submitted in enrichment broth, plated on gelatin agar (GA) and bile slat - potassium tellurite agar (SP).

2. Streaked directly on GA and SP plates, and also MacConkey and SS plates. Duplicate swabs placed in T_1 N_1 and/or BPT broth and selenite F, later plated on appropriate media.

TABLE 2

CHARACTERISTICS OF EL TOR VIBRIOS OF DIFFERENT ORIGINS

Origin	Donor's Identifying No.	Haemolysis			Voges Proskauer	Chicken Cell Agglutination	Sensitivity to Phage Group IV	Slide Agglutination
		Plate	Tube 1 cc	Tube 0.1 cc				
Hongkong	1	+	+	+	+	+	Not Attacked	Ogawa
Hongkong	24	+	W	+	+	+	"	"
Hongkong	50	+	W	+	+	+	"	"
Water El Tor (Calcutta)	1	+	+	+	+	+	"	"
Djakarta	2/16	+	+	+	+	+	"	"
Indonesia	9/61	+	+	+	+	+	"	"
Bandung	10/61	+	+	+	+	+	"	"
Philippines	14/62	+	+	+	0	+	"	"
"	56/61	+	+	+	+	+	"	"
Bangkok	50	+	+	W	0	+	"	"
Burma	B	+	0	W	+	+	"	"
"	J	+	0	W	+	+	"	"
"	1R	+	0	W	+	+	"	"
"	6R	+	0	W	+	+	"	"
"	297	W	0	W	+	+	"	"
"	315	W	0	W	+	+	"	"
Malacca	3734	W	0	W	+	+	"	"
"	3735	W	0	W	+	+	"	"
"	13	W	0	W	+	+	"	"
"	27	W	0	W	+	+	"	"
"	45	W	0	W	+	+	"	"
"	106	W	0	W	+	+	"	"
"	352	W	0	W	+	+	"	"
"	656	W	0	W	+	+	"	"
Muar	1360	W	0	W	+	+	"	"
Kuala Lumpur	13162	W	0	W	+	+	"	"
Water El Tor (Dacca)	107W/63	W	0	W	0	+	"	"
"	152W/63	W	0	W	0	+	"	"
"	153W/63	W	0	W	0	+	"	"

Key: + indicates Positive
W indicates Weakly Positive
0 indicates Negative

TABLE 3

SALMONELLA & SHIGELLA ISOLATIONS

	Stool Cultures			Blood cultures
	Diarrhea Patients	CRL staff	Contacts (Enrichment media only)	Mitford Typhoid Suspects
Shig. A (dysenteriae)	2	-	-	-
Shig. B (flexneri)	9	-	-	-
Shig. C (boydii)	1	-	-	-
Shig. D (sonnei)	5	2	-	-
Salm.paratyphi A	1	-	-	2
Salm.paratyphi B	1	-	-	-
Salm. C	1	-	1	-
Salm.typhi	7	-	-	8
Salm.E	8	-	-	-
Salm. unidentified	1	-	-	-
Total examined:	2406	135	1116	144

continued.....

TABLE 4

ISOLATION OF PATHOGENIC E. COLI
(Among 180 diarrhea specimens)

Type	Number isolated
026	3
055	1
0111	1
0119	4
0126	5
0128	2

TABLE 5

COMPARISON OF T_1 N_1 AND BILE PEPTONE TELLURITE ENRICHMENT MEDIA

Total examined	2097
Total positive	102
Positive in both media	57
Positive in BPT only	32
Positive in T_1N_1 only	13
% Total positive found by BPT	87
% Total positive	69

References:

1. Greig, E.D.W. Indian J. Med. Res. 2:623 (1914)
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5. Monsur, K.A. (1962) Fed. Proc. 21:394
6. Smith, J. L. Jr. and Goodner, K.J. Bact. 76:662 (1958)
7. Monsur, K.A. Trans. Roy. Soc. Trop. Med. Hyg. 66:440 (1961)

STUDIES ON CHOLERA PHAGE

During the year the laboratory has been engaged in the f studies on cholera phage:

1) Phage typing of V. cholerae.
2) Phage sensitivity of the El Tor vibrios.
3) Isolation of cholera phage from the environment.
4) Studies on the behaviour of a new phage isolated in the laboratory.
5) Studies on the therapeutic possibility of choler phage.

These studies have only begun and will require more work be

1. Phage typing of V. cholerae:

Over 500 strains of V. cholerae have been tested for the using Mukerjee's typing phages. Table I shows the criteria for t type of a cholera strain (1).

Table I

V. cholerae Strain No.	Sensitivity to phage group I	II	III	IV
V 154	+	+	+	+
PR 508	-	+	+	+
PR 528	+	-	+	+
PR 542	-	-	+	+
Rg 29	+	+	-	+

All the cholera vibrios so far tested have belonged to type III except for 18 strains (Table II.).

Table II

Phage type	1		2		3		4	
Sero-type	Inaba	Ogawa	Inaba	Ogawa	Inaba	Ogawa	Inaba	(
	0	1	0	0	378	93 + 19* = 112	2	

* See text.

Although the majority of the strains are similar in their sensitivity to Mukerjee's typing phages, individual strains may show variations in the degree of this sensitivity. Thus, nineteen strains appeared to be insensitive to Mukerjee's Ø I and Ø II when tested against routine test dilution of these phages; because of this apparent insensitivity, the strains were first thought to belong to Mukerjee's phage type 4. Later, these strains were tested against concentrated suspensions of phages and were found to be sensitive to Ø I and resistant to Ø II only. Therefore, the strains were grouped as phage type 3.

Similarly, a few of the strains which have been recorded in the Mukerjee's table as belonging to phage type 4 showed slight indefinite clearing, somewhat resembling opaque lysis, when tested against a concentrated suspension of Mukerjee's Ø I. If these few strains are tested against still higher titre phage, they might also prove to be sensitive to Mukerjee's Ø I and would, therefore, have to be recorded as Mukerjee's phage type 3. In any case we have evidence of only a few strains which do not belong to Mukerjee's phage type 3.

Not capable of distinguishing strain dif erences, Mukerjee's phage typing is not adequate for local epidemiological studies. It is, however, still probable that Mukerjee's phage typing may be helpful in special cases and in relation to the global spread of cholera. For example, we have recently isolated from water three vibrios of Ogawa serotype which gave a weakly positive Grieg test. Since we have not so far isolated any El Tor vibrios in East Pakistan and the result of the Grieg test was weak, the question whether these could be El Tors became a pertinent. When these strains were tested against Group IV phage, all the three strains were found to be insensitive to this phage. The present observation, therefore, fits in with Mukerjee's claim (2) and Mukerjee's phage IV appears to be an additional tool for distinguishing between true cholera and El Tor vibrios.

Although the behaviour of the majority of the strains is similar, individual strains may show a difference in the degree of sensitivity to the different phages. Mukerjee seems to have ignored this difference for his typing purposes. Further study may show that this difference in the degree of sensitivity offers a means of subdividing the strains into sub-types.

2. Phage sensitivity of the El Tor vibrios:

Our studies on phage sensitivity of the El Tor strains are still too limited to make generalizations. Most of the El Tor strains are attacked by Mukerjee's Ø III, and by Ø 175 and Ø 326; the latter two have been isolated in this laboratory. A few of the El Tor strains are also attacked by Mukerjee's Ø I, and none so far by Mukerjee's Ø IV.

Regular cholera phages have a much lower titre against the El Tor strains than against true cholera vibrios. Similarly, three phages isolated in this laboratory from El Tor strains have not attacked any of the cholera vibrios tested.

From a small number of unselected strains of El Tor in our collection, isolation phage from three has been possible. Probably, a large percentage of the El Tor strains are either lysogenic or have carried phage. Raising the titre of the El Tor phages has been difficult. One of thephages actually was lost during our effort

to enrich it. For the three other phages, we were able to raise the titre only sufficiently high to get semi-confluent lysis in 10^{-1} dilution. The phage sensitivity of the different groups of El Tor strains is not identical. In properly controlled experiments, distinguishing between different groups of El Tor strains by their phage sensitivity pattern may be possible.

3. Isolation of phage from the environment:

250 samples of water and 100 samples of cholera stool were tested for the presence of phage. Phage was isolated from only 3 samples of water and 12 samples of stool. One of these phages has been described in this report.

4. Studies on the behaviour of a new phage isolated in the laboratory:

During the course of routine investigation, a phage with special characteristics ($\emptyset$ 326) was isolated from water. This phage has a wide spectrum of activity, attacking not only the true cholera vibrios but also most of the El Tors and NAG vibrios. When titrated on a lawn grown on a nutrient agar plate, this phage shows a gradual change from continuous lysis to no lysis without the formation of discrete plaques at any stage. This phenomenon initially led to a confusion whether we were dealing with a phage or an antibiotic substance which gave a confluent lysis when present in a concentrated form, and no lysis when sufficiently diluted out. Alternatively, we would have to assume that the plaques produced by this phage lysis could only be detected by the group effect of the plaques. Since plaque sizes tend to be bigger when tested by the agar layer method, we decided to use the agar layer technique to titrate this phage. With this technique, the plates with concentrated phage had no visible lysis, whereas plaques began to appear as dilution progressed, first in the semi-confluent manner and then as discrete forms which finally diluted out. By the agar layer technique, the plaque sizes were fairly large. We attempted to purify the phage by three successive picks of single plaques from appropriate dilutions. This attempt at purification did not change the behaviour of the phage. The phenomenon at least superficially resembles the zone phenomenon seen with agglutination reactions.

5. Therapeutic possibilities of cholera phage:

Investigation of the possibilities that phage may have as a therapeutic or prophylactic agent against cholera seemed worthwhile. Previous literature on the subject is confusing and does not stand strict scientific scrutiny. This problem merits critical study under properly controlled laboratory conditions so that the real position may be ascertained once and for all. This phase of study has only just begun in our laboratory and results are presented only to invite criticism and guidance from the members of the audience. The following points deserve consideration:

1. Sterilization of a culture of V. cholerae by adding phage is seldom possible, even when the inoculum is large. Such a culture will generally contain a large number of surviving V. cholerae, as can be demonstrated by inoculating a loopful on nutrient agar plates. What is more peculiar is that the resultant colonies which will grow on nutrient agar plates are usually still sensitive to the parent phage used in the inoculum. Our previous phage therapy was based on the theory of adding phage which would multiply in the V. cholerae and ultimately sterilise the

medium. However, this process does not seem to work experimentally. In the test tube, bacteria and phage will multiply together. As a rule, bacteria will be present in the medium in large numbers despite sensitivity to the phage, which is also abundantly present in the medium. At present, we cannot explain this reaction.

2. To improve the reaction, we used a mixture of 7 or 8 phages instead of a single phage. When serial dilutions of the pooled phage are added to a young culture of V. cholerae in measured quantities within 2-3 hours, visible complete lysis takes place in the tubes which contain higher concentrations of the phage. As the dilution of phage increases, turbidity begins to appear increasingly. In the tubes containing visible turbidity, large concentrations of phage and bacteria will be present simultaneously: If a loopful from the clear tubes is plated out on nutrient agar plates, no growth of V. cholerae can be detected.

If V. cholerae are still present in the clear tubes, the bacteria are not capable of giving rise to colonies when plated out on nutrient agar plates. This finding led to the assumption that if phage has any therapeutic or prophylactic value, such a mixture would be more effective than a single phage.

When mixed with normal human stool and left overnight, cholera phages were found to be partially inactivated and had a fall of titre to the extent of 2 to 3 logs. Phage fed to normal monkeys in 4 ml. quantities in the morning and evening could be recovered from the stool in about 24 hours and also showed inactivation to the extent of 2 to 3 logs. In these cases, the inactivation produced by normal human stool is of the same order as that suffered by the phage during passage through the monkey intestine. Hence, most of the inactivation of phage probably took place in the large gut.

Because phage lysates fed to monkeys and men did not exhibit any evidence of toxicity, the effect of phage was tested on actual cholera cases. We succeeded in making a preliminary trial in one case only. The results of this experiment are as shown in Table III.

TABLE III

Time (hours)	Viable V. cholerae per ml. stool	Phage titre of stool**
0	8×10^7	negative
1*	1×10^8	negative
2*	5×10^7	negative
3*	5×10^5	10^3
4*	5×10^4	10^1
5	5×10^5	10^1
7	2×10^7	10^0

* 30 ml. pooled phage (titre: semi-confluent lysis at 10^{-4} dilution) given at hours 1, 2, 3, and 4.

** Dilution of stool yielding semi-confluent lysis.

A sharp drop in viable count of cholera vibrios in the stool occurs at a period which coincides with the administration of cholera phage. The drop is followed by a rise after the administration is completed. The phage used in this experiment was of comparatively low titre. Thus far, we have not been able to obtain good titre cholera phage in this laboratory. Most of the phages have a titre of the order of 10^8 and some of 10^9 plaque forming particles per ml. If a phage titre of 10^{11} or 10^{12} were obtained, the results might prove highly significant.

References:

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(2) Mukerjee, S. "Host-Range of Cholera Bacteriophages Other than the Vibrio Cholerae." Ann. Biochem. & Exp. Med. 20, 317 (1960).

HOUSEHOLD & FAMILY STUDIES

Introduction:

This is a preliminary report of studies of cholera in families and households of Dacca City, Narayanganj City, and suburbs thereof during the period October 1962 to July 1963. Differences which have been observed in the distribution of cholera among family members in East Pakistan, an endemic cholera area, and non-endemic nations which recently have experienced epidemics of this disease, have made family studies particularly desirable. Multiple cholera cases occur frequently in families of East Pakistan; however in Hong Kong, the Republic of the Philippines, Taiwan and Thailand, rarely is more than one family member striken. The high incidence of multiple cholera-case families in East Pakistan provides unique opportunity to examine the mode of transmission of disease and to study the characteristics which distinquish persons who become cholera victims from thosewho do not. In additionto the family, the second potential study unit is the compound which is commonly marked by a bamboo-fenced boundary enclosing a highly variable number of houses and families.

Materials and Methods:

Definitions:

Households: A household consists of two or more occupied houses which share a common courtyard and have a boundary recognized by the inhabitants.

Family: A family consists of those persons who live in the same house or apartment and whose food is prepared in the same kitchen. Members of the family may or may not be blood related.

Cholera and cholera immunization history: The dates recorded are the most recent attack or immunization preceding onset of diarrhea in the index case of the family. Previous attacks of diarrhea were accepted as being cholera only if a definite history of rice water stool was obtained.

Confirmed Cholera Case: The diagnosis of cholera was applied when patients in the study had one or more watery stools and a rectal swab specimen taken 1 day before or 5 days after the onset of diarrhea was positive for Vibrio cholerae of the Inaba or Ogawa type.

Probable Cholera: In some instances, diarrhea attacks reported by patients or members of their family yielded positive bacteriological results; however, if the bacteriological evidence was not obtained from the individual during the interval specified for confirmation of cholera, the case was classified as probable cholera. For example, a case from which positive bacteriological results were obtained 5 or more days following onset of symptoms was called probable cholera. In addition, for families with positive bacteriological evidence of cholera, the term probable cholera was applied to unexamined family members who died of diarrheal disease and to family members not examined bacteriologically during the course of their illness.

Diarrhea cause Unknown: This diagnosis was applied to diarrhea cases which had only negative bacteriological examinations for Vibrio cholerae during the interval of 1 day before to 5 days after onset of symptoms. Diarrhea patients yielding

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NAG organisms have been included in this group.

Asymptomatic Infections: Application of this diagnosis requires the isolation of Vibrio cholerae, Inaba or Ogawa from individuals who reported no diarrhea symptoms and did not subsequently become cases of confirmed cholera or other diarrheal diseases.

Procedures:

The index cases which identified households or families to be included in the study were selected from among cases of suspected cholera admitted to the cholera treatment ward at the Mitford Hospital, Dacca, East Pakistan, the CRL Ward and the Victoria Hospital, Narayanganj. At each institution, ward physicians identified cholera patients admitted during the previous 24 hours. Final selection of the index case was made by sociologists of the CRL epidemiology section. Each consecutive case admitted to Mitford Hospital was selected for inclusion in the study until a full work load was obtained. Thereafter new index cases were selected only after dropping a family or household from the study. Acutely ill cases then on the ward were assigned numbers and the final selection was made by drawing a member blindly. Families found to be living in inaccessible locations were excluded and a replacement selected.

Case sheets providing information concerning place of residence, employment, date and time of onset of symptoms, description of diarrhea, and history of possible exposures to infection were completed on the index case of new households or families and on any new cases admitted to the hospital from families already under study. After collecting these data, bacteriological cultures were taken from patients in the family study. The teams then proceeded to workup new families and followup others already added to the study. The 1st step on the initial visit to a new family or household, was the identification of the number and location of each family within the compound. Once each family was identified by house and kitchen, the census was taken of each family. Individual members of each respective family were listed in order by age, from oldest to youngest. Items recorded concerning each person included, household, family, and individual members, name, religion, age, sex, occupation, educational status movements, history of cholera, cholera immunization and identification of the individuals who prepared or assisted in the preparation of meals. Histories were obtained directly from each adult whenever possible or, in the case of absentees, from another adult intimately acquainted with that individual. Children's histories were obtained from parents.

All the members present were asked to permit collection of rectal swab specimens. Adults and adolescents in general were reluctant to have swabs inserted by strangers. Under the circumstances, each person was asked to collect his own specimen after being instructed in the use of the swab stick. CRL personnel selected a room of the house to be used for this purpose rather than the latrine, in order to minimize the risk of extraneous contamination. Swab sticks were inspected for evidence of fecal staining before being placed in the culture media. If the cotton remained clean the individual was asked to try again. Specimens from small children were obtained more easily and were collected either by a member of the CRL staff or a parent under supervision of CRL personnel.

Followup of households was continued during November for 21 days with daily visits being made to identify new cases of diarrhea and cholera. On about every

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fifth day rectal swab specimens were collected from all family members who would consent.

Because uncooperative attitudes developed among study families toward the end of the 21-day followup, on 15 January the policy was adopted of obtaining rectal specimens daily for 7 days and as often as possible thereafter. Followup was to be discontinued as soon after the 10th day as V. cholerae could no longer be found in either the persons or the environment.

The household was the basic unit studied during the period from 27 October 1962 to 5 February 1963. On all households, at least one family had a suspected case of cholera. Other families within the household compound were followed even though devoid of diarrhea cases.

From February 5, 1963, to 19 June, 1963, individual families having a suspected cholera case were the predominate basic study units. Households were again accepted for study in preference to family during the period of low incidence from 19 June to 30 September.

Results:

During the period from 27 October, 1962 to 31 July 1963, 406 families with a total population of 2,240 persons were entered into household or family studies. (Table I).

Households: The 81 households are classified as having suffered multiple cholera cases, single cholera cases or diarrhea of unknown cause. By definition, households include 2 or more families within their boundaries. Each family within the household may have a diarrheal disease classification distinct from the disease classification given the household of which it is a component part.

In Table I classifications of households are given in the upper portion of the left hand margin. Classifications of families are given at the top of the table. There is little difference in the overall average size of families within the households, but the average size of both households and families appears to vary depending upon their cholera classification. Families and households with 2 or more cholera cases are largest average size in their respective groups, and the smallest mean family size is among families free of diarrheal disease.

The lower portion of Table I presents characteristics in average family size and diagnostic group of suspect cholera families selected for study from among others on the same compound. These families show the same type of relationship between disease classification and mean family size as was observed in families within the household study and these similarities of the two groups in this respect is reflected in the overall mean figures.

Household units appear to be well suited to inter-family transmission of cholera. Table II presents the frequency with which cholera progressed from one family to another in cholera affected households. The occurrence of confirmed cholera cases in more than one family sharing the same compound is limited to 6 (14.1%) of the 64 households studied. The intervals between onset of the first case

continued .....

in the first and second families affected varied from 2 to 9 days, except in one instance when the first case in each family occurred on the same day. Confirmed cholera developed only once in a third family, the interval after involvement of the first family being only 2 days. The remaining families which comprise 70% of the total studygroup had no diarrheal disease, although the high-risk age-group was well represented by 175 males and 199 females under 10 years of age out of a total non-diarrhea group of 1042 persons.

The distribution of cholera and non-cholera family members by diagnosis and type of family is presented in Table III. Ten asymptomatic V.cholerae infections were detected among 1042 members of families devoid of diarrhal diseases.

Tables IV and V reveal the attack rate of cholera to be significantly higher among young children than among adults.

Table VI shows the intervals in days between index and subsequent confirmed cholera cases in 36 multiple case families. Other diarrheal diseases included in the original coding of case order have been excluded from Table VI, resulting in the apparent absence of second cases in two families.

In one instance the second case in a family appeared ten days after the index case. Asymptomatic V. cholerae infections were present in other members of this family and may have been the connecting links in the chain of transmission.

TABLE I

CENSUS BY DIAGNOSIS GROUPS OF HOUSEHOLDS AND FAMILIES WITH DIARRHEAL DISEASE

Dacca, East Pakistan

October 1962 - July 1963

nit tu- ied	Diarrhea Status	Diarrhea Status of Families								Families		Households		Total Persons at risk
		Two or more cholera cases		1 Cholera case only		No Cholera				Total Families	Mean Fam. Size	Total HH*	Mean HH Size	
						Diarrhea present		No Diarrhea						
		No. of Fam.*	Mean Size	No. of Fam.	Mean Size	No. of Fam.	Mean Size	No. of Fam.	Mean Size					
	2 or more Cholera cases	17	7.1	16	5.9	9	4.0	79	4.3	121	4.9	26	22.9	596
	1 Cholera case only	-	-	31	6.0	7	5.3	108	5.1	146	5.3	38	20.4	775
	Diarrhea Cause Unknown	-	-	-	-	19	6.2	36	4.4	55	5.0	17	16.2	275
) Total		17	7.1	47	5.95	35	5.5	223	4.7	322	5.1	81	20.3	1646
	2 or more Cholera cases	19	9.4	-	-	-	-	-	-	19	9.4	-	-	179
	1 Cholera case only	-	-	29	7.3	-	-	-	-	29	7.3	-	-	213
	Diarrhea Cause Unknown	-	-	-	-	36	5.6	-	-	36	5.6	-	-	202
) Total		19	9.4	29	7.3	36	5.6	-	-	84	7.2	-	-	594
NL TOTAL		36	8.3	76	6.5	71	5.6	223	4.7	406	5.5	81	20.3	2240

* Fam. = Families
HH = Households

TABLE II

ATTACK RATES OF CHOLERA AND UNDIAGNOSED DIARRHEAL DISEASE AMONG FAMILIES IN HOUSEHOLDS WHERE AT LEAST 1 CHOLERA CASE HAS OCCURRED

Dacca, East Pakistan

October 1962 - July 1963

Diarrhea Disease Status of Family	No. of Families	Total Family Members	Confirmed Cholera						Diarrhea Cause Unknown			
			Lived		Died		Outcome Unknown		Lived		Died	
			No.	Rate per 100	No.	Rate per 100	No.	Rate per 100	No.	Rate per 100	No.	Rate per 100
1st Families with Cholera	54	334	59*	17.5	14+	4.1	3	0.9	9	2.7	1	0.3
2nd Families with Cholera	9	54	8	14.8	1	1.9	1	1.9	1	1.9	2	3.7
3rd Families with Cholera	1	8	1	12.5	0	0.0	0	0.0	0	0.0	0	0.0
Noncholera Families with Diarrhea	16	76	1*	1.3	2**	2.6	0	0.0	9	11.8	2	2.6
Families without Diarrhea	187	895	0	0.0	0	0.0	0	0.0	0	-	0	0.0
TOTALS	267	1371	69	5.0	17	1.2	4	0.3	19	1.4	5	0.4

* Each cell includes one case diagnosed "Probable Cholera."

\+ Includes 3 cases diagnosed "Probable Cholera."

** Includes 2 cases diagnosed "Probable Cholera."

TABLE III

DISTRIBUTION OF CHOLERA, OTHER DIARRHEAL DISEASE, AND ASYMPTOMATIC VIBRIO CHOLERAE INFECTIONS IN FAMILIES

Dacca, East Pakistan

October 1962 to July 1963

Families			Diagnosis Group										Total Persons	
Type of Family	No of families	Mean family size	Confirmed Cholera Cases		Probable Cholera Cases		Diarrhea Cholera Unknown		Carriers of Inaba or Ogawa		No Disease or Infection			
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
ltiple olera ses	36	8.3	92	30.7	5	1.7	11	3.7	10	3.3	181	60.6	299	100.0
ngle Chole- Case	76	6.5	76	15.4	2	0.4	13	2.6	22	4.5	380	77.1	493	100.0
Cholera arrhea esent	71	5.6	-	-	26	6.6	49	12.4	4	1.0	317	80.0	396	100.0
Diarrhea	223	4.7	-	-	-	-	-	-	10	1.0	1042	99.0	1052	100.0
TALS	406	5.5	168	7.5	33	1.2	73	3.3	46	2.1	1920	88.0	2240	100.0

TABLE IV

AGE AND SEX DISTRIBUTION OF DIARRHEAL DISEASE IN FAMILIES WITH 2 OR MORE CONFIRMED CHOLERA CASES

Dacca, East Pakistan

October 1962 to July 1963

e in ars	Confirmed & Probable Cases				Diarrhea Cause Unknown				No Diarrhea				Total			
	Male		Female		Male		Female		Male		Female		Male		Female	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1	2	3.8	2	4.5	1	20.0	0	0.0	0	0.0	3	3.3	3	1.9	5	3.5
-4	18	33.9	7	15.9	2	40.0	3	50.0	12	12.1	13	14.1	32	20.4	23	16.2
-9	14+	26.4	12*	27.3	1	20.0	1	16.7	21	21.1	13	14.1	36	22.9	26	18.3
-19	9*	17.0	12	27.3	1	20.0	0	0.0	15	15.2	25	27.2	25	15.9	37	26.1
-29	1	1.9	8	18.2	0	0.0	1	16.7	20	20.2	14	15.2	21	13.4	23	16.2
-39	3	5.9	2	4.5	0	0.0	1	16.7	15	15.2	13	14.1	18	11.5	16	11.3
) +	6*	11.3	1	2.3	0	0.0	0	0.0	15	15.2	10	10.9	21	13.4	11	7.7
known	0	0.0	0	0.0	0	0.0	0	0.0	1	1.0	1	1.1	1	0.6	1	0.7
AL	53	100.0	44	100.0	5	100.0	6	100.1	99	100.0	92	100.0	157	100.0	142	100.0

+ Includes 2 cases diagnosed "Probable Cholera."

* Each cell includes 1 case diagnosed "Probable Cholera."

TABLE V

AGE AND SEX DISTRIBUTION OF DIARRHEAL DISEASE IN FAMILIES WITH ONLY ONE CONFIRMED CHOLERA CASES

Dacca, East Pakistan

October 1962 to July 1963

in rs	Confirmed & Probable Cases				Diarrhea Cause Unknown				No Diarrhea				Total			
	Male		Female		Male		Female		Male		Female		Male		Female	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
	0	0.0	0	0.0	0	0.0	0	0.0	2	0.9	8	4.2	2	0.7	8	3.3
4	16	47.1	*11	25.6	2	25.0	2	40.0	25	11.8	25	13.1	43	16.9	38	15.9
9	3	8.8	13	30.2	2	25.0	0	0.0	35	16.5	32	16.8	40	15.7	45	18.8
19	8	23.5	*6	14.0	2	25.0	2	40.0	40	18.9	44	23.0	50	19.7	52	21.6
29	3	8.8	7	16.3	0	0.0	0	0.0	32	15.1	39	20.4	35	13.8	46	19.2
39	2	5.9	3	7.0	1	12.5	1	20.0	39	18.4	18	9.4	42	16.5	22	9.2
+	2	5.9	3	7.0	1	12.5	0	0.0	39	18.4	25	13.1	42	16.5	28	11.7
AL	34	100.0	43	100.1	8	100.0	5	100.0	212	100.0	191	100.0	254	99.8	239	99.7

* Each cell includes 1 case diagnosed "Probable Cholera."

TABLE VI

TIME INTERVAL BETWEEN INDEX CASE AND SUBSEQUENT CONFIRMED CHOLERA CASES IN 36 FAMILIES WITH MULTIPLE CASES*

Onset in days after index case	Case Order Within Family									Totals		Cumulated Percent
	2	3	4	5	6	7	8	9	10	No.	%	
0	8	1								9	16.1	100.1
1	6	3	1							10	17.9	84.0
2	7	1	1							9	16.1	66.1
3	5	1	1	1	1	1				10	17.9	50.0
4	4	1								5	8.9	32.1
5	1	2	2				1			6	10.7	23.2
6	2			1				1		4	7.1	12.5
7												
8									1	1	1.8	5.4
9												
10	1			1						2	3.6	3.6
TOTALS	34	9	5	3	1	1	1	1	1	56	100.1	

* Cases of Probable Cholera and Diarrhea cause Unknown have been excluded.

CHOLERA IN EAST PAKISTAN FAMILIES 1962 - 1963

Introduction: The South East Asia Treaty Organization initiated a cholera research program in 1959 and a permanent laboratory was established in Dacca, East Pakistan in 1960. The endemic and epidemic pattern of cholera in this part of the world is well-known. However, the precise manner in which cholera is spread and the disease perpetuated is not clear.

Since little is known about the behavior of cholera in specific subgroups of the population, such as families, studies of spread of infection in families of cholera patients in Dacca District were begun in 1962. This report describes the epidemiologic features of multiple cases occurring in a group of 85 families of hospitalized patients with proven cholera. 57 secondary cholera cases were observed in 33 of the 85 households. The present study provided an opportunity to examine the relationship between familial and individual characteristics and likelihood of the occurrence of multiple cases within households.

Method: Families located within a 10-mile radius of Dacca were selected for study usually within two days of hospitalization of a family member with bacteriologically proven cholera. Families were defined as units consisting of two or more individuals who lived and dined together. Members were not necessarily blood related. Cholera was defined as an acute diarrheal disease associated with recovery of V.cholerae (Inaba or Ogawa) organisms from feces within five days of onset of symptoms. Six individuals were included for study who died following acute diarrheal disease without positive cultures. There were fatal cases occurring in families in association with one or more bacteriologically proven cases.

The families were visited at daily intervals excluding weekends for at least two weeks by teams comprised of physicians and sociologists. Most of the recording data was performed by sociologists. On the initial visits questionnaires were completed to provide the following information about the families: location, type of housing, religious affiliation, travel away from home, recent contact with diarrhea and cholera cases and past cholera experience. Individual characteristics were recorded such as, age, sex, marital status and occupation. At each visit inquiry was made concerning the health of each family member. Whenever diarrhea or vomiting was reported a fecal specimen was collected for bacteriological investigation, and details of the illness recorded. These included the following symptoms: diarrhea, vomiting and abdominal pain. Specimens were obtained by means of rectal swabs. These were placed in bile peptone transport media and incubated overnight at 37°C. Subcultures were made on gelatin agar and Monsur's bile peptone tellurite medium and incubated overnight at 37°C. Suspicious colonies were tested for agglutination with specific antisera. Significance levels were calculated using the Chi-square (X^2) test.

Results: The families of 85 hospitalized patients were selected for study during the interval from late October 1962 through June 1963. The patients and their families lived in Dacca District in 4 localities all within approximately a 10-mile radius of Dacca City, (see Map). Subsequent cholera cases developed in 33 of the 85 families during the 2-week observation periods. Families with more than one case were encountered throughout the study interval. The frequency distribution of the number of affected individuals in families is shown in Table 1.

continued.....

The intervals between onset of illness in index cases and the appearance of subsequent cases in families are shown in Table 2. The majority of secondary cases developed within 3 days. A few cases continued to appear each day thereafter. In 6 of 57 cases, intervals exceeded 7 days. Since multiple cases in families may result from a common source, transmission of infection from primary cases, or by a combination of these routes, the relationship between intervals from onset of illness in index cases to removal from the household and the occurrence of multiple cases was examined (Table 3). Families experienced fewer secondary cases when index cases were hospitalized in less than 12 hours from onset of illness than in those with hospitalization 12 or more hours after onset. ($P<.05$). The age and sex distribution of index cases, secondary cases and family members at risk is shown in Table 4. The ratio of individuals <15 to those 15 years of age and above in the total study population was 1.1 to 1. The excess number of index cases <15 years of age cannot be interpreted in terms of incidence rates owing to bias introduced by selection of hospitalized cases. The number of secondary cases and population at risk were used to calculate secondary attack rates (Table 5). The number of affected males and females below 15 years of age was significantly higher than that for adults ($P<.01$).

The average family size in those 52 households without subsequent cases was 6.1 individuals per family and in the 33 multiple case families was 8.5. The distribution of cholera cases in families by family size is shown in Table 6. The number of secondary cases in families was directly related to family size ($P<.05$). The occurrence of secondary cases in families was not related to religious affiliation or to age or sex of index cases.

Three of the 85 index cases gave a past history of cholera; all were adults. The prior "cholera illnesses" preceded the present episodes by 1,4, and 25 years, respectively. 20 of the remaining 517 family members at risk reported previous cholera, and none of the 20 were attacked in the present episodes.

Discussion: The infrequent occurrence of several simultaneous cases of cholera in individual family units was reported by Morgan et al in a study of mild cholera in Bangkok in 1959. In the present study, 13 family contacts developed cholera on the same day symptoms appeared in the corresponding 85 index cases. These 13 cases and possibly the 23 other illnesses occurring within 3 days of the onset of disease in index cases, may represent spread from a common source, infection due to contact with primary cases, or a combination of both. Several findings in the present study suggest that spread within families did occur. These include the appearance of cholera in family contacts up to 10 days following primary cases and an increase in secondary cases associated with prolonged household contact with index cases prior to hospitalization. The occurrence of more secondary cases among children in large families is consistent with intrafamilial spread in a crowded setting, although multiple introductions cannot be excluded. High incidence rates among children would be expected in a densely populated developing country in which cholera is a disease of continued high prevalence. Snow stated that, "it is amongst the poor, where a whole family live, sleep, cook, eat and wash in a single room, that cholera has been found to spread when once introduced... In the asylum for pauper children at Tooting, 140 deaths from cholera occurred amongst 1000 inmates...when it is remembered that children get their hands into everything, and are constantly putting their fingers in their mouths, it is not surprising that the malady spread in this manner..."

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It is generally held that cholera may occur more than once in the same individual. Proof of actual re-infection confirmed by bacteriologic and serologic study is, however, lacking. Three index cases in the present study provided a history of prior cholera. In the absence of supporting laboratory evidence, little significance can be attached to these histories.

It is proposed that studies of cholera in families in Dacca District be extended to provide further data on the clinical and subclinical manifestations of the disease as well as test the efficacy of preventive measures such as chemoprophylaxis and vaccine.

Summary: The families of 85 cholera patients hospitalized during 1962-3 in Dacca, District, East Pakistan were investigated. Secondary cases were observed in 33 of the 85. Secondary attack rates were higher among children than adults. Spread of cholera within these families was suggested by the distribution of intervals between primary cases and subsequent ones and the relationship between lengthy home stay of index cases and increased numbers of secondary cases.

TABLE 1

Number of cases per family	Number of families
1	52
2	24
3	4
4	2
5	1
6	1
11	1
Total	85

Number of cholera cases in families

TABLE 2

	Intervals in days										
Subsequent cases	same	1	2	3	4	5	6	7	8	9	10	Total
Daily total	13	9	8	6	8	3	3	1	3	-	3	57
Cumulative total	13	22	30	36	44	47	50	51	54	54	57	57

Intervals between onset of illness in index cases and appearance of subsequent cases in families

continued.....

TABLE 3

Intervals (hours)	Number of families with Secondary cases		Total
	YES	NO	
0-3	2	10	12
4-7	7	15	22
8-11	1	7	8
12-15	4	6	10
16-19	1	2	3
20-23	2	1	3
24+	13	11	24
UNK.	3	-	3
Total	33	52	85

Relationship of length of home stay of index cases and occurrence of secondary cases in families

TABLE 4

Population	Age group 0 - 4		5 - 9		10 - 14		15+		UNK.	Total
	M	F	M	F	M	F	M	F		
Total	60	56	53	60	41	43	142	140	7	602
Index cases	18	9	3	14	7	4	8	19	3	85
At risk	42	47	50	46	34	39	134	121	4	517
Secondary cases	10	8	9	8	6	5	6	5	-	57

Age & sex distribution of index cases, secondary cases and population at risk in families

continued.....

TABLE 5

Age (years)	0 - 4		5 - 9		10 - 14		15+		Total	
Sex	M	F	M	F	M	F	M	F	M	F
Rate	23.8	17.0	18.0	17.3	17.6	12.8	4.4	4.1	16.9	10.2

Secondary attack rates, by age and sex

TABLE 6

Number of persons in family	Number of cases in family: One	Two or more	Total
2 - 5	29	8	37
6 - 9	18	14	32
10+	5	11	16
Total	52	33	

Relationship between family size and

and occurrence of multiple cases

DACCA DISTRICT

Administrative Divisions

Scale 1"=10 Miles

REFERENCES

SUB DIVISION BOUNDARY — — — —

P. S. BOUNDARY · - · - · -

DISTRICT H. Q. Dacca

SUB DIVISION H.Q. N.Ganj

POLICE STATION Tejgaon

TANKS AND CHOLERA

In deltaic regions of East Pakistan, the endemicity of cholera, attributed chiefly to water sources, gives emphasis to the abundant presence of surface water reserviors such as tanks, ponds, and ditches. Indiscriminate use of these water sources makes them suspect in the spread of disease. Previous study has not been made of tanks in East Pakistan. The aim of thepresent study is to classify the tanks according to their use by the community and to note the chemical and bacterial composition, changes in seasonal pattern, and their possible relation to cholera as a reservoir or means of transmission of thedisease.

Tanks were given the following classification: reserve, bathing, stagnant and periodical.

1. Reserve tanks are used primarily for drinking purposes and are common in areas with a scarcity of good water or where salinity of water is a major problem (southern coastal regions). Washing in these tanks is prohibited. The tanks are usually fenced or guarded against entrance of household animals. The water of these tanks is generally rain and surface water which through storage undergoes natural purification.

2. Bathing tanks are numerous and widely used by much of the population for laundering, washing of utensils, and bathing.

3. Stagnant cesspools or dhobas often have latrines built directly over them, and urinals and privys on the embankment drain into them. These tanks are usually covered with green slime. Though the practice is not openly accepted, children and housewives often use these tanks for various purposes.

4. Periodic tanks generally exist from July or August to January or February, and then go dry. These tanks are found in riverine belt areas such as Kaliganj and Narayanganj. In the monsoon season, the tanks connect other reserviors which in the dry season are isolated. During the monsoon, distinguishing between a cesspool, ditch, or bathing tank is difficult. Periodic tanks are used for many purposes, though usually not for drinking. Housewives commonly wash utensils and bathe in them, and carry water home for household use.

Tanks were selected in three types of areas, i.e. Dacca Town, Narayanganj municipal area, and Kaliganj, a riverine village. These areas have a drinking water supply system either from the municipality or from tube wells. They have few reserve tanks and a great many bathing and stagnant tanks. The sampling of water was done as part of the surveillance program or in connection with occurrence of cholera cases. Bacteriological findings are shown in Table I. None of the tanks is entirely free from faecal pollution. The tanks which have the highest coliform density have also shown the presence of cholera vibrios.

The data collected on chemical analysis of these tanks are tabulated in Table II. Reserve tanks are moderately alkaline with a pH range of 6.2 to 7.8 (average 7.1). As gauged by specific conductance, the mineral content is low. The bathing tanks usually have a high pH, ranging from 7.5 to 9.1 (average 8.1). Laundering and washing would cause the high pH. These tanks often show a high rise in pH during bright sunny days, as observed by Dr. Cockburn and Mr. Cassanos.

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Stagnant tanks generally have a low pH, ranging from 6.2 to 7.4 (average 6.6), but a high specific conductance because of the high content of faecal matter and other waste material. Periodic tanks vary too greatly to define any chemical pattern.

TABLE I

Representation of Bacteriological Quality of 4 types of Tanks

Figure in ( ) = Number of Tanks with V. cholerae

Types of Tanks	No. of Tanks Exam.	Coli Count per 100 mls of water					Percent Positive for	
		1 - 10	11 - 500	501 - 2000	2001 - 10,000	10,000	V.cholerae	NAG
Reserve	9	3	6				0	56%
Bathing	44	1	7	9	19 (3)	8 (4)	16%	80%
Stagnant	47		1	4	17	25 (3)	6%	85%
Periodic	8		1	2	4 (1)	1	12%	87%

TABLE II

Chemical data on 4 Types of Tanks

Types of Tanks	pH			Sp. Cond.			Alkalinity			Chloride		
	Max	Ave	Min	Max	Ave	Min	Max	Ave	Min	Max	Ave	Min
Reserve	7.8	7.1	6.2	350	134	37	98	45	5	24	9	4
Periodic	8.1	7.1	6.6	1160	437	122	302	136	28	135	53	16
Stagnant	7.4	6.6	6.2	1740	599	340	490	165*	105	245	89	21
Bathing	9.1	8.1	7.4	1246	388	150	380	154	40	120	36	5

* High free CO_2 contributing to the total Alkalinity.

References:

(1) GOHAR, M.A. 1953 Draft Report

(2) ARBUTHNOT on Cholera, WHO Report 1953

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EVALUATION OF METHODS

Sanitary Bacteriology

The standard methods procedure to determine (presumptive test) was found to be unnecessarily time such as tanks, ditches, and dug wells because of their membrane filter technique for determining coliform org but the dilution factor was always a matter of guess, was frequent. To know the actual magnitude of faecal cy of faecal pollution, the determination of faecal co a change in the routine method was necessary.

With the help of Mr. A. A. Crispe (from Sydr Sewerage Board, deputed by Mr. Flynn), we modified our broth for coliform density. The time factor was reduce to those obtained with the membrane filter technique.

The test for incidence of true E. coli was a test, the optimum incubation temperature for faecal E. be 45.4°C. Now the routine procedure used in this labc the Eijkman test at 45.5°C and making faecal E. coli cc

Use of membrane filters for E. coli at 45.5°C with Endo medium (specific for coliform) and MacConkey m satisfactory. The Endo mediumfails to produce the typi temperature; on MacConkey medium,yellow colonies are tc in a few cases have samples from storage jars, with tub as source, given results worth considering.

Occassionally, KF mediumhas been used to dete but shortage of personnel and vast numbers of routine sa regular use.

Isolation of Vibrios from Waters

Rare isolation of V. cholerae, even during th frequent isolation of non-agglutinable vibrios after en water and bile peptone media necessitated the evaluati observation was made when a sample of water poured dire count of V. cholerae as 8 organism per ml of sample, bu V. cholerae except NAG was isolated. A growth competiti between V. cholerae and NAG in enrichment media. The l enrichment media finally helps the NAG to overgrow. A cultures from enrichment media were made at different t presented below indicate why water having 8 V. chol any agglutinable colony from enriched subculture.

Direct Plate	No V.
4 hours enriched	+ In
6 hours enriched	++ I
8 hours enriched	++ I
12 hours enriched	+ NA
16 hours enriched	++ N
24 hours enriched	+++

The technique was changed as of January 1963. Subcultures from enrichment broth were made twice, once after 6-8 hours incubation and again after 20-24 hours enrichment. Alkaline peptone water and bile peptone were evaluated to find which would yield more isolations (Table III).

TABLE III

Vibrio Isolations	6-8 Hours enrichment		20-24 Hours enrichment	
	BP	P.W.	BP	P.W.
V. cholerae	28	21	17	10
NAG	4	5	15	18
NEGATIVE	7	13	7	11
Total Samples	39	39	39	39
% V.cholerae	72	54	43.5	25.6

Reduction in T.C. isolation in 24 hours

$$= \frac{72 - 43.5}{72} = 39.6 \text{ or } 40\%$$

Reduction in T.C. isolation in P. W. (in 6 hours enriched culture)

$$= \frac{72 - 54}{72} = 25\%$$

Reduction in T.C. isolation in P.W. (in 24 hours enriched culture) = 41%

The method now used as a routine is described in Appendix I. With this change in methods, the isolation of cholera vibrios from water has increased in comparison to the previous year. In 1962, isolation of V. cholerae was only 2.4%, but as of September 1963, isolation was 8.5%.

SURVEILLANCE AND STUDIES

In addition to the Kaliganj studies in 1962, water surveillance has continued, bringing in more areas with incidence of cholera. The new areas include Bander Refugee Colony in Narayanganj Municipality, Deobogh and Paikpara, the slum and cholera endemic localities of Narayanganj City, and Mudafa, an agricultural community.

In addition to the main sources of water (tanks, tube wells, open dug wells and rivers) household water, i.e. water stored in earthen pitchers, was also kept under surveillance in two riverine belt areas of Kushairbagh and Agahnagar, both with cholera incidence. Data have been collected on the water's seasonal variations in chemical constituents, changes in bacteria and magnitude of faecal contamination. The human body seems the source of vibrios and water contamination. Surface

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water sources and dug wells show positivity as long as the disease persists in the community or locality. When known disease is over, the cholera vibrios also vanish and reappear only when there is a fresh incidence of clinical cholera. So far our studies have not shown contamination of tube wells with cholera vibrios even when there are cholera cases in the area.

Surveillance of storage jar waters indicate that there is occasionally transmission of the organisms from an individual to the water. In the absence of any disease, no cholera vibrios have been isolated from storage jars in the households, irrespective of the source of water from which the jars were filled. Isolation of NAG's was not infrequent. Unfortunately, adequate correlated culturing of the individuals in the households has not been performed sufficiently to establish the true relationship of vibrios found in jars.

The results of surveillance can be seen in Table IV.

TABLE IV

Source		Vibrios			Faecal Contamination	
		No. of Examination.	% of V. cholerae found	% of NAG found	No. of Examination	% of Faecal contamination
Dug Well	Surveillance	104	0	41	56	98
	Case Investigation	301	19	60	131	96
	Total	405	14	55	187	97
Tank	Surveillance	195	0	64	133	97
	Case Investigation	249	18	52	131	99
	Total	444	10	58	264	98
River	Surveillance	81	2.4	61	39	90
	Case Investigation	8	25	50	4	100
	Total	89	4.4	60	43	91
Canal	Surveillance	14	0	36	12	83
	Case Investigation	12	17	75	8	100
	Total	26	7.7	54	20	90
Tube Well	Surveillance	45	0	2	38	2.6
	Case Investigation	90	0	1	65	3.1
	Total	135	0	1.4	103	3
Municipal Supply	Case Investigation	30	0	0	28	7.2
Jar	Surveillance	226	0	21	137	47
	Case Investigation	545	7.5	10	294	40
	Total	771	5	13	431	41

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This year, waters of the Buriganga River, which passes through Dacca, and of the Lakhya River in Narayanganj have also been studied. The Lakhya River has shown high faecal contamination in comparison to last year, and has also shown presence of cholera vibrio on two occasions.

The Buriganga River has always shown more faecal pollution than the Lakhya River. The Buriganga is a smaller river and its banks more heavily populated than those of the Lakhya. Graphs I and II show the seasonal variation in chemical and bacteriological contents, and the presence of vibrios in the Lakhya and Buriganga Rivers.

HOUSEHOLD AND FAMILY STUDIES

In order to establish the role of water in spread of cholera, household and families have been studied where cholera cases occurred. These studies were conducted in close collaboration with the Epidemiology Section. Households known to have cholera cases were visited at the first opportunity. The water used by the family concerned (storage jars), by the other family members in the same household (dug wells), and by the community (tanks, tube wells, municipal supply sources, ditches, canals, rivers) was all sampled for vibrio isolation and coliform count. Occasionally, swabs of latrines, drains, and mud samples were also taken. Any of the water found positive for cholera vibrio was continually re-examined until that time when no more cholera vibrios were found in it. Though waters from many households have been examined, no definite relationship between water positivity and occurrence of cases has evolved.

In households with confirmed cholera, a comparative study was made based on presence or absence of a household dug well. In 48 households with dug wells, seven households had more than one family with confirmed cholera; in 48 households without dug wells, only one household had more than one family with confirmed cholera (Table V). This finding suggests that the dug well is a possible means of transmission of disease within the compound.

TABLE V

	With Dug well	Without Dug well
Total No. of Households	48	48
Total No. of families within households	155	157
No. of Households where only one family involved	41	47
No. of Households where more than one family involved	7	1

If water is the agent in spreading the disease, the hazard within the family, household, and community is great. Nevertheless, three households with water sources

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positive for vibrio and contiguous to diseased households did not develop a single case in either the household or the family.

The custom of storing leftover rice, that was cooked for the evening meal, and eating this rice the next day offers a possible source for spread of multiple cases within the family. Most of the multiple cases have occurred within one family who share common food and water sources.

A detailed study was made of "panta bhat" (cooked rice stored in water), testing the following variables:

(a) differences in quality of rice
(b) effect of storage at various temperatures
(c) effect of storage for various time intervals
(d) effect of storage with and without salt

After being cooked, therice was cooled and the pH noted. Tap water was then added in measured quantity (200 to 300 cc), and again the pH of the mixture was taken. Inoculum of cholera vibrio was given after a 10-fold serial dilution of fresh broth culture of the organism to give a count of 10 to 10^2 or multiples of 10^2 organisms per cc of "panta bhat." At fixed time intervals, viable counts were taken from this rice after mixing and taking an aliquot of supernatant liquid and plating directly, or after making 10-fold serial dilutions in normal saline. At each specified time interval, the pH was also observed (Table VI).

The results show that "panta bhat" of cheaper and inferior quality (aus rice) serves as a better enrichment mediumfor cholera vibrio than more expensive and better quality rice (boro rice). The difference may possibly be attributed to the pH, the better quality having a lower pH after cooking.

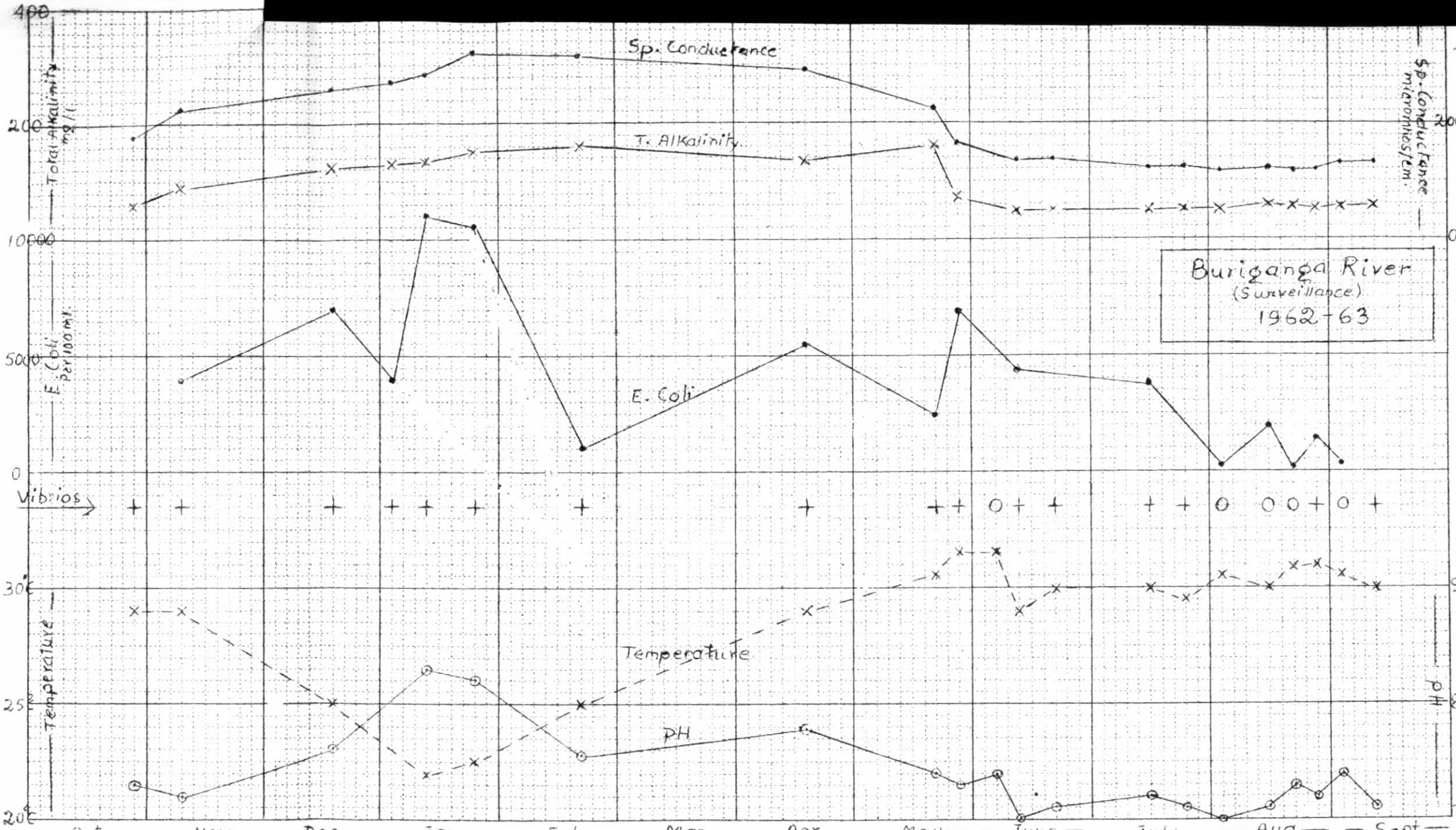
"Panta bhat" kept at atmospheric temperature (29 to 33°C, as it is usually kept in the home) has a quicker and greater rise in vibrio count than rice kept at room temperature (25 to 27°C). Addition of salt, even in as small a quantity as 0.1%, makes the "panta bhat" a better enrichment medium, and the rise in count of Vibrio cholerae is more rapid and higher.

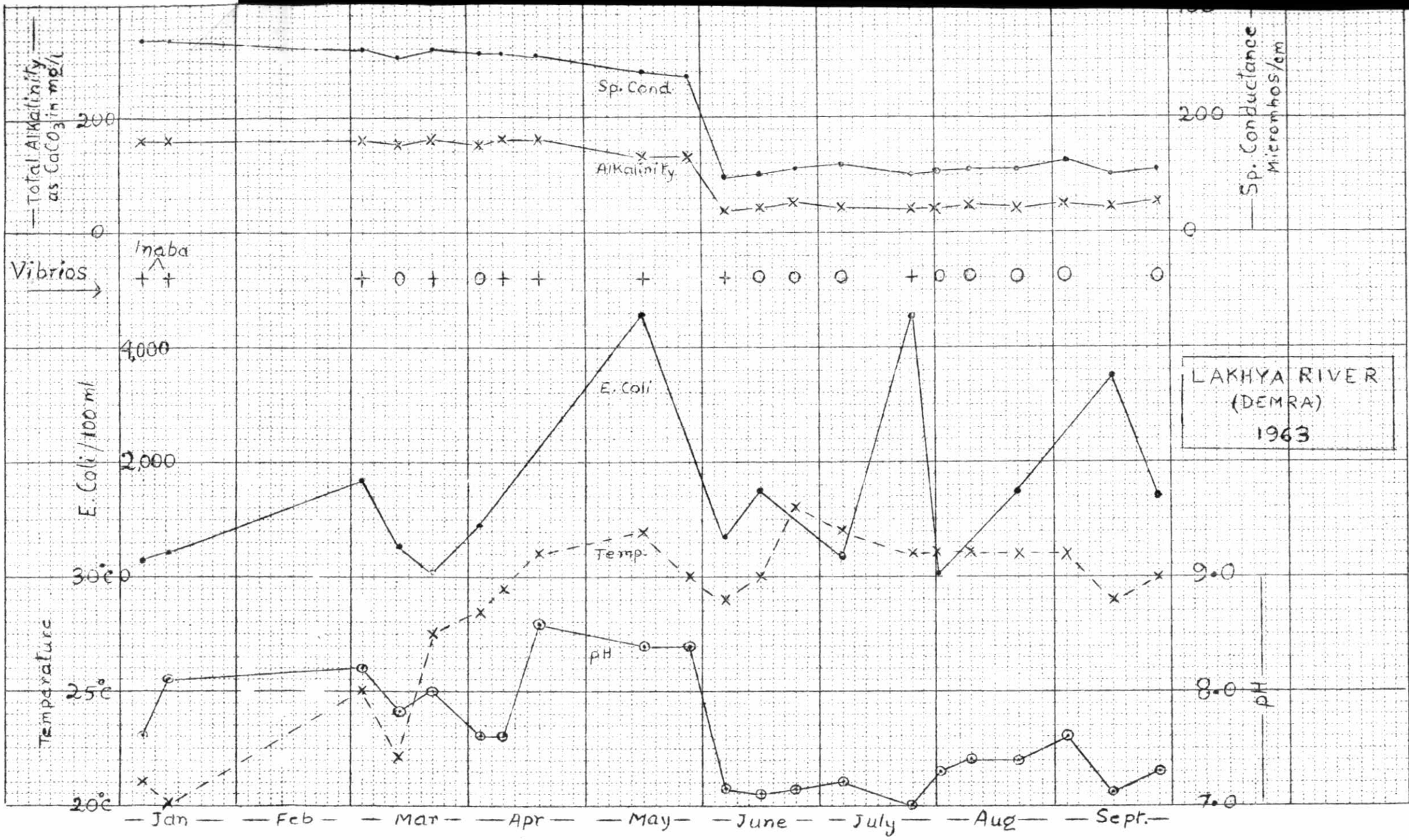
In these experiments, the rice was contaminated with only one organism. In the presence of other organisms, such as are found in water, the growth of Vibrio cholerae may be retarded; with the drop in pH from growth of several bacterial species, vibrio in water might be killed within the storage period. Under the right circumstances, "panta bhat" appears to be a likely source of transmission of disease, perhaps even more so than water sources within or outside the family.

TABLE VI

Showing growth of Vibrio cholerae in "Panta Bhat" (Cooked Rice) under variable conditions

uality f Rice		Temperature	Viable count per cc at - time intervals						pH range
			0 hr.	6 hr.	8 hr.	12 hr.	16 hr.	24 hr.	
us ice	Without Salt	Atmosphere 29-33°C	1.4×10^2	1.4×10^2	-	7×10^3	4×10^3	3×10^3	6.9-5.6
		Room 25-27°C	1.5×10^2	1.5×10^2	-	5×10^2	2×10^3	3×10^3	6.9-6.0
		Incubator 37°C	1.2×10^2	2.2×10^2	-	1.1×10^4	2.4×10^4	3×10^4	6.9-5.6
	With 0.1% Salt	Atmosphere 29-33°C	20	2×10^2	2.6×10^3	4×10^4	-	3.5×10^3	7.1-4.6
		Room 25-27°C	10	60	$<10^3$	$>10^3$	-	6×10^4	7.1-5.2
	With 0.5% Salt	Atmosphere 29-33°C	30	2×10^4	10^5	-	-	4×10^3	7.0-4.5
oro ice	Without Salt	Atmosphere 29-33°C	2×10^2	10^3	-	-	-	Profuse Growth	6.6-6.0
		Room 25-27°C	2×10^2	1.2×10^2	-	-	-	4×10^2	6.6-5.8
		Incubator 37°C	4×10^2	2×10^3	5×10^3	10^3	-	4×10^2	6.6-5.5
	With 0.5% Salt	Atmosphere 29-33°C	3×10^2	6×10^2	10^3	-	Profuse Growth	Profuse Growth	6.8-5.0
		Room 25-27°C	3×10^2	8×10^2	2.2×10^4	4.5×10^4	-	5×10^2	6.8-5.5


Buriganga River
(Surveillance)
1962-63
Sp. Conductance
T. Alkalinity
E. Coli
Temperature
PH
Total Alkalinity mg/l
E. Coli per 100 ml.
Temperature
Sp. Conductance micromhos/cm.
pH
400
200
10000
5000
0
Vibrios
30°C
25°C
20°C
200
0
9.0
8.0
7.0
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
June
July
Aug
Sept


— Total Alkalinity as $CaCO_3$ in mg/l
200
0
Sp. Cond.
Alkalinity
Sp. Conductance Micromhos/cm
200
0
Inaba
Vibrios
+ + + 0 + 0 + + + + 0 0 0 + 0 0 0 0 0
4,000
2,000
E. Coli/100 ml
E. Coli
Temp.
LAKHYA RIVER
(DEMRA)
1963
30°C
25°C
20°C
Temperature
pH
9.0
8.0
7.0
pH
Jan
Feb
Mar
Apr
May
June
July
Aug
Sept.

July 11, 1963.

TECHNIQUE FOR ISOLATION OF CHOLERA VIBRIOS FROM WATER SAMPLES.

This presents the technique currently in use in the Pakistan-SEATO Cholera Research Laboratory for the isolation of cholerae vibrios from natural water supplies.

I. Transport medium.

Triple Strength Bile peptone water

Bile peptone	3%
Sodium chloride	3%
Sodium taurocholate	1.5%
Sodium hydroxide (1 N solution)	37.5 ml. per liter

25 milliliters are bottled in 100 milliliter containers; autoclave at 15 lbs pressure for 15 minutes time, pH after autoclaving, 9.1 - 9.2.

II. Solid Plating Media.

Gelatin agar (GA) plates.

Tripticase	1%
Sodium chloride	1%
Bacto Gelatin	3%
Bacto agar	1.5%

The medium is autoclaved at 15 lbs / square inch pressure for 15 - 20 minutes. Final pH after autoclaving, 7.1 - 7.2.

Bile Salt Tellurite Gelatin Agar (SP) plates.

Tripticase	1%
Sodium chloride	1%
Sodium hydroxide (1.ON solution)	15 ml. per liter
Bacto gelatin	3%
Bacto agar	1.5%

Medium is autoclaved at 15 lbs / square inch pressure for 20 minutes After cooling, but before pouring into plates, 0.5% potassium tellurite is added to give a final concentration of 1 - 200,000.

Procedure:

Direct plating: When contamination is heavy, semi-quantitative counts can be performed by direct plating of the test water on SP plates; 0.25 ml

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and 0.1 ml of the water is added to the relatively dried plate. The water is spread by a glass spreader so that the entire surface of the plate is evenly covered. The plate is incubated at 35 - 37°C overnight and typical colonies are counted.

Enrichment techniques: To the 25 ml of triple strength bile peptone, 50 ml of test water are added, diluting the medium to its normal concentration. These are subcultured on to GA and SP plates after not more than 8 hours incubation (including the time from sampling under normal tropical or subtropical conditions). The bottles are subcultured again after 18 - 24 hours incubation at 35 - 37°C.

Plating: Each water is subcultured by streaking one GA plate and one SP plate. The plates are incubated overnight (18 - 20 hours) at 35 - 37°C.

GA medium is not suppresant and therefore must be relatively thinly streaked. Vibrio colonies must be differentiated from other species by their typical colonial appearance. On this medium, they are round, clear, bluish (not white) and each colony is surrounded by a halo due to the gelatinase produced. The characteristic colonies are more easily recognized by examining the plate against black background with side illumination.

The SP medium is highly suppresant and may completely inhibit many intestinal organisms of the Pseudomonas, Klebsiella and Escherichia groups; Proteus grows only as small colonies. Alkaligenes faecalis is not inhibited, but, against the black background its colonies present an iridescent white appearance. This is in contrast to the round and clear vibrio colonies which have a bluish grey tinge, usually with a central grey spot of precipitated tellurite in each colony. On standing at room temperature for 24 - 48 hours or after prolonged incubation, on both media the colonies become large and more prominent.

Identification:

Suspect colonies are picked and tested by slide agglutination using "group" "Inaba" and "Ogawa" anticholera sera. Agglutinating strains are further characterized by chicken cell agglutination, hemolysis and phage testing. All other colonies considered to be vibrios are checked by sugar fermentation tests and by Gram strain, confirming their identity as non-agglutinable vibrios and establishing their Heiberg grouping. Non-agglutinable vibrios are almost always present in water samples in East Pakistan and overgrow true vibrios if incubated in fluid medium for more than 8 hours. In their absence, a low level of contamination with true cholerae vibrios may become manifest after 24 hours when the 8 hours reading was negative.

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Nutritional Status of Cholera Patients

Introduction:

Many observations suggest that there are physiologic and biochemical variables that affect the likelihood of development of overt disease in a subject exposed to cholera infection. Since the endemic cholera area is a region of overpopulation, and cholera tends to attack primarily the poorer classes, malnutrition must be considered as a possible predisposing factor. An investigation of relationships between nutrition and cholera susceptibility has therefore been undertaken at the CRL. It is fortunate for this study that the work of the Nutrition Survey of Pakistan has made available definitive information on the nutritional status of the population as a whole.

Investigations to date have been focussed on four vitamins - thiamine, ascorbic acid, vitamin B_{12}, and riboflavin. The possibility that thiamine deficiency might play a part in the pathogenesis of cholera was suggested primarily by bizarre cardiac disturbances encountered in a number of the first patients admitted to the CRL ward. These included ECG abnormalities which were consistent with, though not diagnostic of, beri-beri heart disease, and evidence of functional myocardial impairment in a few cases. Although the Nutrition Survey indicates that thiamine deficiency is not a problem in the population, we thought that an acute deficiency might develop in those eating poorly cooked fresh-water fish (thiaminase effect), and that these few might be the cholera victims. The supposition was supported by initial findings of low thiamine excretion in active cases. With riboflavin and ascorbic acid, there were more definite indications for a careful study. Deficiencies of these factors are widespread, and it appears that the seasons of maximum deficiency coincide with the seasons of greatest cholera incidence. Clinical signs of riboflavin deficiency have been manifest in cholera patients, but overt scurvy has not been encountered. Vitamin B_{12} and folic acid are agents that might be implicated in cholera, especially since deficiencies of these factors are so closely associated with gastrointestinal disturbances. Studies on folic acid are beginning, but have not yet progressed sufficiently to permit inclusion in this report.

Procedures, Findings, and Interpretations:

Thiamine: Transketolase activity of red cell hemolysates from 24 cases of typical acute cholera were measured. The procedure was that of Brin, *et al.* (1960), in which the consumption of ribose is measured with and without the addition of thiamine pyrophosphate. In thiamine deficiency, addition of TPP stimulates the consumption of ribose by more than 20%. This was not found to be the case in any of the samples investigated.

In twelve cases, it was possible to determine urinary thiamine excretion immediately prior to the onset of symptoms, using urine samples obtained in connection with work on household contacts. As these were casual specimens, we followed the procedure of the Nutrition Survey in expressing results as ratios of thiamine to creatinine. In only one of these twelve cases was the output suspiciously low, 68 micrograms of thiamine per gram of creatinine.

These observations indicate that thiamine intake prior to onset of disease, and functional status at the time of hospitalization, were normal in the individuals

studied. These facts, together with the Nutrition Survey results, point to the conclusion that thiamine deficiency is not an important determinant of cholera susceptibility in this area. They cannot be construed to show that thiamine deficiency has no effect on cholera in areas where beri-beri is common.

Thiamine determinations on the urine of patients under treatment for established cholera showed a rapid fall of excretion rate, with a minimum at about the fifth day. The details of these studies are available in a separate report (Rosenberg, 1963). It is felt that this is merely a reflection of the decrease in thiamine intake that results from the inability of the patient to consume a normal diet, and does not pose a therapeutic problem unless the resumption of normal food intake is unusually delayed.

Ascorbic Acid: Serum levels of ascorbic acid were measured on admission in 32 cholera cases, using the procedure of Shaffert and Kingsley (1955). Bloods were not available from household contacts before onset of disease, and the use of ascorbic acid in the therapeutic protocol precluded systematic study of serum levels after rehydration. Of the 32, 8 had initial levels in the deficient range (below 0.2 mg.%). An even larger proportion of patients who proved to have non-cholera diarrhea had low levels, although the difference did not approach statistical significance. The Nutrition Survey found the incidence of deficiency to be 21% in field work during the same season (spring, 1963). It therefore appears that cholera does not tend specifically to select those members of the population whose vitamin C nutriture is poor.

In a pilot study, 23 guinea pigs were rendered scorbutic by a vitamin C deficient diet. Without utilizing any other measures to increase cholera susceptibility, we tried to infect them with oral and intra-intestinal doses of 1 to 2 ml. of an overnight broth culture of V. cholerae. Only one developed a fatal disease characterized by diarrhea and a fluid-distended gut, even though some were virtually moribund from scurvy when challenged. This investigation fails to implicate vitamin C deficiency in the etiology of cholera. Formal and Lowenthal (1963) have also tested the relationship of ascorbic acid deficiency to cholera in guinea pigs, with negative results.

Vitamin B_{12}: Serum vitamin B_{12} levels in 28 patients with cholera were assayed with Lactobacillus leichmanii, according to the procedure of Spray (1955). Serum levels of this vitamin have been shown to correlate well with overt deficiency in a variety of clinical situations, and to become abnormal before tissue stores are exhausted or anemia and symptoms appear (Spray, 1962). The majority of the determinations were done on blood obtained during the first hospital week, after correction of dehydration. The B_{12} level was subnormal in only one patient, a woman in the third trimester of pregnancy. This is a common finding in otherwise normal pregnancies. In 24 patients the B_{12} levels were normal, and in three they were elevated. In two of the latter cases, the serum had been obtained on admission, while hemoconcentration was present. The cause of the elevated level in the third case is not known.

As this assay has not previously been available in Dacca, there are no figures on the incidence of B_{12} deficiency in the general population. However, it appears improbable that this deficiency will be found to be common, and quite unlikely that either dietary lack of vitamin B_{12}, or gastrointestinal abnormalities interfering with its absorption, will prove to be a factor in the development of cholera in a significant number of cases.

Riboflavin: Technical difficulties have plagued our attempts to determine urinary riboflavin excretion, and the data remain inconclusive. The photofluorimetric procedure of Slater and Morell (1946) has been used, and the results expressed as a ratio to creatinine. The only conclusion that seems justified at this time is that some individuals who have developed cholera had a normal urinary riboflavin output in the days immediately before onset, and that riboflavin deficiency is not a prerequisite for cholera. It may be desirable to resume this study when improved methods for assessing riboflavin nutriture become available.

Summary and Conclusions:

Although the hypothesis that dietary deficiency may predispose to cholera infection is an attractive one, the investigations that have been completed so far provide no support for it. Specific deficiencies of thiamine and vitamin B_{12} appear not to occur among the population of East Pakistan afflicted with endemic cholera. Biochemically demonstrable ascorbic acid deficiency is common in the area, but cannot be shown to be related to cholera. Data that have been obtained on riboflavin are not conclusive, but give little encouragement for continued effort.

There are other deficiencies which occur in East Pakistan, the relationships of which to cholera susceptibility remain to be studied. These factors include protein-calorie malnutrition, deficiencies of essential minerals, poor intake of vitamin A, and probably also lack of folic acid. Investigations of the role of these deficiencies may play in the epidemiology of cholera are either under way or are under consideration for the future.

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Potassium Deficiency in East Pakistan: Preliminary Evidence from a Survey of Urinary Electrolyte Excretion.

Introduction:

Among the body constituents lost in the diarrhea of acute cholera, one of the most important is potassium. Cholera stools have been found to contain from 15 to 70 mEq. potassium per liter, with most containing about 20 mEq/l. During the course of the disease, cumulative losses may approach one-third of the total body content (Watten, Blackwell, & Phillips, 1960). Unless replacement of potassium is properly effected, severe deficits may be incurred, and renal tubular lesions may result (Benyajati, et al. 1960). Although we are not aware of any controlled clinical studies designed to evaluate the effect of potassium replacement on the course of acute cholera, it would seem likely that cases in which potassium therapy is neglected would do less well overall. That this is true in the diarrheal diseases of infants has been known for many years (Darrow, et al. 1949).

As a corollary of the above supposition, one might expect that a patient who had incurred a potassium deficit before onset of cholera might have more severe symptoms, or a less favorable prognosis than one whose body potassium was originally normal. The clinical aspects of cholera in Dacca, as observed on the CRL ward in the months that have elapsed since its opening in November 1962, have been discussed earlier. Certain of the findings suggested the possibility of a greater potassium deficit than one would have expected on the basis of the history of illness prior to admission. These included electrocardiographic abnormalities, intolerance to glucose, hyperactive deep tendon reflexes, and signs of paralytic ileus. These considerations led us to raise the question whether or not potassium deficits might occur naturally among the population from which the CRL cases are drawn, and whether potassium deficiency, if indeed it exists, might play any role in determining either the probability of cholera infection, or the severity of the case once infection has occurred.

Although it has long been known that potassium is an essential constituent of protoplasm, and that an intake of potassium is necessary for all animal life, the element has received scanty attention from nutritionists. As potassium is so widely distributed in all plant and animal tissue, it is virtually impossible to conceive of a diet that would not provide more than adequate potassium for the maintenance of balance under ordinary circumstances. Perhaps for the same reason, the mammalian kidney, which can conserve sodium with such tenacity, has not developed a corresponding ability to retain potassium when body stores are low (Relman and Schwartz, 1958). In recent years potassium deficiency has come to be recognized more and more frequently as a complication of one or another disease condition in which abnormal potassium losses occur. These fall into two categories - those in which the loss is through the GI tract, as in malabsorption, chronic diarrhea, abuse of laxatives, and intestinal surgery; and those in which loss is through the urine. The latter category includes cases of hyperadrenalism, either natural or therapeutically induced, certain types of renal disease, and the many conditions in which diuretic drugs are employed for the treatment of edema. Many of these conditions respond well to the administration of supplementary potassium.

Another channel for loss of potassium, which is usually of little importance in temperate climates, is through sweat. Sweat contains from 5 to 30 mEq. potassium per liter (Randall, 1961), the exact value depending on heat acclimation,

salt intake, and other factors. Since men doing heavy work in the hot sun may lose sweat at rates exceeding one liter per hour, it is apparent that cutaneous losses may be of significance in a tropical situation. Leithead (1963) says that such losses may be comparable to those in chronic diarrhea.

The staple of the diet of East Pakistan is rice, which of all the cereals has the lowest potassium content, a mere 3 mEq. per 100 gm. dry weight. Most of the dietary potassium of the populace, therefore, must be derived from meats, fish, milk, fruits, and vegetables, the quantity of which will vary with seasons, and with the economic level of the family concerned. The combination of possible low intake and considerable losses through sweat during the hot seasons might well result in potassium deficiency among the people of this area.

The most unequivocal evidence of potassium deficiency that may be obtained is the demonstration of a reduction of body potassium content, either by isotope dilution with K - 42, or by whole body counting of the K - 40 present in natural potassium. Simpler indices of potassium status, such as determination of serum potassium level or electrocardiographic examination are available, but neither correlates as well as might be wished with potassium content. Before attempting to apply any of these procedures on a wide scale, however, it seemed advisable to study the average values of electrolyte excretion in the population, using urine samples made available through the Nutrition Survey. This should serve to indicate to what extent average potassium intake exceeds obligatory extra-renal losses, and would provide reference data useful in the further study of potassium metabolism in cholera cases. However, potassium depletion that might develop as a consequence of urinary loss, as with excessive mineralocorticoid secretion by the adrenal gland, would not be evident from a study of urine alone.

Unfortunately, there are few established criteria for the normal, or ideal, urinary potassium excretion. Dauphinee (1962) states that the normal western diet provides from 2 to 4 gm (50 to 100 mEq.) of potassium per day, most of which is eventually excreted in the urine. Elkinton and Danowski (1955) mention an intake of 100 mEq. per day as normal. Relman and Schwartz (1958) make the following statements: "Only after two weeks on a potassium intake of 10 to 15 mEq. does urine potassium approach this figure, at a time when the total potassium deficit is perhaps 250 to 300 mEq.", and "Thus a very low excretion of potassium (less than 10 mEq. per day) usually indicates severe and fairly long-standing potassium depletion..." In the present study, the impossibility of procuring timed 24 hour urine collections has made it necessary to relate potassium and sodium excretion to creatinine in casual specimens, but as the average adult male excretes approximately 20 mgm. creatinine per kg. body weight per day (Peters and Van Slyke, 1946), the quantity of electrolyte excreted per gram of creatinine should approximate the daily output of a 50 kg. man.

Procedures and Results:

Beginning in April, 1963, an aliquot of each urine specimen procured by the Nutrition Survey of Pakistan has been analyzed for sodium, potassium, and creatinine. The cations were measured in the EEL flame photometer, and creatinine by the method of Folin and Wu (1919). Ratios were then calculated, and these data analyzed by accepted statistical methods. In addition, urine specimens have been obtained from CRL cholera patients at follow-up visits after recovery was complete, and analyzed in the same way. The influence of age, sex, and other variables on these data has not yet been tested.

The findings are presented in the table. It may be seen that mean urinary sodium excretions are high, and that specimens with a low sodium content are quite rare. On the other hand, mean potassium excretion is slightly low, judging by the Western averages cited above. The fraction of specimens with potassium content falling into the lowest ranges is considerable.

There are statistically significant differences between the mean values of urinary potassium/creatinine and sodium/creatinine ratios when one compares survey results in certain locations with those in others. It is not yet possible to determine the cause of these differences, as many variables, such as local preferences in eating habits, electrolytes in the water supply, and differences in weather with the change in seasons might influence the results.

The specimens obtained from follow-up visits of cholera patients are noteworthy, as they show lower mean levels of potassium excretion than do all but one of the population groups tested in connection with the Nutrition Survey, and the highest percentage of specimens with low potassium output. It would appear that these differences are not due to a persistent potassium deficit incurred during the attack of cholera, as the cases tested within one month of discharge (12 individuals) do not differ in mean potassium output from those seen at a later time (25 cases).

Discussion:

The results of the study to date indicate that mean sodium intakes in East Pakistan are high, and that cases with low urinary sodium excretion are extremely rare. The ability of the normal human kidney to conserve sodium under conditions of salt restriction is well known, so that this datum can be taken as reliable evidence that sodium deficiency is not a problem. As salt depletion has been blamed for a number of disturbances that develop in hot environments, it is of interest to note that it apparently does not occur among the indigenous inhabitants of this province.

Potassium deficiency, on the other hand, probably exists in a fraction of the population which would appear to be as high as 5% in some of the groups surveyed. As has been stated, this should be regarded as a minimum figure, as deficiency consequent to inappropriate urinary excretion would not be detected by these procedures. On the basis of the results to date, the continuation of the present survey procedure, and the initiation of appropriate additional studies to evaluate the magnitude of the potassium depletion problem appear indicated.

Study of potassium balance in the tropics may have great significance beyond its possible importance in relation to cholera. It seems quite probable that potassium deficits develop under heat stress in many geographic areas, and that this may be responsible for some of the physiologic disturbances that occur in hot climates. Ladell, Waterlow, and Hudson (1944) described a condition developing in British service personell in the Middle East that they called "heat exhaustion, type II". What is undoubtedly the same condition has been recorded since, and it is now known as tropical anhydrotic asthenia (Ladell, 1957). A relation to hypokalemia was suggested by Good (1957). Features of the condition which are consistent with the belief that it is due to potassium depletion are muscular weakness, a pitressin-resistant polyuria, and a decrease in plasma chloride levels. The condition develops slowly, and is usually seen at the end of a period of hot weather. Recovery, with rest and avoidance of heat stress, requires some weeks.

Knochel et al. (1961) have raised the question whether or not potassium deficiency might occur prior to the onset of acute symptoms in heat stroke. Disturbances of potassium balance that occur with the onset of the acute syndrome confuse the issue, just as with cholera, but the speculation remains a reasonable one. Ladell (1957) has commented on the possibility of a relationship between heat stroke and tropical anhydrotic asthenia, each of which is characterized by a decrease in sweating and by elevation of body temperature. Knowledge of the effects of heat stress on potassium metabolism is seriously deficient (Leithead, 1963).

The results of analyses of specimens from follow-up visits of former cholera patients are consistent with the hypothesis that there may be a relationship between potassium deficiency and cholera. The fact that these subjects were more prone to show low values for urinary potassium may be taken to reflect less satisfactory eating habits, greater potassium losses, or a combination. It is unlikely that the previous bout of cholera would have influenced habits of diet, occupation, or the likelihood of heat exposure. It also seems unlikely that the low potassium excretion represents the persistence of a deficit incurred during cholera, as all cases received potassium supplements during therapy, and those cases seen within a month of discharge did not have a lower mean potassium output than those seen at intervals of up to eight months.

It will be important to correlate the epidemiology of cholera with the distribution of potassium depletion, as more data become available. It may be only fortuitous that a cholera outbreak was reported from Jamalpur in September, 1963, only a few weeks after the completion of the survey showing unusually low mean urinary potassium excretion, and a high percentage of the population in the range that probably indicates depletion.

Data already at hand indicate that climate influences the incidence of cholera, with the propagation of epidemics being favored by hot and/or humid weather, especially if rainfall is poor. Snow (1855) observed that the usual cholera outbreak in Britain tended "to increase gradually during.... summer, reach its climax at the latter part of the summer, and decline somewhat rapidly as the cool days of autumn set in." Pollitzer (1959) has reviewed many epidemiological studies, and concludes that cholera epidemics occur primarily in hot seasons. In recent work, Yen (1962) has shown a correlation between heavy sweating due to heat exposure or strenuous exertion and the development of clinical cholera as opposed to the asymptomatic carrier state. It is possible that the influence of heat on cholera epidemics is exerted by way of alterations in the potassium metabolism of the potential host.

Summary:

Low values of urinary potassium excretion have been found among the people of East Pakistan. The figures indicate that potassium depletion due to an intake inadequate to compensate for extra-renal losses may be expected in up to 5% of the individuals in some groups studied. Urinary potassium losses may aggravate the situation, but cannot be detected by the procedures employed here. The group of former cholera patients showed a higher incidence of low urinary potassium values than did the general population, but it is only a supposition that potassium deficiency preceded cholera. However, the hypothesis that potassium depletion might predispose to cholera, or at least make the disease more severe if it develops, is a tenable one in view of the evidence that potassium deficiency actually occurs.

Electrolyte excretion (mEq. per gm. creatinine) in East Pakistan, 196

Number of Samples	Location	Dates of Survey	POTASSIUM				SO	
			Mean	Std. Error of Mean	% Samples 30 or less	% Samples 15 or less	Mean	Std. Error of Mean
18	Dinajpur	16-23 April	58.4	15.5	28	6	241	32
82	Comilla	16-22 May	72.5	4.1	10	1	185	13
53	Faridpur	10-15 June	89.1	8.2	4	0	263	19
64	Bogra	26-30 June	66.0	5.4	14	0	239	17
85	Pabna	3- 8 July	55.5	4.5	25	4	158	9.5
78	Jessore	25-31 July	63.0	3.9	9	1	210	17
77	Jamalpur*	23-29 Aug.	44.1	3.7	38	5	222	25
72	Kushtia	19-25 Sept.	56.9	4.3	22	6	244	30
529	Total	Apr. - Sept.	62.5	1.9	18**	2.4	214	5.7
37	Post - cholera	July - Sept.	53.2	7.9	35**	8	268	58

* Survey followed by cholera outbreak.

** Difference significant by Chi - square test (P = .01)

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A CHOLERA FOCUS IN DACCA CITY

At the outset I must admit that I am not an expert and in fact, I have not studied anything on scientific lines. But being born in an endemic zone of most of the tropical diseases I have had my due share of them which I shall not narrate here to burden you. I shall only relate what I have been able to gain in my association with the Pakistan-SEATO Cholera Research Laboratory during the last 3 or 4 months. I am grateful to all members of this great organization for offering me every facility and all kindness.

Now I presume, you accept the theory that several thousands of years ago in an epoch of earth-tilling cultures along the Brahmaputra and the Ganges rivers, social and natural conditions were suitable for the evolution of a new species of organism, the cholera vibrio. The dense settlement by cave and hut-dwelling people in this area facilated the spread of infection among human beings. How many years ago this evolution happened, no one can say. However, all records show that the vibrio cholera thrived here well and settled down comfortably. Perhaps, because of pressure of space, the vibrio spread to other areas, but did not find them congenial for its existance; as a result, we now find it confined only to its birth-place. Koch in 1885 stated in Berlin, that Bengal alone was the home of cholera, and many others have confirmed it.

According to some scientists, the germ of cholera is excreted through feces and vomit of the patients and must have an entry into the mouth of man to cause illness in him. However, the question arises why all people who partake of the same food and drink do not get the disease. I am puzzled to see and eager to know why suddenly a case of cholera comes when no evidence of the disease has been seen in surrounding areas for a long period. I believe, that nothing happens in nature by chance. Pakistan-SEATO Cholera Research Laboratory is not here by chance; neither am I here in this world by chance. If cholera appears at a place, then the vibrio comma existed somewhere nearby. If that is so, the question arises where does it exist, what are its habitats, and how does it travel?

It is postulated that cholera persists in an area where the absolute humidity is high. However, if we take the case of Dacca, we find that cholera prevails in the old city throughout the year, but almost no cholera occurs in the surrounding areas such as Ramna, though climatic factors are exactly the same.

The whole of Indo-Pak sub-continent has always been considered to be the endemic focii of cholera since human beings settled down here. However, Swaroop showed in his study of cholera mortality in India that cholera is endemic only in areas which -

1. lie along the banks of big rivers,
2. have high population density,
3. lie in low-lying areas,
4. have high absolute humidity.

These conditions prevail in many other places besides India and Pakistan. Some areas of the Indo-Pakistan sub-continent have these conditions, but have no cholera. Let us take the case of New Orleans. Cholera reached the port but did not succeed to establish itself, though factors noted by Swaroop prevailed there.

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Certainly some factors exist in New Orleans which either disfavoured the growth of vibrio outside the human body or created a barrier between the patients and the susceptibles. Perhaps such factors also play the same role in many parts of Indo-Pakistan sub-continent. Therefore, it is incorrect to think that the whole Indo-Pakistan sub-continent is the cholera focii irrespective of incidence of the disease. Kamal elaborated the findings of Swaroop by stating that main characteristics of endemic areas are low lying land studded with as many pools, tanks and ditches, for supply of water. While I agree that these conditions are not necessary for prevalence of cholera in an area, I fail to understand how cholera vibrios can persist in areas if the fundamental necessities of animal life, particularly nutrients are available to them.

I am, therefore, inclined to believe that additional factors governing endemicity of cholera in any locality are the following :

1. That the vibrio cholera exists in the locality

2. That the physical environmental conditions are such that they favour growth of vibrio outside the body.

3. That such socio-economic factors exist in the community for easy transmission of the vibrios.

For survival and growth of vibrio environmental conditions must be, as with other living beings, of the same nature under which the vibrio was born. Any variation in these conditions leads to transformation and to death. Conditions noted by Swaroop and Kamal, are of course, essential, but cannot provide requisite nutrient for the growth of vibrio. I believe that only the human excreta can provide this kind of food. Like other living organisms, however, vibrio does also try to survive for sometime under unfavourable circumstances. Again human excreta alone will not be enough. Moisture is necessary to liquify the nutrient and perhaps for motility of the vibrio. The natural water of rivers, tanks, canals, and ditches provides moisture. The waste water from houses and latrines is a source of moisture, in the city. Dumping of refuse further helps to provide nutrient to the vibrio by decomposition of organic substances. For persistence of vibrio in any place, I believe that the following conditions should also be prevalent:

a. the area should be a dumping place for human excreta;
b. the soil should receive sufficient moisture, preferably from urinals;
c. the area may also be a dumping place for household refuse.

Again for the endemicity of cholera in a locality the socio-economic environment should be such that the vibrio can easily get entry into the mouth of healthy persons from the sick or their physical environment. Not only high density of population but also social customs, food habits, and ignorance about health and hygiene, influence the spread of cholera.

1. <u>Vibrio cholera</u>:

Does vibrio cholera exist in Dacca city? To answer this question I have only to refer you to the records of the Infectious Ward of the Mitford Hospital

continued.....

Dacca. These records reveal that cholera prevailed in the city every year, every month, and may I say, every day of the year without break (Table I).

TABLE I

Year	Attack	Death	Percentage of death
1953	364	21	5.7%
1954	451	47	10.4%
1955	496	93	18.7%
1956	291	105	36.1%
1957	693	129	18.6%
1958	667	61	9.0%
1959	940	148	15.7%
1960	868	157	23.5%
1961	481	75	15.6%
1962	4	54	?

Table I also shows that the number of cholera cases steadily increased until 1959, perhaps with the gradual increase of population. From 1960, the attack rate began to decline. Then another disease appeared in the field, gastro-enterities which together with cholera, maintained the usual increase, as may be seen from Table II. With the introduction of Martial Law in the country, the Royal Bengal Tiger suddenly changed itself into a timid East Pakistani lamb. Never the less, the people suffered as usual and in increased numbers. The demon of death was not afraid of Martial Law and was as active as ever and took its usual toll of lives. In 1962, though only 4 cases of cholera appeared on admission records, not less than 54 deaths were certified as cholera.

TABLE II

	Cholera		Gastro-enteritis		Cholera and Gastro-enteritis combined	
Year	Attack	Death	Attack	Death		
1953	364	21	-	-	364	21
1954	451	47	-	-	451	47
1955	496	93	- -	-	496	93
1956	291	105	-	-	291	105
1957	693	129	-	-	693	129
1958	667	61	-	-	667	61
1959	940	148	266	8	1206	156
1960	668	157	412	10	1060	167
1961	481	75	792	25	1273	100
1962	4	54	219	26	223	80

Admittedly, differentiating a case of cholera from a case of acute diarrhea is difficult without bacteriological examination. The hospital records show that another series of cases diagnosed as acute diarrhea were admitted in the infectious ward in increased number during the same period. The death rate of these

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cases was high enough to suggest that they were actually cholera instead of acute diarrhea. Including these cases with the combined cholera and gastro-entritis figures, gives better indication of the probable attack rate of cholera, as shown in Table III.

TABLE III

Cholera			Gastro-enteritis		Diarrhea		Grand Total	
Year	Attack	Death	Attack	Death	Attack	Death	Attack	Death
1953	364	21	-	-	51	3	416	24
1954	451	47	-	-	189	14	640	61
1955	496	93	-	-	297	9	793	102
1956	291	105	-	-	305	10	596	115
1957	693	129	-	-	427	19	1120	148
1958	667	61	-	-	234	11	901	72
1959	940	148	266	8	360	14	1466	170
1960	668	157	412	10	496	22	1576	189
1961	481	75	792	25	512	25	1785	125
1962	4	54	219	26	1683	106	2225	186

These figures are not collected by any hired agents. Patients themselves reported to the hospital for relief. Unlike advanced countries, persons do not come to the hospital for a medical check-up, especially not for diarrheal disease. Only those people come to the hospital who have not the means to call in a private physician or have not any shelter of their own. These cases which come perhaps represent only a small percentage of the total cases of diarrhea.

The number of cases suffering from diarrheal diseases has been steadily increasing in Dacca. Though the population of the city has only doubled itself during the last ten years the number of diarrheal cases has increased more than 5 times. The distribution of cases of cholera (1959) and diarrheal diseases (1962) admitted in Mitford Hospital shows distinct peaks in April-May and again in October-November (Table IV).

TABLE IV

		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
Cholera	1959	23	33	35	89	60	21	9	13	25	539	123	54	940
Diarrhea	1962	86	72	172	225	208	154	128	88	126	111	150	381	1683

These peaks vary slightly from year to year. Many of these patients came from the outside of the city causing hospital admission to rise. Some cases admitted

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as cholera were probably not genuine cases of cholera. Dr. Stockard's figures show that at least 70% were caused by vibrio cholera.

II. Physical environment

Though correct statistics are not available about the day-to-day temperature, humidity, rainfall, and wind-movement, all the climatic factors which favour growth of vibrio are prevalent here throughout the year. In fact, bacteria thrive here under natural conditions, and no incubator is necessary to cultivate them. Further, a visit to any area of old Dacca city will convince anyone that nutrient necessary for the growth of vibrio is scattered about in enormous quantity.

Primitive service latrines are still in use, and excreta over flows the receiver to the nearest drain, tank, or ditch. Waste water from the houses, urinals, and latrines, freely drains into these ditches and pools and stagnates there. House and street refuse are dumped into any available vacant land. An examination of the water from the dugwells, ditches, and urinals shows that they are most suitable for the growth of vibrio.

III. Socio-economic environment

Presence of vibrio in a locality many not cause the area to be endemic unless suitable communication exists between vibrio, the diseased, or his environment, and healthy persons. In Lutfar Rahman Lane, which was closely studied by sociologists of epidemiology section, the residents of the area belong to a group among whom infection can be transmitted easily. Most of them are very poor and live huddled together in small sheds. A high percentage are illiterate and have no knowledge of health and hygiene. Many cook their own food, but some take their meals in any roadside eating place. Sweets, ice cream, and other prepared foods are always exposed to dust and flies. Children indulge in these foods without restriction. Drinking water is obtained from any nearby source. I do not like to mention here the findings of Abou Garib, who traced the spread of infection of cholera from Nakhoda mosque in Calcutta. All known methods of transmission of cholera are prevalent in the old City.

During the period from 27 October 1962 to the 30 July, 1963, an intensive study of a series of 67 affected households in Dacca City was made by the Epidemiology section under direct supervision of Dr. Stockard. Of these 67 households, only two were situated in the newly built area of Dacca; the rest were situated in the old city. Of three cases which occurred in the new area, 2 were positive and 1 negative. In the 15 households of the old city, no cases positive were found. In the remaining 50 households there were 103 cases of which 90 were positive and 13 were negative. The rate of confirmed cases was 87.0%. (Table V). There were 27 Ogawa asymptomatic and 11 NAG asymptomatic cases.

The population of the new area is about 188137 against 362006 of the old city. Sporadic cases occur all over the area and throughout the year, but some of the areas are more affected than others. Lutfar Rahman Lane of Siddique Bazar Union is one of the worst areas of the city. Most of the houses are kutcha huts of one room with hardly any provision for ventilation and sunlight. Drinking water is obtained from street hydrants and is always in short supply, so water is also taken from tanks and dugwells. Some houses have service latrines which are hopelessly inadequate. All conditions favourable for prevalence of cholera throughout the year are present.

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TABLE V

STUDY OF HOUSEHOLDS IN DACCA

October 1963 to July 1963

	No. of households	Total case	Inaba	Ogawa	NAG	Neg.	N.C. diarrhea	Percent positive case	Asymptomatic carriers	NAGasymptomatic carriers
Old Area										
Positive households	49	103	56	34	-	13	-	87	24	10
Negative households	16	19	-	-	5	13	1	-	3	1
Sub Total	65	122	56	34	5	26	1	73.7	27	11
New Area										
Positive households	2	3	2	-	-	1	-	-	-	-
Total	67	125	58	34	5	27	1	73.6	27	11

In a recent outbreak of cholera in this area, an attempt was made under supervision of Dr. Islam of the Epidemiology Section to follow up cases of diarrhea until they proved to be negative. The study continued from 20 June to 3 October, during a period when the incidence of diarrheal diseases is at its lowest in the city. 20 households with 74 families consisting of 419 persons were included in the study. Among the members of these households, 43 cases of cholera were suspected, and 30 proved positive (7% of all family members). One case, probably of cholera, died before the study began. In this group of houses, another 20 persons were detected as asymptomatic carriers of vibrios: 13 Ogawa and 1 Inaba. Thus, 3.3% wereasymptomatic carriers of cholera vibrios.

To determine the prevalence of vibrios among this population, a diarrheal disease clinic was operated in this area by Dr. Rahman of the Epidemiology Section. During the period 11 July to 30 August, 557 persons sought help. Of these, 183 gave complaints of diarrhea; 74.8% were under age 10. 374 patients complained of fever and other non-diarrheal symptoms; only 52.6% of this group were under 10. In 74 persons, the clinical manifestations were considered compatible with a possible diagnosis of cholera, and rectal swabs were taken for vibrios. Three were positive for Ogawa V.cholerae; these three (all under age 10) later developed full-blown symptoms of cholera. From 5 to 13 September, all who reported to the clinic were cultured for enteric pathogens. 71 individuals were examined with the recovery of only two true pathogens; S. typhi was recovered from one 7-year-old girl with high fever and Shigella flexneri from a 2-year-old boy with diarrhea. No cholera vibrios were isolated; Heiberg type II non-agglutinable vibrios were recovered from a 6-month-old male with

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diarrhea. 54 individuals were studied for the presence of entropathogenic E. coli; five strains were isolated from 4 individuals of types 026, 055 and 0111. Only one of these patients complained of diarrhea, this was the child who was also infected with Shigella flexneri. Although this study did not cover enough people to measure the cholera vibrio rates in the community at large, the concentration of symptoms of diarrhea and of the few positive bacteriological findings in those under 10 years of age is significant. The low rate of isolation of pathogens is surprising in view of the sanitary conditions which pertained.

ENVIRONMENTAL SANITATION STUDIES

Because cholera is usually associated with low socio-economic groups and poor sanitation, study of the environment as well as of the water sources of endemic areas is imperative. An investigation was made of the presence and persistence of cholera organisms in the environment of areas where water surveillance was also maintained.

The occurrence of cholera in epidemic form in a section of Dacca's old town provided opportunity to study some environmental factors related to the disease. This locality, Lutfar Rahman Lane, includes both slum and hygienic surroundings. The lane is situated along famous Dolaikhal Canal, which previously served as the main flood drainage for old Dacca. Urban development has caused partial filling of Dolaikhal, blocking the natural washway of refuse and filth. The locality's population is composed of both educated persons, accustomed to modern ways of living, and illiterate ones, having little concern for modern hygienic practices. Some of the dwellings have sanitary facilities; others have none. The common water source is shallow, open dugwells, used by all the populace, irrespective of their mode of living.

The official source of drinking water is the municipal supply, but this is inadequate to meet the inhabitant's needs. Hence, dugwells which are used extensively for all purposes except possibly drinking, supplement the inadequate official supply. Tubewells, serving as emergency sources for drinking water, are very few and usually are inactive because of lack of maintenance. Even the municipal supply is not available directly in the household; instead water is carried into the house from communal fawcets.

The sewage disposal system is principally the old fashioned service latrines. These latrines are inadequately attended; as a result the faeces usually overflow the collection pots and are washed away into a nearby ditch or tank. The sewer pipe lines which were laid a few years ago do not serve their purpose. Few households are connected to these pipes; moreover, connections that are made are water seal latrines without a flushing arrangement. As a result, the faecal matter remains in the pipe lines. Further, the dumping of faeces collected from service latrines into the nearby manholes blocks the sewer lines. An additional complicating factor occurs when the ground water rises above the level of the sewer , flooding the lines and eliminating any gradient for flow. Drainage of wash and refuse water is also inadequate. The main natural drain (Dolaikhal) being blocked, the smaller drains remain water logged all the time.

Refuse Disposal:

Arrangements for refuse disposal are inadequate. The few dustbins supplied by the Municipality remain unattended most of the time; garbage and filth are spread all around by fowl and household animals, creating unhygienic surroundings and good breeding places for insects, particularly those, which are instrumental in spread of disease. Because disease was found in households with dugwells showing positivity for cholera vibrio, the possibility of a channel between sewer lines and dugwells was

investigated. At the time of study, dugwells had a high water level, only 4 or 5 feet from the surface. In addition, the wells were close to the latrines (average distance 8 to 10 feet). A survey of the sewer lines was conducted (see appendix 1). The sewer lines of the affected area were found to be blocked because new connections were being made. This situation made the earth layer soft and permeable. The waste inside the pipelines naturally tried to find cracks in the soil layer; with little distance between the dug wells and the pipeline, the sewage may have entered the dugwells.

This assumption was justified by the finding that V. cholerae was isolated from 9 of 12 dugwells which were contiguous to blocked sewer lines. However, in an area of unblocked sewer line, only 3 out of 10 dugwells were positive for the vibrio.

To verify this apparent relationship, fluorescein solution in .1% concentration was poured into the latrines of the affected households which were connected to the sewer line. Well waters were tested for fluorescence with ultra violet light before and after pouring the solution. Positive indications of fluorescence were observed in most of the dug wells.

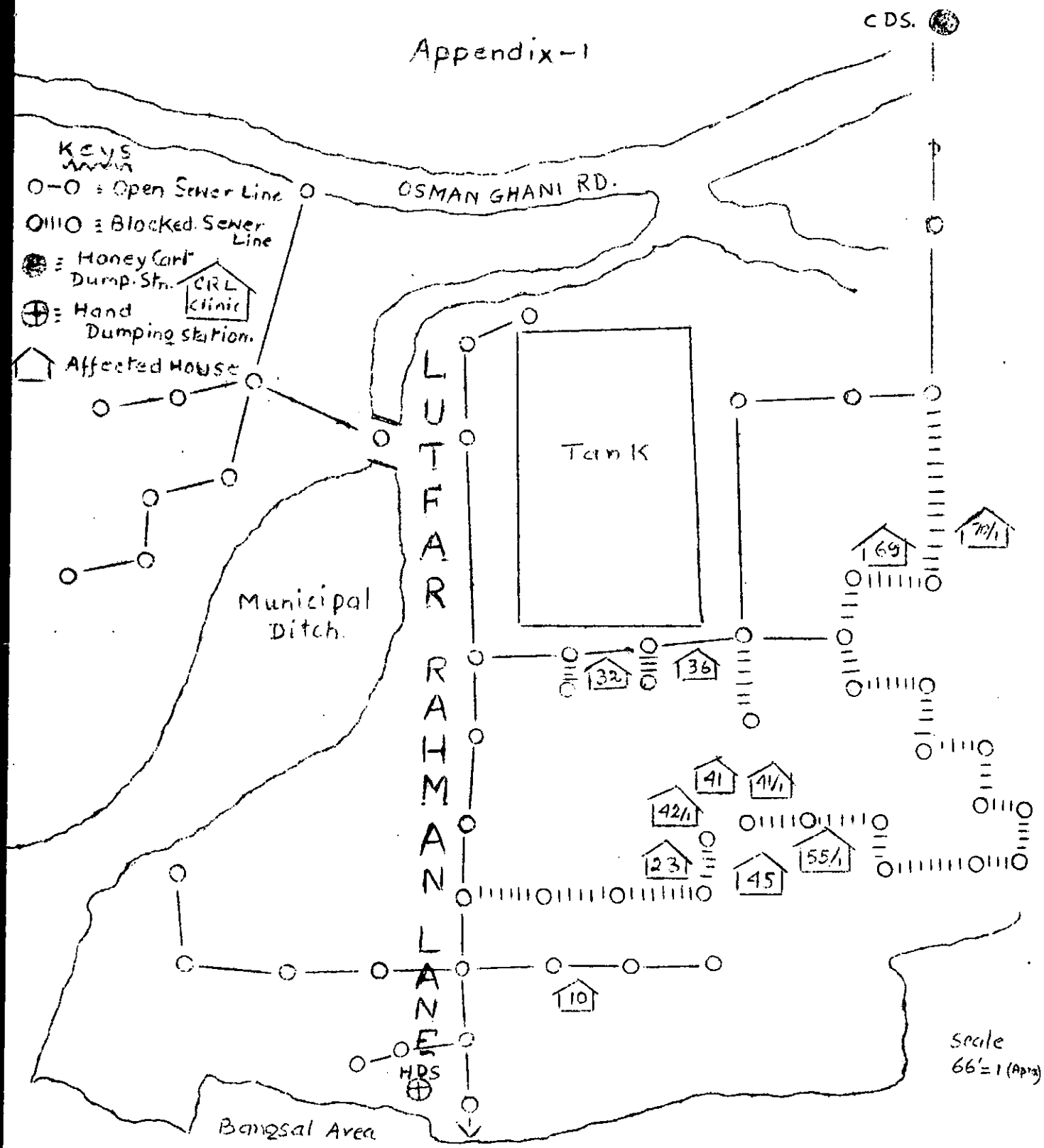
The chemical and bacteriological test results (Table I) also gave evidence of faecal pollution in these dugwells. High incidence of E. coli, high nitrates, and chlorides indicated entry of sewage into the dugwells.

Summary and Conclusions:

Poor environment sanitation has been found to prevail in an area of old Dacca in which an outbreak of cholera occurred. The especially dangerous situation was the proximity of dug wells to latrines and blocked sewer lines, which gave rise to a serious degree of fecal contamination of the well water.

TABLE I

Address	CRL Epid. No.	Latrine Type	Approx.Dist of Well from Latrine	Duration of Positive V.cholerae isolation		E.coli/100 ml. in Dug Well	pH	Cl^- P.P.M.	NO_3^- P.P.M.
				from Inhabitants	from Dug Well				
41/1 Lutfar R. Lane	H.A. 147	Service	5 ft.	6 days	3 days	10^5	7.1	229	71
41 " " "	H.A. 148	Sewer	6 ft.	1 day	9 days	4.6×10^4	6.7	211	160
55/1 " " "	H.A. 156	Sewer	8 ft.	6 days	1 day	2.4×10^4	6.8	220	239
42/1 " " "	H.A. 157	Service	5 ft.	1 day	7 days	1.5×10^4	6.8	315	108
23 " " "	H.A. 160	Service	25-30 ft.	7 days	2 days	2.4×10^4	6.8	200	142
69 " " "	H.A. 161	Sewer	10 ft.	8 days	4 days	4.6×10^5	6.8	310	133
32 " " "	H.A. 162	Sewer	6 ft.	1 day	1 day	10^5	6.8	124	97
109 " " "	H.A. 163	Service	8-10 ft.	1 day	-	2.3×10^3	6.8	190	155
139 " " "	H.A. 164	Service	8-10 ft.	1 day	-	$>10^5$	6.9	220	4.0
45 " " "	H.A. 165	Service	8-10 ft.	1 day	-	$>10^5$	6.8	530	566
36 " " "	H.A. 166	Service	10 ft.	1 day	-	$>10^5$	6.7	210	168
95 " " "	None	Service	10 ft.	3 days	3 days	2.4×10^4	6.4	170	151


Appendix-1
C.D.S.
Keys
O-O : Open Sewer Line
OIIIO : Blocked Sewer Line
: Honey Cart Dump. Stn.
: Hand Dumping station.
Affected House
CRL Clinic
OSMAN GHANI RD.
LUTFAR RAHMAN LANE
Tank
Municipal Ditch
69
70/1
32
36
41
41/1
42/1
23
145
55/1
10
HDS
Bangsal Area
Scale 66' = 1 (Apx)

Sera were obtained from ward patients on admission and, so far as possible periodically and on discharge. These were stored in the deep freeze and all sera from a patient were tested at the same time for the presence of agglutinating antibodies against living Inaba and Ogawa antigens using Dr. Goodner's methods. Most of the sera were also tested with NCV 696 and, when a NCV was isolated, the homologous organism. In the beginning, each serum was tested with three different Ogawa and three Inaba strains. Since no significant difference in titers was observed, the test battery was reduced to the standard strains Ogawa 465 and Inaba 466. After an exchange of strains with Dr. Goodner, we added Inaba J89, which produces a more clean-cut agglutination, usually one tube higher than 466. Titers we obtain are several dilutions lower than those obtained by Dr. Feeley; we have just received, but have not yet tested, the strains he uses - Ogawa VC12 and Inaba VC13.

TABLE I

	Vibrio Isolation			
	Positive	Inaba	Ogawa	None or NCV
Total number	106	92	14	50
4 fold or greater rise	88	76	12	2
% Positive	83%	82.6%	85.7%	4.0%
No. with Inaba titer equal to Ogawa titer		36	7	
Inaba > Ogawa		37	1	
Ogawa > Inaba		3	4	

Serological Diagnostic Results on C.R.L. Cases with Adequate Sera

In the first 255 admissions to the C.R.L. ward, sera adequate for serological interpretation were obtained from 156 patients. 106 of these were proven by bacterial isolation to be cholera - 83% of these showed a fourfold rise in titer (Table 1). In roughly half, the titer against the infecting organism is slightly higher; this is more frequently noted when the antibodies first begin to appear.

59 bacteriologically confirmed cases had no antibody detectable at a 1:40 dilution early in the disease; 55 of these (93%) developed antibodies at a level of 1:80 or higher. As can be seen in Table 2, the response begins on the 4th day and, in this group, all have developed a titer of 80 or more by the 7th day. The highest geometric mean titer in this group is found in the 8-10th day period, but the cases are too few for this to be reliable. There is a fall-off in the few cases tested more than one month after onset; two have dropped below the 1:40 level.

No effect of the administration of antibiotic can be demonstrated on the serological response. Among those with adequate sera for diagnosis, 31 had been treated with tetracycline intravenously and orally, and 58 had not. A significant

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rise in titer occurred in 81 and 84%, respectively. Comparing the mean antibody levels on the 8th to 15th days after onset of those with an earlier negative serum, there was no significant difference between those who had or had not received antibiotic.

In some cases, agglutinating antibody was present during the first three days after onset of disease and is considered to have existed before infection. In 35% of 92 proven cholera patients with early sera, antibodies were present at levels up to 4 tubes (1:320). No influence of these antibodies was evident on the severity of the clinical disease, judging by mean stool volume in the first 24 hours.

TABLE 2

Tubes Positive	Titer	Titers on days after onset in 55 serologically positive proven cholera patients with no pre-existing antibodies										
8	5120								1	1		
7	2560								3	2	1	
6	1280							1	7	1	1	
5	640					1	2	3	4	4	–	
4	320					–	1	3	7	7	2	
3	160					1	3	2	3	3	2	3
2	80				1	–	3	1	–	–	1	1
1	40				1	1	1	–	–	1	–	1
0	40	18	13	19	12	2	2	–	–	–	–	2
Geom. Mean Tubes		0	0	0	0.2	1.8	2.5	4.1	5.1	4.7	4.1	1.7
		1	2	3	4	5	6	7	8–10	11–15	16–30	30

Days after onset of illness

The source of these antibodies is not clear. Only 5% of our cholera patients and 9% of the non cholera patients claim to have had prior attacks of cholera. 34% of patients who deny cholera immunization have pre-existing titers. These are found in both the cholera patients and the non-cholera patients (30% vs 36%). By contrast, among those who do report prior cholera vaccine, 46% have antibody on admission.

All vaccine histories obtained from the patients admitted to the C.R.L. ward are presented in Table 3.

There is a highly significant (chi square 5.96) difference between cholera and non-cholera groups in the percentage having received vaccine 10 days to 6 months before illness.

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TABLE 3

VACCINATION HISTORY OF CHOLERA AND NON-CHOLERA CRL PATIENTS

	Vibrio Isolations			
	Cholera		NCV + Negative	
	No.	%	No.	%
No vaccine	117	58.5	85	53.5
Less than 10 days	15	7.5	4	2.5
10 days to 6 months	23	11.5	37	23.3
6 months to 2 years	36	18.0	28	17.6
Over 2 years	10	5.0	5	3.1
History of prior cholera	(11)	5.5	(15)	9.4
Total	201		159	

An effect of vaccine on the frequency of pre-existing titers can be seen in comparison of the findings in cholera and non-cholera patients. (Table 4).

TABLE 4

PRE-EXISTING TITERS IN CRL CHOLERA AND NON-CHOLERA PATIENTS

	Vibrio isolations					
	Cholera		NCV or Nonc		Total	
History of vaccine	13/39	(33%)	20/33	(60%)	33/72	(46%)
No history of vaccine	19/53	(36%)	7/23	(30%)	26/76	(34%)
Total	32/92	(35%)	27/56	(48%)	59/148	(40%)

Although the history of vaccination is not significantly different among those with cholera, it can be claimed that the non-infected group reflect the protection of the vaccine. However, it may as well reflect attributes of the individual which makes them accept or seek immunization and which also brings them into a cholera hospital when they develop diarrhea. No difference in severity of the clinical disease, as judged by stool volume in the prior 24 hours, can be related to history of vaccination.

Animal studies:

The dearth of locally available rabbits, and the much better supply of monkeys (and their relatively low cost) suggested their use as a possible model for the study of immunity in cholera. Review of the literature offered two very old and promising reports, which are appended in translation from the original Spanish and French. Both these reports suggest that oral administration of vibrios results in a clinical symdrome similar to that seen in man, and that "weak doses" (1/5 to 1/10 of a Petri dish) would result in mild diarrhea with subsequent immunity to "strong doses" ("thecontent of a 9 cm Petri dish").

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Preliminary studies have been conducted using the methods described by these authors. Our experience in reproducing the typical clinical disease is unfortunately not as successful. Only four of thirty-two monkeys challenged developed the fatal disease described even after the administration of challenges as high as 5×10^{11} organisms by stomach tube. Recently isolated strains with as few as two passages on artifical media were used. A two-passage strain isolated from one of the fatal monkey exposures was no more successful, and rice water stool directly from cholera patients which contained 4.5×10^{8} Ogawa organisms, had no perceptible effect.

The oral administration of vibrios does, however, result in a transient intestinal infection as evidenced by bacteriologically positive stools continuing for two to seven days. For the first two days, the administered organisms are so prevalent that a rectal swab streak spread by loop over the surface, results in vibrio colonies throughout the streaked areas. Usually, a second dose of vibrios resulted in fewer days of positivity and few organisms on the plate. An Inaba challenge to nine monkeys following an Ogawa exposure resulted in an average of one day less of vibrio passage. One monkey was never bacteriologically positive, but, by way of contrast, one died from this second exposure. A subsequent third exposure with Inaba for the second time failed to infect three of five monkeys. Antibody response to these exposures was generally poor; success or failure cannot be related to the humoral titer at the time of challenge (see monkey 29). Some typical protocols are shown in Table 5. Monkeys 7 and 8 represent the more usual finding, monkey 29 represents the exceptional finding.

TABLE 5

Effect on Stool Positivity of Repeated Exposure of Monkeys to Oral Cholera Challenge

Monkey No.	Exposure No.	Date	Challenge	Titer Og	Titer In	Rectal swab Results by day 1	2	3	4	5	6	7
7	1	28 June	Og 4.5×10^{9}	0	0	++++	+++	+	+	0	0	0
	2	12 July	In 7.5×10^{11}	2	0	++	+	++	0	0	0	0
	3	25 July	In 9×10^{11}	3	1	0	0	0	0	0	0	0
8	1	28 June	Og 4.5×10^{9}	0	0	++++	+++	+	+	0	0	0
	2	12 July	In 7.5×10^{11}	0	0	+	+	0	0	0	0	0
	3	25 July	In 9×10^{11}	0	0	0	0	0	0	0	0	0
	4	14 Aug.	In 3×10^{9}	0	0	0	0	0	0	0	0	0
29	1	29 July	In "2 Petri Dishes"	0	1	+	0	0	0	0	0	0
	2	31 July	Og 8×10^{7}	0	1	0	0	+	+	+	+	0
	3	19 Aug.	In 6.5×10^{9}	4	5	0	+++	+	+	0	0	-

That these are not reflections of inadequate challenge is shown by the results of one exposure, that of 25 July, in which 9×10^{11} Inaba organisms were administered by stomach tube. (Table 6).

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STOOL BACTERIOLOGY AFTER ORAL CHOLERA CHALLENGE
INABA 9×10^{11} ORGANISMS BY STOMACH TUBE 25 JULY 1963

Monkey No.	Prior Exposures	Titer Og	Titer In	Rectal swab results by day 1	2	3	4	5	6	7	8
31	None	1	0	++++	++++	+++	+	+	+	0	0
32	None	2	0	++++	++++	+++	+	+	+	-	0
24	1	2	2	0	0	0	0	0	0	0	0
7	2	3	1	0	0	0	0	0	0	0	0
8	2	0	0	0	0	0	0	0	0	0	0
9	2	2	0	0	0	0	0	0	0	0	0
15	3	-	-	0	0	0	0	0	0	0	0

In summary:

Serological confirmation of cholera can be expected in over 80% of cases; in only two out of fifty bacteriologically negative cases is there a significant rise in antibodies. Pre-existing antibodies are demonstrable at a 1:40 or higher level in 40% of cases admitted to the CRL ward for diarrhea, and in 35% of the confirmed cholera cases. 41.5% and 46.5% of cholera and non-cholera cases give a history of prior vaccination, respectively; indeed, 5.5% of cholera patients and 9.4% of non-cholera patients claim to have had the disease of cholera in past.

Significantly, more non-cholera than cholera patients claim to have received cholera vaccine more than 10 days and less than 6 months before onset; serological confirmation of this claim is only seen among the non-cholera patients. No relation can be established between prior immunization or presence of antibodies at the onset and severity of disease. Antibiotic therapy has no evident effect on antibody response.

Preliminary work on monkeys suggests that this species may offer a satisfactory model for the study of the factors involved in intestinal infection.

Note about Experimental Cholera in the Monkey

by A. Mendoza

(Bulletin of the National Institute of Hygiene of Alfonso XIII. Madrid 30 June 1913. Vol. 9 number 34 pp 131-133.)

The work which Messrs. Pottevin and Violle have just published, giving an account of the production of experimental cholera, which they have accomplished in Cynomologus and Rhesus monkeys, whose work is clearly summarized in the Bulletin of the International Office of Public Hygiene (T. V., number 8), obliges me to record that as early as 1886 I had used the monkey for the study of the specificity of the cholera germ, and had succeeded in reproducing in them a fatal disease, accompanied by symptoms of algidity very similar to those of man.

As a result of the publication of the study in a professional journal which did not survive long, very few authors have been aware of it, and I wish to give here a summary of the principal result.

The above mentioned periodical was called International Review of Medical Sciences, and the work was published in the third number, toward the beginning of 1886.

In that work we were concerned also with the morphology of the vibrio, and were rebutting the arthrospore theory of Hueppe; but here we wish only to describe the part relative to experimental cholera in monkeys.

In 1885 and 86, in the period when Dr. R. Koch, after a peritoneal injection of alcoholic tincture of opium, infected guinea pigs by the gastric route, using a tube; in which Nicati and Riestch did the same, by means of laparotomy and injection through the bile ducts; in which Doyen, believing that in the technique originated by Dr. Koch, the alcohol of the tincture of opium was the active agent, used as a substitute intraperitoneal injection of alcohol, we were of the opinion that the experimental manipulations were excessive and were being carried out in animals very different from man. Finding ourself in possession of ten monkeys (Cercopithecus), we began a series of experiments by the gastric route, avoiding manupulations which could obscure or complicate the results and their interpretation.

Following this criterion, we proceeded as follows:

In infections by the gastric route, we began by giving the monkeys bicarbonate water ad lib to drink (sterilized water 100 grams; sodium bicarbonate 3 grams) one or two days before the infection. Subsequently we prepared a 24 hr. culture in broth, of which we administered 8 or 10 cubic centimeters by means of a gastric tube. Afterwards, we left the animal on ordinary diet (cooked potatoes, bread, etc.) and continued the use of the alkaline water.

The monkeys became ill, presenting at the end of 12 or 24 hours, clearly defined symptoms. First hypothermia was evident, temperature dropping from

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the normal (39°) to 36°, and subsequently, progressively, to 35° or 34° at the moment of death. In addition, aphonia was noted very early, with a hoarse and weak cry; flexed position of all the limbs, cyanosis manifest in all mucous membranes, sunken eyes, hair standing on end, and complete passivity. The duration of this state was approximately 60 to 72 hours.

At autopsy, the picture was as follows: The peritoneum was greasy and identical in aspect to that seen in human cases dead of cholera; the small intestine had extensive capillary hyperaemia, the extent increasing from above downward; that is to say pink in the duodenal segment, more reddish in the jejunum, becoming violet in the ileum and above all in the vicinity of the ileo-caecal valve. This appearance of the intestine was the same as that seen in the cases of human cholera. The colon was distended with content very similar to rice water stool, although somewhat less fluid.

The content of the small intestine was liquid in consistency, whitish in colour, with particles which, examined in the microscope, were seen to be formed of food residues, desquamated epithelial cells, and an almost pure culture of vibrios, typical in all their characteristics, and whose vitality we tested many times by direct examination as well as by cultures and by experimentation in guinea pigs.

The content of the large intestine showed in the same way the presence of the cholera vibrio of equal vitality.

In another series of monkeys we carried out experiments with subcutaneous infection with cultures of the vibrio, grown in broth for 24 hours. We injected as much 200 cubic centimeters without being able to produce serious symptoms of intoxication or death.

It may be seen, therefore, by these experiments, that we, in the year 1886, succeeded in reproducing in monkeys the human disease cholera, without resorting to any sort of vigorous preparatory operation, merely allowing the specific organism to carry out in the intestine its own pathogenic functions.

Translation by Dr. R. Gordon
29 July, 1963.

Experimental Cholera in Lower Monkeys

H. Pottevin and H. Violle, introduced by M. Roux, Comptes rendus de l 'Academia des Sciences, (Paris). Volume 157, page 343, (1913).

A number of attempts have been made in various places to infect lower monkeys with cholera, by absorption of virulent products, without any positive result so far. These attempts have been continued because of the obvious value, from the point of view of development of research on therapy, vaccination, and prophylaxis of cholera, of being able to produce in the monkey an illness which is initiated and evolves under the same conditions as the human disease.

Recently we announced (1) that by oral infection with cultures of a toxigenic cholera vibrio, we produced an experimental cholera in monkeys (Cynomolgus and Rhesus) the characteristics of which, clinical and pathologic, correspond exactly to those of human cholera. New experiments have permitted us to extend and confirm our earlier results.

We have learned that the animals may be made receptive by administering to them a dose of sodium sulphate capable of producing in three or four hours a vigorous purgative effect: in general 7-8 grams of salt was sufficient, but in some cases it is necessary to repeat the dose to a total of 12 grams or 15 grams. Larger doses, 20 grams and more, leave the animals without ill effects after the purgative effect is over.

After the diarrhea was clearly established, we administered to the monkeys by stomach tube a certain quantity of a 24 hour agar culture, suspended in 20 cc of a broth culture of the same age.

When the dose of culture administered was as great as the content of a 9 centimetre Petri dish, the animals always died after a delay of 18-48 hours. In this case, there is intoxication by preformed toxins rather than true infection, because one obtains essentially the same results using cultures killed by ether. With lower doses of living culture (one fifth or even one tenth of a Petri dish) death always occurs, but after an appreciably longer illness, from 2 to 4 days. If the dose is further reduced, by substituting fresh broth for the 24 hour culture, one can obtain an infection which kills only a few of the animals inoculated.

In all our experiments we have used, as the infecting strain, the Italian vibrio previously described by one of us (2). Preliminary experiments with even much greater doses of another strain of cholera vibrio, which was not toxigenic remained negative.

We have produced fatal cholera using, as infectious material, a very small quantity of intestinal content of an animal which has just died. We intend to study the alterations which may occur in the cholera vibrio by uninterrupted passage from animal to animal. At this time, we may say that a first passage certainly has not reduced its virulence.

When the illness is established, hypothermia develops early; the yellowish diarrhea, due to the action of the purgative, is replaced little by little by

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watery stools, whitish, which present (particularly those which one finds in autopsy in the intestine), with surprising accuracy, the classic aspect of rice water stools of human cholera. Preparations made from the mucus flakes show vibrios in abundance, in their characteristic arrangement as a "school of fish". At autopsy, one finds the large intestine distended, and in the small intestine the usual lesions of cholera. Cultures demonstate: with the intestinal content, a strongly positive culture of vibrios identical with the infecting dose; with heart's blood, negative culture. Blood suspended in physiologic saline and kept at 37° for four hours shows no hemolysis.

The problem of vaccination against cholera is surely the most important issue in cholera prophylaxis. We have instituted a series of experiments to define the results. These experiments, which should reproduce as closely as possible the conditions of practise, will require considerable time, but we can already indicate some results.

The animals easily tolerate subcutaneous and intravenous injections of live cultures.

An antitoxic and bacteriolytic serum of high activity, prepared with the same vibrio which we use as an infecting culture, has been shown to have protective value. One monkey which had received 20 cc of serum subcutaneously, and was infected 5 days later with a strong dose (one Petri dish full in 20 cc of broth culture), became ill but survived. A second animal, which had received 10 cc of serum subcutaneously at the same time as a weaker infecting dose, (one fifth of a Petri dish in fresh broth) died after three days.

A first attack of the illness confers immunity. Four monkeys which, having received weak doses of culture, had had only mild cholera, survived a challenge given ten days later, with strong doses which invariably killed new animals.

(1) Societe de Pathologie exotique, 9 July 1913.

(2) Pottevin, Toxine et antitoxine choleriques (Comptes rendus, 26 mai 1913)

Translated by

Dr. R. S. Gordon, Jr.
Mrs. D. Dunham

3 July, 1963

VACCINE STUDIES OF MARCH - APRIL, 1963

Vaccine:

A pilot vaccine trial was carried out in the 3 communities (Bandar, Mudafa, and Kaliganj) which had been under study by the Epidemiology Section, and discussed last year. Each house in the three communities had been identified and given a number, and each individual within the house identified by age, name and sex. This information was recorded on a census family form. The family forms were arranged in order and each member of the community assigned sequential numbers. Those given even numbers were to receive cholera vaccine and the odd numbers the control vaccine.

The cholera vaccines used were American products. The typhoid vaccine was prepared for us by the Institute of Public Health in Dacca. Because of the difference in shape of the 2 bottles it was obvious to the operating personnel which was which; however, the labels were removed so that this information would not be emphasized. The vaccines were administered by jet injectors, one being used for the even numbered individuals and one for the uneven. The numbering was accomplished by marking the forearm with the assigned number in the house. At the vaccination site (this was moved frequently), 0.25 ml of cholera vaccine was given to those under 2 years of age, 0.5 ml to those from 2 to 13 years of age, and 1 ml to those over 13. The typhoid vaccine was used initially in a similar 0.5 ml dose for all but because of the rather marked reactions in smaller children, those under ten years of age were later given only 0.1 c.c.

Results:

55% of the population in these three communities were inoculated despite the fact that no preliminary education program was undertaken. In the rural community, 72% of the total population were immunized, and in the two semi-urban communities the acceptance rate was 51 and 49%, respectively. It is to be noted that females accepted vaccine under the conditions of this study more satisfactorily than males. The poorest response was found in males over 20 years of age. The table below shows the age and sex characteristics of the vaccinated and non-vaccinated populations:

AGE AND SEX DISTRIBUTION OF VACCINATED AND UNVACCINATED POPULATIONSIN MUDAFA AND KALIGANJ

Age Group	Typhoid Vaccine			Cholera Vaccine			No Vaccine		
	M	F	Total	M	F	Total	M	F	Total
0-4	118	148	266	124	134	258	182	178	360
5-9	168	152	320	155	183	338	118	110	228
10-19	121	174	295	117	152	269	246	170	416
20-39	119	222	341	97	200	297	511	276	787
40-59	63	86	149	71	63	134	240	112	352
60 +	30	21	51	28	22	50	83	42	125
Total	619	803	1422	592	754	1346	1380	888	2268
Unknown			22			10			24
Total			1444			1356			2292

PERCENT OF POPULATION GROUPS ACCEPTING VACCINE

Age Group	Bandar (Population 1345)			Mudafa (Population 1314)			Kaliganj (Population 2334			All		
	M	F	Total	M	F	Total	M	F	Total	M	F	Total
0-4	54	65	60	76	69	72	49	56	53	57	61	59
5-9	70	78	74	83	89	86	69	65	67	73	75	71
10-19	50	54	52	64	78	71	41	67	53	49	66	57
20-39	25	52	39	48	82	64	23	55	39	30	61	45
40-59	29	47	36	60	77	67	28	51	38	36	57	45
60 +	31	52	40	55	60	56	33	42	37	41	51	45
Total	43	59	51%	64	79	72%	39	58	49%	47	64	55%

The results compare very favorably with previous experience in these communities. The 72% volunteer rate in Mudafa compares with 50% response to smallpox vaccination achieved by the Government vaccinators in a door-to-door campaign in March 1963. 49% in the Kaliganj population compares with the 29% quoted by Dr. Ataur Rahman in his uncontrolled vaccine trial conducted in the year 1956, which followed an intensive health education program.

Follow up:

In order to learn of any cases of possible cholera within the immunized group, a local man of prominence was hired in each community. It was his job to visit each home within the study group daily, and report to us births, deaths, doctor or hospital visits, diarrheas with and without vomiting. In case of illness consistent with cholera, he was to call CRL for the ambulance or otherwise bring the patient to the ward or advise us of the case. Later he was to submit to CRL rectal swabs on diarrhea cases. The form used is appended.

Reactions:

Over the period 6 to 12 days after the immunization program in Bandar, the first community, the surveillance man reported 41 individuals with reactions to the inoculations. Investigation disclosed that 34 of these had occurred in those who had been given cholera vaccine. After minor initial fever and discomfort had improved or completely disappeared, a reaction reappeared at the vaccination site in the 6 - 8 day period after injection. There was recurrence of pain and swelling and complaints of feverishness.

In the immunization of this community, the cholera vaccine of one American manufacturer was used on the first two days (vaccine B), that of a second producer (vaccine A) was used on the remainder of the group. Review of the records revealed this distribution of delayed reactors as terms of age group and vaccine.

Age Group	Vaccine A		Vaccine B	
	No. injected	No. reacting	No. injected	No. reacting
0-4	51	0	24	0
5-9	63	0	21	0
10-19	47	8	25	0
20-39	43	13	22	0
40-59	28	12	8	0
60 +	4	1	6	0
Total	236	34	106	0

The implication of vaccine A is obvious. The reactions are restricted to those over 10, and 7 of the 8 in the 10 - 19 year group were 14 or over, and therefore had received 1.0 ml of vaccine. Dr. Joseph was unable to relate the phenomenon to a previous history of cholera or previous anticholera immunizations or sex.

Similar delayed reaction were also observed in Mudafa. All occurred in adults. Because of injector problems, part of the population had been given 0.5 ml, in which others received 1.0 ml of vaccine A. The reaction rate was approximately twice as high in adults who had received the 1.0 ml dose of cholera vaccine. Distribution of delayed reactors by age group and vaccine dose is shown in the following table:

VACCINE A

Age Group	0.5 ml dose		1.0 ml dose	
	Total	Reactors	Total	Reactors
10-19	22	0	67	3
20-39	56	4	44	10
40-59	22	1	30	2
60 +	14	1	10	1
Total	114	6	151	16

Severe delayed reaction also occurred in adults in Kaliganj, but these reactions were not documented.

Surveillance Findings:

Up to 15th October 1963, 184 cases of diarrhea were reported from these three communities and rectal swabs obtained for bacteriological examination (Table X). There were no isolations of cholera vibrios from the recipients of cholera vaccine; there were, however, 2 INABA isolations among the recipients of typhoid vaccine. Two OGAWA and one INABA strains were recovered from the non-recipient group.

Eleven of these 184 individuals with diarrhea were hospitalized at CRL and INABA cholera vibrios were recovered from 3 of the 11; two of these had received the typhoid control vaccine and one had not been immunized.

Discussion:

The data to date are too few to permit any valid conclusions; surveillance is being maintained during the fall in anticipation of further cases.

This pilot study did demonstrate the feasibility of conducting a controlled vaccine study in East Pakistan. It disclosed a potential problem in the high incidence of delayed reactions, and the need for further studies of the vaccine to be used in any project concerning a large population group.

ocation	Groups by vaccine status								
	Cholera vaccine			Typhoid vaccine			No vaccine		
	No.of isolations		No.of patients with diarrhea	No.of isolations.		No. of patients with diarrhea	No.of isolations		No.of patients with diarrhea
	V.cholera	NAG		V.cholera	NAG		V.cholera	NAG	
andar	0	1	68	1(1)*	0	43	2(1)*	1	45
udafa	0	0	3	1(1)*	0	9	0	0	3
aliganj	0	0	2	0	2	5	1	1	6
Total	0	1	73	2	2	57	3	2	54

Recovery of vibrios from 184 patients with diarrhea in vaccine trial.

* Numbers in parenthesis represent 3 proven cholera cases among 11 patients with diarrhea admitted to CRL hospital.

RESPONSE AND REACTIONS TO CHOLERA IMMUNIZATION

In the cholera vaccine trials carried out in three communities near Dacca, East Pakistan in April, 1963, it was observed that many individuals developed severe delayed reactions to cholera vaccine A. Reactions of fever and local swelling, pain and induration were observed 5-8 days following the injection. No children experienced the delayed reaction. Reactions were twice as frequent with the 1cc dose as with the ½ cc dose. The delayed reactions did not occur following the administration of cholera vaccine B administered at the same time by the same method to other members of the same population groups. There was no correlation between the delayed reactions and history of cholera immunization or attacks of cholera.

A study was undertaken by Dr. Paul Joseph in collaboration with Lt. Col. M.S. Mullick and his staff at the Combined Military Hospital, Dacca Cantonment, to study more carefully the delayed reactions, and to determine possible relationships of vaccination, antibody level, antibody response, attributes of the vaccine itself and the development of reactions.

87 adult male Pakistani civilian employees of the Combined Military Hospital volunteered as subjects. A questionnaire was administered to the men and information obtained on age, number of years in East Pakistan, past experience with cholera and number of previous injections of cholera vaccine. A copy of the questionnaire is included at the end of this report. After collection of the information, each man was at random given a number from 1 to 90. On the basis of their numbers, the men were divided into 6 groups, each of which was to receive a different immunization schedule. (Individual number 1 was placed into Group A, 2 into B, 3 into C, 4 into D, 5 into E, 6 into F, 7 into A and so on) The immunization schedules for the 6 groups are shown in the following table:

Immunization Schedules

Group	Day 0		Day 7
A	0.5 ml cholera vaccine	A	1 ml cholera vaccine A
B	0.5 ml cholera vaccine	B	1 ml cholera vaccine B
C	0.5 ml typhoid vaccine		1 ml typhoid vaccine
D	0.5 ml cholera vaccine	A	0.5 ml typhoid vaccine
E	0.5 ml cholera vaccine	B	0.5 ml typhoid vaccine
F	0.5 ml typhoid vaccine		0.5 ml saline

Vaccines A & B were the products o two different US manufacturers; both satisfy the minimal requirements of the Division of Biologics Standards. The typhoid paratyphoid vaccine was prepared at the Institute of Public Health, Dacca, in March, 1963. Vaccines were administered by jet injector into the left arm, and one week later into the right arm. Blood samples of 5-10 cc were obtained from all men on days 0, 7, 14, and 28. Each man was examined daily for 17 days and pertinent observations recorded by the same CRL research nurse.

continued .....

80 men completed the study. Each of the six groups was essentially comparable. Ages ranged from 10 to 52 as follows:

	Age Group					
Group	1-19	20-29	30-39	40-49	50+	Total
A	-	3	4	5	1	13
B	1	8	1	2	1	13
C	-	3	9	1	-	13
D	-	2	7	5	1	15
E	-	2	7	5	-	14
F	-	6	5	1	-	12
not complete	-	3	2	2	-	7
Total	1	27	35	21	3	87

This group had extensive previous contact with cholera antigens. One quarter of the group had had over twenty previous doses of vaccine; one man reported 65 prior doses of cholera vaccines, and this figure was not inconsistent with his record of military service. When the preimmunization sera were examined for cholera antibodies by the standard technique using living organisms, the titers of this group were relatively high - the overall geometric mean titer was 2.6 tubes. (Titers will be presented as geometric means expressed as tubes of agglutination in a doubling dilution starting at 1.20). There is an unimpresive upward trend with greater numbers of the injections:

Number of Previous Injections	Subjects	Mean Titer	
		Ogawa	Inaba
0 - 19	62	2.6	2.5
20 - 39	15	2.7	2.6
40+	5	3.0	3.0
Total	82	2.58	2.57

A more convincing relation between the titer and the interval since the last dose of vaccine is shown below:

	Subjects	Mean Titer	
		Ogawa	Inaba
Less than 5 months	34	2.9	2.8
5 months or more	53	2.2	2.3

Antibody response to Cholera Vaccines:

The titer of agglutinating antibodies, both before and after immunization,

continued.....

was essentially the same, whether determined with an Ogawa or Inaba strain of V. cholerae. For simplicity, only Ogawa titers will be presented. 30 and 28 men were given 0.5 ml of vaccines A & B, respectively; one week later half of each group was given 0.5 ml of typhoid vaccine while the other half received a second dose of 1.0 ml of the same cholera vaccine. Typhoid and saline groups were included to control reaction data; no rise in cholera antibodies occurred in these groups. The serological response to the specific vaccines follows. Mean titers show standard error of the means:-

Day	0.5 ml Vaccine A (30 men)	).5 ml Vaccine B (28 men)
0	2.9 (± 0.28)	3.1 (± 0.24)
7	4.9 (± 0.26)	3.9 (± 0.21)

Day	0.5 ml typhoid(17)	1.0ml vaccineA (14)	0.5ml typhoid (15)	1.0ml vaccineB (13)
7	5.3 (± 0.30)	4.5 (± 0.40)	3.5 (± 0.33)	4.4 (± 0.21)
14	5.7 (± 0.26)	5.5 (± 0.35)	3.7 (± 0.40)	4.7 (±
28	5.6 (± 0.27)	5.3 (± 0.39)	3.4 (± 0.36)	4.5 (± 0.33)

It is evident that Vaccine A is much more potent in eliciting an antibody response. 0.5 ml evokes a serological response not significantly different than follows 1.5 ml of Vaccine B; an additional 1.0 ml dose of Vaccine A has not apparent effect on the titer.

Early Reactions

The pattern of reactions present one day after each of the various injections is recorded in Table 1. The saline control group reaffirms the need for a base line in a study of injection reactions - essentially as many reported malaise follow-ing saline as following the injection of 0.5 ml vaccine A or 0.5 ml typhoid vaccine. Obviously, 1 ml of any of the three active vaccines did evoke malaise, evident by the clearly higher complaint frequency. The immediate reaction pattern of vaccine A is very similar to that of the typhoid vaccine. Both cholera vaccines proved to be pyrogenic; the first dose would appear to have created tolerance for the second larger dose. Headache was more frequently reported following injection of vaccine A, as was the local reaction at the site of injection, manifested objectively by swelling erythema and induration, and subjectively by complaints of pain and tenderness. These reactions had generally subsided by the third day; occasionally the indurated area became a slowly resolving nodule.

Delayed Reactions

In four of the 30 individuals who received 0.5 ml of Vaccine A, the early reaction completely or partially disappeared only to reappear with local signs and symptoms of pain, tenderness, erythema, swelling and induration. These symptoms appeared or intensified on the third, fourth, sixth, and twelvth day after the vaccine had been given; the reactions were maximal on the fifth, seventh, eighth, and twelvth day, respectively. Only in the first two individuals were these reactions marked enough to have been noted without close examination. Both subjects reported headache and malaise, and had oral temperatures of 99.2 and 100°F.

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TABLE I

EARLY REACTIONS AFTER VACCINE INJECTIONS

		Vaccine A		Vaccine B		Typhoid		Saline
		0.5 ml	1 ml	0.5 ml	1 ml	0.5 ml	1 ml	0.5 ml
Number of individuals		30	14	27	13	54	13	12
No. of men losing duty time	Whole	0	5	0	2	0	4	0
	Partial	0	4	0	7	9	7	1
No. of duty days lost	Whole	0	8	0	2	0	4	0
	Partial	0	12	0	8	13	12	1
Fever	99°-99.9°F	17	6	18	6	26	8	3
	100°F or	2	0	1	1	3	4	0
Number with Chills	Mild	6	5	0	1	2	3	0
	Severe	0	0	0	0	0	0	0
Number with Malaise	Mild	13	11	1	11	17	10	6
	Severe	1	2	0	0	0	2	0
Number with Headache	Mild	16	12	10	5	20	10	3
	Severe	0	1	0	1	1	3	0

continued .....

A fifth individual, who received both doses of vaccine A, developed pain, tenderness, erythema and a 3 inch area of induration at the site of the 0.5 ml dose, (left arm) ten days after it had been administered (3 days after 1 ml of vaccine A had been injected into the other arm). The reaction had cleared when next examined two days later. On the eighth day after the second dose, he developed a sharp reaction to the second injection (right arm), with malaise, an oral temperature of 99.6°, pain, tenderness, erythema and a four inch area of induration. The reaction resolved completely within three days. Four other recipients of the 1.0 ml dose of vaccine A developed comparable reactions - one quite mild on the fifth and one on the seventh day, and the other two beginning on the fifth day and persisting through the tenth day. One of these men was absent sick on the seventh day, the other reported malaise from the third day to the tenth day, and headache from the fifth to the ninth day.

There were no delayed reactions following the injection of cholera vaccine B or typhoid, in 0.5 ml or 1.0 ml doses, but there were persisting nodules of incompletely resolved early reactions.

		Vaccine A		Vaccine B		Typhoid		Saline
		0.5 ml	1.0 ml	0.5 ml	1.0 ml	0.5 ml	1.0 ml	0.5 ml
Number injected		30	14	28	13	54	13	12
Slight reaction		3	2	0	0	0	0	0
Significant reaction		2	3	0	0	0	0	0
Number with	0 - 1/2"	17	6	7	4	12	4	0
induration at	1/2 - 1"	2	2	0	0	1	1	0
site of injec-	1" - 2"	1	2	0	0	1	0	0
tion after 5th day	2" - or	3	4	0	0	0	0	0

No relation can be established between objective reaction, early or delayed, and the serological titer at the time of injection, or the titer rise after injection. However, severe delayed reactions may possibly be expected in those with the higher preexisting titers. (Table II). The reaction dose relates less well with rise in titer; among the severe reactors, three had a one tube rise, one a four tube rise, and one, no rise. Among those with no reactions, four individuals had four to six tubes rise; one subject with a four tube rise had a slight reaction.

Vaccine Studies

These delayed reactions have only been seen in those who received vaccine A. Therefore, a study of the vaccines themselves was indicated. Dr. Margaret Pittman of the Division of Biologics Standards, National Institutes of Health, Dr. John F. Barbero of the Department of Immunochemistry, Walter Reed Army Institute of Research, and the manufacturers of Vaccines A and B cooperated in these studies.

The organisms for vaccine A were grown on a solid medium, from which they were harvested with physiological saline containing 0.5% phenol. Vaccine B is a whole broth culture diluted with phospate buffered saline to the required organism concentration; it has a final phenol concentration of 0.45%. In the mouse potency

continued .....

TABLE II

DELAYED REACTION AND TITER BEFORE AND AFTER INJECTION

Last Tube Positive	Vaccine A								
	0.5 ml dose			1.0 ml dose			All		
	No Reactions	Slight React.	Signi-ficant React.	No Reactions	Slight React.	Signi-ficant React.	No Reactions	Slight React.	Signi-ficant React.
	Number with given titer at time of injection								
0	1	–	–	–	–	–	1	–	–
1	5	1	–	–	–	–	5	1	–
2	5	–	–	1	–	–	6	–	–
3	5	1	1	3	1	–	8	2	1
4	5	1	–	3	–	1	8	1	1
5	2	–	1	1	–	–	3	–	1
6	2	–	–	1	1	1	3	1	1
7	–	–	–	–	–	1	–	–	1
Total	25	3	2	9	2	3	34	5	5
	Number with given titer 7 days after injection								
1	–	–	–	–	–	–	–	–	–
2	1	–	–	–	–	–	1	–	–
3	3	–	–	–	–	–	3	–	–
4	8	1	–	3	–	–	11	1	–
5	3	1	1	2	1	1	6	2	2
6	5	1	–	3	–	–	7	2	–
7	3	–	1	1	1	1	4	–	2
8	–	–	–	–	–	1	–	–	1
Total	23	3	2	9	2	3	32	5	5

continued ———

vaccine A has proven to be much more antigenic than vaccine B.

Microkjedahl determinations were performed on the whole vaccines, on the 5% trichloracetic acid precipitates (protein precipitant) and on the precipitates after addition of 1.5 and 3 volumes of acetone (technique advocated for measure of bacterial material). In addition, the manufacturers provided medium blanks which simulated the vaccine, but had not been inoculated with bacteria; these were not the medium lots used to prepare our vaccines, but are analogous in material method. The nitrogen values in mg/ml follow:-

Nitrogen Determinations mg/ml

	Vaccine A			Vaccine B		
	Bottle #1	Bottle #2	Medium Blank	Bottle #1	Bottle #2	Medium Blank
otal N	1.3104	1.3440	0.6160	1.6744	1.6744	2.7048
% TCA ppt N	0.0765	0.0873	0.0019	0.0425	0.0429	0.0033
cetone ppt N 1.5 vol	0.0971	0.0859	0.000	0.0448	0.0471	0.0042
" " " 3 vol	0.1097	0.1120	0.0023	0.0499	0.0551	0.0121

It is evident that despite a higher total nitrogen, vaccine B contains only about half as much protein and about half as much bacterial somatic substance as does vaccine A. The medium blanks permit evaluation of the changes in the nitrogen partitioning. In terms of percentage total nitrogen:

	Vaccine A			Vaccine B		
	Bottle #1	Bottle #2	Medium Blank	Bottle #1	Bottle #2	Medium Blank
% TCA ppt N	5.8%	6.5%	0.3%	2.5%	2.6%	0.1%
cetone ppt N 1.5 vol	7.4	6.4	0.0	2.7	2.8	0.2
" " " 3 vol	8.4	8.3	0.4	3.0	3.3	0.4

Both medium blanks are comparably low in protein or acetone precipitating material, but finished vaccine A contains more than twice as much protein and acetone precipitable material.

These serological and chemical studies show that vaccine A contains much more antigenic substance than vaccine B. A single 0.5 ml dose elicits an antibody response after 28 days greater than two doses totaling 1.5 ml of vaccine B; indeed, the single dose elicits a response not improved by a second 1.0 ml dose one week later. The human response occurs largely within the first seven days after injection; one wonders whether similar findings would pertain in a non-endemic area. It is to be noted that one individual who derived prior immunization and had no antibodies detectable at a 1:20 level, developed positivity thru 6 tubes seven days after 0.5 ml of vaccine B.

Delayed reactions to cholera vaccine have been reported before (Tewari, Lancet 1 : 572, 1936.) These findings suggest that the delayed reactions are a

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reflection of antigen excess. Similar reactions had been reported with alcoholized typhoid vaccine by Felix Rainsford and Stokes (British Med. Jour. 1:435, 1941) and by Climie (J. Hyg. 42:411, 1942). These workers reported that a reduction in the dose administered eliminated the delayed reactions.

These studies indicate that vaccine A is indeed a potent preparation antigenically. It can be administered in a single 0.5 ml dose without eliciting significant adverse reactions, and without reducing the antibody response in Pakistan. At this dose level, it probably is as antigenic a vaccine as can be tolerated. It therefore constitutes a satisfactory vaccine for use in a field trial designed to test the degree of protection against natural infection with cholera afforded by the best available vaccine.

DISTRIBUTION OF CHOLERA PATIENTS ADMITTED TO MITFORD HOSPITAL FROM DACCA CITY AND KERANIGANJ

Hopefully, a vaccine study should be carried out in the area within which the base laboratory is situated. In order to maintain cognisance of the cholera situation in the Dacca area, an attempt has been made to culture all admissions to the cholera ward at Mitford Hospital. To seek a good focus of cholera as a potential study area, these admissions were related to their place or residence. Dacca City itself would be a poorer area for a vaccine study than a rural area; in the urban area a large floating population would make surveillance very difficult.

Keraniganj Thana, situated directly across the river from Dacca City, is a more rural area. As indicated in tabular form, the overall rate per thousand during the last year was 0.89, but this was based on rates ranging from 0.06 to 1.88 per thousand. This variation may bear more relation to the distance from the hospital than to disease incidence.

The highest rate was found in Zinzira, the area directly across the river. This is one of the areas in which the pilot study of March - April was carried out. The people in this area largely work in Dacca City, commuting daily. They proved to be poorly cooperative, complicating surveillance. Only 7 cases of diarrhea were reported from a population of 2334 who had taken vaccine, in contrast with 123 cases of diarrhea reported among the 2659 vaccinees in the other two communities.

It seemed therefore, that a successful field trial would require a study site where the population was stable (which essentially requires a rural area), and where the people would be truly cooperative, rather than merely acquiescent.

BACTERIOLOGICAL RESULT OF CHOLERA PATIENTS ADMITTED TO MITFORD HOSPITAL FROM DACCA CITY AND KERANIGANJ

October 1962 - July 1963

Month	Inaba		Ogawa		Inaba & Ogawa		Ogawa & NAG		NAG		Negative		Total	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Oct. '62	1	1	-	-	-	-	-	-	-	-	21	19	22	20
Nov. '62	32	16	-	-	-	-	-	-	2	1	38	22	72	39
Dec. '62	91	49	-	-	-	-	-	-	-	-	52	28	143	77
Jan. '63	11	14	-	-	-	-	-	-	-	-	39	19	50	33
Feb. '63	5	1	-	-	-	-	-	-	-	-	32	14	37	15
Mar. '63	7	3	-	-	-	-	-	-	1	-	97	52	105	55
Apr. '63	3	2	4	1	-	-	-	-	6	1	72	35	85	39
May '63	8	10	7	9	1	-	-	-	2	3	63	26	81	50
June '63	3	5	18	17	-	-	-	1	6	1	57	39	84	63
July '63	-	-	6	8	-	-	-	-	8	2	65	24	79	34
TOTAL	161	101	35	35	1	-	-	1	25	10	536	278	758	425
Unknown	-	-	-	-	-	-	-	-	2	-	27	18	29	18
GRAND TOTAL	161	101	35	35	1	-	-	1	27	10	563	296	787	443

CONFIRMED CHOLERA CASES ADMITTED TO MITFORD HOSPITAL

FROM KERANIGANJ THANA

October 1962 - July 1963

Name of Union	Population 1961 Census	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Un-known	Total Cases	Rate per 1000
Kalatia	17544	-	-	-	1	-	-	-	-	-	-	-	1	.06
Konda	21404	1	-	4	1	-	-	-	-	-	-	-	6	.28
Tegharia	13767	1	-	3	-	-	-	-	-	-	-	1	5	.36
Sakta	17606	-	-	5	1	-	-	-	-	-	-	1	7	.40
Subhoya	20992	-	14	23	5	-	-	-	-	-	-	1	43	1.10
Zinzira	18659	-	2	24	6	1	-	-	-	-	-	2	35	1.88
TOTAL	109972	2	16	59	14	1	-	-	-	-	-	5	97	.89
Rate per 1000	-	.02	.15	.54	.13	.01						.05		

DISTRIBUTION OF CHOLERA PATIENTS ADMITTED TO MITFORD HOSPITAL FROM DACCA CITY AND KERANIGANJ

Hopefully, a vaccine study should be carried out in the area within which the base laboratory is situated. In order to maintain cognisance of the cholera situation in the Dacca area, an attempt has been made to culture all admissions to the cholera ward at Mitford Hospital. To seek a good focus of cholera as a potential study area, these admissions were related to their place or residence. Dacca City itself would be a poorer area for a vaccine study than a rural area; in the urban area a large floating population would make surveillance very difficult.

Keraniganj Thana, situated directly across the river from Dacca City, is a more rural area. As indicated in tabular form, the overall rate per thousand during the last year was 0.89, but this was based on rates ranging from 0.06 to 1.88 per thousand. This variation may bear more relation to the distance from the hospital than to disease incidence.

The highest rate was found in Zinzira, the area directly across the river. This is one of the areas in which the pilot study of March - April was carried out. The people in this area largely work in Dacca City, commuting daily. They proved to be poorly cooperative, complicating surveillance. Only 7 cases of diarrhea were reported from a population of 2334 who had taken vaccine, in contrast with 123 cases of diarrhea reported among the 2659 vaccinees in the other two communities.

It seemed therefore, that a successful field trial would require a study site where the population was stable (which essentially requires a rural area), and where the people would be truly cooperative, rather than merely acquiescent.

BACTERIOLOGICAL RESULT OF CHOLERA PATIENTS ADMITTED TO MITFORD HOSPITAL FROM DACCA CITY AND KERANIGANJ

October 1962 - July 1963

Month	Inaba		Ogawa		Inaba & Ogawa		Ogawa & NAG		NAG		Negative		Total	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Oct. '62	1	1	-	-	-	-	-	-	-	-	21	19	22	20
Nov. '62	32	16	-	-	-	-	-	-	2	1	38	22	72	39
Dec. '62	91	49	-	-	-	-	-	-	-	-	52	28	143	77
Jan. '63	11	14	-	-	-	-	-	-	-	-	39	19	50	33
Feb. '63	5	1	-	-	-	-	-	-	-	-	32	14	37	15
Mar. '63	7	3	-	-	-	-	-	-	1	-	97	52	105	55
Apr. '63	3	2	4	1	-	-	-	-	6	1	72	35	85	39
May '63	8	10	7	9	1	-	-	-	2	5	63	26	81	50
June '63	3	5	18	17	-	-	-	1	6	1	57	39	84	63
July '63	-	-	6	8	-	-	-	-	8	2	65	24	79	34
TOTAL	161	101	35	35	1	-	-	1	25	10	536	278	758	425
Unknown	-	-	-	-	-	-	-	-	2	-	27	18	29	18
GRAND TOTAL	161	101	35	35	1	-	-	1	27	10	563	296	787	443

CONFIRMED CHOLERA CASES ADMITTED TO MITFORD HOS

FROM KERANIGANJ THANA

October 1962 - July 1963

Name of Union	Population 1961 Census	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June
Kalatia	17544	-	-	-	1	-	-	-	-	-
Konda	21404	1	-	4	1	-	-	-	-	-
Tegharia	13767	1	-	3	-	-	-	-	-	-
Sakta	17606	-	-	5	1	-	-	-	-	-
Subhoya	20992	-	14	23	5	-	-	-	-	-
Zinzira	18659	-	2	24	6	1	-	-	-	-
TOTAL	109972	2	16	59	14	1	-	-	-	-
Rate per 1000	-	.02	.15	.54	.13	.01				

MATLAB BAZAR

Several possible sites for a cholera vaccine trial have been explored during the past two months. Some of these endemic areas are not easily accessible, requiring as much as 24 hours to reach. Barisal, including Bhola Island, a site located in the south central part of the province, was visited by means of river steamer and launch. Cholera case registers with records dating back to the 1930's were available for study. In jhalakati, a community along the river 13 miles south of Barisal, the Sanitary Inspector had a careful description of cholera morbidity and mortality for the past 30 years. These records gave ample evidence of the regular yearly occurrence of cholera. On the average, between 1946 and 1951, cholera deaths were recorded 10 months per year. All seasons were affected, but the greatest concentration of cases was observed each spring.

We visited the health clinic at Tongi, a town less than an hour's autoride north of Dacca. The clinic is staffed by 2 physicians and a health educator who handle an ambulatory case load of 90 to 100 patients daily. Study of the diagnostic categories of illnesses observed during the week preceding our visit revealed that no instances of acute diarrheal disease were recorded. The Sanitary Inspector assigned to the area said that only one cholera patient sought aid at the clinic in the preceding year and that individual was promptly referred to CRL for hospitalization. Thus to date, the clinic has functioned chiefly as a dispensary, and acute diarrheal disease has been minimal.

The CRL is presently cooperating with Dr. M.R. Majumdar in planning a cholera vaccine field trial in several riverside villages located in Matlab Thana. East Pakistan is divided administratively in 17 districts, and each of these into smaller sub-units, the thanas or police station jurisdictions. These in turn comprise unions containing several towns or villages. Matlab Thana is located southeast of Dacca city along the western edge of Comilla district at the confluence of the Meghna and Buriganga rivers. It can be reached in an hour and a quarter by jet boat. The population is approximately 200,000. Several villages near Matlab Bazar, the administration headquarters for study, have been selected in the southeastern part of Matlab because of the continued high prevalence of cholera. The population of these villages is 26,000. As part of the smallpox eradication program, the office of the Sanitary Inspector has a card on each family in this area; the information on this card actually constitutes effective census data, listing all family members with age and sex.

Dr. Fahimuddin, at present a consultant to the CRL, provided cholera mortality data for East Bengal during the interval from 1945 to 1951 for a WHO cholera control project conducted by J.B. Arbuthnot. Some of these data (Table 1) indicate that the District of Comilla ranked sixth among the 17 districts regarding both the average death rate and lowest death rate per 100,000 during this 7-year interval. One method used by Arbuthnot in defining endemic areas was examination of the average number of months per year during which no cholera deaths were reported. Column 3 of Table 1 shows that the average number of months without cholera deaths in Comilla was 4.5 and that in Matlab Thana, 4.4.

continued.....

Recent data covering the past 5 years have been kindly provided by Dr. Majumdar, (Table 2). Changes in cholera reporting practices after 1960 probably account for the paucity of cholera cases in 1961 and 1962. Examination of deaths caused by diarrhea as well as cholera during this interval seemed useful. The average annual death rate for cholera in Comilla District was 0.2/1000 and for cholera and diarrhea combined was 0.7/1000. The average annual death rate for cholera in Matlab P.S. was 0.3/1000. (See Table 3). Reporting in the lower age groups is evidently incomplete. Cases of cholera, which are certainly frequent in children, are often simply classified as "diarrhea", the term cholera being reserved for older age groups. A mortality rate of 74% also implies that many cases had not been reported. In view of changes in cholera reporting practices after 1960, as noted above, the actual rates based on the entire five-year period are probably higher than those calculated.

The annual incidence of cholera in Matlab very probably exceeds 2 or 3 cases per 1000; in a population of 25,000, at least 75 cases of disease should be recognised by present criteria. Including bacteriologically positive diarrheas, the total number may well achieve significance. A limited vaccine trial in this area is justified by the local support and disease incidence, and will provide a more accurate estimate of the occurrence of cholera than has been available for any defined population group in East Pakistan. Experience will be gained (1) in applicability of census data derived from smallpox vaccination records already in use; (2) in practical problems of enlisting community participation and performing door-to-door immunization; (3) in maintaining an effective surveillance system; and (4) in providing adequate medical care to those who develop the disease. It should be possible to measure both the incidence of diarrhea associated with Vibrio cholerae in immunized and non-immunized individuals and the contact carrier prevalence in affected families according to immune status.

The trial is proceeding at present in the following stages:

1. Enumeration of the population by family within villages. This census by the staff of the local sanitary inspector is proceeding at present, bringing up to date lists previously prepared for smallpox eradication.

2. A door-to-door family-by-family inoculation program conducted by the sanitary inspector's staff employing a potent cholera vaccine alternating with a typhoid vaccine as a control. The attributes of the cholera vaccine were described in XII. Response and Reactions to Cholera Immunization.

3. Subsequent cholera surveillance by a health assistant assigned to observe village groups not to exceed 2000 persons per assistant. The CRL will maintain personnel in the field whose function will be periodic visits of villages and continuous collaboration with health assistants in observation for illness, collection of fecal samples in the event of illness, and keeping of records.

4. The provision by CRL of trained physicians centrally located in Matlab Bazar. These physicians will be available to treat acute cholera locally using modern methods adopted to field conditions, as well as search out cases of mild and sub-clinical infection.

continued.....

TABLE I

	'45-51 Average death rate per 100,000	'45-51 Lowest death rate per 100,000	'45-51 Average No. months/year no cholera deaths
1. Barisal	145	90	3.7
2. Faridpur	95	44	5.7
3. Khulna	91	37	4.6
4. Jessore	63	35	5.4-10
5. Dacca	62	35	5.5
6. Comilla	49	22	4.5 (Matlab PS=4.4)
7. Kushtia	49	4	9.6
8-16 -	-	-	-
17. Chittagong Hill tracts	13	0	11.3

Frequency of cholera in East Bengal, modified from J.B. Arbuthnot

TABLE 2

Comilla District.

No. of deaths due to:	1958	1959	1960	1961	1962
Diarrhea	593	1409	2683	4046	2237
Cholera	726	1616	1237	100	154
Diarrhea & cholera	1319	3025	3920	4146	2391
All centers	36078	33081	35280	33721	30504

Mortality associated with diarrhea and cholera in Comilla 1958 to 1962 modified from Dr. N.K. Majumdar.

TABLE 3

Matlab P.S. 1958-1962
Age groups

	<1	1-5	5-10	10-20	20+	Total
No. Attacked	-	6	26	145	246	423
No. deaths	-	2	16	111	207	336

Mortality and Morbidity due to Cholera in Matlab P.S., 1958 - 1962

LIST OF PAKISTAN-SEATO CHOLERA
RESEARCH LABORATORY STAFF

—

DIRECTOR'S OFFICE

			Joined
Dr. Abram S. Benenson		Director	28 June 1962
	2	Steno/Secretaries	

CLINICAL RESEARCH SECTION

Dr. Robert S. Gordon Jr.		Scientific Director	18 October 1961
Dr. W.B. Greenough		Assistant Chief	18 July 1962
Dr. John Lindenbaum		"	19 August 1963
Dr. Jalaluddin Ahmed		Senior Physician	1 October 1963
Dr. (Mrs.) Rezia Akbar		Junior Physician	1 Feb. 1963
Dr. Rafiqul Islam		"	13 Feb. 1963
Dr. S. Zoha		"	1 March 1963
Dr. Matleb Ali		"	22 March 1963
Dr. A.K.M. Jamiul Alam		"	21 August 1963
	4	Senior Research Asstts.	
	1	Research Assistant	
	1	Senior Lab. Technician	
	6	Junior Lab. Technicians	
	1	Senior Research Nurse	
	1	Radiology Technician	
	2	Lab. Attendants	
	1	Medical Record Secretary	

CRL WARD:

Miss Dorothy G. Torrance		Supervisor	16 July 1963
	10	Senior Nurses	
	1	Junior Nurse	
	8	Aid Nurses	
	2	Cooks	
	1	Cook's Helper	
	7	Orderlies	
	2	Ward Boys	
	1	Chief House Keeper	
	1	Glass Washer	
	1	Laundryman	

BACTERIOLOGY SECTION:

		Joined
Dr. Saiyid Sibte Hasan Rizvi	Chief	27 August 1963
Mr. Imdadul Haque	Deputy Chief	16 July 1962
Mr. J.J. Brennan	Chief Technician	18 April 1963

1 Senior Res.Assistant
2 Bacteriological Asstts.
2 Lab. Technicians
1 Lab. Assistant
1 Sample Taker
3 Media Makers
6 Lab.Attendant(Glass Washers)

EPIDEMIOLOGY SECTION:

Dr. Joe L. Stockard	Deputy Director	3 September 1960
Dr. Robert O. Oseasohn	Chief	2 September 1963
Dr. A.Q. Khan	Asstt.Chief	1 August 1962
Dr. Robert M. Glasse	Anthropologist	18 September 1963
Mrs. R.M. Glasse	Anthropologist	18 September 1963
Dr. Md. Ashraful Islam	Physician	23 April 1963
Mr. A.S.M. Mizanur Rahman	Physician	29 April 1963

2 Senior Res. Asstts.(Sociologist)
9 Research Asstts (Sociologist)
1 Field Nurse
1 Lady Health Visitor
2 Statistic Clerk-cum-Operators
2 Junior Lab. Technicians

WATER STUDIES SECTION:

Mr. Syed Zafar Ahmed	Chief	1 October 1961

2 Senior Research Asstts.
1 Junior Research Asstt.
1 Senior Lab. Technician
1 Junior Lab. Technician
1 Sanitary Surveyor
2 Asstt.Sanitary Surveyors
1 Glass Washer
1 Lab. Attendant

ADMINISTRATIVE SECTION:

		Joined
Mr. M. R. Bashir	Administrative Officer	1 November 1961
1	Property & Supply Officer	
1	Administrative Assistant	
1	Accountant	
3	Junior Administrative Asstts.	

GENERAL SERVICES SECTION:

Mr. M. R. Bashir	Chief
1	Medical Editor (Part time)
1	Maintenance Supervisor
1	Maintenance Asstt.
1	Librarian
1	Typist/Clerk
1	Telephone Operator/Receptionist
1	Dispatcher
1	Mechanic
1	Electrician
11	Drivers
5	Darwans
2	Night Guards
1	Care Taker Asstt.
1	Veterinarian
1	Animal Care Taker
1	Animal Assistant
1	Animal Handler
1	Animal Cage Cleaner
1	Gardener
4	Sweepers

CONSULTANTS:

Dr. K.A. Monsur	Bacteriology
Dr. A.K.M. Abdul Wahed	Clinical Research
Dr. Md. Fahimuddin	Epidemiology

CONSULTANTS	=	3
PROFESSIONAL & EXECUTIVE	=	23
TECHNICAL & SUPPORTING	=	140
TOTAL	=	166