

CHOLERA RESEARCH LABORATORY

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TECHNICAL COMMITTEE MEETING
FEBRUARY 22 - MARCH 1, 1977

Dacca, Bangladesh

SCHEDULE FOR ELEVENTH MEETING OF THE SCIENTIFIC REVIEW

AND TECHNICAL ADVISORY COMMITTEE

CHOLERA RESEARCH LABORATORY, DACCA, BANGLADESH

FEBRUARY 22 TO MARCH 1, 1977

Tuesday 22 8:00 P.M. Meeting with Acting Director at Hotel Intercontinental.

Dr. Mujibur M. Rahaman, Acting Director, and Head, Clinical Division.
Dr. George T. Curlin, Head, Epidemiology Division.
Mr. Philip O. Weeks, Staff Assistant to the Director.

Review of Agenda: Committee assignments
Role and composition of the Directing Council. Relationship between CRL and USAID, GOB, ODM, Australia, CDC and Johns Hopkins ICMR.

Wednesday 23 8:00 A.M. Session I - ORAL THERAPY

Dr. Mujibur M. Rahaman.
Dr. George T. Curlin

2:00 P.M. Session II - E COLI STUDIES

Dr. Michael Merson, Epidemiology Division Head Designate.
Dr. K. Ahmad, Pharmacologist, Institute of Post Graduate Medicine & Research.
Dr. K. Aziz, Head, Laboratory Division.

6:30 P.M. Reception - Hotel Intercontinental

Thursday 24 8:00 A.M. Session III - CLINICAL STUDIES OF DYSENTERY

Dr. Robert Gilman, Resident Co-ordinator, JHU/ICMR.
Dr. Mujibur M. Rahaman.
Dr. Md. Yunus,

2:00 P.M. Session IV - POPULATION STUDIES

Mr. Alauddin Chowdhury, Head, Statistics Branch.
Dr. Douglas H. Huber, Medical Epidemiologist.

Friday 25	8:00 A.M.	<u>Session IV - ENVIRONMENTAL EPIDEMIOLOGY</u> Dr. Mujibur M. Rahaman. Dr. M. Khan, Head, Community Studies. Dr. George T. Curlin. Dr. John Briscoe, Sanitary Engineer.
	1:00 P.M.	Consideration of Cholera Vaccine Trials.
	3:30 P.M.	Technical Committee meeting with Directing Council.
Saturday 26	8:00 A.M.	Technical Committee meeting with Prof. K.A. Khaleque, Secretary, Ministry of Health, representing Col.(Ret'd) M.M. Huq, President's Advisory Council, in-charge of Ministry of Health, Labour and Social Welfare.
	9:30 A.M.	JHU/ICMR Program Reports.
	2:00 P.M.	Conversion of CRL to an International Institute.
	3:00 P.M.	Technical Committee discussion and report preparation.
Sunday 27	7:00 A.M.	All day trip to Matlab.
Monday 28	9:00 A.M.	Preparation of Committee report.
Tuesday 1	8:30 A.M.	Review of draft of report.
	11:00 A.M.	Meeting with Acting Director and new Director.

Jordan, William S. Jr., M.D.

Chairman, United States,
Director, Microbiology of Infectious
Diseases Program
National Institute of Allergy and
Infectious Diseases
National Institutes of Health
Bethesda, Maryland 20014

Mackay, Donald M., M.D.

Member, United Kingdom,
Deputy Director
London School of Hygiene and Tropical
Medicine
Ross Institute of Tropical Hygiene
Keppel Street (Gower Street)
London WC1E 7HT
England

Latif, M.A., B.Sc., M.B.B.S.,
D.Ph., Ph.D.

Member, Bangladesh,
Officer-On-Special Duty
Institute of Public Health
Mohakhali Medical Complex
Mohakhali, Dacca-12

Rowley, Derrick, M.D. PhD.

Member, Australia,
Department of Microbiology and
Immunology
The University of Adelaide
South Australia 5001

Craig, John P., M.D.

Consultant,
Chairman, U.S. Cholera Panel,
Department of Microbiology and
Immunology,
Downstate Medical Center
State University of New York
450 Clarkson Avenue, Brooklyn
New York 11203

Gangarosa, Eugene J., M.D.

Consultant,
Chief, Enteric Diseases Branch
Deputy Director
Bacterial Diseases Division
Epidemiology Bureau
Center for Disease Control
Atlanta, Ga.

Benenson, Abram S., M.D.

Special Consultant,
Member, U.S. Cholera Panel
Department of Community Medicine
University of Kentucky
Texington, Ky. 40506

Greenough, William B., M.D.

Observer,
Director of Clinical Studies
Designate, CRL
Director, The Clinical Scholars Office
Johns Hopkins Hospital
Baltimore, Maryland 21205

Merson, Michael, M.D.

Observer,
Epidemiology Division Head Designate,
CRL
Career Development Officer
Department of Medicine
Baltimore City Hospital
Johns Hopkins University
4940 Eastern Avenue
Baltimore, Maryland

Miller, Carl E. D.V.M.

Observer,
Enteric Diseases Program Officer
National Institutes of Health
Room 749, Westwood Building
Bethesda, Maryland 20014

Mosley, Wiley H., M.D.

Observer,
Member, U.S. Cholera Panel
Director Designate, CRL
Chairman, Department of Population
Dynamics
Johns Hopkins School of Hygiene and
Public Health
Baltimore, Maryland 21205

Sack, R. Bradley, M.D.

Director, Johns Hopkins University
International Center for Medical
Research
Associate Professor of Medicine
The Johns Hopkins University School of
Medicine
Baltimore, Maryland

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ORAL HYDRATION IN NON-CHOLERA DIARRHOEA: SUPERIORITY
OF FLAVOURED VERSUS UNFLAVOURED SOLUTIONS

M. Mujibur Rahaman
M. A. Majid
W. F. Verwey

Cholera Research Laboratory
Dacca, Bangladesh

ABSTRACT

Oral Hydration in Non-cholera Diarrhoea: Superiority of Flavoured Versus Inflavoured Solutions

M. Mujibur Rahaman, M. A. Majid and W. F. Verwey

Intake of plain and orange flavoured Dacca Oral Solution was measured in children and adults during the maintenance phase of non-cholera diarrhoea in hospitalised patients for 8-48 hours. All were kept successfully hydrated and none required intravenous infusion during the oral hydration. When either plain or flavoured solution was given to 64 patients in strict alternation, there was little difference in the volume of intake of either of the solutions. However when both the solutions were made available to each of the patients in another series of 29 patients, the intake of the orange flavoured solution was significantly higher than the intake of the unflavoured solution. In this group the sum of intake of both the plain and flavoured solutions were substantially higher for a given period than the intake of any single solution for a comparative period. In 24 patients of all ages during the prolonged drinking lasting 48 hours intake of flavoured solution was significantly higher than the unflavoured type except in children during the first 24 hours. The study suggests that the flavoured solution is superior to the unflavoured solution by its ability to enhance the quantity drunk during a given period and by reducing fatigue during prolonged drinking. However this superiority must be weighed against increased cost involved in adding flavouring and colouring agents.

Oral hydration by glucose-electrolyte solution is a cheap and effective method of correcting dehydration following diarrhoeal attacks. Studies carried out at Cholera Research Laboratory (CRL) had shown that unflavoured Dacca Oral Solution is acceptable to the large majority of dehydrated patients. A small number of the patients however find the salty taste unacceptable. Many among those who drink the solution avidly on admission become "fatigued" later which is manifested by unwillingness to drink or development of nausea and vomiting and also by "falling behind" or drink an inadequate quantity of fluid to match the output.

A study was carried out to find out the role of a suitable flavouring agent in enhancing the acceptability of the Dacca Oral Solution (DOS) and reducing fatigue during prolonged drinking after initial hydration by routine intravenous method. The present report is based on this study.

MATERIALS AND METHODS

Patients were selected from hospitalized cases admitted with a history of acute diarrhoea of short duration. They were initially hydrated by Dacca Intravenous Solution. After full hydration was achieved in 4-8 hours, they were supplied with oral solutions as described below. Intravenous infusion was stopped after full hydration was achieved. Flavouring agent: Natural orange flavour was used throughout the study. Colour (yellow-6) was also added to give the solution its orange colour.

Regime I: Single Solution Study:

Only one type of solution, either plain DOS or added with orange flavour and colour was supplied to alternate patients. A quantity of 500 ml was given to each of the patients initially and they were told to drink the solution frequently in small sips. Supply was replenished as frequently as required. Routine records of intake and output as well as vital signs and hydration status was kept at hourly intervals for an eight hour period after full hydration.

Regime II: Double Solution Study:

Under the regime each patient was provided with a choice of both flavoured and unflavoured solution in equal quantity.

Consumption of each type of solution was recorded at hourly intervals for a period of 8 hours.

Regime III: Prolonged drinking:

Under this regime both the types of solutions were given to all the patients, one of which was the plain DOS and the other flavoured. The observation and drinking of the solutions however was continued for the duration of diarrhoea or for a period of 48 hours.

RESULTS

None of the cases yielded any pathogenic organisms by routine bacteriological methods. All were successfully hydrated during the phase of the study and none required intravenous therapy.

Regime I - Single Solution Study:

When no choice was given, the flavour and colour of the solutions made little difference to the patients. Table 1 shows this clearly. The intake of the solutions were only marginally better with the flavoured type.

Regime II - Double Solution Study:

If the patients were given a choice between two types of solution as was done in this case, the quantitative intake

was much higher in favour of the flavoured solution. Table 2 shows this to be true for both the children as well as adults. The difference were significant.

The other significant finding was the higher intake of the solutions by the patients when a choice was provided. The sum of intake of the plain and the flavoured solution was higher than those shown in the table 1. Although the degree of severity of diarrhoea might be quite different, there is a suggestion in the table 2, that patients may drink a larger quantity of solution by switching from one type to another to avoid fatigue.

Regime III: Prolonged Drinking:

Only cases who had diarrhoea for a period of 48 hours after initial hydration were analysed. Table 3 shows the volume of intake for maintenance during the first 24 hours and the second 24 hours after hydration. Again a choice was provided to each of the patients. It is obvious from the table 3, that under this circumstances the intakes were higher in favour of the flavoured solution. The differences were also significant. The results suggest that flavoured solution would be preferred if a patient is given the choice.

TABLE 1
INTAKE OF PLAIN FLAVOURED ORAL SOLUTION
(IN ML/KG/HR) DURING THE MAINTENANCE PHASE OF
DIARRHOEA WHEN A SINGLE SOLUTION WAS PROVIDED

	Children		Adults	
	(n = 16)	(n = 16)	(n = 16)	(n = 16)
	PLAIN	FLAVOURED	PLAIN	FLAVOURED
2 hrs	4.9	7.3	6.3	6.4
6 hrs	4.3	5.8	4.5	4.4

TABLE 2
 INTAKE OF PLAIN AND FLAVOURED ORAL SOLUTION
 (IN ML/KG/HR) DURING THE MAINTENANCE PHASE OF
 DIARRHOEA WHEN TWO SOLUTIONS WERE SUPPLIED TO EACH OF THE PATIENTS

	Children (n = 12)		Adult (n = 17)	
	<u>PLAIN</u>	<u>FLAVOURED</u>	<u>PLAIN</u>	<u>FLAVOURED</u>
2 hrs	2.7	7.9	2.2	5.3
6 hrs	1.9	4.9	1.4	3.4

TABLE 3
INTAKE OF PLAIN AND FLAVOURED ORAL SOLUTION
(IN ML/KG/HR) DURING THE MAINTENANCE PHASE OF
DIARRHOEA FOR PROLONGED DRINKING WHEN TWO SOLUTIONS
WERE SUPPLIED TO EACH OF THE PATIENTS

	Children (n = 6)		Adult (n = 18)	
	PLAIN	FLAVOURED	PLAIN	FLAVOURED
Ist 24 hrs	2.8	6.7	0.6 ^b	6.4 ^b
2nd 24 hrs	1.3 ^a	5.5 ^a	0.8 ^c	4.4 ^c

a $p < 0.005$

b and c: $p < 0.001$

Success of Dacca Oral Solution as the Sole Rehydrating
Fluid in Moderately Severe Dehydration due
to Non-cholera Diarrhea

M. Mujibur Rahaman
W.F. Verwey
Safiqul Islam

Cholera Research Laboratory
Dacca, Bangladesh

Abstract

Success of Dacca Oral Solution as the Sole Rehydrating Fluid in Moderately Severe Dehydration due to non-cholera Diarrhoea

M. Mujibur Rahaman
W.F. Verwey
Shafiqul Islam
John Middaugh

Attempts were made to rehydrate 451 patients exclusively by Dacca Oral Solution. However, only 277 were found to have a plasma refractive index of 1.029 and above indicating moderately severe dehydration. Rehydration was carried out successfully at the hospital as well as at home in 215 cases with a minimum of professional instruction and supervision. Full rehydration was shown by clinical improvement and drops in plasma solids. There were 62 (22%) failures at hospital requiring further treatment including intravenous infusion. This included 17 cases who were hydrated successfully but developed pyrexia of 100°F and above and were cancelled from the study. If these pyrexial cases were excluded, the rate of failure was found to be 16%. Inadequate drinking for correcting dehydration and persistent vomiting were the principal reasons for the remaining failures; a small percentage showed a dislike for the taste. In spite of drinking oral solution at home during the followup, 6 out of 215 patients had to be brought back to the hospital for intravenous therapy because of development of severe dehydration. Although oral method of hydration may be successful in over 80% of moderately severe dehydration, caution should be exercised in recommending its use as the sole method of rehydration in all types of diarrhoea without backup facility for intravenous infusion.

The usual method of correction of moderately severe dehydration following diarrhoea consists of infusion of intravenous fluid. A large number of such patients come to the outpatient of the Cholera Research Laboratory (CRL) daily. If oral hydration could be adopted for these patients, a substantial saving of skilled nurse's time and expensive intravenous fluid could be achieved. The present report is based on an attempt to evaluate the success of glucose-electrolyte fluid or Dacca Oral Solution in correcting moderately severe dehydration in patients who had hitherto been infused with the intravenous solution.

MATERIALS AND METHODS

Patients:

Subjects for the study were selected from those attending the outpatient department of the CRL with moderately severe dehydration following a bout of liquid diarrhoea. Patients of all ages were selected and those with complications like high fever, pneumonia, infection or frank dysentery were excluded. On their arrival, patients were examined for the severity of dehydration. Those who were pulseless were given fluid immediately by intravenous route and therefore were not included in the study.

Management:

After examination and recording of vital signs the patients were transferred to a special area for the oral hydration. Finger tip blood was collected for total serum solids. Dacca Oral Solution was supplied in 500 ml quantity at a time to each patient and the supply replenished as required. An aid-nurse in plain dress told the patient to drink the solution. No attempt however was made to force intake of the fluid. Intake and output were recorded

hourly along with the vital signs for a period between 4-5 hours. The patients were kept under surveillance during this time for any deterioration of their clinical condition including the status of dehydration. If the patients showed any signs of deterioration or pyrexia above 100°F, the study was cancelled and the patient was transferred to the ward. If the patient had persistent vomiting or was unable to take the fluid by mouth or dehydration remained unchanged or showed signs of actual deterioration, he or she was taken out of the study and was transferred to the ward for intravenous infusion. A blood sample was collected for electrolytes whenever possible from these cases. After 4-5 hours, a finger tip blood was collected for serum solids along with the recording of vital signs and evaluation of the clinical condition.

Rectal swabs were collected and plated on Monsur's, TCBS, SS and MacConkey's agar and subjected to routine culture and identification.

Follow up:

Those patients who were considered to have been successfully hydrated by oral route as shown by improvement

of clinical hydration and confirmed by drop in the value of the serum solids were selected for follow up. Before they were sent home, 6 packets of "Oralyte" was supplied by UNICEF, Dacca and contains sodium of 90 mEq/l in contrast to Dacca Oral Solution which has a sodium content of 120 mEq/l were given to each patient. Verbal instructions were given to the patients or their attendants on methods of mixing and drinking the oral solution if the diarrhoea continued. Instructions were provided to discard leftover solutions after 12 hours. A member of the follow-up team accompanied the patients to their homes to facilitate finding of house during subsequent follow-up visits.

The patients were followed-up daily for a period of five days. During each visit a proforma was filled out, vital sign taken, temperature recorded and a finger tip blood obtained for serum solids. If the patient's condition was considered to have remained unchanged and the diarrhoea continued, advice was given and transportation provided to bring the patient to the hospital for further treatment. No antibiotic was given to any of the patients during this study and follow-up.

RESULTS

A total of 451 cases were selected initially for rehydration on the basis of clinical dehydration of moderate severity. However, cases of mild dehydration were excluded during the final analysis because of insufficient elevation of serum solids. An arbitrary value of 1.029 as the refractive index of serum was selected for defining the lower limit of moderately severe dehydration in these patients. Therefore 277 were subjected to the final analysis. Table 1 shows the breakdown of the patients under study.

Only one case was positive for cholera inaba El Tor, one yielded non-agglutinating vibrio and three were shigella positive, two for S.flexneri and one S.dysenteriae type 1.

Successful Cases:

A total of 187 out of 277 cases (68%) were successfully hydrated in the outpatient department (OPD). Table 2 shows their results. All of the patients showed a drop in serum solid values from their admission figures. It was also found during the follow-up that these patients were able to maintain an adequate intake by mouth so that despite continuing diarrhoea, they did not develop dehydration at home.

Inability to drink an adequate amount to correct dehydration was the most important cause which was followed by persistent vomiting as a cause of failure.

A further analysis of causes of failure at the OPD was carried out by obtaining samples of blood for analysis. An estimation of blood electrolytes was carried out. A total of 13/31 cases where serum electrolytes was done showed acidosis as reflected by serum bicarbonate of ≤ 16 mEq/litre. No other abnormality except a high level of serum slid was found.

Success at OPD but failures at home:

Out of total of 277 patients, 215 were successfully hydrated in the OPD before being sent home for followup. During the followup 14 or 6.5% could not be traced due to change of address and 4 patients did not like to cooperate. Therefore a total 8% of patients were unavailable during followup. On arrival at home, 6 or 3% of patients were found to have a deterioration in their status of dehydration and were brought back to CRL for intravenous therapy. Only 4 patients did not like the taste of "Oralyte" which contains vanilla as a flavouring agent.

Out of 174 mild cases 2 were admitted from the OPD due to pyrexia and the rest were sent home and successfully followed up. There were a total of 12 cases in this group where followup was not successful due to non-availability of patients or non-cooperation from the family.

DISCUSSION

To our knowledge this is the first time that a systematic effort was made to evaluate the practicability of oral hydration in moderately severe dehydration under relatively unsupervised setting. The usual method of dealing with these patients was give an initial infusion of intravenous fluid and maintenance by the oral route. Since there was a potential danger that some of them may go into severe dehydration while attempts were being made to correct the initial dehydration, the study could not have been ethically done outside the hospital. It is obvious that oral hydration is a very successful method of correcting dehydration in these patients. However, some 16% (excluding these developing pyrexia) of the cases may still require intravenous therapy in the setting

we describe. If intense efforts were made to hydrate these patients by repeated instruction from a physician or a uniformed nurse, the failure rate might have been lower. Since one of the objectives of this study was to mimick as much as possible, an unsupervised situation, pursuasion was left entirely to the judgement of the patient's attendant.

Once the patients were sent home, 3% were brought back for intravenous therapy. This is a small but significant number as these are the patients who potentially might go into severe dehydration. While recommending unsupervised or home-based oral therapy for correcting dehydration in diarrhoea no one should lose sight of these facts. It is also important to remember that this observation was carried out almost exclusively in non-cholera diarrhoea where the severity of dehydration is usually less. It is expected that with heavy purging in cholera oral therapy at home may be subjected to a test which is bound to be severer in non-cholera diarrhoea.

TABLE 1

SUCCESS AND FAILURE OF DACCA ORAL SOLUTION
AS THE SOLE REHYDRATING FLUID

SUCCESS OR FAILURE	NO.	%
1. Total Patients Selected	451	100
2. Mild cases excluded from analysis	174	39
3. Patients under study	277	61
Success at O.P.D. & Followup	187	68
Failure at O.P.D. only	62	22
Success at O.P.D. but failure at home	28	10

TABLE 2

SUCCESSFUL USE OF ORAL HYDRATION IN 187 PATIENTS OF MODERATELY SEVERE DEHYDRATION
BY DACCA ORAL SOLUTION

Age Group	No.	Age	Weight	Admn.	Total Serum Solids			Intake ml/kg/1 hr	Stool output ml/kg/1hr	Hour spent at OPD
					5 Hours	Ist Day	2nd Day			
≤ 2	25	1.18 (0.52)	7.47 (1.4)	1.030 (0.001)	1.027 (0.001)	1.026 (0.002)	1.026 (0.001)	14.4 (5.9)	5.5 (3.4)	5.2 (0.8)
3 - 7 yrs.	36	4.38 (1.62)	12.63 (3.2)	1.030 (0.002)	1.027 (0.002)	1.026 (0.002)	1.026 (0.001)	22.8 (8.4)	6.3 (5.7)	5.3 (0.9)
8 - 15	22	10.55 (0.36)	23.8 (7.7)	1.031 (0.002)	1.029 (0.002)	1.026 (0.001)	1.026 (0.001)	21.6 (11.4)	5.1 (4.0)	5.3 (0.8)
16+	104	28.8 (11.39)	43.61 (6.91)	1.031 (0.002)	1.027 (0.002)	1.027 (0.002)	1.027 (0.002)	16.7 (6.1)	4.5 (3.2)	5.3 (0.8)

(Figures in parenthesis indicate standard deviations)

TABLE 3

CAUSES OF FAILURE OF ORAL HYDRATION BY DACCA ORAL SOLUTION
IN MODERATELY SEVERE DEHYDRATION IN 62 PATIENTS

Causes	AGE GROUP				Total	%
	≤ 2	3 - 7	8 - 15	16 ⁺		
Pyrexia > (100°F)	6	4	1	6	17	27.0
Persistent vomiting	3	2	3	7	15	24.0
Inability to correct dehydration	7	4	1	9	21	34.0
Refusal to drink	2	-	-	3	5	8.0
Dysenteric symptom	1	1	-	2	4	6.0
	19 ^I	11 ^{II}	5	27 ^{III}	62	100.0

I and II = *Shigella Flexneri* three cases

III = *Shigella Shiga* in one, NAG in one and cholera in one.

TABLE 4
CAUSES OF FAILURE OF DACCA ORAL SOLUTION
DURING FOLLOWUP AT HOME IN 28 PATIENTS

Causes	≤ 2	3 - 7	8 - 15	16+	Total	%
Brought to O.P.D. for giving I.V.	2	-	-	4	6	21.4
Lost to follow up	2	-	1	11	14	50.00
Dislike of oral fluid	1	-	2	1	4	14.3
Non cooperation from the family	-	1	1	2	4	14.3
Total	5	1	4	18	28	100.0

SHIGELLOSIS AS A CONTINUING CLINICAL PROBLEM IN 1976

M. Mujibur Rahaman

Shigellosis in 1976 continued to be a major clinical problem in the hospitals of the Cholera Research Laboratory (CRL). For the first time (table 1) isolation of shigella exceeded the isolation of cholera by a factor of greater than three. Although this was partly due to the failure of cholera to break out in big epidemic, the importance of shigellosis as a continuing challenge should not be underestimated. Table 1 also shows the importance of shigellosis as a cause of death in CRL where 30% of all deaths were associated with a positive diagnosis of shigellosis. Figure 1 shows the monthly trend of admission from different serotypes of shigellosis in both 1975 and 1976. The most interesting departure from the last year was the continuing increase of admission due to *S. flexneri* and a decrease for *S. dysenteriae* 1 from a peak in April. Table 2 shows the breakdown of isolation of the shigella serotypes. The overall ratio between *S. flexneri* and *S. dysenteriae* 1 seem to be the same as in 1975. The pattern of mortality were also similar. Malnutrition associated with *S. flexneri* was the most important factor contributing to the higher mortality in this serotype. Table 3 shows the breakdown by age in the admission and mortality pattern of all shigellosis. It is clear that mortality was highest in the youngest age group, particularly in those below 5 years of age where the rates of admission were also the highest. Leukemoid reactions as shown in table 4 continues to plague us suggesting that the severity has not decreased among the admitted cases. *S. dysenteriae* 1 continues to be the major contributor for the leukemoid reaction.

PROBABLE PATHOGENESIS OF HAEMOLYTIC AND HAEMOLYTIC- UREMIC SYNDROME IN SEVERE SHIGELLOSIS

1. EXTENSIVE AND SEVERE ULCERATION IN THE COLON.
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RESULTING IN LEUKEMOID REACTION IN SOME CASES.
3. SEVERE TENESMUS GIVING RISE TO INCREASED POSITIVE
PRESSURE IN THE COLON.
4. BACTEREMIA AND/OR ENDOTOXEMIA THROUGH THE ULCERATED
AREAS CAUSING "PRIMING" OF THE SUBJECTS.
5. FURTHER BACTEREMIA AND/OR ENDOTOXEMIA CAUSES
SCHWARTZMAN TYPE REACTION BY DEPOSITION OF FIBRIN ON
THE MICROVASCULATURE.
6. FIBRIN DEPOSITS CAUSE MICROANGIOPATHIC - HAEMOLYTIC
ANAEMIA.
7. EXTENSIVE INVOLVEMENT OF KIDNEYS BY DEPOSITS CAUSE
HAEMOLYTIC-UREMIC SYNDROME.

TABLE 1

ADMISSION AND DEATHS IN CRL, 1976

Admission		
6785		
Shigellosis	Cholera	Other
1126	358	5301
(17%)	(5%)	(78%)
Deaths		
225 (3.3% of total admission)		
Shigellosis	Cholera	Other
67	2	156
(30%)	(0.9%)	(69%)

* A substantial proportion had symptoms of dysentery not confirmed by bacteriology.

TABLE 2

ADMISSION AND DEATHS STATISTICS IN SHIGELLOSIS - 1976

TOTAL SHIGELLA ADMISSION

1126		
SHIGA	FLEX	OTHER
317	605	204
(28.2%)	(53.7%)	(18.1%)

DEATH

67		
SHIGA	FLEX	OTHER
15	39	13
(22.4%)	58.2%	19.4%

% of total death

% of total respective serotype

% of total shigella admission

4.7%

1.3%

6.5%

3.5%

6.4%

1.2%

TABLE 3

ADMISSION AND DEATHS IN SHIGELLOSIS ACCORDING
TO AGES IN HOSPITALIZED PATIENTS IN 1976

Age group	F L E X N E R I			S H I G A			O T H E R		
	Total Cases	Death	%	Total Case	Death	%	Total Case	Death	%
< 1	122	13	11	22	3	14	36	2	6
1 - 2	145	14	10	60	6	10	37	4	11
3 - 5	116	10	9	87	4	6	37	5	14
6 -10	48	1	2	43	1	2	28	2	7
11 -19	25	-	-	38	-	-	12	-	-
20+	150	1	1	67	1	1	53	-	-
All ages	606	39	6	317	15	5	203	13	6

TABLE 34

LEUKEMOID CASES IN SHIGELLOSIS IN CRL, 1976

77 (6.8 of total Shigella Admission)		
Shiga	Flex	Others
(79.2)	14 (18.1)	2 (2.6)
9.2 of total shiga admission)	(2.3 of total flex admission)	(1% of total other shigella admission)
DEATH		
20 (25.9 of total leukemoid in shigella)		
(29.8% of total death in shigella)		
Shiga	Flex	Others
(65%)	6 (30%)	1 (5%)
6.7% of total death in shiga)	(15.4% of total death in flex)	(7.7% of total death in other shigella)
0.1% of total shiga admission)	(1% of total flex admission)	(0.5% of total other shigella admission)

DISTRIBUTION OF LEUKEMOID CASES (1976), ACCORDING TO AGE AND SEROTYPE

Age Group	F L E X N E R I			S H I G A			O T H E R		
	Total case	Leukemoid	%	Total case	Leukemoid	%	Total case	Leukemoid	%
< 1	122	3	2	22	8	36	36	1	3
1 - 2	145	4	3	60	22	37	37	-	-
3 - 5	116	5	4	87	19	22	37	-	-
6 - 10	48	2	4	43	9	21	28	1	4
11 - 19	25	-	-	38	1	3	12	-	-
20+	150	-	-	67	2	3	53	-	-
All ages	606	14	2	317	61	19	203	2	1

DISTRIBUTION OF LEUKEMOID CASES (1976)

ACCORDING TO AGE AND SEROTYPE

Age group	Flexneri	Shiga	Other	Cholera	Bacteriologically negative	Total	%
<1	3	8	1	1	15	28	23
1 - 2	5	23	-	-	9	37	31
3 - 5	5	20	-	2	4	31	26
6 -10	2	9	1	-	2	14	12
11 -19	-	1	-	-	2	3	2
20 +	-	2	-	1	5	8	7
All ages	15	63	2	4	37	121	100

ENTEROTOXIGENIC ESCHERICHIA COLI (ETEC) DISEASE IN BANGLADESH: CLINICAL, THERAPEUTIC AND LABORATORY ASPECTS

Michael H. Merson, R. Bradley Sack, Sirajul Islam, Golam Saklayen,
Nural Huda, A.K.M. Golam Kibriya, Abdullah-al-Mahmud, Qazi Shafi Ahmed,
Anwarul Quder, Robert H. Yolken, Mujibur Rahaman, Albert Z. Kapikian.

Johns Hopkins University, Baltimore, Maryland,
The Cholera Research Laboratory, Dacca, Bangladesh,
and the National Institutes of Health, Bethesda, Maryland.

Many studies have demonstrated the importance of enterotoxigenic E. coli (ETEC) as a cause of diarrhea. In Bangladesh 3 previous studies have shown these organisms to be responsible for many adult cases of cholera-like diarrhea (4, 6, 7). In this study we attempted to define, in hospitalized patients, detailed clinical and laboratory features of ETEC disease, the effect of tetracycline on its clinical course, and an efficient means of its diagnosis.

MATERIALS AND METHODS

One hundred seventy six adult males presenting to the Cholera Research Laboratory Hospital in Dacca, Bangladesh, from October to December 1976 with a history of acute watery diarrhea, appearing to have lost 5% or more of their body weight, were studied. All had admission stools examined for Vibrio cholerae by darkfield microscopy and were negative. On admission a history and physical were performed, stool was obtained by rectal catheter for culture, microscopic analysis, and electrolyte determination, and a venous blood sample was drawn for measurement of electrolytes and specific gravity and for acute serology. Replacement intravenous fluid therapy was administered within four hours at which time a fingertip blood specimen was obtained for measurement of specific gravity and a complete blood count. Two hours after admission tetracycline or placebo therapy was initiated with 500 mg capsules for patients weighing 30 or more kilograms and 250 mg capsules for those weighing less than 30 kilograms. Capsules were given at 6 hour intervals for 72 hours. All subsequent stool losses were replaced with intravenous fluid, and stool volume and fluid replacement were recorded every 2 hours. Follow-up stool cultures were obtained by rectal swabs every 24 hours for 4 days, and 7 and 14 days after admission. Convalescent sera were obtained at 14 days from 102 cases.

All stools were examined for salmonellae, shigellae, and vibrios

by standard CRL procedures and for rotavirus by the ELISA assay (11). Patients with those organisms were dropped from further analysis. Up to ten lactose-positive colonies with typical E. coli morphology were individually picked from the MacConkey plate of each stool culture. The first 5 colonies were repicked to form a 5 pool and all 10 colonies were then repicked to form a 10 pool. Isolates and pools from admission cultures were grown in trypticase soy broth with 0.6% yeast extract (TSBY) in a shake (60-70 shakes/min) culture at 37°C and examined for production of heat-labile toxin (LT) by the CHO assay (2) and heat-stable toxin (ST) by the infant mouse assay (5). Isolates and pools from follow-up cultures of LT-ST and LT E. coli cases were grown in syncase broth in a resting 48 hour culture and examined for LT production in the adrenal cell assay (8). Isolates and pools from follow-up cultures of ST cases were tested for ST production after 18-24 hour growth on a roller drum (22rev/min).

The Sereny test (10) for invasiveness was used to screen lactose-positive and negative isolates from 100 patients.

One lactose-positive colony from each patient was confirmed as E. coli and serotyped at the WHO Reference Center in Copenhagen. Antibiotic sensitivity tests were carried out by the Kirby-Bauer method (1) on these enterotoxigenic isolates and a sample of non-toxigenic E. coli.

In an attempt to detect LT and ST in the stools of cases 2-3 ml aliquots of admission stools that had been stored at -40°C for approximately 5 months were defrosted and spun at 10,000 RPM for 30 minutes. Supernatants were then tested in the adrenal cell and infant mouse assays. Specimens were run by 2 different procedures. In the first the stools were kept at room temperature for 45-90 minutes after centrifugation and prior to toxin testing (Method A); in the second they were kept at all time at 4°C (Method B).

Anti-toxin neutralization titers were determined for the 102 paired sera by the adrenal cell method (9). Antibody titer measurements to rotavirus were measured by complement fixation (3) and ELISA assays (12).

RESULTS

A diagnosis was made in 147 (86%) of the 176 cases. Twenty-three (13%) were mixed infections. Data in Table 1 shows the number of cases

in which each organism was found as a single pathogen and the total number of cases in which the organism was isolated. ETEC were isolated from 109 (62%) cases and was the only pathogen in 95 (54%). These 95 will be referred to as E. coli cases. Other pathogens isolated included Vibrio cholerae, Vibrio parahaemolyticus, non-cholera Vibrios (NAG), Shigella Flexneri, Shigella boydii, Shigella sonnei, Salmonella C and invasive E. coli. In 8 of the 9 rotavirus infections, the virus was detected in stool. Nine (64%) of the 14 NAG infections, 12 (75%) of the 16 shigella infections and 8 (89%) of the 9 rotavirus infections were mixed infections.

Table 1

FREQUENCY OF ORGANISMS ISOLATED FROM 176 STUDY CASES

<u>Organism</u>	<u>Isolated</u>		<u>Total Infections</u>	
	<u>No.</u>	<u>%</u>	<u>No.</u>	<u>%</u>
Enterotox. <u>E. coli</u>	95	54	109	62
<u>V. cholerae</u>	12	7	14	8
<u>V. parahaemolyticus</u>	6	3	6	3
NAG (Non-cholera vibrio)	5	3	14	8
<u>Shigellae</u> *	4	2	16	9
Invasive <u>E. coli</u>	1	1	1	1
Rotavirus	1	1	9	5
<u>Salmonella C</u>			1	1

* Includes 11 cases with S. flexneri, 3 cases with S. boydii and 2 cases with S. sonnei.

Of the 95 E. coli cases 62 (65%) had LT-ST producing strains, 30 (32%) had ST producing strains, and 3 (3%) had LT producing strains. The clinical features of disease caused by LT-ST E. coli and ST E. coli were similar (Table 2). Almost all the E. coli cases had vomiting with onset on the average 3.8 hours after onset of diarrhea. Two thirds had muscle cramps, 51% had abdominal pain and 19% had fever lasting an average of 4 hours.

Selected laboratory features of these cases are shown in Tables 3 and 4. For all cases the mean admission serum sodium (134 meq/L), potassium (4.8 meq/L), and chloride (102 meq/L) and the mean hematocrit value (37.9%) measured 4 hours after admission were normal. The mean total white count 4 hours after admission was elevated (14,600); many.

patients had a shift to the left. Stool microscopic examinations revealed 0 to 5 polys per high powered field in 82% of cases, but 8(9%) cases had 6 to 10 and 7 (8%) cases had greater than 10 polys per high power field. Over 90% of patients had parasitic infestation. For all these values there was no difference between LT-ST and ST E. coli cases. Data in Table 4 shows that the mean stool electrolyte and osmolarity values for the LT-ST and ST E. coli cases were similar to each other and to those of the cholera, other vibrio, and unknown etiology cases.

Table 2

CLINICAL FEATURES OF ENTEROTOXIGENIC E. COLI CASES

	LT - ST*		ST		All**	
	(N=55)		(N=30)		(N=88)	
	No.	%	No.	%	No.	%
Vomiting	53	96	28	94	84	96
D - V (hrs)***	4.1		3.7		3.8	
Muscle cramps	37	67	20	66	58	66
Abdominal pain	25	46	18	60	45	51
Temp. (over 100°F axillary)	9	16	8	27	17	19
Fever dur. (hrs)	3.7		4.4		4.0	

* Excludes 7 cases who took tetracycline prior to admission

** Includes 3 LT cases

*** Interval from diarrhea to vomiting

Table 3

ADMISSION LABORATORY FEATURES OF ENTEROTOXIGENIC E. COLI CASES

(Mean Values)

	LT - ST*		ST		All**	
	(N=55)		(N=30)		(N=88)	
Serum Na ⁺ (meq/L)	133		134		134	
Serum K ⁺ (meg/L)	4.9		4.6		4.8	
Serum Cl ⁻ (meg/L)	102		102		102	
Hct*** (%)	37.7		38.4		37.9	
WBC*** (cells/mm ³)	15,100		13,100		14,600	
Fecal Leucocytes (No./hpf)		*		*		*
0 - 5	44****		25		72	
6 - 10	6		2		8	
> 10	5		2		7	

* Excludes 7 cases who took tetracycline prior to admission.

** Includes 3 LT cases.

*** Measured 4 hours after admission.

****No. of cases

Table 4

STOOL ELECTROLYTES* BY ETIOLOGY

<u>Diagnosis</u>	<u>Na</u> <u>(meq/L)</u>	<u>K</u> <u>(meq/L)</u>	<u>Cl</u> <u>(meq/L)</u>	<u>HCO₃</u> <u>(meq/L)</u>	<u>OSMOL</u> <u>(osm/L)</u>
Enterotox. <u>E. coli</u>					
LT-ST (N=50)	108±4	27±2	78±3	45±4	306±3
ST (N=23)	108±6	24±3	72±4	37±2	327±14
LT (N=3)	129±4	18±3	89±3	42±4	309±3
Cholera (N=11)	115±7	27±4	88±6	38±3	322±16
Other Vibrios (N=10)	124±2	14±2	82±4	36±4	344±15
Unknown (N=26)	114±4	21±2	77±4	39±3	322±8

* Mean values ±1SE

Twenty three (61%) of the 38 LT-ST cases and 3 of 4 LT cases had 4-fold or greater rise in titer to LT. None of 22 ST patients had a titer rise. Only 1 of 38 other patients with diarrhea due to NAGs, Shigella, rotavirus or of unknown etiology had a titer rise, and this case had a NAG infection and only 1 E. coli isolated from his admission stool specimen which was not enterotoxigenic.

Table 5 presents data that attempts to compare the severity of disease in 31 LT-ST and 16 ST cases. Although none of the differences are significant, the LT-ST cases tended to be older, had greater loss of body weight from their illness, more often appeared severely dehydrated on admission, and had a higher admission serum specific gravity and a lower serum bicarbonate. Although the duration of diarrhea before hospital admission was almost identical, the total duration of diarrhea in the LT-ST cases (32.6 hours) was significantly greater than that in the ST cases (22.5 hours). LT-ST cases also purged a significantly greater volume of stool than ST cases (35cc/Kg versus 11cc/Kg) and required more intravenous fluid (111cc/Kg versus 81 cc/Kg). The duration of excretion of organisms in stool was almost the same: 2.4 days for LT-ST cases and 2.1 days for ST cases.

The effect of tetracycline on 20 LT-ST cases was compared with 25 cases who received placebo (Table 6). Persons in both groups had tetracycline-sensitive strains and purged for at least 4 hours after admission. In the 2 groups there was no significant differences in clinical or laboratory parameters on admission, although the placebo group had a slight trend toward a more severe illness with a lower mean systolic pressure, greater percent weight loss, more cases appearing severely dehydrated, and a higher admission specific gravity and lower bicarbonate. Compared to the placebo cases the tetracycline cases had a shorter duration of illness in the hospital (25 hours

versus 18.8 hours), less stool volume (43 cc/Kg versus 28 cc/Kg), and less intravenous fluid requirement (120 cc/Kg versus 105 cc/Kg), although these differences were not statistically significant. There was a highly significant difference in mean duration of excretion in the 2 groups - 2.2 days in the placebo group and 0.6 days in the tetracycline group. This effect of tetracycline on bacterial excretion was apparent after only one day of therapy (Table 7). Twenty-four hours after admission 25 of 29 placebo cases were still culture positive for LT-ST organisms compared to 9 of 22 in the tetracycline group. This difference became more apparent over the next 72 hours. One case in each group was positive on day 7 or 14 follow-up cultures.

Table 5

COMPARATIVE FEATURES OF LT - ST AND ST CASES*

	LT-ST (N=31)	ST (N=16)	
Age (yrs)	33	24	P=.07**
Discharge weight (kg)	41.6	38.5	
Admission systolic BP (mmHg)	76	79	
Weight loss (%)	7.5	6.3	P=.4 **
Clinical severity (cases)			
Moderate	10	9	
Moderate-severe	13	6	P=<.02****
Severe	8	1	
Admission serum spec. gravity	1.0334	1.0327	P=.09**
Admission serum bicarbonate (meq/L)	16.0	17.8	P=<.1***
Duration of diarrhea (hrs)			
Pre-admission	11.9	11.7	
Total	32.6	22.5	P=<.05***
Stool Volume (cc/Kg)	35	11	P=.03**
I.V. Volume (cc/Kg)	111	81	P=<.05***
Stool excretion (days)	2.4	2.1	

* All values are means except these for clinical severity.

** Mann-Whitney U Test

*** t test.

**** chi square

Table 6

EFFECT OF TETRACYCLINE ON LT-ST*
ENTEROTOXIGENIC E. COLI DISEASE

	<u>Placebo</u> <u>(N=25)</u>	<u>Tetracycline</u> <u>(N=20)</u>	
Age (years)	34	30	
Discharge weight (Kg)	41.9	39.4	
Admission systolic BP(mmHg)	72	88	
Weight loss (%)	17.8	6.4	
Clinical severity (cases)			
Moderate	6	5	
Moderate-severe	11	11	
Severe	8	4	
Admission serum spec. gravity	1.0347	1.0338	
Admission serum bicarbonate(meq/L)	16.1	16.8	
Duration of diarrhea (hrs)			
Pre-admission	11.8	11.7	
Hospital	25.0	18.8	P<.2**
Stool volume (cc/Kg)	43	28	P=.34***
I.V. Volume (cc/Kg)	120	105	P<.3**
Stool excretion (days)	2.2	0.6	P<.001**

* All values are means except for clinical severity.

** t test

*** Mann Whitney U test.

Table 7

NUMBER OF CASES EXCRETING LT-ST E. COLI

<u>Culture</u>	<u>Placebo</u> <u>(N=29)</u>	<u>Tetracycline</u> <u>(N=22)</u>	
24 hrs.	25	9	P<.01**
48 hrs.	21	3	P<.001**
72 hrs.	16	1	P<.001**
96 hrs.	5	0	
7 days	1(24)***	0(14)	
14 days	0(17)	1(11)	

* Includes 6 cases who did not purge in hospital

** t test

*** Numbers in parenthesis is number cultured.

In an attempt to further assess the effect of tetracycline on duration of diarrhea, Figure 1 shows the number of cases in the placebo and tetracycline groups by duration of diarrhea in the hospital. Almost all of the cases that purged 28 hours or longer were in the placebo group. As shown in Table 8 there was a significantly greater number of cases in the placebo group than in the tetracycline group whose duration of illness was greater than 28 hours.

Figure 1

See Page -57(a)

Table 8

DURATION OF DIARRHEA IN LT-ST CASES
IN PLACEBO AND TETRACYCLINE GROUPS

<u>Duration</u>	<u>Placebo</u>	<u>Tetracycline</u>
≤28 hours	17	19
>28 hours	8	1

P = 0.03, Fischer's exact test.

The percentage of cases in the placebo and tetracycline groups who had a 4-fold or greater rise in anti-LT titers and the geometric mean acute and convalescent titers in the 2 groups are shown in Table 9. Thirteen of 18 (72%) placebo cases and 6 of 12 (50%) of tetracycline cases had a 4-fold or greater titer rise; the difference was not significant. Similarly, the mean convalescent titer in the placebo group (21.2 units) was greater than that in the tetracycline group (16.4 units), but this difference was also not significant.

Table 9

RELATIONSHIP BETWEEN TETRACYCLINE THERAPY AND
ANTITOXIN RESPONSE IN LT-ST CASES

	<u>Placebo</u> <u>(N=18)</u>	<u>Tetracycline</u> <u>(N=12)</u>
Four-fold or greater titer rise	13/18 (72%) ¹	6/12 (50%) ¹
Geometric mean titer (unit/ml)		
Acute	3.0	6.1
Convalescent	21.2 ²	16.4 ²

1.2

Difference in placebo and tetracycline groups not significant

Only 18 cases were included in the assessment of tetracycline on ST disease - 10 received placebo and 8 received tetracycline. Except for age and clinical severity these groups were well matched (Table 10). Tetracycline had no effect on disease duration, stool volume or intravenous fluid requirement. However tetracycline did significantly shorten the duration of bacterial excretion from 1.8 to 0.4 days. As was the case of LT-ST disease this effect was seen within 24 hours after admission (Table 11). 12 of 14 placebo cases were positive for ST organisms at 24 hours compared with 4 of 10 tetracycline treated cases. By 72 hours only placebo patients were still culture positive. No cases were culture positive on day 7 or day 14.

Table 10

EFFECT OF TETRACYCLINE ON ST DISEASE*

	Placebo (N=10)	Tetracycline (N=8)	
Age (years)	25	40	P=.03**
Discharge weight (Kg)	41.0	43.3	
Admission systolic BP (mmHg)	88	93	
Weight loss (%)	5.2	4.8	
Clinical severity (cases)			
Moderate	8	4	
Moderate-severe	1	4	
Severe	1	0	
Admission plasma spec. gravity	1.0326	1.0320	
Admission serum bicarbonate (meq/L)	18.3	19.2	
Duration of diarrhea (hrs).			
Pre-admission	10.5	8.0	
Hospital	17.2	15.5	
Stool volume (cc/Kg)	17	24	
I.V. Volume (cc/Kg)	85	86	
Stool excretion (days)	1.8	0.4	P=.01**

* All values are means except for clinical severity.

** Mann Whitney U test

Table 11

NUMBER OF CASES EXCRETING ST E. COLI

<u>Culture</u>	Placebo (N=14)	Tetracycline (N=10)	
24 hours	12	4	P<.05*
48 hours	8	1	P<.05*
72 hours	6	0	P<.05*
96 hours	2	0	
7 days	0(12)**	0(9)	
14 days	0(11)	0(7)	

Includes 6 cases who did not purge in hospital.

* t test.

** number in parenthesis is number cultured.

Table 12 shows the antibiotic resistance patterns of the ETEC and a group of 76 nontoxigenic E. coli isolated from admission stool specimens of 11 ETEC and 63 non-E. coli cases. The most common resistance pattern was sulfa/strep in the LT-ST E. coli, tet alone and sulfa/strep in the ST E. coli and sulfa/strep/tet in the control strains. Eleven (16%) of 69 LT-ST E. coli were resistant to one or more antibiotics compared to 11 (32%) of 34 ST E. coli and 28 (37%) of 76 control strains. The difference in resistance between LT-ST and ST strains was of border-line statistical significance ($p=.06$); the difference between the LT-ST and controls was highly significant ($p<.01$). Significantly more of the LT-ST E. coli (96%) were sensitive to tetracycline than the ST E. coli (80%) ($P<.05$ by chi square analysis) or control strains (67%) ($P<.001$ by chi square analysis).

Table 12

ANTIBIOTIC SENSITIVITY OF ENTEROTOXIGENIC
E. COLI AND CONTROL STRAINS

<u>Antib. Res.</u>	<u>LT/ST(N=69)</u>	<u>ST(N=34)</u>	<u>LT(N=6)</u>	<u>Control(N=76)</u>
S/ST	7	4		3
S/ST/TET	2	1		14
S/ST/TET/CH		1		
S/ST/TET/KA	1			
S/ST/TET/AMP			1	2
S/ST/AMP/KA	1			
S/ST/SM/CH/TET				4
TET		5		3
TET/S				1
TET/CH				1
Total:	11*#	11*	1	28#
Percent:	16	32	17	37

* chi square = 3.34, $p=.06$

chi square = 7.00, $p<.01$

The 0 serogroups of the ETEC strains are shown in Table 13. Although the 69 LT-ST strains included 11 serogroups, 59 (85%) of the strains were in one of 4 serogroups - 06, 08, 078, 0115. In contrast the 34 ST strains were distributed among 15 0 serogroups and the two most common serogroups were 078, one of the 4 groups that included the majority of the LT-ST strains, and 0128, a classical enteropathogenic serotype. The 6 LT strains were distributed among 6 0 serogroups.

Table 13

O GROUP AND ENTEROTOXIN TYPE OF E. COLI STRAINS
ISOLATED FROM 109 ENTEROTOXIGENIC E. COLI

<u>O Group</u>	<u>LT-ST</u>	<u>Enterotoxin Type</u>	
		<u>ST only</u>	<u>LT only</u>
04		1	
06	9		
07		1	
08	19 (1)	1	1
015	1		
020	1	3 (2)	
025	2		
029		2	
034		2	
048			1
063	1	2	1
078	20 (3)	5	
085	2	1	
096			1
0114		1	1
0115	11	2	
0123		1	
0126	1	1	
0128		5	
0148		1	
0159			1
OX2	1		
Neg 01-0163	1	3	
Rough		2	

(1) Includes one 08:060

(2) Includes three 020:0153

(3) Includes three 078:044 and one 078:0137

To determine the most expedient way to diagnose ETEC disease in these patients we compared results from toxin testing 10 individual isolates, a pool of 5 isolates and a pool of 10 isolates from each stool culture. Of the 109 E. coli cases detected on admission by testing 10 individual picks the 10 pool was positive in 105 (96%) and the 5 pool in 104 (95%). If only the first pick from each patient had been toxin-tested, 100 (92%) of the cases would have been identified; if the first 2 picks had been tested 104 (95%) would have been diagnosed.

Table 14

SENSITIVITY OF POOLING ISOLATES FOR LT TESTING*

<u>Positive Picks (of 10)</u>	<u>Cases (no.)</u>	<u>Pool Positive No.</u>	<u>%</u>
1	6	9	56
2	9	6	66
3	4	3	75
4	6	5	83
5	2	1	50
6	7	5	71
7	6	6	100
9	7	7	100
9	7	7	100
10	24	24	100

* Resting 48 hr. culture in syncase broth; adrenal cell assay;
36 LT-ST and 2 LT only strains.

Table 15

SENSITIVITY OF POOLING ISOLATES FOR ST TESTING*

<u>Positive Picks (of 10)</u>	<u>Cases (no.)</u>	<u>Pool Positive No.</u>	<u>%</u>
1	5	2	40
2	9	8	89
3	2	2	100
4	1	1	100
5	1	1	100
6	2	2	100
7	2	1	50
8	8	8	100
9	2	2	100
10	6	6	100

* Roller drum culture (22 revol/min) in trypticase soy broth with
0.6% yeast extract

Results of the follow-up cultures in which most patients had less number of toxin-positive organisms provided an assessment of the sensitivity of pooling 10 isolates for LT and ST testing. As shown in Table 14 when 7 or more of the 10 isolates were positive, the 10 pool was always positive. The diagnostic yield gradually decreased

as less picks became positive and was only 56% when 1 of 10 picks was positive. Similar data for ST testing was more limited because of the few number of cases (Table 15). Except for 1 pool of 7 positive picks, all pools with 3 or more of 10 ST positive isolates were positive. Eight of nine pools with 2 positive isolates and 2 of 5 pools with 1 positive isolate were toxin-positive.

Results of testing directly for LT and ST in admission stools are shown in Tables 16 and 17. Significantly more stools known to contain LT-ST organisms were positive for LT by Method B (85%) than by Method A (51%) ($p < .01$). One of 6 cases with LT E. coli and 11 of 13 cholera cases were also positive. None of 33 ST cases, 22 cases with other diagnoses, or 29 cases with no known diagnosis were positive. Twelve (36%) of 33 LT-ST cases were positive for ST by Method A and 14 (44%) of 32 by Method B. Six (21%) of 29 ST cases were positive. No LT or cholera cases were positive but 2 cases with mixed shigella and rotavirus infections and 4 (17%) of 24 cases with no known diagnosis were positive.

Table 16

DETECTION OF LT IN STOOL SUPERNATANTS

<u>Laboratory Diagnosis</u>	<u>Specimens (No.)</u>	<u>Positive No.</u>	<u>%</u>
Enterotox. <u>E. coli</u>			
LT-ST (Method A)	35	18	51*
LT-ST (Method B)	33	28	85*
ST	33	0	0
LT	6	1	17**
Cholera	13	11	77**
Others	22	0	0
No diagnosis	29	0	0

* Difference in Method A and Method B significant, chi square = $P < .01$

** All positive stools run by Method B.

Table 17.

DETECTION OF ST IN STOOL SUPERNATANTS

<u>Laboratory Diagnosis</u>	<u>Specimens (No.)</u>	<u>Positive</u>	
		<u>No.</u>	<u>%</u>
Enterox. <u>E. coli</u>	33	12	36
LT-ST (Method A)	33	12	36
LT-ST (Method B)	32	14	44
ST	29	6*	21
LT	5	0	0
Cholera	13	0	0
Others	10	2**	20
No diagnosis	26	4***	15

* 1 of 7 positive by Method A and 5 of 21 positive by Method B

** Includes two with mixed shigellae and rotavirus infections positive by Method B.

*** 0 of 6 positive by Method A and 4 of 20 by Method B.

CONCLUSIONS

This study has demonstrated that in the time period under study a diagnosis could be made in 86% of adult cases of cholera-like diarrhea admitted to the Cholera Hospital with moderate to severe dehydration. In up to 62% of cases ETEC were responsible. In 13% of the cases more than 1 pathogen was isolated. More than 60% of the cases in which rotavirus, shigellae or NAG organisms were isolated were mixed infections.

Of the ETEC cases LT-ST strains were isolated from about 2/3, ST strains from about 1/3 and LT strains were found in less than 5%. Frequent clinical features of the E. coli cases included vomiting, muscle cramps and abdominal pain; fever was documented in 20% of cases. Serum electrolytes were normal and stool electrolytes were similar to those seen in cholera. Antibody rise to LT was found in 61% of LT-ST cases but in none of the ST cases. LT-ST E. coli disease was clinically more severe than ST E. coli disease.

Tetracycline treatment of LT-ST disease resulted in a slightly earlier termination of diarrhea. Tetracycline had no effect on ST disease. Tetracycline did however shorten the duration of excretion of organisms in both diseases. Tetracycline had no significant effect on antibody response to LT. LT-ST strains had significantly less antibiotic resistance than ST or non-toxigenic strains; this data was not controlled for serotype. LT-ST strains but not ST strains

appeared to be clustered in a few O serogroups.

For laboratory diagnosis of ETEC disease pooling was a less sensitive method than testing individual strains for LT when less than 6 of 10 isolates were enterotoxigenic and for ST when less than 3 were toxigenic. However, for work up of admission specimens in our hospitalized patients there was no advantage to pooling as diagnosis could be made with nearly equal frequency by testing only 1 or 2 isolated colonies. As an alternative diagnostic approach we found LT could be detected in stool supernatants in 80-90% of our hospitalized cases. Testing for ST in supernatants appeared to be less sensitive and perhaps less specific.

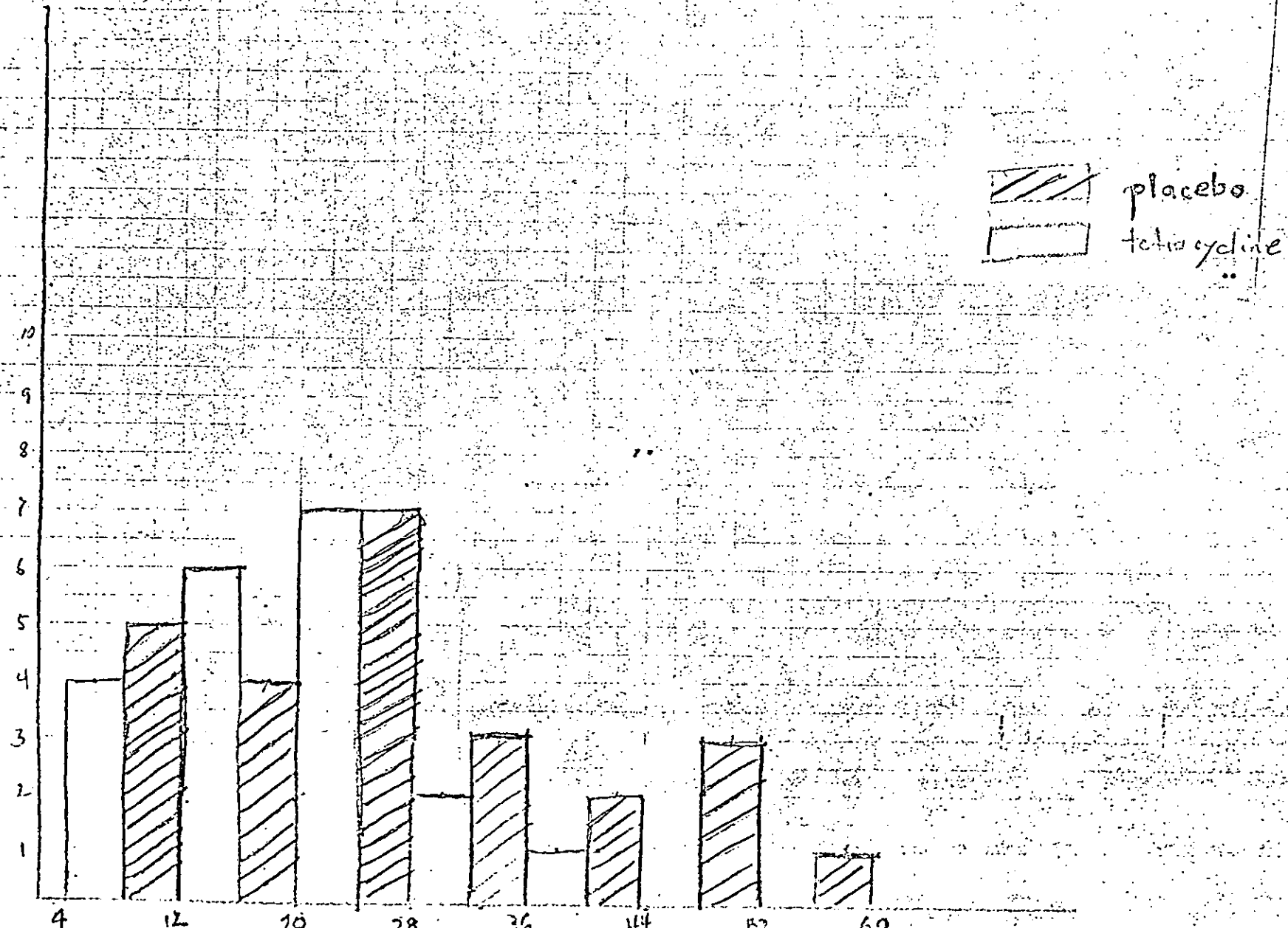
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DURATION OF DIARRHEA AFTER HOSPITAL ADMISSION IN ENTEROTOXIGENIC E. COLI LT-ST Cases.

NUMBER OF CASES



RECENT TRENDS IN FERTILITY AND MORTALITY
IN A RURAL BANGLADESH

BY

A.K.M. Alauddin Chowdhury*
George T. Curlin**

* Head, Statistics Branch, Cholera Research Laboratory
G.P.O. Box 128, Dacca-2, Bangladesh

** Head, Epidemiology Division, Cholera Research Laboratory
G.P.O. Box 128, Dacca-2, Bangladesh

ABSTRACT

One of the major difficulties encountered in assessing the severity of population problems in developing countries and in evaluating the effects of programs and policies on population trends is the lack of accurate data on a variety of demographic variables. Efforts to supply the needed information have generally met with only qualified success. The Cholera Research Laboratory in Dacca, Bangladesh, has developed a data collection system for a densely settled rural population which appears to generate reliable data. This paper presents the trend of some of the demographic data produced in the last ten years by this system.

INTRODUCTION

The Cholera Research Laboratory was established in Dacca, Bangladesh in 1960 to develop, improve and demonstrate measures for the prevention and eventual eradication of cholera. An essential component of this program is controlled field trials of cholera vaccines which require accurate denominator data. Villages in Matlab Thana, Comilla District, located in the south central area of Bangladesh, were selected for these studies. The basic design of these field trials involves taking a complete census in the villages under study and assigning an identifying census number to every individual. Since field work was begun field workers have visited every household daily to make inquiries about the occurrence of acute diarrheas. This frequent contact with the inhabitants has remained an essential feature of the CRL program.

The first field work began in 1963 and initially covered 23 villages with a population of 28,000 individuals. In 1964, the trial area was expanded to include an additional 35 villages covering a total population of 60,000. The area was further expanded in 1966 to cover an additional 74 villages, giving a total population of 112,000 in 132 villages under surveillance. This area is named as the Old Trial Area (OTA).

In 1968, 101 more villages with a population of 109,000 added to the study. These 101 villages are called New Trial Area (NTA). Since the expansion of the Old Trial Area in March-April 1966, the field staff have been maintaining a regular registration of all births, deaths, and migrations, in addition to carrying out their regular surveillance for acute diarrheal diseases. The surveillance of vital events has been maintained in the New Trial Area since its inclusion in 1968.

This paper describes the trends in fertility and mortality rates of the last 10 years of Old and New Trial Areas separately.

THE STUDY AREA

Matlab is located in the deltaic area of Bangladesh, which is intersected by innumerable tidal rivers, canals, and branches of the major rivers; the Ganges, Brahmaputra and Meghna. The climate is subtropical, with the Tropic of Cancer passing through the area.

The people are almost entirely indigenous Bengalis. Over 80 percent are Muslim; the remainder are Hindu. Less than 25 percent of the adults are literate. The principal occupations are agriculture and fishing, the latter being mainly a Hindu occupation. Most of the farmland is given to rice, the dietary staple, and jute, the cash crop.

Villages have an average population of 1,000 persons. Each village is divided into many bars, a unit of two or more patrilineally-related families. Each family has its own one or two-room house with a mud floor, jute stick walls, and a thatched grass or galvanized iron roof. The houses in bars are arranged around a central courtyard. The average population density is approximately 2,000 per square mile. During the monsoon, when most of the land is under water, the actual population density is much higher. This area has only one road; internal communication is

accomplished primarily by country boat or on foot. A few motorised passenger launches provide transport to Dacca and other large towns.

METHODS

In the Old Trial Area the census was taken in March and April of 1966 by four teams, each consisting of two trained field workers. The workers went from house-to-house collecting the basic information from each family on a simple form. In the New Trial Area census was taken in March - April, 1968 by a similar method.

A family was defined as a hearth unit, i.e. a group which eats together. Families were identified by the name of the family head, barl and the location. The family members were listed by name, age and sex. Efforts were made to obtain reasonably accurate ages by beginning with the age of the youngest child in the family and then asking the ages of the older children and of the parents and other family members. The census workers made an effort to correct any obvious discrepancies in the ages reported by the informants. No effort was made, however, to verify the reported ages by such means as dating of historical events.

At the completion of this census, triplicate copies of the census books were made from the family census sheets arranged in geographical order. Every individual was assigned the village census number and an individual serial number within the village. One copy of the census book was returned to the field worker who used it to issue individual family census cards to every family. At this time any discrepancies which were noted by the field workers were reported to the central office and corrected.

Surveillance for births and deaths is maintained by several levels of workers. A local female resident of each village visits each household daily and inquires about births and deaths. A male field assistant supervises from 10 to 15 of these

lady field workers. These men, with the equivalent of a high school education (matriculates), visit each family an average of once monthly and register all births, deaths, and migrations on standard forms. Supervision of this phase of the work is maintained by senior field assistants (usually Sanitary Inspectors) who visit each household approximately once in three months to check on the completeness of birth and death registration by independently enquiring about these events. In turn, these workers are supervised by the Field Surveillance Supervisor and his deputy who are responsible for the co-ordination of the field work.

RESULTS

Mortality

Table 1 shows the crude death rate and infant mortality rate of the last ten years in the Old Trial Area and the last eight years in the New Trial Area. Two distinct patterns were observed in the crude death rate. In the first five-year period (1966-70), the death rates were stable with only slight year-to-year variation. In the second five-year period (1971-76) these have exhibited an upward trend with significant year-to-year fluctuations. Once in 1971 and again in 1974, death rates rose sharply to levels over 25 percent of the first five-year average.

Infant mortality rates both for the Old and New Trial Areas have shown a more distinct upward trend than that found for crude death rates, however similar peaks were observed in 1971 and 1974. Unlike crude death rates, infant mortality rates remained high in 1975. These patterns were seen in both the Old and New Trial Areas as shown in figure 1.

Table 2 shows the crude birth rates, total fertility rates and sex ratio at birth for the last ten years in the Old Trial Area and the last eight years for the New Trial Area. Except for 1973, both crude birth rates and total fertility rates showed

a very slow downward trend until 1974. In 1975, the decline was dramatic. As noted in figure 2, prior to 1974, the trend in age-specific fertility rate there was virtually no decline in any but the 10-19 year age group. In the 10-19 year age-group a pronounced decline was observed prior to 1974. In 1975, the age-specific fertility rates for all age-groups declined dramatically as noted in the crude birth rate and total fertility rate.

Table 3 shows the rate of natural increase in recent years in Matlab. A steady declining trend in natural growth was seen. This is partly the reflection of a decline in fertility of young women, (but it is mostly due to increasing crude death rates). During the period 1966 through 1970 the yearly growth rate was 3.0 percent which declined to 2.5 percent during 1971-74. In 1975 the growth rate was 0.9 percent, a remarkable decline by any standards. This was due primarily to the dramatic decline in fertility for that year. For the last ten years, the average annual growth rate was 2.6 percent. Similar trends were observed in both the Old and New Trial Areas.

Intrinsic growth rates were estimated as 3.2 percent for the period 1966-70; these declined during 1971-74 to 2.8 percent.

DISCUSSION

The first issue which deserves comment is the quality of registration data. Remarkable conformity of yearly rates and sex ratio at birth between two areas suggests the data were highly reliable throughout the period of observation. Although the data are not presented here, two other techniques to estimate fertility in this population - an analysis of birth intervals of 2,000 women and a pregnancy prevalence survey - have independently confirmed a crude birth rate of approximately 28/1,000.

The abrupt change in the pattern of mortality rates which began with the period of civil unrest in 1971 is striking. From a five-year period of relatively low, stable

rates a 35% increase in the crude death rate was noted in the Old Trial Area. A similar increase of 33% over the previous 3 years was observed in the New Trial Area. Previous analysis of these observations showed the excess deaths to be concentrated in the children and elderly. Although the period of unrest in 1971 lasted only nine months an analysis of the pattern of mortality, which included broad classifications of cause of death, suggested the increase in mortality was due to a variety of specific causes.

Crude death rates returned to low levels in the two years after independence, but some age groups continued to experience high mortality rates. Thus, the mortality rate for the 5 - 9 year age-group was higher in 1972 than 1971. The excess mortality was attributed largely to dysentery which might have been related to a decreased level of nutrition.

However, crude death rates in both areas combined jumped dramatically in 1974 to levels which were almost 50% higher than those of the previous year. Although we have not analysed in any detail the deaths for 1974, the period was marked by severe economic hardship and food shortages for those on a money economy. There was no war, no mass migration out of the country. The hospital functioned smoothly and there were no apparent epidemics which could account for a large part of the excess mortality. The only factor in common between 1971 and 1974 was disruption in the intricate food-grain distribution system in a rice deficit area. Although our analysis is incomplete we are left with the working hypothesis that shortages in food availability was the common denominator for both 1971 and 1974. Defining the mechanism by which the lack of availability of food was reflected in higher death rates is of primary concern.

The qualitative change in the pattern of mortality is very revealing. One must emphasise the instability which describes the crude death rates in the past five years. It demonstrates society is able to recover from significant insults, but it also shows how little reserve exists in the system to deal with stress. There appears

to be a tremendous inelastic relationship between the complex foodgrain marketplace and mortality in Matlab at the present time.

The decline in fertility among young women is probably due to the increasing age of marriage. The median age of marriage was 14.8 years in 1968 and rose to 17.4 years in 1975. The drastic fall in birth rate in 1975 is due to variables which are yet to be explored. However, the food shortage observed in 1974, which caused a large net out-migration, postponed marriages, increased the number of divorces and separations in this population, undoubtedly affected the fertility rates in 1975 (Appendix I). In addition to these major social disruptions the shortage in availability of food probably decreased the nutritional status of women which caused a reduction in fecundity and lengthened the period of temporary infertility. Thus the food shortage might have affected both biological and social factors regulating fertility in a non-contracepting society (Appendix II).

We expect the downward trend in fertility to be reversed in the future as the Matlab area recovers from the disruptions of 1974. But we are unsure if fertility rates will rebound to the high levels observed in the past. It is reasonable to assume we may be entering an era of unstable fertility rates and significant fluctuations in both fertility and mortality may be the pattern in the future as the cushion of reserve resources available to families to deal with social and economic disruptions is exhausted.

These data illustrate the composite nature of changes in the crude rate of natural increase. During the last two years of observation 75% of the decrease in the rate was due to the decline in fertility and 25% due to an increase in mortality, but in 1974 changes in fertility and mortality shared equally in the decline.

We feel confident our data accurately reflects the true picture of mortality and fertility in Matlab, but we cannot document to what extent the Matlab experience

represents the overall picture of population growth in Bangladesh. We are at a loss to identify factors unique to Matlab which are sufficient to explain either the significant increases in mortality or the striking fall in fertility. Matlab is simply not that different from other rural areas of the delta. Therefore we feel the Matlab data probably does reflect significant changes which occurred elsewhere in Bangladesh until proven otherwise.

Figure 1

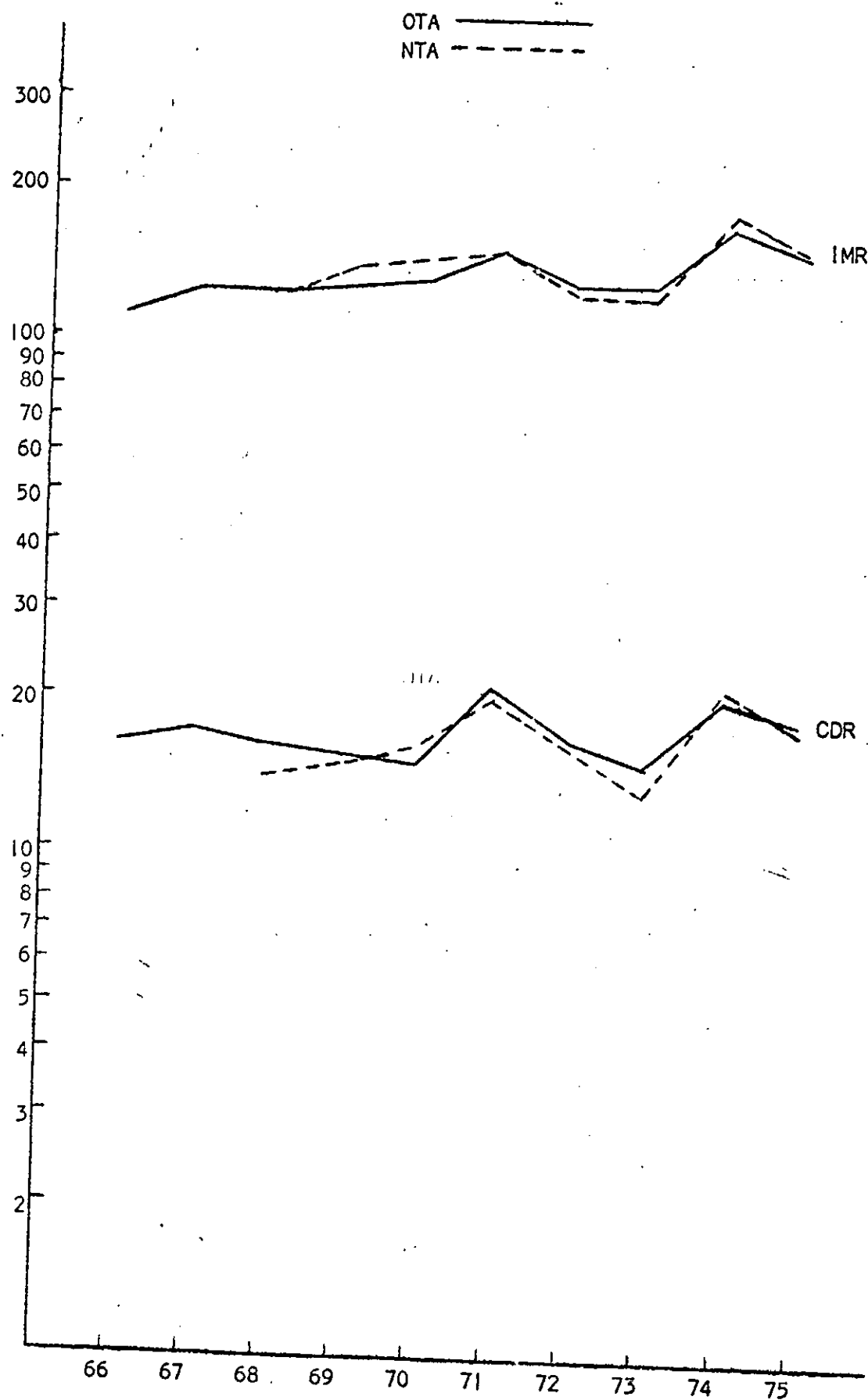


Figure 2

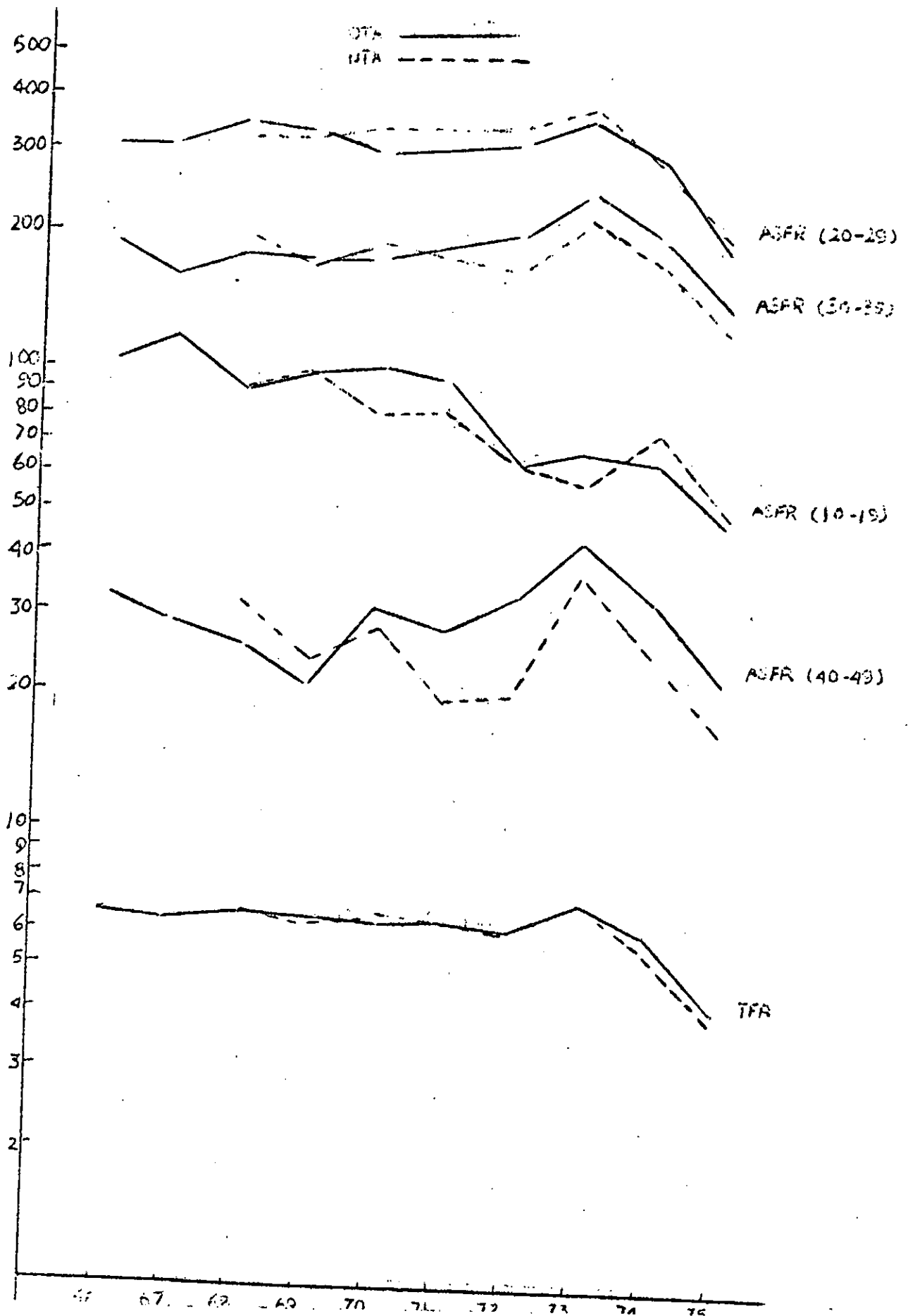


TABLE I

CRUDE DEATH RATES AND INFANT MORTALITY RATES OF OLD AND NEW
TRIAL AREAS IN MATLAB FOR LAST TEN YEARS

Year	Old Trial Area		New Trial Area	
	CDR	IMR	CDR	IMR
1966	16.0	110.7	-	-
1967	17.2	125.4	-	-
1968	15.7	123.8	14.0	121.3
1969	15.1	127.5	14.7	139.3
1970	14.6	131.3	15.9	144.5
1971	21.3	146.6	19.7	147.3
1972	16.4	129.2	16.0	125.9
1973	14.6	128.8	13.2	122.0
1974	20.0	167.2	21.3	180.5
1975	18.2	150.4	17.6	153.6

TABLE 2

CRUDE BIRTH RATES, TOTAL FERTILITY RATES AND SEX RATIO AT BIRTH OF
OLD AND NEW TRIAL AREAS IN MATLAB FOR LAST TEN YEARS

Year	Old Trial Area			New Trial Area		
	CBR	TFR	Sex: Ratio	CBR	TFR	Sex: Ratio
1966	46.8	6.66	104	-	-	-
1967	45.2	6.43	104	-	-	-
1968	46.4	6.68	104	44.9	6.72	106
1969	45.2	6.56	105	44.0	6.47	106
1970	43.6	6.40	101	44.8	6.58	101
1971	44.5	6.54	103	43.8	6.40	106
1972	41.8	6.06	102	41.8	6.08	106
1973	47.8	7.25	104	46.6	7.19	104
1974	40.1	6.11	105	39.0	5.72	103
1975	27.6	4.15	106	26.5	3.94	107

TABLE 3

RATE OF NATURAL INCREASES AND INTRINSIC GROWTH RATE OF OLD AND
NEW TRIAL AREA IN MATLAB FOR LAST TEN YEARS

Year	Rate of Natural Increase in Percent	
	OTA	NTA
1966	3.1	-
1967	2.8	-
1968	3.1	3.1
1969	3.0	2.9
1970	2.9	2.9
Mean (upto 70)	3.0	3.0
Intrinsic Growth (upto 70)	3.2	3.2
1971	2.3	2.4
1972	2.5	2.6
1973	3.3	3.3
1974	2.0	1.8
Mean (71-74)	2.5	2.5
Intrinsic Growth Rate (71-74)	2.8	2.7
1975	0.9	0.9
Mean (upto 75)	2.6	2.5
Intrinsic Growth Rate (upto 75)	2.8	2.8

RECENT MIGRATION TREND
IN MATLAB

Appendix I

Year	68	69	70	71	72	73	74	75
Migration-In (Total)								
OTA	3673	3100	3840	4315	3074	1891	1974	3131
NTA	2912	3104	3610	2948	3099	1716	1850	3075
Migration-Out (Total)								
OTA	3058	3664	2751	4439	4525	2300	5548	4632
NTA	4162	3620	2871	3093	4251	2041	5275	5010
Migration-In (Marriage)								
OTA	455	494	458	263	563	438	222	445
NTA	388	470	441	303	545	372	227	532
Migration-Out (Marriage)								
OTA	517	522	494	325	581	508	229	569
NTA	477	528	562	323	647	487	285	548
Migration-In (Divorce & Separation)								
OTA	105	81	74	58	86	42	148	135
NTA	79	102	86	74	99	73	155	163
Migration-Out (Divorce & Separation)								
OTA	80	95	81	76	97	56	131	136
NTA	60	97	98	91	87	44	164	134

Appendix II

LENGTH OF POST-PARTUM AMENORRHEA AND WAITING TIME FOR CONCEPTION BY THE BODY WEIGHT OF THE WOMEN (1975 - 1976)

Body Weight Kg.	Length of PPA			Waiting time for Conception			Percent Developed Sterility
	Closed Intervals	Closed and Open Intervals (months)		Closed Intervals (months)	Closed and Open Intervals (months)		
	$\bar{x}$	$x:F(x)=.5$	ex	$\bar{x}$	$x:F(x)=.5$	ex	
<38.5	17.9 (137)	26.3 (318)	24.9 (318)	11.3 (120)	40.0 (380)	33.5 (380)	17.6
38.5-42.4	17.5 (191)	23.1 (376)	22.6 (376)	10.7 (138)	33.4 (405)	31.1 (405)	6.7
42.5+	16.8 (174)	21.9 (349)	21.4 (349)	10.0 (161)	23.6 (383)	26.3 (383)	8.4

$\bar{x}$: mean closed interval

$x : F(x)=.5$: median interval

ex : expected life of interval at the beginning of interval

Percent developed sterility: Percent of women who are exposed to conception for 60 months and over since resumption of her menstruation failed to conceive for any reason.

CONTRACEPTIVE DISTRIBUTION PROJECT
IN RURAL BANGLADESH - ONE YEAR EXPERIENCE

Douglas H. Huber
Atiqur Rahman Khan
George Curlin
Ray Langsten

CRL Technical Committee Meeting
February 24, 1977

Introduction

Experience with the family planning programme for the last 11 years shows that although the past programme was able to spread the message of family planning to a large fraction of the population, it achieved very little success in promoting practice of family planning. This low rate of practice was in sharp contrast with the need for contraception as expressed by the desire to limit childbearing (1). The impact survey found that while 55% rural and 61% urban women did not want any more children and thus needed contraception, only 1.9% rural and 3.7% urban women were actually using a modern method of contraception (2). Such a large gap between need, knowledge and actual use, raised the concern to understand the underlying reasons, and it was believed that lack of convenient and easily available contraceptives is an important factor responsible for the low use rate (3). The relative magnitude of this factor can be determined by making contraceptives adequately available in the household and noting the extent of contraceptive practice it promotes.

Inadequacy of clinical facilities and shortage of medical personnel constitute additional justification for implementing a household contraceptive distribution system.

The Cholera Research Laboratory thus has undertaken a contraceptive distribution project, providing oral contraceptives and condoms on a house to house basis in the CRL Field Surveillance Area (FSA) in Matlab Thana (4).

Objectives

The project envisages to accomplish the following research objectives:

1. To assess the level of contraceptive practice in rural Bangladesh when these contraceptives are made available at the doorstep.
2. To evolve an efficient delivery system for contraceptives in Bangladesh.

3. To assess the demographic impact of a household contraceptive distribution system.
4. To identify factors determining acceptance and continued use.

Matlab was selected for implementation of this project because of the demographic information available there which will allow us to measure fertility variations in the future. The FSA comprising 234 villages with about 260,000 people was divided into two areas, half being the contraceptive distribution area and the other half serving as a control area, part of which was served by the Fertility Research Project clinic (FRP) (5). Demarkation between the distribution and the control area was somewhat arbitrary. As the FRP clinic had pill acceptors from neighbouring villages who needed continued supervision and surveillance under the clinic, the villages around Matlab Bazar were included in the control area. Thus the control area people previously had a greater access to family planning clinic service.

This differential access to family planning clinic service is reflected in slightly higher base-line contraceptive practice rate for the control area than the distribution area (2.9% against 1.1% table 1). Because the overall practice rate was very low, this difference was not considered likely to affect the experimental purpose.

Personnel

The distribution project utilized the services of Lady Village Workers (LVWs)/or dais, and Field Assistants (FA) working with CRL for field survey work in infectious diseases and other health problems. LVWs are mostly illiterate village women assigned to an area (usually one village) comprising roughly 1,000 population. Although the word "dai" means indigenous birth attendant; in fact, only 15% of the LVWs are true birth attendants. The Field Assistants (FAs) are males educated up to matriculation (10th grade).

The training given to the FAs and dais comprised only what was considered minimally necessary. The LVWs were given training for 6 part/days with one day of supervised field work. FAs received 5 days' training and periodic supervision in the field.

Distribution phase

The distribution of oral contraceptives started in October 1975 with 8 FAs and 150 dais. One dai worked with one FA during the distribution in her village. They had been previously supplied with standardized printed instructions. In the initial round of distribution the FAs distributed the pills and gave instructions with the assistance of the dais.

- ✓ All women of reproductive age (15-44 years) present in the house were given the information about oral contraceptives (OCs) and were offered the supply. If they agreed to receive the pill a supply of 6 cycles was given. In many instances, a number of women in the same "bari"* were given instructions together.

In the subsequent rounds of distribution the FAs were gradually withdrawn from the job of actual distribution to a role of supervision, and the dais alone carried out the re-supply and distribution work. Dais carried a red plastic bag with contraceptives, instruction sheets and a small register to record names of clients and quantities supplied.

Although the FAs and dais were instructed not to provide any motivation beyond saying that longer child spacing can be good for health, in actual practice it was found very difficult to separate motivational efforts from simple information. Usually the husbands were absent from the household during the visit, but when present (in about 20-25% of instances) they were included in the discussion because without the husband's approval the wives were found hesitant to take the supplies.

*Bari - a bari is a collection of about 5-6 households sharing a common courtyard but with separate kitchens. The 25-35 persons living in a bari are blood related.

Base-line KAP Survey.

An extensive KAP survey was conducted immediately before the distribution to provide bench mark information, in relation to which a changing level of contraceptive behaviour can be determined in the future with its social, psychological, economic and programmatic correlates. The size of the sample was 1077 women equally from both distribution and control areas.

Preliminary analysis showed 2.4% of the women using a modern method and 4.9% using traditional methods. 23% knew where to get contraception, 16% naming the FRP clinic in Matlab as the place to go. 35.7% indicated a willingness to use contraception at some time, 24.9% of whom named pills as the method they would use. Injectable was the second most popular and IUD was named by none.

Contraceptive use prevalence surveys.

A contraceptive prevalence survey was planned to be carried out before the distribution and thereafter every three months in the first year and every six months afterwards. The base-line prevalence was conducted in October 1975 and the 2nd through 5th surveys were repeated at roughly 3 month intervals after the distribution of contraceptives. Only data from a 20% sample of villages in the contraceptive and control areas will be presented here. Six items of information were collected on the first survey; these were parity, date of last pregnancy termination, menstrual status, current contraceptive use, contraceptive use in the last three months and the source.

Contraceptive Practice

The first round of distribution took 62 days. Out of the total 23,395 eligible women in the distribution area 19,027 (81.3%) were contacted and 4,368 (18.7%) were absent from the household. An additional 8.2% were contacted on a second round. Of the 19,027 women contacted initially 13,987 (68.8%) accepted the supply of 6 cycles of pills. These women we will categorize as contraceptive recipients because they agreed to receive the supply without any commitment to use them.

The baseline rate of contraceptive practice for all women contacted who were married and not menopausal (fecund) was 1.1% for the contraceptive area and 2.9% for the control area (Table 1). This 20% sample control area use rate is slightly higher than the rate for the entire area probably due to the chance inclusion of more women close to the Matlab clinic. Oral contraceptive use rose appreciably in the first few months following distribution and this rate has steadily declined since the second survey. Condom use increased after a special distribution and instruction effort following the 4th survey. Related to this the overall use rate increased again slightly in the contraceptive area to 15.0% at 12 months. The control area was served by a one week nation-wide oral contraceptive campaign between the 1st and 2nd surveys. However, the results do not seem appreciable. During the year more women have been receiving injectable contraceptives at the Fertility Research Clinic in Matlab.

At 12 months after the distribution area Hindus have a 2.5% higher rate of use than Muslims (Table 2). However, in the control area the differential is much greater. This may relate to Hindu women having greater morbidity and are therefore able to attend the Matlab Fertility Research Clinic.

Menstrual status has been recorded for each woman when interviewed on the quarterly surveys. Because of seasonal fluctuations in fertility a direct comparison is difficult between the contraceptive and control area, except for the 12 month survey. On this survey both areas were covered simultaneously whereas previous survey proceeded sequentially, first in the contraceptive area and second in the control. The proportion reporting pregnancy was 15.3% in the contraceptive area and 17.8% in the control. There is an excess in the artificial category "menstruating on the pill" for the contraceptive area, as we would expect. Correspondingly, fewer contraceptive area women report they are menstruating naturally and fewer are in the period of post-partum amenorrhea. Presumably some of these women have been shifted into the "menstruating on pill category". These data suggest women in the contraceptive area may be experiencing pregnancy at a rate 14% below the control area. However, the possibility of reporting bias exists, and therefore, birth data must be compiled before conclusions can be drawn.

If under-reporting of pregnancy (or delayed reporting of the pregnant condition) exists, this may become apparent by examining sequential surveys for the same women. Table 4 shows the menstrual status for those women who were present for interviews on each of the five surveys. The contraceptive area generally shows fewer women reporting pregnancy for each survey after the contraceptive distribution; however the difference in timing between areas again makes direct comparison difficult. The proportion of all married women who were present on all 5 surveys was the same (60.8%) for both areas. However, the large number of one-or-more-time absentees still leaves open another question of bias due to possible differentials in pregnancy among this group.

Women not reporting an early pregnancy on survey two would be obviously pregnant on survey three. Bias in the contraceptive area might present as a relatively greater increase in reported cumulative pregnancies on survey 3 and 4 in comparison with the control area. However, figure 1 shows the rate of increase is greater for women in the control area. Between April 24 and Nov.12 an estimated 14.8% of contraceptive area women reported a new pregnancy compared with 18.5% for the control area.

Women becoming pregnant in this period would therefore be 20% fewer than in the control area. A reduction this great seems implausible and may be related to pregnant women having a higher rate of absenteeism in the contraceptive area. Another possibility of course remains that the fertility behaviour in the two areas may be inherently different for other reasons.

Another way of looking at the occurrence of pregnancy is by the number of new pregnancies reported on each survey per 1000 woman months of exposure to married life (Table 5). Again the risks of pregnancy appears higher for the control area except in the last interval. As the risks of pregnancy was higher in the summer months for both groups and the last interval begins earlier for the contraceptive area, this may explain the higher rate. Alternatively some pregnancies may have simply been temporarily delayed for these women.

Two special groups deserve mention, even though complete data is not yet available. Six villages of the 20% sample in the contraceptive area received special instructions. Women were told not to begin taking oral contraceptives until this breastfeeding child was 18 months old. If the child died or their menses started before 18 months post-partum, then they may begin oral contraceptives. The object was to avoid the substitution effect of oral contraceptives for the fairly effective fertility control measure of lactational amenorrhea. This period of infertility has a median duration of 18-20 months for Matlab women and roughly 5-8% of pregnancies usually occur before menstruation begins. Therefore, if women began OCs on this instruction schedule only 2.5-4% theoretically would become unintentionally pregnant before starting. The benefit could be that women predestined to use oral contraceptives for only a few months would still manage to delay the next pregnancy somewhat. Another consideration is the argument that OCs may decrease breast-milk flow, cause the woman to stop breastfeeding, and if she then discontinued contraception her period of post-partum infertility would be shortened. In this situation OCs could actually make fertility increase. The best way to address the potential program and biologic effects seemed to be a direct application of different instructions. Oral contraceptive use has remained lower in these villages as one would expect (Table 6). The proportion of women in various menstrual categories may be somewhat adversely affected, although the numbers may be too small to draw conclusions at present (Table 7).

The other sub-group is those villages accessible to Matlab in the control area. These three villages (20% of the control area population) account for over half the control area users and their contraceptive use rate is approaching the contraceptive area. Over one fourth of these users receive injectables from the FRP clinic.

Introduction of injectable contraceptives:

With increasing interest shown in injectable contraceptives offered through a number of Government and non-Governmental programmes we decided, at the request of Government, to

assess the potential of this method in our programme when offered in addition to pills and condoms. Accordingly we provided this method along with pills and condoms on a house-to-house basis on 6 villages.

A male field worker, accompanied by the CRL dai for each of the 6 villages, introduced the new method, explaining potential side effects and avoiding any implication that either the pill or the injectable was superior. Women were allowed to select either the 3 month or 6 month dose and the male worker gave the injection in the arm. The male field worker (with the dai) again visited 2 weeks after the initial canvas and thereafter at 1 month intervals. On each visit he offered pill, condom, or injectable to those who had not accepted, and he enquired about any complaints.

The addition to injectables in August 1976 raised the contraceptive use rate of 50% in these 6 villages. In November these villages remained 33% above the sample villages in the distribution area (Table 9).

In addition the menstrual status categories compare favourably with the other areas on the last survey (Table 9). Previous surveys did not reveal these villages to have a ^{8.0} mortably low proportion pregnant.

One program variable which does not appear to improve contraceptive use in this population is the color of the condom (Table 10). Areas where colored condoms were distributed do not demonstrate a higher rate of condom use 4 months after distribution.

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5. Fertility Research Programme is a collaborative venture between the Johns Hopkins University and the Government of Bangladesh and is undertaking contraceptive research in Dacca and Matlab.

Table 1

Contraceptive Use Rate for Married Fecund Women Aged 15-44 on
Quarterly Surveys, 20% Sample, Matlab, Oct.1975 - Nov.1976

	Contraceptive Distribution										Control Area									
	1st		2nd		3rd		4th		5th		1st		2nd		3rd		4th		5th	
Women Contacted	4583		4072		4157		4141		4253		3660		3618		3482		3407		3495	
Current Use:	No.	@	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Oral Contra- ceptive	34	0.7	694	17.0	642	15.4	512	12.4	439	10.3	81	2.2	106	2.9	110	3.2	88	2.6	74	2.1
Condom	1	.02	16	0.4	36	0.9	50	1.2	160	3.8	8	0.2	6	0.2	9	0.3	8	0.2	6	0.2
IUD	1	.02	11	0.3	12	0.3	14	0.3	13	0.3	9	0.2	7	0.2	7	0.2	7	0.2	9	0.3
Injection	4	.09	-	-	1	.02	-	-	2	0.05	4	0.1	10	0.3	10	0.3	23	0.7	28	0.8
Sterilization	4	.09	7	0.2	10	0.2	11	0.3	18	0.4	5	0.1	4	0.1	3	0.09	7	0.2	8	0.2
Others	6	.13	3	.07	4	0.1	5	0.1	4	0.09	-	-	1	0.03	1	0.03	1	0.03	2	0.06
Total	50	1.1	731	17.9	705	17.0	592	14.3	636	15.0	107	2.9	134	3.7	140	4.0	134	3.9	127	3.6

Table 2

Contraceptive Use Rate for Hindu and Muslim Women on 12 Months
Survey Matlab, November, 1976

	Contraceptive Distribution Area						Control Area					
	H	%	M	%	T	%	H	%	M	%	T	T
Eligible Women*	597	14.0	3656	86.0	4253	100.0	314	9.0	3181	91.0	3494	100.0
Current Use:												
Oral Contra-												
ceptive	60	10.1	379	10.4	439	10.3	26	8.3	48	1.5	74	2.1
Condom	32	5.4	128	3.5	160	3.8	1	0.3	5	0.2	6	0.2
IUD	6	1.0	7	0.2	13	0.3	3	1.0	6	0.2	9	0.3
Injection	1	0.2	1	0.03	2	0.04	5	1.6	23	0.7	28	0.8
Sterilization	2	0.5	16	0.4	18	0.4	2	0.6	6	0.2	8	0.2
Others	1	0.2	3	0.8	4	0.09	1	0.3	1	0.03	2	0.06
Total	102	17.1	534	14.6	636	14.9	38	12.1	89	2.8	127	3.6

* All married fecund women, aged 15-44

Table 3

Distribution of Menstrual Status for All Married Women
Aged 15-44 on 12 month Survey, Matlab,
November, 1976

<u>Menstrual Status</u>	<u>Contraceptive Distribution Area</u>		<u>Control Area</u>	
	<u>Frequency</u>	<u>%</u>	<u>Frequency</u>	<u>%</u>
Menstruating	1558	32.9	1401	36.4
P.P.A.	1204	25.4	1091	28.3
Pregnant	725	15.3	685	17.8
Menopausal	358	7.6	254	6.6
Amenorrhea for other reason	259	5.5	208	5.4
Menstruating on pill	455	9.6	79	2.1
Undetermined	52	1.1	31	0.8
N.A.	128	2.7	101	2.6
Total Present	4739		3850	

Table 4

Distribution of Menstrual Category for Contraceptive Distribution and Control Area
for the 5 Quarterly Surveys. All women Present on Each Survey,
October 1975 - November 1975

Menstrual Status	Contraceptive Distribution									
	Base- line	%	3ms	%	6ms	%	9ms	%	12ms	%
Menstru- ating	1108	34.3	907	28.1	901	27.9	957	29.7	1049	32.5
Post-partum Amenorrhea	1315	40.8	872	27.0	821	25.4	847	26.3	871	27.0
Pregnant	396	12.3	417	12.9		15.3	555	17.2	461	14.3
Menopause	251	7.8	292	9.1		8.4		8.1	269	8.3
Amenorrhea for other	143	4.4	188	5.8	208	6.4	139	4.3	184	5.7
Menstru- ating on Pill	-	-	540	16.7	527	16.3	413	12.8	357	11.1
Undeter- mined	13	0.4	10	0.3	7	0.2	53	1.6	35	1.1
Total	3226*	100.0	3226	100.0	3226	100.0	3226	100.0	3226	100.0

*60.77% of total eligible

Control Area									
Base- line	%	3ms	%	6ms	%	9ms	%	12ms	%
993	37.1	1106	41.3	1004	37.5	929	34.7	984	36.8
1091	40.8	696	26.0	680	25.4	721	27.0	778	29.1
265	9.9	459	17.2	538	20.1	576	21.5	466	17.4
174	6.5	176	6.6	184	6.9	187	7.0	197	7.4
110	4.1	129	4.8	159	5.9	118	4.4	138	5.2
-	-	87	3.3	85	3.2	78	2.9	67	2.5
42	1.6	22	0.8	25	0.9	66	2.5	45	1.7
2675**	100.0	2675	100.0	2675	100.0	2675	100.0	2675	100.0

**60.77% of total eligible

Table 5

Risk of Pregnancy in Contraceptive and Control Areas,
Matlab Oct. 1975 - Nov. 1976

<u>Contraceptive Area</u>		<u>Control Area</u>	
<u>Interval</u> <u>between</u> <u>Surveys</u>	<u>Pregnancy</u> <u>Rate*</u>	<u>Interval</u> <u>between</u> <u>Surveys</u>	<u>Pregnancy</u> <u>Rate*</u>
Oct. 4 - Feb. 28	22.6	Nov. 8 - Apr. 24	30.2
Feb. 29- May 7	37.8	Apr. 25- June 10	52.4
May 8 - Aug. 7	38.1	June 11- Sept. 11	44.0
Aug. 8- Nov. 12	22.2	Sept. 12- Nov. 12	19.8

*New pregnancies reported per 100 woman months
for married women 15-44 present on each of 5 survey

Table 6

Contraceptive Use Rate for Married Fecund Women in
Villages Receiving Special Instructions and
Villages with Access to FP Clinic, Matlab,
5th Survey, November 1976

	<u>Contraceptive Area</u> <u>"Start at 18 mo."</u> <u>Villages*</u>		<u>Control Area</u> <u>Villages</u> <u>Accessible</u> <u>to Matlab**</u>	
Women Contracted	746		653	
Current Use:	No.	%	No.	%
Oral Contraceptive	48	6.4	49	7.5
Condom	32	4.3	4	0.6
IUD	2	0.3	3	0.5
Injection	-	-	22	3.4
Sterilization	3	0.4	2	0.3
Others	-	-	-	-
Total	85	11.4	80	12.3

* Women instructed to begin oral contraceptives when youngest child aged 18 months (instead of 6 mo. for remainder of contraceptive area) unless menses begin earlier.

** Adjacent to or within 2 miles by water transport of Matlab Family Planning Clinic.

Table 7

Distribution of Menstrual Status for Married Women in
Special Instruction Villages and Villages Accessible
to Matlab F.P. Clinic, 5th Survey, Nov. 1976

<u>Menstrual Status</u>	<u>Contraceptive Area</u> <u>"Start at 18 mo."</u> <u>Villages</u>		<u>Control Area</u> <u>Villages</u> <u>Accessible</u> <u>to F.P. Clinic</u>	
	<u>No.</u>	<u>%</u>	<u>No.</u>	<u>%</u>
Menstruating	278	33.3	250	35.1
P.P.A.	218	26.1	182	25.5
Pregnant	147	17.6	118	16.5
Menopause	56	6.7	34	4.8
Amenorrhea for Other Reason	37	4.4	33	7.4
Menstruating on Pill	46	5.5	48	6.7
Undertermined	20	2.4	2	0.3
N.A.	33	4.0	26	3.6
Total	835	100.0	713	100.0

Table 8

Contraceptive Use Rate for Married Fecund Women 15-44 in
Villages Offered Injectable Contraceptives*,
Matlab, Oct. 1975-Nov. 1976

	Six Injectable Villages					
	1st		2nd		3rd	4th 5th
Women Contacted	1042		947			970
Current Use:	No.	%	No.	%		No. %
Oral Contracep- tive	10	1.0	151	16.0		33 3.4
Condom			1	0.1		10 1.0
IUD			1	0.1		2 0.2
Injection	1	0.1				140 14.4
Sterilization			1	0.1		5 0.5
Others						4 0.4
Total	11	1.1	154	16.2		194 20.0

* Injectable (Depo-Proval added in August 1976)

7

were put into buffered-glycerine media which were transported to Teknaf for streaking and incubation.

The result of one year of observation between January 1 and December 31, 1976 are presented here.

RESULTS

Attack rates from dysentery and diarrhoea: The attack rates from dysentery and diarrhoea as based on the history and bacteriologically confirmed are shown in the table 1. It was obvious that incidences of both clinical dysentery and shigella positive cases were extremely high in the youngest age group and fell rapidly with the advancing age. The high attack rate in Teknaf was partly due to the presence of the treatment facility close by which was responsible for more than 80% of reported cases. This treatment facility was not available in Shahpuridwip which was a definite factor in underreporting. Shigellosis rate was three times higher in those living in Teknaf as the swabbing there was more prompt and more comprehensive.

Deaths due to dysentery and diarrhoea: Table 2 shows the total number of deaths due to dysentery and diarrhoea as well as from all causes in the villages during the calendar year 1976. Total deaths in all age group from all causes was 240, 144 being in Shahpuridwip and 96 in Teknaf, which gave a crude death rate of 16.0 and 12.5 per 1000/year respectively. A total of 35 deaths due to dysentery and diarrhoea occurred in these two villages, 25 out of 35 or 71% occurred in children below 5 years of age. These deaths constituted 35% of deaths due to all causes in this age group. Age specific deaths rates are also shown in this table.

The number of deaths due to dysentery and diarrhoea fell with the increasing age but tended to go up again in the elderly persons. The peak age for mortality from dysentery and diarrhoea was 1 - 4 years in this population. This is also the age for the peak incidences of malnutrition.

Impact of the treatment centre on mortality: There was a large difference in the mortality rates due to dysentery and diarrhoea between the Shahpuridwip and Teknaf. The former village having no treatment facility but an apparently lower attack rate had a higher mortality compared to the later

village having a higher attack rate but a lower mortality. This difference was statistically highly significant, $p < 0.001$.

Medicines used in the treatment of Dysentery and Diarrhoea:
Table 3 shows the list of medicines used for the treatment of dysentery and diarrhoea. A substantial amount of sulphas like sulphaguanidine and sulphadiazine were used, mostly in adult patients with mucoid diarrhoea. Tetracycline was the most frequently used antibiotic against these diseases. Ampicillin was used less frequently. As mentioned before, only those showing severe dysentery were given ampicillin. Intravenous hydration was used in the centre only for a small number of dysentery patients. A large percentage of diarrhoea patients required intravenous infusion. OralYTE was used for oral hydration in dysentery cases. This apparently high rate of use might have been due to the fact that the dysenteric patients were kept in the treatment centre for sometime to observe them and enable them to pass stool for inspection and examination. OralYTE salt solution were given to them while waiting.

Cost of medicines: The total average cost of medicine supplied was Taka 2/- per patient contact. This does not include the salaries paid to the staff.

CONCLUSION

A simple regimen followed in Teknaf showed that mortality from dysentery and diarrhoea could be significantly reduced by employing trained paramedical personnel even in the face of a high attack rate of shigellosis. The expenses involved was reasonable and within the purchasing ability of the majority of the rural population of Bangladesh.

TABLE 1

AGE SPECIFIC DIARRHOEA/DYSENTERY AND SHIGELLOSIS ATTACK
RATE (PER 1000/YEAR) IN SHAHPURIDWIP (SD) AND
TEKNAF (TK): TWO RURAL VILLAGES IN BANGLADESH IN 1976

AGE (YR.)	DYSENT./DIARRHOEA		SHIGELLOSIS	
	SD	TK	SD	TK
0 - 4	469	489	40	89
5 - 9	98	222	8	44
10 - 14	45	136	4	29
15 - 19	36	67	2	12
20 - 24	46	100	9	30
25 ⁺	74	117	6	19
ALL	137	203	12	38

TABLE 3

PERCENTAGE DISTRIBUTION OF DYSENTERY
AND DIARRHOEA PATIENTS ACCORDING
TO TYPE OF DRUGS SUPPLIED

DRUG	PERCENT OF PATIENTS	
	DYSENTERY	DIARRHOEA
SULPHAS	33.2	28.3
TETRACYCLINE	35.7	49.3
AMPICILLIN	14.8	3.8
MIXTURE CARMINATIVE	9.2	12.1
I. V. FLUID	0.9	8.6
"ORALYTE" *	12.9	7.6
OTHER	2.6	5.5
NO MEDICINE	2.5	-

* ORAL REHYDRATION SALT PACKETS SUPPLIED BY UNICEF, DACCA

TABLE 2

DEATHS DUE TO DYSENTERY-DIARRHOEA AND FROM ALL CAUSES
 ACCORDING TO AGE IN SHAHPURIDWIP (SD), POP.=9063 AND TEKNAF (TK), POP.=7652.....

DYSENTERY/DIARRHOEA DEATHS						DEATHS FROM ALL CAUSES					
AGE	SD	TK	TOTAL	ASDR ^C		% DEATHS	SD	TK	TOTAL	%	
				SD	TK						
< 1 MTH	0	1	1	0	3.0	2	33	31	64	27	
1 - 11 MTHS	3	2	5	8.0	6.1	10	23	25	48	20	
1 - 4 YRS	15	0	19	12.5	0	35	41	13	54	23	
5 - 9 YRS	2	0	2	1.1	0	12	14	3	17	7	
10 - 14 YRS	0	0	0	0	0	0	1	0	1	4	
15 - 19 YRS	0	1	1	0	1.2	25	2	2	4	2	
20 - 49 YRS	5	0	5	7.5	0	22	13	10	23	10	
50+	2	0	2	0.7	0	7	17	12	29	12	
ALL AGES	27	4	35	2.97	0.52	15	144	96	240	100	
CDR/1,000	2.97 ^A	0.52 ^B	2.09 ^C	-	-	-	16.0	12.5	14.4	-	

A VS B $P < 0.001$

C AGE SPECIFIC DEATH RATE

HEALTH CONSCIOUSNESS IN TEKNAF:
A RURAL BANGLADESH VILLAGE

M. Mujibur Rahaman
K. M. S. Aziz
Abdul Quddus Mondal
Yakub Patwary
M. Mizanur Rahman

Teknaf Dysentery Project
Cholera Research Laboratory

The most important health problems of Bangladesh are common infectious diseases particularly diarrhoea, dysentery and respiratory infections which are responsible for the large bulk of morbidity and mortality. Poor environmental and personal hygiene are responsible for a high attack rate from infectious diseases which in turn bring about under-nutrition and malnutrition in an already impoverished population. The literacy in Bangladesh is approximately 20% and the level of general education is also poor. Very little health education is imparted in school where large majority of the population never go. Attacks from diarrhoea and dysentery are closely linked with the standard of hygienic practices, which in turn depends on an awareness of the knowledge of oral-faecal contamination. It was, therefore, decided to carry out a survey of health consciousness in Teknaf population. Attempts are being made to impart health education, supply safe water and instal sanitary privies in this community. It was therefore felt necessary to carry out a baseline survey on the existing state of health consciousness, current hygienic practices, and attitudes towards the proposed improvement in Teknaf so that any future changes could be evaluated.

MATERIALS AND METHODS

Population: The study families were from areas Jaliapara and Kulalpara situated close to Teknaf. The total population was 4,324 divided into 659 families. Two hundred families were selected on a random basis. One hundred of the respondents were females, almost all of whom were wives of the head of the families. The other 100 were male head of the families which were different from those of the female interviewee.

Questionnaire: The questionnaire was set up in Bengali and where appropriate local terms of some diseases were used. There were two interviewers involved in asking the questions. One of them was a lady who asked the questions to the female respondents; the other was a male interviewer who interviewed men only. Both the interviewers were well known to the community and were able to converse with them freely in the local dialect. A pre-test was carried out before the full survey was undertaken. On the basis of the pre-test a number of modifications were made and the modified questionnaire is shown in Appendix I.

The interviews were carried out over a period of two months as if during normal rounds of visits. The routine for carrying out interviews was so arranged that no neighbouring families were interviewed on the same or within a few days.

RESULTS

The final questionnaire had as many as 43 questions. Answers to only 26 questions dealing with hygienic practices, knowledge of enteric diseases, parasitic infestation and nutrition are presented here.

Age, sex, occupation, income and education:

Table 1 - 5 show the age and socio-economic condition of the two hundred families interviewed. Their ages had wide range, but the majority were young adults. Almost half were below 30 years of age and only 10% were above 55. Nearly 1/5th of the male head of families had no fixed jobs and were daily labourers. Twenty six were fishermen. There was a high proportion of petty-businessmen in this community mainly because of the closeness of the Teknaf Bazar which acts as a trading centre. Table 4 shows the range of monthly income of the families. Half of the families had an income between Taka 200-500 per month which will be about US \$400 per year. Since the average family size is 6.5, the per capita income per year was less than \$60 dollars.

Table 5 shows the educational level of the respondents. Half of the male respondents and 94% of the females never went to any modern schools at any time in their lives. Some 13% of men and 21% of women, however, went to religious schools where Arabic and religious practices were taught.

Sources of Water: Table 6 shows the sources of water used for drinking, washing and bathing. Concrete ring-wells are shallow dug-wells of less than 5 - 7 metres depth and their walls are protected by concrete rings. These wells were the principal sources of drinking water. Almost all of the ring wells are community water sources and serve a large number of families. Tubewells or handpumps serve a relatively smaller number of families due to difficulty in boring them through the subsoil rocks in this area. Water for washing comes from many sources. Half of the families get water for general washing purposes from ringwells and over one third of them go to the two tanks or ponds situated in these areas. Nearly half of the families use ringwell water for bathing and almost a similar proportion go to tanks or ponds.

Places of defaecation: Table 7 shows the stated places of defaecation by adult males, females and children. An equal proportion of adult male and female used the fixed latrines which were most often an open pit. Only a small number of children (5.5%) however, used these latrines. The bank of the river situated nearby was the most frequently cited place for defaecation for the adult men. For the adult women on the other hand open places besides the houses were preferred more often than the bank of the river. Sides of the houses

were also more frequently used by children than the bank of the river. There were very few bushes around the house in this community. Therefore only a small number of families cited using the bushes. Going to the fields involved walking some distance from the house through the open fields which was responsible for its less frequent use as the places of defaecation by Teknaf women and children. The most significant feature of this table was the fact that majority of children did not use any fixed place for defaecation. Since attack rates from shigellosis is known to be the highest in children, the significance of this habit in spreading the disease is obvious.

A small proportion of families (7.5%) told us that they allow their children to defaecate in a fixed place within the compound of the house. These faeces were later collected and thrown out into the neighbouring fields or bushes.

Methods of cleaning anal area after defaecation:

Table 8 shows the information obtained regarding this practice. Almost all of the adults carry water in a pot with them while they go for defaecation. Only 3.5% go to the tanks or ponds to wash their anal areas after defaecating in the fields.

After cleaning their anal areas 26.5% of the respondents rinse their hands using nothing but water. Nearly 2/3rd of them rub their hands and fingers on mud and grass and then wash with water. Only 9% told us that they use soap regularly.

Knowledge and beliefs about hygienic practices and incidence of diseases:

The next series of tables show the beliefs of the population about some hygienic practices which have close relationships with enteric diseases, parasitic infestations and development of malnutrition.

Harmful effects of indiscriminate defaecation:

When the question was asked about any real harm that could come from indiscriminate defaecation, over $\frac{1}{4}$ of men and $\frac{2}{3}$ of the women gave a negative answer. Only 6.5% of both sexes told us that it could result in spreading of intestinal diseases; another 20% had some vague ideas that the practice could spread any disease (table 9).

On the other hand, when the question was asked whether any real harm could arise from handling food without washing hand soiled by cleaning the anal area after defaecation, 62.5% said that the habit may cause intestinal disease (table 10). Another 15.5% said that the habit could also be responsible for causing other diseases. Only 4% said that there is no harm if they handle food after defaecation without washing hands.

Knowledge about the role of flies in spreading diseases:

Only 7% of male and 24% of female thought that flies were harmless (table 11). Over half of the respondents, 74% of the male and 24% of the female thought that they are responsible for spreading some diseases. Only about 15% think that foods

are made unclean by flies sitting on them and a similar proportion thought that they lay eggs on food and made them unwholesome by sitting on them.

When question was asked on the places of origin of flies, almost all the men and 80% of the women had definite knowledge (table 12).

Knowledge about harmful effects of walking bare-foot:

Question on harmful effects of walking without shoes or sandals elicited a variety of answers without relationship to any particular disease (table 13). One fourth of the respondents saw no harmful effects at all and another 40% thought that it could cause soiling of the feet or cause some form of ulcer on the legs. Nearly 30% thought that it could cause some physical injury. Only 3.5% of the respondents told us that it could be responsible for infestation by some worms although no one mentioned hookworm as one of them.

Roundworm is considered to be the cause of many diseases in children in rural Bangladesh. When a question was asked on the mode of becoming infested by these worms, the answers (table 14) were found to be very interesting. Nearly half of the females and three of the males considered eating fish as a cause of infestation by the roundworms. Since Teknaf has many fishermen in the community and whose population is accustomed to eating fish almost round the year, the psychological association between the two is not surprising. Why men did not consider eating fish as a cause for infestation by worms compared to the female was

intriguing. Eating sweets including biscuits and banana, spoiled food and eating uncooked rice as cause of infestation by worms were also cited. Ingestion of dust was considered to be a cause in only 2% of the respondents.

Attitude towards building latrine near home:

Latrines or privies in rural areas of Bangladesh are usually a shallow hole on the ground, smelly and poorly maintained. Therefore, people in general do not like to use them or build them near their homes. Question was asked as to whether the Teknaf villagers would like to have one near their homes if they are of the type that does not smell. Table 15 shows the response. The respondents were almost unanimous on their approval for such latrines. Only one man and three women had a negative attitude towards the latrines. Two of them gave shortage of space as the reason and the other two thought that drinkingwater will go "bad" if a latrine was built near their homes.

To find out how seriously the population wanted latrine near their homes they were asked about their willingness to pay for it. No exact amount was specified but it was made apparent that the total cost of materials and labour would be above Taka 300/-. Thirty five percent (table 16) of the population were still willing to pay for the latrines. Another 45.5% were willing to contribute by labour, as they stated that they were poor. Only 15.5% of those who wanted the latrines said that they were unwilling to pay either money or contribute labour.

Knowledge about dysentery and diarrhoea:

The next series of questions were directed to find out the degree of awareness about dysentery and diarrhoea and whether or not they could distinguish them, as well as their knowledge about their modes of spread.

As far as the symptoms of dysentery were concerned, 62% of the population cited passage of both mucous and blood in stool as the main symptoms of the disease (table 17). Passage of mucous, blood with pain and frequent diarrhoea were considered to be the symptoms of dysentery by an additional 20.5% of the respondents.

Passage of watery motion alone was considered to be a symptom of diarrhoea (table 18) by 71.5% of the respondents. Watery stool and vomiting were cited as symptoms of diarrhoea by an additional 12.5% of the population. Vomiting alone without diarrhoea was thought to be a symptom of diarrhoea by 2.5% of the population. Abdominal cramps rather than pain was cited as a cause of diarrhoea by 1% of the population. Less than 5% of the respondents did not know the predominant symptom of diarrhoea.

Mode of spread of diarrhoea and dysentery:

Half of the respondents did not have any idea on the mode of spread of diarrhoea or dysentery (table 19). Only 15% and 23.5% of the respondents could associate diarrhoeal stool with the cause of spread of diarrhoea and dysentery respectively.

Only 10% of them thought that contact with patients or eating their leftover food could cause the diseases. Another 10% considered flies as responsible for the spread of these diseases. Only 3.5% cited water as responsible for spreading diarrhoea.

Knowledge about practice of infant feeding:

Breast feeding is almost universal in rural Bangladesh. One of the main causes of failure of growth in a child between 6 - 18 months of age is almost total dependence on breast milk.

The belief of the population and the level of consciousness on some aspects of infant feeding are shown in the next series of tables.

When question was asked about the right age for stopping the breast feeding, only 4% of the women thought that it should be done at 18 months of age (table 20). Even at 24 months of age, only 41% of the women thought that breast feeding should be discontinued. Additional thirty-five and 21 percent of the women considered that the right ages for stopping the breast feeding was between 30-36 months respectively. Male respondents on the other hand, thought that the breast feeding should be discontinued a few months earlier than those mentioned by the women.

Effect of adding boiled rice to a breast-fed infant before reaching one year of age showed that 29% of the women and 12% of men thought that it could cause no harm to the child (table 21). The respondents gave various reasons against the practice which ranged from an inability to digest rice, cause of intestinal

illnesses, making children weak, giving them a large belly to causing worm infestation.

A question was asked about the right age at which a child acquires an ability to digest rice, meat and fish, the common adult type diet in Bangladesh. It showed that only 10% of the respondents (both men and women) considered that a child at 18 months acquires this ability (table 22). Only $\frac{1}{4}$ of the respondents thought that this capacity was acquired by 24 months of age. Nearly half of the respondents did not consider that a child's digestive system was able to handle these foods even at three years of age.

Beliefs on the causes of malnutrition in a two year old child:

Malnutrition was commonly observed in Teknaf villages. It was predominantly a protein-calorie deficiency. When asked to pass an opinion on factors causing "lada", the term used to denote a malnourished child, over half of the women (57%) and 1/3rd of men (table 23) did not have any opinion. Nearly $\frac{1}{4}$ of the women and 8% of the men thought it could be due to "bad" effects of prolonged breast feeding, a surprising piece of information. Insufficient milk, a child weak and sick from birth were considered to be factors also responsible for malnutrition by nearly $\frac{1}{4}$ of the men but only 3% of women. Worm infestation was cited as factors by a small proportion of both men and women.

Ratings on some possible items for development which could benefit the Teknaf villagers:

Table 24 shows the ratings of the villagers when some items for possible developments were mentioned. It was clear that hospital, water supply and latrine were the priority items despite the fact that these items for improvements were mixed with a large number of other items, some of which were more attractive in nature.

Table 1

TEKNAFHEALTH CONSCIOUSNESS SURVEYDistribution of Respondents According toTheir Age and Sex

Age	Male	Female	Both	Percent
15 - 24	9	32	41	20.5
25 - 34	24	23	47	23.5
35 - 44	28	26	54	27.0
45 - 54	24	15	39	19.5
55 +	15	4	19	9.5
All Ages	100	100	200	100.0

TABLE 2

TEKNAFHEALTH CONSCIOUSNESS SURVEYRelationship of the Respondents to theHead of the Families

	Male	Female	Both	Percent
Self	87	8	95	47.5
Other	13	92	105	52.5
Total	100	100	200	100.0

TABLE 3

T E K N A FHealth Consciousness SurveyDistribution of Respondents and Head of theFamilies According to OccupationHead's Occupation

Own Occupation	None	Labour	Cultivation	Business	Fishing	Service	House wife	Other	Total	Percent
None	1	1	1	1	-	-	-	-	4	2.0
Labour	-	13	1	-	-	-	-	-	14	7.0
Cultivation	-	-	-	-	7	-	-	-	7	3.5
Business	2	2	2	39	-	-	-	-	45	22.5
Fishing	-	-	-	3	21	2	2	-	26	13.0
Service	-	-	-	2	-	14	-	2	18	9.0
House Wife	1	6	3	46	18	9	1	-	84	42.0
Other	-	-	-	2	-	-	-	-	2	1.0
Total	4	22	7	93	46	23	3	2	200	100.0
Percent	2.0	11.0	3.5	46.5	23.0	11.5	1.5	1.0	100.0	

TABLE 4

TEKNAFHEALTH CONSCIOUSNESS SURVEYDistribution of The Respondents According To GrossMonthly Income Of The Families

Monthly Income (in Tk.)	Number	Percent
≤ 100	2	1.0
100 - 200	28	14.0
201 - 500	103	51.5
501 - 1000	35	17.5
Above 1000	30	15.0
Not Known	2	1.0
Total	200	100.0

TABLE 5

T E K N A FHEALTH CONSCIOUSNESS SURVEYDistribution of Respondents According toTheir Educational Status

Education	Male	Female	Both	Percent
No Education	33	73	106	53.0
Religious Education	13	21	34	17.0
Primary School	39	-	39	19.5
Secondary and higher	13	4	17	9.0
Not Known	1	2	3	1.5
Total	100	100	200	100.0

TABLE 6

T E K N A FHEALTH CONSCIOUSNESS SURVEYDistribution of Families According to Their Water Sources

Using Type		Ring Well	Tube Well	Tank	Ditch Within home	Not Known	Total
Drinking	No.	170	24	-	5	1	200
	%	85.0	12.0	-	2.5	0.5	100.0
Washing	No.	99	14	72	14	1	200
	%	49.5	7.0	36.0	7.0	0.5	100.0
Bathing	No.	91	14	83	11	1	200
	%	45.5	7.0	41.5	5.5	0.5	100.0

Table 7

T E K N A FHEALTH CONSCIOUSNESS SURVEYDistribution of Families According to Places of Defaecation

	Fixed Latrine	Bank of River	Side of House	Bushes	Field	No Fixed Place	Defaecate at home and thrown to the side of the house later	Not Applicable	Total
ADULT MALE									
Number	41	71	46	11	28	2	-	1	200
Percent	20.5	35.5	23.0	5.5	14.0	1.0	-	0.5	100.0
ADULT FEMALE									
Number	41	56	84	12	5	1	-	1	200
Percent	20.5	28.0	42.0	6.0	2.5	0.5	-	0.5	100.0
CHILDREN									
Number	11	14	26	5	4	115	15	12.0	200
Percent	5.5	7.0	13.0	1.5	2.0	57.5	7.5	6.0	100

TABLE 8

T E K N A FHEALTH CONSCIOUSNESS SURVEYDistribution of Respondents According to Cleaning Habits
of Anus and Hands After Defaecation

	Male	Female	Both	Percent
To wash anus				
a) Carry Water	94	99	193	96.5
b) Go to Tank	6	1	7	3.5
Wash hands with				
a) Only Water	25	28	53	26.5
b) Rubbing with Mud	67	62	129	64.5
c) Rubbing with soap	8	10	18	9.0

TABLE 9

T E K N A FHealth Consciousness SurveyKnowledge About Harmful Effects (Other Than Bad Smell)Of Indiscriminate Defaecation Near Home

	Male	Female	Both	Percent
No Harm	27	70	97	48.5
Helps Spreading Disease	30	10	40	20.0
Helps Spreading Intestinal Disease	5	8	13	6.5
Causes Problem in Movement	21	2	23	11.5
Ugly to Look At	4	5	9	4.5
Flies Grow	1	-	1	0.5
Combination of two of above	5	-	5	2.5
Other	-	2	2	1.0
Do not Know	3	1	4	1.0
Not Known	4	2	6	3.0
Total	100	100	200	100.0

TABLE 10

TEKNAFHEALTH CONSCIOUSNESS SURVEYKnowledge About The Effect Of Handling Food Without
Washing Hands After Defaecation

	Male	Female	Both	Percent
No harm	1	7	8	4.0
Intestinal disease	59	66	125	62.5
Disease	26	5	31	15.5
Dirty	8	18	26	13.0
Do not know	2	3	5	2.5
Not known	4	1	5	2.5
Total	100	100	200	100.0

TABLE 11

TEKNAFHEALTH CONSCIOUSNESS SURVEYKnowledge About The Role Of Flies In Causing Harm

	Male	Female	Both	Percent
Do not harm	7	24	31	15.5
Spread disease	74	29	103	51.5
Food becomes unclean	9	19	28	14.5
Lay eggs on food	6	21	27	13.5
Other	-	1	1	0.5
Do not know	3	6	9	4.5
Not known	1	-	1	0.5
Total	100	100	200	100.00

TABLE 12

T E K N A FHEALTH CONSCIOUSNESS SURVEYKnowledge About The Origin Of Flies

Origin of Flies	Male	Female	Both	Percent
Bush/Dustbin	50	18	68	34.0
Cowdung and Faeces	32	36	68	34.0
Rotten Fish	16	26	42	21.0
Others	1	1	2	1.0
Do not know	1	17	18	9.0
Not known	-	2	2	1.0
	100	100	200	100.00

TABLE 13

T E K N A FHEALTH CONSCIOUSNESS SURVEYKnowledge About The Effects Of Walking Barefoot

Effect	Male	Female	Both	Percent
No Harm	29	27	49	24.5
Soiling of feet and ulcer on legs	46	35	81	40.5
May cause injury	28	29	57	28.5
Worm	4	3	7	3.5
Other	-	4	4	2.0
Not Known	-	2	2	1.0
Total	100	100	200	100.0

TABLE 14

T E K N A FHEALTH CONSCIOUSNESS SURVEYKnowledge About The Mode of Infestation of Worms in Children

Causes	Male	Female	Both	Percent
Do not Know	78	12	90	45.0
Eating Fish	3	50	53	26.5
Eating sweets/ biscuits/banana	8	6	14	7.0
Spoiled Food	3	7	10	5.0
Eating uncooked rice	4	5	9	4.5
Ingesting dust	2	2	4	2.0
Combination	1	3	16	8.0
Not Known	1	3	4	2.0
Total	100	100	200	100.0

TABLE 15

HEALTH CONSCIOUSNESS SURVEYWillingness of making latrine near home

	Male	Female	Both	Percent
Yes	99	97	196	98.0
No	1	3	4	2.0
Short of space	1	2		
Water goes bad	1	1		

TABLE 16

T E K N A FHealth Consciousness SurveyDistribution of Respondents Willing to Pay ExpenseAs Labour For Building Latrine Near Home

	Male	Female	Both	Percent
Pay Expense	51	19	70	35.0
Pay by Labours	43	48	91	45.5
Un willing	4	27	31	15.5
No Comment	2	6	8	4.0
	100	100	200	100.0

TABLE 17

T E K N A FHEALTH CONSCIOUSNESS SURVEYKnowledge About the Symptoms of Dysentery

Symptoms	Male	Female	Both	Percent
Do not Know	2	-	2	1.0
Mucous in stool	5	1	6	3.0
Blood in stool	-	6	6	3.0
Mucous and blood in Stool	59	65	124	62.0
Abdominal Pain	2	-	2	1.0
Mucous and Abd. Pain	8	2	10	5.0
Blood and Abd. pain	1	1	2	1.0
Mucous + Blood + pain + frequent diarrhoea	19	22	41	20.5
Not Known	4	2	6	3.0
Total	100	100	200	100.0

TABLE 18

T E K N A FHEALTH CONSCIOUSNESS SURVEYKnowledge About the Symptoms of Diarrhoea

Symptoms	Male	Female	Both	Percent
Do not Know	5	2	7	3.5
Watery Stool	69	74	143	71.5
Watery stool & Vomiting	11	14	25	12.5
Vomiting	4	1	5	2.5
Watery stool + Frequent diarrhoea + patient becoming weak	2	4	6	3.0
Abdominal Cramp	-	2	2	1.0
Watery Stool + Vomiting + Patient becoming weak	8	3	11	5.5
Not known	-	1	1	0.5
Total	100	100	200	100.0

TABLE 19
TEKNAF

HEALTH CONSCIOUSNESS SURVEY

Knowledge About the Mode of Spread of Diarrhoea and Dysentery

Spread by		Male	Female	Both	Percent
Do not Know	Dia.	48	53	101	50.5
	Dys.	51	45	96	48.0
Walking over or near diarrhoeal stool	Dia.	16	14	30	15.0
	Dys.	18	29	47	23.5
Eating left over food of patient/ contact with patient	Dia.	13	12	25	12.5
	Dys.	13	8	21	10.5
Flies	Dia.	10	12	22	11.0
	Dys.	9	9	18	9.0
Bad food	Dia.	7	4	11	5.5
	Dys.	4	4	8	4.0
Polluted water	Dia.	5	2	7	3.5
	Dys.	3	2	5	2.5
Other	Dia.	1	3	4	2.0
	Dys.	2	3	5	2.5

TABLE 20

T E K N A FHEALTH CONSCIOUSNESS SURVEYBelief About The Right Age For Stopping Breast Feeding

Age (months)	Male	Female	Both	Percent	Cum. percent
6	1	-	1	0.5	0.5
12	12	2	14	7.0	7.5
18	17	2	19	9.5	17.0
24	44	37	81	40.5	57.5
30	14	35	49	24.5	82.0
36	10	21	31	15.5	97.5
48 ⁺	2	2	4	2.5	99.5
Not Known	-	1	1	0.5	100.0
Total	100	100	200		

TABLE 21

T E K N A FHEALTH CONSCIOUSNESS SURVEY

Believes of Respondents on Effects of Feeding Boiled Rice
To Infants Before 1 year of Age

Effects	Male	Female	Both	Percent
No harm	12	29	41	20.5
Can not digest/will cause intestinal disease	40	17	57	28.5
Make a child weak/have large belly	10	15	35	17.5
Cause worm infestation	17	10	27	13.5
Combination of above	20	18	38	19.0
Not known	1	1	2	1.0
Total	100	100	200	100.0

TABLE 22

T E K N A FHEALTH CONSCIOUSNESS SURVEY

Belief of Respondents About The Age of Children
Acquiring Ability to Digest Rice, Meat and Fish

Age (months)	Male	Female	Both	Percent	Cum. Percent
6 - 12	2	-	2	1.0	1.0
13 - 18	8	9	17	8.5	9.5
19 - 24	11	20	31	15.5	25.0
25 - 30	10	16	26	13.0	38.0
31 - 36	26	10	36	18.0	56.0
37 ⁺	43	43	86	43.0	99.0
Not Known	-	2	2	1.0	100.0
Total	100	100	200	100	

TABLE 23

T E K N A FHEALTH CONSCIOUSNESS SURVEYBelieves of the Villagers on Causes of MalnutritionIn Children of 2 yrs. of Age

Cause	Male	Female	Both	Percent
Do not Know	34	57	91	45.5
Bad Effect of Breast Milk	8	23	31	15.5
Insufficient milk/ weak/sick at birth	23	3	26	13.0
Worm	14	6	20	10.0
Uncleanness	2	-	2	1.0
Combination of any two	16	9	25	12.5
Not Known	3	2	5	2.5
Total	100	100	200	100.0

TABLE 24

TEKNAF HEALTH CONSCIOUSNESS SURVEYRatings by the Villagers on Some Suggested Items for Development

Items	Essential	Useful	Useless	Total
Electricity	77.0	20.5	2.5	100.0
Cinema Hall	7.5	29.5	63.0	100.0
Rice husking machine	87.5	11.0	1.5	100.0
Hospital	99.0	1.0	-	100.0
Water supply	97.0	2.0	1.0	100.0
Mosque	80.5	17.0	2.5	100.0
Tank	88.5	11.5	-	100.0
College	62.5	36.5	1.0	100.0
Road to Shahpuridwip	70.0	29.5	0.5	100.0
Market	67.5	31.5	1.0	100.0
Latrine	95.0	4.0	1.0	100.0
Loan	30.5	44.5	25.0	100.0

ENVIRONMENTAL EPIDEMIOLOGY. I. ENVIRONMENTAL AND PROSPECTIVE
EPIDEMIOLOGICAL INVESTIGATION OF CHOLERA OUTBREAKS

W.M. Spira
M.U. Khan
Y.A. Saeed
A. Sattar

Introduction

The association of water and food, particularly the former, with the occurrence of cholera outbreaks is beyond questioning. However, the specific role played by these vehicles, especially in endemic areas, has not been documented. One question is whether or not a multiplication step in food, drink or fomites contaminated with infected water or feces is either necessary for disease transmission or even a frequent mode of transmission. Another question is whether contamination of water, food and fomites totally within the household of one infected person is an important mode of transmission.

A major problem in studying this question is that the uses of water are so many and so pervasive that distinctions needed for meaningful analysis are blurred. Thus, the usual methods used have been unable to penetrate beyond a broad picture of the transmission cycle.

The approach we have taken to this problem is to study actual outbreaks as they are occurring using techniques of environmental microbiology and prospective epidemiological surveillance. The use of both methodologies should allow us to pinpoint potentially critical points in the transmission of cholera. We can then ascertain the significance of each risk factor and quantitate the cholera vibrios actually present in each vehicle involved at that

point. In this way, we will be able to "track" the organism through the environment and relate the extent of its presence to the incidence of infection in the population at risk.

Materials and Methods

Patients admitted to the Matlab hospital with cholera were used as "pointers" to areas in which V. cholerae was likely to be found. The morning after an admission a team of epidemiology field staff and microbiology laboratory personnel would visit the bari from which the patient came. The water use pattern of each member of all families was determined by questionnaire as was the occurrence of diarrhea. Rectal swabs were taken from everyone in the index family or who shared a water source for any reason with the index family. Environmental samples taken for analysis included those from water sources, cooking and drinking water jars in houses, contact plates of food preparation surfaces, enrichment broth rinses of left hand fingers and of empty cooking pots and utensils, and samples of left over food. These last were collected just prior to their being heated for the evening and morning meal. All food samples were held in ice boxes until they could be processed. All specimens were examined for V. cholerae using standard CRL procedures for direct detection and enrichment, except that direct plates were prepared so as to obtain a count of organisms present in contaminated samples.

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12

REPORT OF THE ELEVENTH MEETING OF THE SCIENTIFIC
REVIEW AND TECHNICAL ADVISORY COMMITTEE
OF THE CHOLERA RESEARCH LABORATORY
DACCA, BANGLADESH

FEBRUARY 22 - MARCH 1, 1977

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Report of the Eleventh Meeting of the Scientific
Review and Technical Advisory Committee
of the Cholera Research Laboratory
Dacca, Bangladesh
February 22-March 1, 1977

INTRODUCTION

The Scientific Review and Technical Advisory Committee (the Committee) of the Cholera Research Laboratory (CRL) met for one week in Dacca, Bangladesh, to review the present and proposed activities of the CRL, and to provide technical guidance to the Directing Council. The members of the Committee, Consultants, and Observers are listed in Attachment No. 1. The Agenda for the meeting is summarized in Attachment No. 2.

The Committee noted with regret that it was meeting in the absence of the Director, Dr. Willard F. Verwey, who had returned to the United States two months earlier because of illness. The Committee extends its best wishes to Dr. Verwey for a speedy recovery, and commends him for his leadership of the CRL since its re-establishment as a research organization in May of 1974. In his Annual Report for Fiscal Year 1976, Dr. Verwey observed that: "It is a source of satisfaction, but not complacency, to state that over this period of time, particularly in FY 76, the Laboratory has moved from a situation of gross underutilization to one where it is now a matter of concern that the research projects are competing with each other for both personnel and facilities." The Committee shares both this satisfaction and concern.

The Director's Report comments on the re-orientations of CRL research activity that have occurred in response to developments in diarrheal disease research programs in other laboratories and in recognition of the needs of Bangladesh and other developing countries. Dr. Verwey's listing of these trends serves as an appropriate introduction to the Committee's appraisal of the research program:

- "1. Greater emphasis on work with the non-cholera diarrheal diseases.
2. A corresponding reduction in the preoccupation with cholera per se.
3. An intensification of the interests of the Laboratory in problems of population control and fertility.
4. An increase of interest in the inter-relationships between nutrition, diarrheal disease and fertility at the biomedical level.
5. In assigning research priorities, a somewhat greater weight applied to research offering possibilities of interventive action."

The Committee also noted that its meeting not only coincided with a change in CRL Directorship, but was being held at a time when negotiations, authorized by the Directing Council in August, 1976, were in progress to convert the CRL to an independent international institution having multilateral financial and technical support. Because this development bears directly on the future research program of the CRL and its successor Institute, a brief summary of the status of the conversion, now targeted for October 1978, has been included in this report.

The work of the Committee was greatly facilitated by the participation of Dr. Henry Mosley, Director Designate, of Dr. William B. Greenough, III, who is to become Director of Clinical Science, and of Dr. Michael Merson, who has been deputed by the Center for Disease Control to succeed Dr. George Curlin as Head of the Epidemiology Division. These three investigators have had prior experience at the CRL, and a smooth and effective transition in leadership seems assured.

The Committee expresses its gratitude to Dr. M. Mujibur Rahaman, Acting Director, for his gracious hospitality, and thanks the entire staff of the CRL, particularly Dr. George Curlin and Mr. Philip Weeks, for facilitating the review process. Both the Committee and the staff benefited from the participation of the consultants, all of whom contributed to the preparation of this report.

The scientific activities of the CRL are reviewed in the following sections in the order in which the research programs were presented by the responsible investigators (Attachment No. 2). In addition, to assure continuity with last year's Committee review and to focus on administrative concerns as they may bear on research productivity, brief sections are devoted to all Divisions other than Maintenance.

RESEARCH PROGRAMS

Oral Replacement Studies in Diarrheal Diseases

Diarrhea constitutes one of the most common and debilitating health problems in Bangladesh. Data from the CRL indicated that in 12 villages during November - December 1975, there were 67 cases of diarrhea per 1,000 person months. Although rates varied, the average incidence of diarrhea was one episode per person per year. About one per cent of cases required hospitalization. Both the incidence and case fatality due to diarrhea were much higher in young children than in adults. Replacement of fluid and electrolyte losses with a solution that may be taken orally has been shown to be effective in treating cholera and related diarrhea under medical supervision. This mode of treatment is very simple and inexpensive and could be applicable on a wide scale.

As proposed to the Committee in 1976, a trial of initial oral rehydration was done in the out-patient service of CRL, with continuing replacement being completed at home. From the approximately 200 patients who attended the OPD each day, cases deemed by objective criteria to be moderately severely depleted as defined by a plasma specific gravity of

1.029 were entered in the study. Of 451 consecutive cases, 174 had specific gravities less than 1.029, and were excluded. Of the 277 remaining, 230 were successfully rehydrated in the clinic and at home. The remaining patients required hospitalization or supplementary intravenous fluids. Of those patients followed at home, six required additional intravenous treatment. Of the 174 milder cases, 12 were considered treatment failures. These data indicate that oral replacement with a special solution in an ambulatory care setting followed by unsupervised treatment at home may be a highly successful and inexpensive approach to treatment; since there was no concurrent control group treated with kaolin mixture, this conclusion required confirmation.

In four villages of the Matlab area a subsequent study was conducted on the usefulness of unsupervised oral replacement therapy for diarrhea. In two villages cases were treated with the standard aqueous kaolin mixture; in two other villages, with the CPL oral replacement solution with the following composition: Na^+ , 120; K^+ , 25; Cl^- , 9; and HCO_3^- , 50 mEq/l, and 20 grams per liter of glucose. Dried salts were packaged to be mixed in a seer* of water. Samples of the mixtures used were assayed later to verify how accurately families prepared the solution. All patients were advised to come to the Matlab Hospital should they feel treatment was not working. Field Assistants visited all cases daily, and took blood specific gravity measurements on the first and third days. Serum sodium was also measured on the third day. All cases were cultured for etiologic diagnosis.

Except for the occurrence of 10 cases of cholera in the standard treatment villages as compared to only one case in the special treatment villages, the pattern of diarrhea was comparable, with an incidence of 20 cases per 1,000 person months. Too few severe cases were seen to judge effectiveness in the prevention of serious morbidity. In the mild cases both forms of treatment worked equally well, but in the oral replacement group there was a significantly greater reduction in blood specific gravity on the third day. There was great variability in composition of the home mixed solution; sodium concentrations ranged from 90 - 230 mEq/Liter. When the Field Assistants showed families how to measure one seer, the accuracy of mixtures was much improved. There was one instance of hypernatremia, presumably due to inadequate intake of salt-free water.

During discussions of these results, it was suggested that to ensure safe and effective use of oral replacement solution in the home, several important steps must be taken. First, it will be necessary to establish a convenient and accurate measure of the amount of water to be added to packaged dry salts by an uneducated person. Second, the salts must be packaged cheaply, yet secure from moisture. Third, the acceptability of the salt solution must be established with respect to color and flavor. Fourth, it must be determined whether prior training or supervision by a health worker is needed. Fifth, it must be shown that

* Seer-a poorly standardized measure, approximately a liter, but varying with the size of available container.

mortality and the need for hospitalization can be averted in a field situation. Finally, it will be necessary to demonstrate the superiority of oral electrolyte therapy over water and food ad lib term. To do this, means must be devised of assuring that the home-mixed solutions are properly prepared.

E. Coli Diarrhea-

An excellent study of the clinical and laboratory aspects of diarrhea associated with toxinogenic E.coli (TEC) was carried out on 176 males over 10 years of age admitted to CRL with acute diarrhea during 10 weeks in the fall of 1976. Cholera and dysentery-like illnesses were excluded. Previous data had shown that the usual enteric pathogens could be isolated from less than 50% of patients admitted to the Matlab hospital. The purpose of this study was to determine how many of the remaining "unknown" diarrheas were associated with TEC and to look for clinical and epidemiologic correlates with these agents. Heat labile toxin (LT) was detected by the Chinese hamster ovary cell assay, and stable toxin (ST) by the suckling mouse assay. Both assays appeared to be well established at the CRL.

Sixty-two percent of the cases yielded either LT or ST producing strains on selective culture. More than half were LT-ST; one-third were ST only; six strains were LT only. Only one isolation of invasive E.coli was made. A few non-cholera vibrios were isolated but usually in conjunction with TEC.

E.coli diarrhea was relatively much more common among adults than is cholera. Clinical analysis suggested that LT-ST disease was slightly more severe than diarrhea associated with ST-only. ST-only cases had significantly shorter duration of stooling and lower stool volume; this is consistent with the activity of ST in animal models. There were too few cases of LT-only to draw conclusions. A controlled study indicated that tetracycline was moderately effective in reducing the duration of LT-ST disease, but had no effect in ST disease. This important observation should be followed by a search for association between ST and RTF plasmids.

Excellent data on the effect of pooling clones for LT detection showed that, in clinically manifest disease at least, picking of one clone was as sensitive as testing a pool of five or 10 clones.

The investigators plan to measure anti-LT antitoxin response, especially in relation to antibiotic therapy, to look for persistent infection following clinical recovery, and to attempt direct LT and ST detection in stool samples. Further studies on antibiotic sensitivity in relation to toxin production are also planned. Rotavirus studies will be carried out on stored serum and stool samples.

It will be important to learn how much inapparent infection there is with enterotoxinogenic E.coli. The effect of pooling of isolates will be an important consideration. Because of the immunologic relationship between cholera and E.coli LT toxins, sero-epidemiologic surveys of antitoxin levels to either toxin may be useful in clarifying the epidemiology of both cholera and TEC diarrhea in Bangladesh, and might provide an understanding of how they relate to one another.

Epidemiologic data collected in Matlab during 1974 and 1975 sought to determine the role of LT+ E.coli in the total diarrheal disease picture. Diarrheas of unknown etiology accounted for 58% of 11,982 cases studied. The case fatality rate in this group was 1.1% in contrast to 5.6% for cases of shigellosis. Disease appeared in spring and fall peaks roughly comparable to cholera. E.coli strains isolated during the latter part of the study were tested for LT production, and about 18% were found to be positive. The isolation rate of LT+ strains was more than twice as high in hospitalized cases as in OPD cases. It was estimated that the overall incidence of LT+ diarrhea which reached Matlab facilities was about 1.7/1000 during a six month period. The rates were highest in children aged 1-4 years, but their illnesses tended to be mild and seldom required hospitalization. The disease became more severe with advancing age; 70% of persons over 15 required hospitalization. This well-designed and well-executed study has provided the first quantitative estimate of the impact of diarrhea associated with TEC in this population. It is hoped that similar data can be obtained on ST disease, and on inapparent infection with organisms producing both LT and ST.

Amoebic Dysentery

A study has described the appearance of the rectum during clinical amoebic dysentery as viewed through a proctoscope. Cases were selected on the basis of blood and mucus in the feces associated with E. histolytica on microscopy of fecal smears. A characteristic pattern emerged in that the earliest visible signs were excessive mucus and swelling of the rectal folds, followed by a lobular appearance in the pink mucosa due presumably to changes in the vascular bed. With suitable treatment these changes could be reversed. A serious complication was the beginning of necrosis in the bowel wall; this signaled a poor prognosis even with optimal treatment. Even in those patients who did recover, the appearance of the rectum returned to normal only after a long delay. The necrotic form appeared to be a different syndrome in that it had a much more acute onset and occurred exclusively in older people. On the basis of this study, cases are now clarified in one of three categories depending on degree of severity of the proctoscopic findings: - mild, lobular, and necrotic. An interesting feature was that severity did not seem to be related to the numbers of E. histolytica found in the rectal swabs.

Two other positive observations were made. When delayed hypersensitivity to a variety of antigens was tested, the mild and lobular forms exhibited similar proportions of positive skin reactions. By contrast, those patients with the necrotic form showed a remarkable and significant depression in their D.H. reactions. Another finding which correlated with necrosis was the level of a serum protein component called prealbumin. On admission to hospital the prealbumin was lowered to about 10% of normal in those patients with necrotic lesions. It is not clear at the moment what the functional significances of these two correlations are, but they are both of considerable interest and further work is required. The Committee thought this work was good, but suggested the inclusion of a control series to determine the prevalence of E. histolytica infection in the general population. Since there was no correlation between severity and numbers of E. histolytica, discussion centered on the casual role of this organism in the production of the rectal changes. For this question it would be useful to know if increases in antibody levels to E. histolytica antigens occur during the course of the disease.

Leukemoid Reaction in Shigellosis

Dysentery due to shigella infection continued to be a major clinical problem at CRL in 1976. More than 1,100 cases were admitted. These had a six per cent case fatality rate, with the rate being highest in young children as usual. The interesting leukemoid syndrome which was described at CRL a few years ago was again a feature in 77 cases. As a result of intensive investigation of such cases, it seems that endotoxin may circulate in the blood stream of patients present with the hemolytic-uremic syndrome, and that those with a leukemoid reaction may possess circulating immune complexes. As a working hypothesis, it has now been suggested that these complications follow the absorption of bacterial endotoxin as a result of severe colonic ulceration. This bacterial antigen then evokes a brisk immune response; at a certain stage in antibody formation, immune complexes may form, circulate and be deposited, particularly in the kidney. This simple hypothesis is at least testable. Preliminary studies indicate that when immune complexes are detectable endotoxin is not. It is therefore likely that the immune complexes are in antibody excess. Thus, it should be possible to detect anti-shigella antibodies at this time, - perhaps by using the hemagglutination test. No doubt this will be done. Real progress has been made in the last year towards resolving the puzzle of this interesting condition.

Shigella toxin

Work on shigella toxins has not advanced much since last year's report was written. The existence of Shiga toxin in filtrates of local strains has not yet been clearly demonstrated. A few studies have been carried out on rabbit ileal loop activity in crude filtrates from one selected strain of Shigella dysenteriae type 1. As expected, shigella filtrates are relatively less active than cholera toxin. Membrane filtration suggests a molecular weight of 50,000 - 100,000 which would be

consistent with shigella neuroenterotoxin. Attempts have been made to raise antitoxin in rabbits, but this has shown only minimal protection in high doses. The toxin appears to be stable at 56C but destroyed at 99C.

Although it was recommended last year that toxin produced by the local strain be tested against the International Standard Dysentery Antitoxin this has not been done. It is important to determine whether the exotoxin(s) elaborated by local strains are immunologically related to classical Shiga neurotoxin and to the toxin produced by recently isolated strains from Central America. Hela cell studies have been dropped, and no attempt has been made to look for antitoxin response in dysentery cases.

Since the pathogenesis of the leukemoid-hemolytic syndrome in shigellosis is still unknown, it would be wise to determine the antitoxin response in these cases, and to look for evidence of involvement of the Shiga exotoxin or derived antigens in the pathophysiology of this disorder. A mouse assay has been described and it was recommended last year that this be used at CRL. Examination of the effect of toxin-containing preparations on blood elements of laboratory animals might also be informative.

Procedures for simple concentration and partial purification of Shiga exotoxin were described long ago, and could be used to advantage at CRL to help answer a few of these basic questions without getting involved in sophisticated purification techniques. If preliminary work with Shiga toxin should suggest promising ways of relating toxin to the pathogenesis of shigellosis, then these studies might be pursued further; to be successful, however, more direction and greater collaboration among members of the CRL staff will be necessary.

Diarrhea Associated with Non-Cholera Vibrios

There has been a recent and sudden increase in the number of diarrhea cases associated with the isolation of non-cholera vibrios of Heiberg Group III. Very preliminary data were presented showing that crude culture filtrates of these organisms caused fluid accumulation in rabbit ileal loops. The filtrates have not been tested for heat stability or resistance to trypsin. Since previously described NCV toxins have been cholera-like, it would be sensible to determine the effect on vascular permeability in skin and on CHO or adrenal cells, and to do neutralization tests with cholera antitoxin. Also, the strains isolated should be sent to a reference laboratory for serotyping so that meaningful epidemiologic data can eventually be obtained. The data presented mean very little as they stand, but a systematic study of non-cholera vibrios which appear to be agents of diarrhea would be worthwhile. Such studies would include:

determination of PF activity in filtrates of non-cholera vibrios isolated from different sources to look for association between toxinogenicity and pathogenicity; confirmation of positive PF tests by ileal loop and neutralization by cholera antitoxin; preparation of antitoxin against immunologically unrelated "new" toxins if they are found; serotyping of representative strains; measurement of antitoxic and specific antibacterial serologic responses in patients from whom organisms were isolated.

Population Studies

The data which have been carefully recorded for the vaccine trial area population of 260,000 persons since 1966 provides demographic information of increasing value each year. Serious disruption of the population over a nine month period occurred as a result of civil unrest in 1971 and again in 1974 when a serious food shortage followed floods and failure of the rice crop. Mr. Alauddin Chowdhury reported the effects of these events on certain demographic markers. Death rates over the period 1966 to 1971 were relatively stable; in 1971 there was a 33% increase over previous levels. In 1974, there was again a sharp rise, about 50% over the previous year. Infant death rates showed a similar picture, with a more distinct upward trend. Increased numbers of deaths were mainly among the children and the elderly; the increased mortality was associated with a markedly increased incidence of shigellosis. The total fertility rates showed a very slow downward trend attributable to a pronounced decline in the age-specific fertility rate of the 10-19 year age-group. A fall in 1975 affected all groups. The validity of these data was confirmed by comparing "Old Trial Area" (those villages studied since 1966) and the "New Trial Area," (those villages added in 1968); the curves were essentially identical.

To test the hypothesis that the declining fertility in the 10-19 year age-group was due to later menarche and later marriage, girls in this age-group living in 13 villages were interviewed. There had been an increasing age of onset of menarche, and this appeared to be associated with the malnutrition experienced during the period 1971 through 1975. Higher body weight was strongly associated with earlier age of onset of menarche, which was in turn associated with earlier age of marriage. Data for Hindu girls was comparable to those for Muslim girls, although their age at marriage was somewhat higher.

To study the relation of maternal nutrition to the length of post partum amenorrhea and to the interval between resumption of menstruation and conception, a longitudinal study of 2,200 married women aged 15-49 years was begun in November 1975. Analysis of the experience of the first year indicated that the duration of post partum amenorrhea was longer for women with lower body weight (26 vs. 22 months); the interval between resumption of menses and conception was even more strikingly

lengthened (40 vs 24 mo.) in these women. Another survey of over 2,000 women found that the median length of post partum amenorrhea in breast-feeding women was 20 months, and that the period of amenorrhea was prolonged with increasing age and/or parity. Higher socio-economic levels were associated with shorter intervals of amenorrhea. Improved nutrition of the mother, measured as ponderal index, decreased the period of amenorrhea; supplementation of the infant's diet reduced the proportion of post partum women who were amenorrheic. These findings indicated a direct relation between nutrition and fertility. This was supported by the interesting practical observation that there was a marked seasonality in the resumption of menses, with a greater proportion of women resuming menstruation in November and December following the harvest, regardless of whether the birth occurred in February-April, or August-September.

A survey of 3,850 married women (in 20% of the villages in the area now included in studies on contraceptive dissemination) compared the number who became pregnant during the famine with the number who became pregnant one year later. After the famine, increased fertility was manifested by the poorest women. Poverty was estimated by possession of minimal living space, and by marriage to landless agricultural laborers. Thus, fertility was markedly reduced in those most affected by the famine.

A contraceptive distribution project was described in which contraceptives were made available at the doorstep in over 100 villages with a population of approximately 130,000; a comparable area served as a control. In this project the cholera program Lady Village Workers (dais) and Field Assistants brought the materials and instructions to each household. A preliminary survey indicated that 2.4% of women were using a modern contraceptive method, and 4.9% traditional methods. Thirty-six per cent indicated a willingness to use contraception, and 25% of these would use pills. The second choice was periodic injections; none chose IUD's. For a 20% sample of women, experience in the first year showed that contraceptive use rose from 1.1% to 18%, falling at the end of the period to 15%. In contrast, use in the control area was reported by 2.9% at the beginning and 3.6% at the end. The higher basic rate in the control area is explained by inclusion of Matlab village where the Johns Hopkins Fertility Research Project is functioning. Use was greater by Hindu women than by Muslim women in the control area, and comparable in the study area. The effectiveness of the program in terms of lowered birth rate was evidenced by a lowered proportion of women who reported pregnancy in the contraceptive area than in the control area in the surveys carried out after April 1976. By a case-control method, symptoms in users were compared to those in non-users from the same village and from a control village. Dizziness, eye problems, and a "burning sensation" were reported more frequently by users. Breast feeding was practiced by significantly more contraceptive users; non-users more frequently wanted more children. Of interest was the fact that 61% of users and 34% of non-users would be willing to undergo induced abortion at two to three months gestation.

This intensive sampling was carried out on a daily basis and more families and water sources were included if they could have been contaminated on succeeding days. This sampling was continued until all samples in a study area were negative for three consecutive days.

The study was carried out from October 1976 through January 1977.

Results and Discussion

The tabulation of this data is far from complete and only a very preliminary analysis can be provided. So far the data of 13 sites has been partially tabulated. This covers 695 individuals in 118 families. The average family size is 5.9 (SD = 1.2). In all 47/695 persons were infected with V. cholerae El Tor (6.7%). The serotype in all cases was Inaba. The gross results of our environmental sampling are given in Table 1. The number of contact plates and utensil rinses has not yet been tabulated but in no case were El Tor vibrios detected.

The cumulative infection rate and the rate as a function of exposure to various sources of infection is given in Table 2. There is a pronounced shift in peak when the infection rate is distributed as a function of exposure, particularly exposure to cooking jars. This shift may be indicative of a significant role

played by these sources in the transmission of cholera at these study sites.

The clinical picture of the 47 infections covered in this study is fairly typical of El Tor outbreaks: 1 hospitalized, 11 with mild to moderate diarrhea and the remainder asymptomatic. The age distribution was also usual for cholera - all symptomatic persons were children as were half of those were asymptomatic.

Fomites and foods were intensively sampled throughout these outbreaks. Absolutely no contamination of either was detected except for two food samples which were not associated with any detectable infection after their ingestion. There is virtually no possibility that these were significant vehicles in cholera transmission. There is no possibility whatsoever that a multi-plication step occurred with any reasonable frequency in food.

The role of water as a vehicle in these outbreaks is incontrovertible. However, the data analysis has not proceeded to a point in which a final decision on the specific mode of transmission can be made. Exposure to contaminated bathing water (Table 3) and contaminated cooking jar water (Table 4) were both highly significant risk factors. Drinking water jars which were filled from tubewells were virtually never contaminated so their role is negligible. Thus, bathing water and cooking water appear to be the most important vehicles at this point. As Table 5 shows,

there is a great deal of overlapping use of the same water source for both of these purposes. When the bacteriological findings are included, the effective difference is only 3.4%. Some indication of the relative importance of exposure to each source on infection rate may be had from the analysis in Table 6. Considering only those persons exposed to contaminated bathing water on day 1, only those who were also exposed to contaminated drinking jar water were at risk of becoming infected. The reverse analysis, i.e. distribution of infection among the 37 persons already exposed to contaminated cooking jar water as a function of concomitant exposure to a contaminated bathing site shows no significant difference. It appears, therefore, that water brought into the house for cooking purposes may be, in some manner yet unknown, the major vehicle for cholera transmission in these outbreaks. This conclusion is highly tentative at present and must await a complete analysis of the data.

We hope to be able to tabulate the data from this study and carry out its analysis using the computer facilities available to CRL in Bangladesh. This activity will represent the remaining future work on this protocol.

Table 1

Detection of V. cholerae El Tor in Environmental Samples

Sample Type	No. Positive/Total Samples
R/S	65/5064
Cooking jars	110/1571
Food samples	2/1511
Finger wash	0/677
Water sources	103/1067

Table 2

Cumulative Attack Rate as a Function of Exposure to Various
Water Sources on Day 1

	<u>Vibrio</u> <u>cholerae</u> <u>isolated</u>	Total infected	Cumulative Infection Rate (%) on day							
			2	3	4	5	6	7	8	9
Cooking	+	37	10.8	40.5	54.0	73.0	83.8	94.6	97.3	100.
jars	-	10	40.	60.	70.	90.	90.	90.	90.	100.
Bathing	+	28	14.3	35.7	50.	71.4	85.7	100.	-	-
site	-	19	15.8	52.6	68.4	84.2	84.2	84.2	89.5	100.
Overall		47	17.0	44.6	57.4	76.6	85.1	93.6	95.7	100.

Table 3

Infection Rate for 8-day Period Following Exposure to
V. cholerae — Positive Bathing Water on Day 1

Bathing	Persons at Risk		Total
	Infected	Not Infected	
Positive	28	192	220
Negative	19	274	293
	47	466	513

$$P (X^2 \geq 5.15) < 0.01$$

Table 4

Infection Rate for 8-Day Period Following Exposure to
V. cholerae — Positive Cooking Jars on Day 1

Cooking Jar	Persons at Risk		Total
	Infected	Not Infected	
Positive	37	122	159
Negative	10	348	358
	47	470	517

$$P (x^2 \geq 53.4) \ll 0.001$$

Table 5

Differences in Water Use for Cooking and Bathing by
Families in Outbreak Study

No. using same site for both	86
No. using different sites	33

<u>V. cholerae</u> isolation (Day 1)	Q/O	25
" isolation "	+/+	4
" isolation "	+/O	} 4
or	O/+	

119

Differential on basis of V. cholerae isolation = $4/119 = 3.4\%$

Table 6

Infection Rate for 8-Day Period Following Exposure to a V.cholerae -
Positive Cooking Jar on Day 1 in that Population Who also Used
a Contaminated Bathing Site on Day 1

Cooking Jar	Persons at Risk		
	Infected	Not Infected	Total
Positive	27	98	125
Negative	1	79	80
	28	177	205

$$P (X^2 \geq 14.8) < 0.001$$

ENVIRONMENTAL EPIDEMIOLOGY. II. ECOLOGICAL STUDIES ON
VIBRIO SP. IN CANAL AND TANK ENVIRONMENTS

W.M. Spira
K.M.S. Aziz
W.F. Verwey

Introduction

Contaminated water sources are strongly associated with the spread of cholera and other diseases transmitted by the fecal — oral route. The environmental transmission of V. cholerae in particular seems to be centered on water. Epidemiologic research on cholera has established a clear picture of the spectrum of the disease and has accurately characterized the general nature of endemic environments, both physical and social. It has, however, been unable to supply definitive answers to several questions. Those of particular concern to this study are the seasonality of cholera and the maintenance of V. cholerae during inter-epidemic periods. Answers to these questions, especially the first, are very likely to require a knowledge of the specific behaviour of V. cholerae in natural waters as well as a broader characterization of seasonal variations in tank and canal ecology.

The approach we have taken in this study is a systematic observation of a small number of water sampling points in an area which has a history of being at high risk for cholera. We have focused mainly on detecting variations in the concentration of Vibrio sp. in different habitats as a function of season. In this regard, we have monitored certain chemical, physical and meteorological parameters as well. We have also included measure-

ment of total microorganism and plantation concentration, of fecal indicator organisms and of other clinically important organism (pathogens and antibiotic resistant bacteria).

The primary objective is to determine the seasonal variation in Vibrio sp. population and to assess the role of various microenvironments (water, sediment, plants, phyto and zoo-plankton in the maintenance of these organisms. In particular, we are looking for conditions or microenvironments which are conducive to the survival and, perhaps, maintenance of V. cholerae. As a secondary objective, we are attempting to assess whether such seasonal variations are part of a more general seasonal effect on tank or canal ecology. The screening for clinically important bacteria has been included to evaluate their extent in the non-human environment in this area.

Materials and Methods

Sampling was carried out at 28-day intervals at selected tank and canal points in Meharon from March 1976 to the present. A shift to fewer samples and more extensive analysis occurred in October. From March to October, water samples only were collected at weekly intervals and only from near the surface. These were analyzed for Vibrio sp. and some physico-chemical properties.

Water samples were taken from the surface, middle of the water column and from the mud/water interface. Plants (primarily water hyacinth) were collected at each point. A plankton sample was obtained by sieving water through a net. Sediment samples were obtained using a core sampling device. Water samples were also collected from points in Baragaon during the period March - July 1976. Water and plant samples were collected from a number of water sources in the Matlab VTS area during the fall of 1976 as well.

The pH, dissolved oxygen tension, (CO_2) and temperature of water point at 0600, 1200 (sampling time) and 1800 was determined on - site. Weather observations were made daily at the Matlab laboratory.

The sites monitored were a large tank (100 x 200') used for all purposes by a population of 100 - 150 and the canal near the main ghat which is extensively used as a site for bathing and gathering cooking water. Water sample points were established in the center of the tank and along the edge at a point 30 ft. from the main ghat and approximately 10 feet from a latrine area used by 4-6 persons. Sampling points in the canal were established at 30 feet directly out from the main ghat and at the edge of this ghat.

Microbiological examination of samples included total aerobic heterotrophic plate count; coliform, E. coli, fecal streptococci and antibiotic resistant bacteria counts; and enrichment for Salmonella and Shigella sp. Vibrio sp. were detected using a membrane filtration technique in which the cells were allowed to grow on starch agar for 4 h at ambient temperature prior to being transferred to TCBS at 37C. This procedure significantly improved the isolation of vibrios over direct plating on TCBS. Vibrio isolates were characterized by reaction in KIA and MIU, fermentation of sucrose, mannose and arabinose, sensitivity to O/129 vibriostatic compound and by agglutination in O Group I antisera. The plankton biomass in water samples was estimated by microscopic count.

Results and Discussion

The weather normals for the Matlab area are given in Figure 1. These three climatic parameters (rainfall, temperature and sunlight) along with nutrient input tend to be the dominant determinants of natural water ecosystems. It is only in relatively extreme situations, such as the presence of industrial wastes, that other factors become equally important. It is possible to distinguish four seasons from the data in Figure 1: June - September (high rainfall and temperature, low sunlight);

September - December (decreasing rainfall and temperature, increasing sunlight); December - February (low rainfall and temperature, high sunlight); and February - June (increasing rainfall and temperature, decreasing sunlight). The weather monitoring in Matlab as well as observation obtained from the weather station at Narayanganj since November has proven to be fairly close to the 30 - year normals. The noon temperature at 60 cm depth in both tank and canal (Figure 2) closely parallels the maximum in temperature during the warmer months but tends to be closer to the temperature minimum during the colder months. The great difference in mixing between the two water sources had no demonstrable affect on water temperature.

We observed a very strong tendency for a DoT gradient to form in the stationary tank water as the day progressed (Figure 3a). DoT at the tank surface was in excess of equilibrium concentration and, hence, unstable while the DoT at 150 cm and below was too low to measure. The oxygenated zone deepened as the day progressed and this effect became more marked in December - January as a result of increased photosynthetic activity in response to more sunlight. Lower temperatures did not appear to inhibit photosynthetic activity although overall oxygen demand is reduced as indicated by the shift in sunrise DoT profile. The well-mixed canal water column failed to develop a significant DoT gradient (Figure 3b), though an increase in DoT at later sampling times

is apparent. The seasonal influence on DoT is also apparent in this system. The thorough mixing, however, prevents the overnight oxygen depletion observed in the tank water column.

A similar pattern exists for pH (Figure 4a-b) which is highly correlated with CO₂ concentration and, thus, strongly linked to the level of photosynthetic activity. The pH of these sampling sites does not appear to be strongly influenced by laundry soaps at the level they are used at present.

Data on the concentration of various life-forms others than Vibrio sp. is available only since November. Analysis is incomplete but data for the seasonal extremes so far will be presented. The concentration of phytoplankton (Table 1) is, in general, higher at the surface than deeper in the water column by about a factor of 10. The concentration is somewhat lower in January than in November and lower in the canal than in the tank. No difference between edge and center of water sources was observed.

The seasonal change in total heterotrophic aerobe concentration (Table 2) shows a similar decrease with colder weather, particularly in the center of water sources. The count at the edges is less affected by season and possibly reflect an increased role of human contamination of these sites in adding to the bacterial population. The concentration is greater at the surface

and mid-water interface than in the center of the water column in the tank. The thorough mixing in the canal prevented this layering to a great extent. Gram negative organisms comprise the bulk of the surface microflora while the mud-water interface population is more than half Gram positive bacteria.

The coliform count (Table 3) shows no layering effect but does show the effect of season. This count is primarily Enterobacteriaceae. The concentration of E. coli varies widely between samples and shows some effect of edge vs center, the edge having a higher concentration. A seasonal effect is not apparent thus far with this data. The fecal coliform/fecal streptococci ratios are consistently in the range of 1-7 indicating that virtually all fecal contamination in our sampling points can be attributed to humans.

Isolations of Vibrio sp. for the past year are shown in Table 4. No V. cholerae biotype El Tor were isolated during this period and Meharon experienced no demonstrated cases of cholera. This "unfortunate" situation points out one difficulty with this type of study. Isolation of the so-called "NAG vibrios" (V. cholerae, NAG) were frequent throughout the study period. Heiberg groups II and V dominated these isolation, though groups I, III and VII were occasionally found as well. A statistically significant decrease in the frequency of isolations occurred in

December - January. Surprisingly, the frequency of isolation during the monsoon period was not lower than during the diarrhea peak periods of Spring and Fall.

The concentration of NAG vibrios in positive water samples (Table 5) shows no significant seasonal trend. It is clear, however, that the frequency and concentration of NAG vibrios in these water sources during the monsoon period are little different than during the peak diarrhea seasons.

Heiberg groups I and II have traditionally been thought to contain most of the human — associated NAG vibrios while those in group V were usually considered water vibrios. Table 6 shows the concentration of group I and II organisms at the various sampling points. No differences are seen in the tank while the samples taken at the canal ghat are significantly higher than those in the center. Other data collected recently at other points in the tank indicate that the concentration of NAG vibrios directly at the main ghat can be extremely high (10^4 /ml) without a noticeable change occurring at the sampling point 30 feet away. Apparently the stratification in this water source is intense both horizontally and vertically. Data for group V organisms show no statistically significant difference between sampling points as would be expected if these were true inhabitants of the water system. This may also be the reason why the group V

organisms managed somewhat better than other groups during the monsoon season (Table 4).

Vibrio sp. were not isolated from sediment, phyto - or zooplankton specimens taken at any time since November. A significant association was found, however, between Group V NAG vibrios and water hyacinths collected at the sampling points (Table 7). This association may be a maintenance factor for these organisms and may act for other vibrios as well. To test this possibility in the case of El Tor vibrios, we collected paired hyacinth and water specimens from tank and canals known to be used by persons with cholera. Initial analysis of this data shows that El Tor vibrios do associate with water hyacinths as do Heiberg Groups I and V of V. cholerae, NAG. Heiberg Group II organisms were not found in association with hyacinths. The behavior of El Tor vibrios and those in groups I and V were identical in our study. The combined results show a significantly greater association of V. cholerae with hyacinths than the water column (Table 8) and a significantly higher concentration in positive specimens (Table 9). The maximum concentration observed for an El Tor isolation was 4800 per gram in a water hyacinth specimen taken from water containing 10 El Tor vibrios/ml. This association with a surface plants may play a role in the dissemination of the cholera vibrio during outbreaks and it may provide a means by which the organism can maintain itself for longer periods than in the water column.

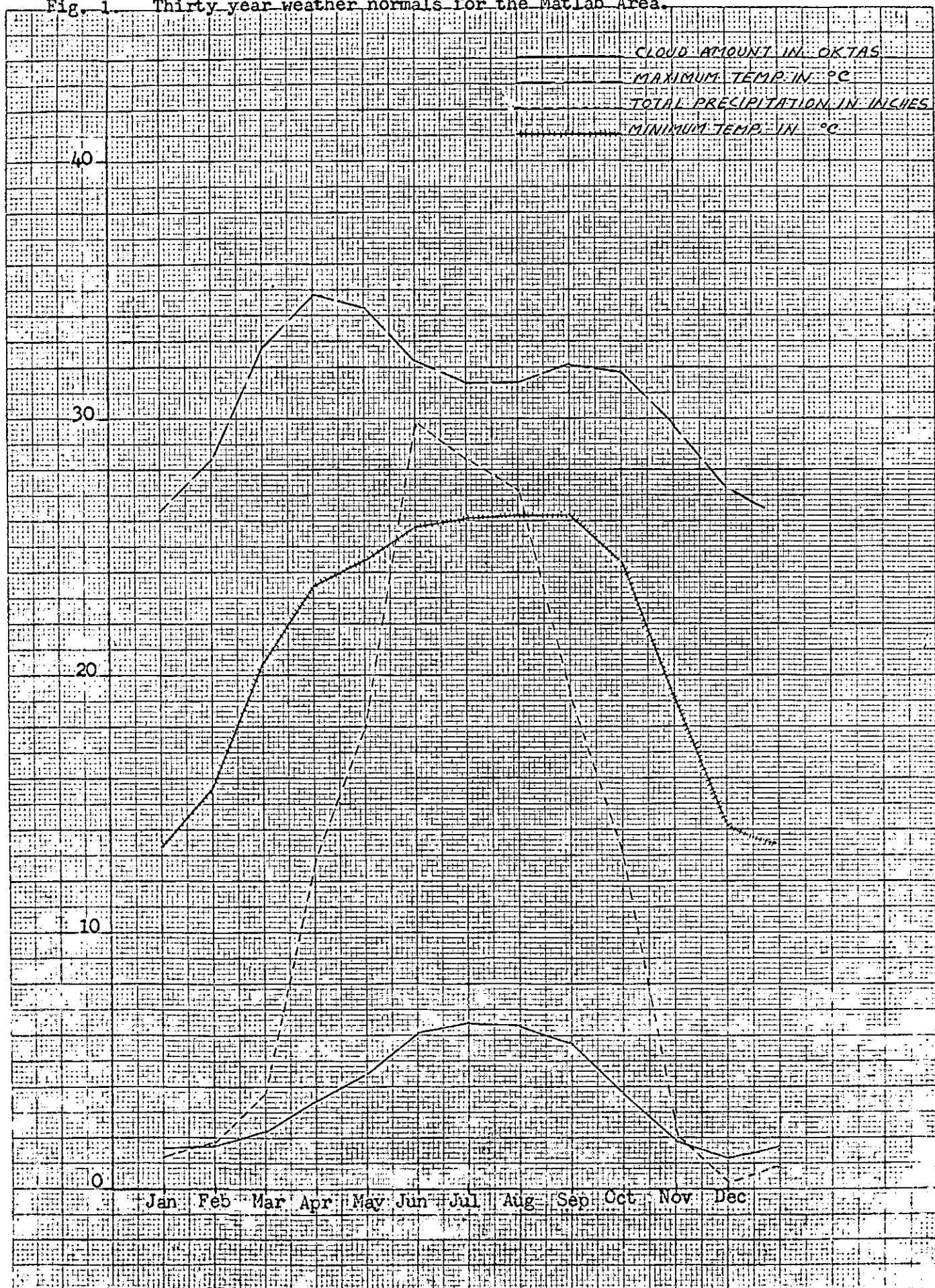
The screen for antibiotic resistance has yielded a high number of resistant isolates. Most are Pseudomonas sp. but about 1-5% of the isolates which appear to be Enterobacteriaceae are resistant to three or all four of the screening antibiotics (penicillin, streptomycin, tetracycline, chloramphenicol). These, too, have tended to occur in higher concentration on surface plants than in the water column.

Future work

The longitudinal surveillance of water sampling points in Meharon will continue through the monsoon season in order to complete one year's observation of seasonal changes in the fundamental parameters affecting these water sources' ecology. Since there is no likelihood of gathering any direct data on the behavior of El Tor vibrios at these sampling sites, the surveillance will be, of necessity, somewhat limited in scope. We will, however, be able to follow seasonal variation in the NAG vibrios.

The association between vibrios and water hyacinth will be examined very thoroughly. In particular we will attempt to determine the mechanism involved in adherence, the range of surface plants in which the phenomenon occurs, and its role in the maintenance and transmission of El Tor vibrios during outbreaks.

Fig. 1. Thirty year weather normals for the Matlab Area.



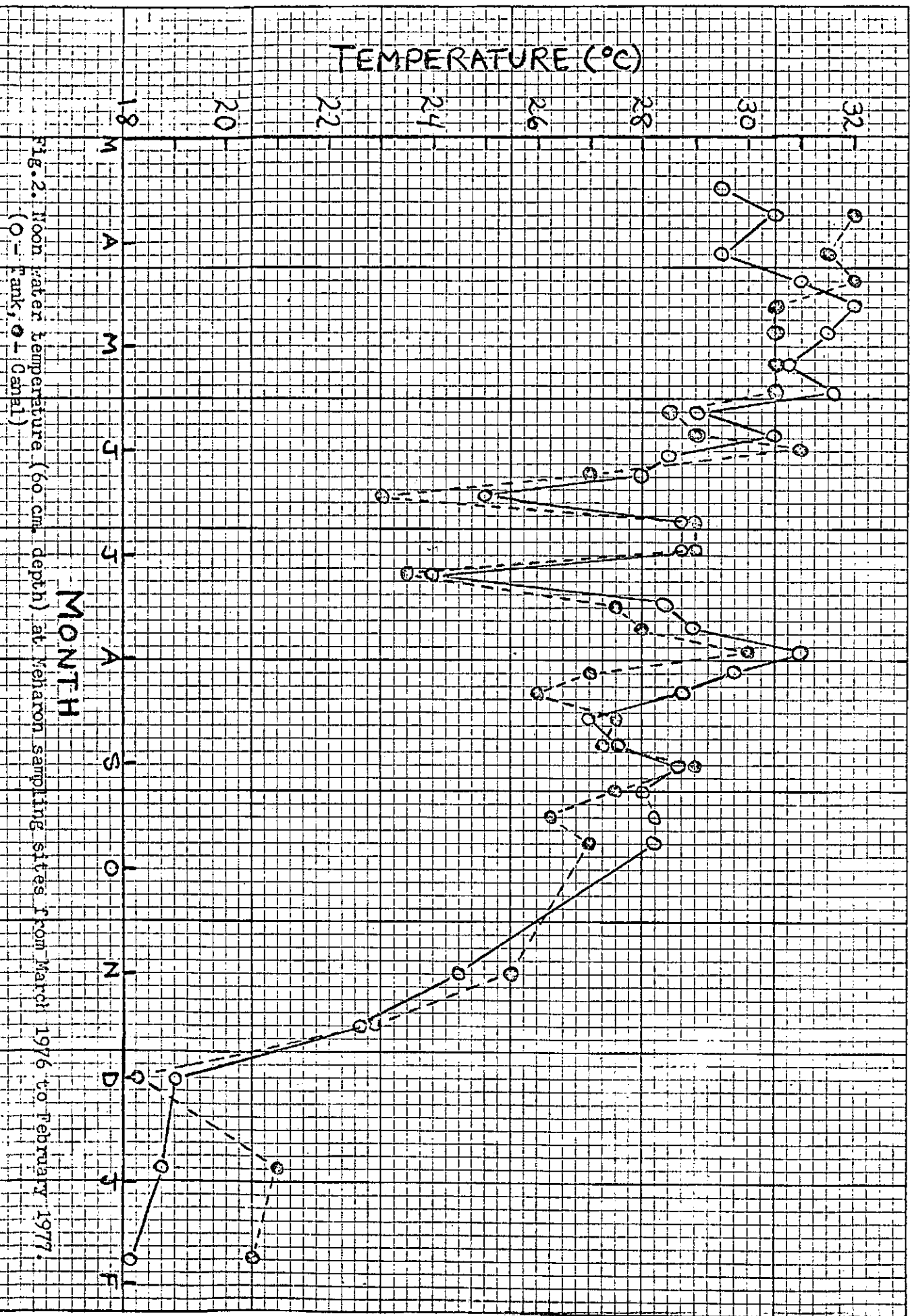
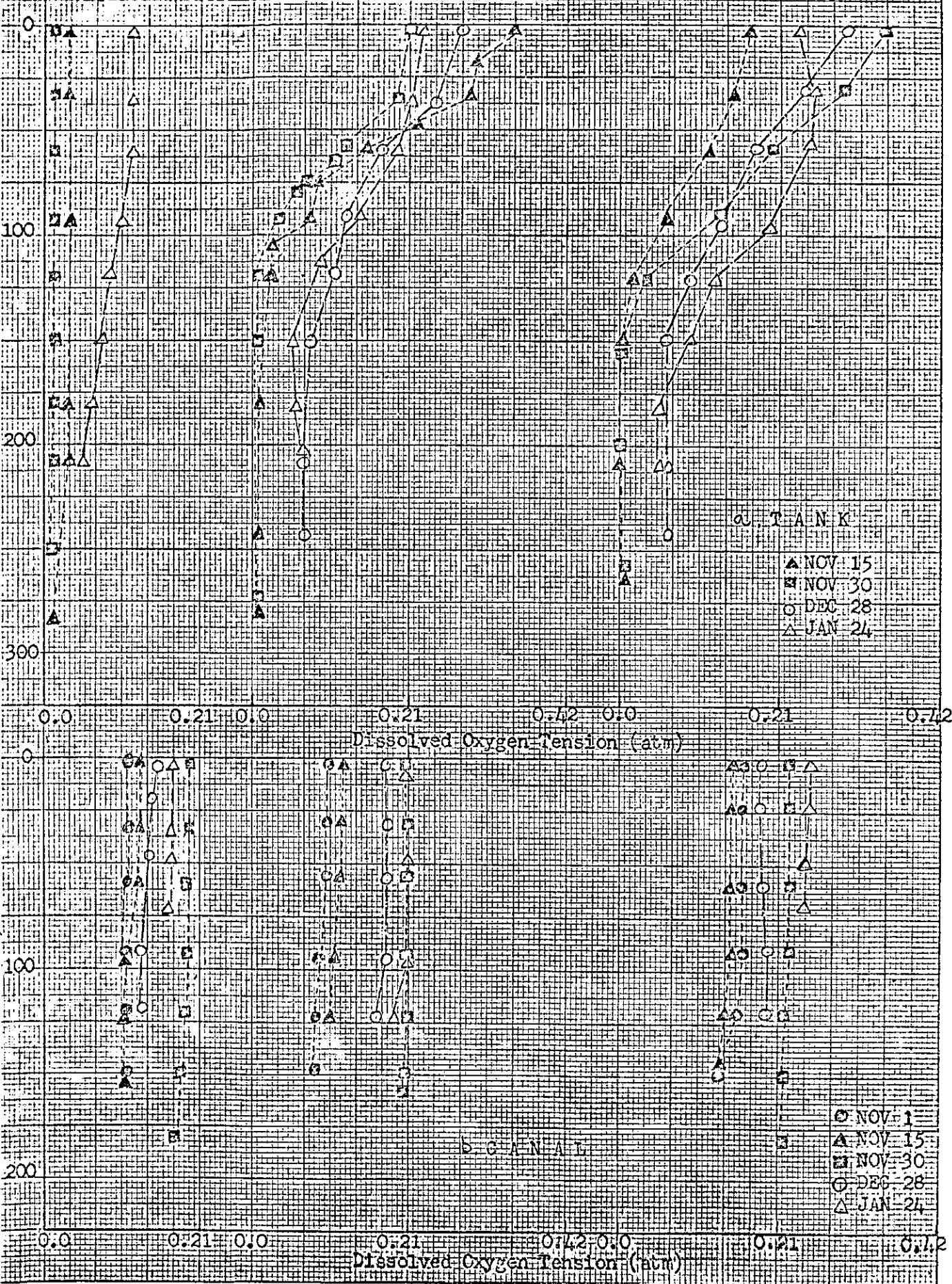


Fig. 2. Noon water temperature (60 cm. depth) at Meharon sampling sites from March 1976 to February 1977.
(O - Tank, ● - Canal)

Fig. 3. Dissolved Oxygen Tension depth profile at Meharon sampling sites on different dates. Profile taken at center of source.



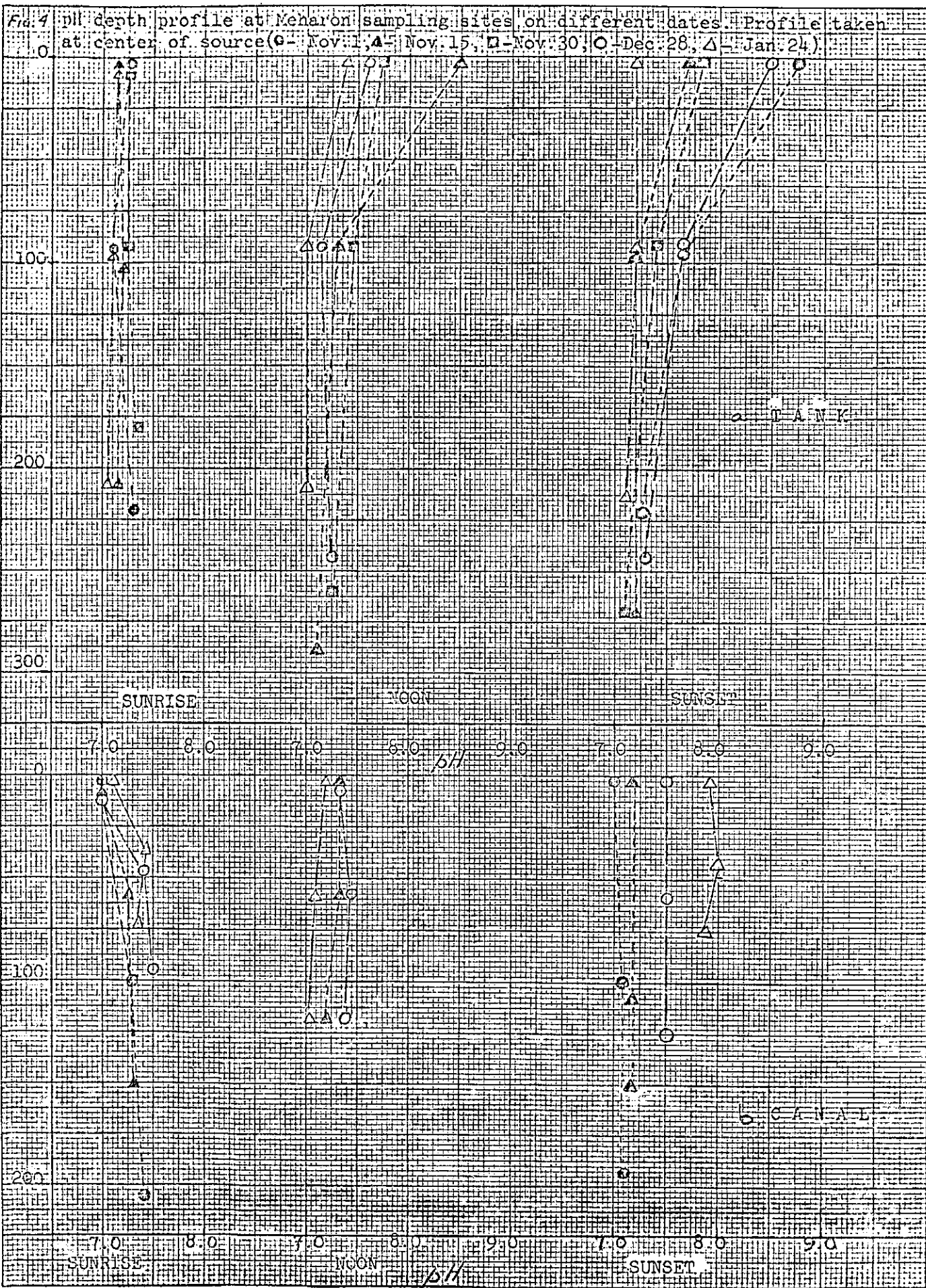


Table 1

Seasonal Variation in Concentration of Phytoplankton at
Different Sampling Points

Sampling Point	Depth	Log No./ml on	
		November 15	January 24
Canal - Edge	Surface	2.1	1.6
	Interface	1.3	1.2
Center	Surface	2.2	1.5
	60 cm	1.4	1.3
	Interface	1.4	1.1
Tank - Edge	Surface	3.2	2.5
	Interface	2.9	2.5
Center	Surface	3.6	2.5
	60 cm	3.2	2.5
	Interface	2.5	2.0

Table 2

Seasonal Variation in Concentration of Heterotrophic
Aerobes at Different Sampling Points

Sampling Point	Depth	Log No./ml on	
		November 15	January 4
Canal - Edge	Surface	4.0	4.0
	Interface	4.1	4.2
Canal - Center	Surface	4.8	3.0
	60 cm	4.3	3.0
	Interface	4.3	3.2
Tank - Edge	Surface	5.2	4.0
	Interface	4.8	4.2
Tank - Center	Surface	5.3	3.4
	60 cm	3.8	2.4
	Interface	4.2	3.5

Table 3

Seasonal Variation in Coliform Concentration at
Different Sampling Points.

Sampling Point	Depth	Log No./ml on	
		November 15	January 24
Canal - Edge	Surface	2.6	1.5
	Interface	3.2	1.4
Canal - Center	Surface	3.5	1.3
	60 cm	3.6	1.4
	Interface	3.6	1.3
Tank - Edge	Surface	2.2	2.0
	Interface	2.0	2.0
Tank - Center	Surface	2.3	1.5
	60 cm	2.0	1.7
	Interface	2.5	2.5

Table 4

V. cholerae, NAG Isolations from Water Column Samples Taken in Meharon During March 76 - January 77 - by Heiberg Group

Time Period	n	I	II	III	V	VII
Mar - May	44	3	22	2	25	0
Jun - Sep	68	4	23	1	32	1
Oct - Nov	20	4	10	0	5	0
Dec - Jan	30	0	5	0	5	0

Two factor ANOVA without replication :

Ho : Frequency of isolation is the same for all groups
 $F = 8.808$ $F_{0.005}(1, 5, 10) = 3.33$ Ho is rejected
 $P(F \geq 8.81) < 0.0025$

Ho : Frequency of isolation is the same at all time periods
 $F = 1.557$ $F_{0.005}(1, 4, 10) = 3.48$ Ho is accepted

Scheffé's S Test

Frequency I = Freq III = Freq VII Freq II = Freq V
 $P(S \geq 4.76) < 0.025$

Overall Freq in Dec - Jan Overall Freq in all other time period
 $P(S \geq 8.08) < 0.0025$

Table 5

Concentration of V. cholerae, NAG in Positive Tank and Canal
Water Column Samples Taken in Meharon During Mar 76 - Jan 77
Colony Forming Units/ml

Time Period	I			II			V		
	n	X	S.D	n	X	S.D	n	X	S.D
Mar - May	3	12.	16.	20.	15.	14.	24.	18.	27.
Jun - Sep	3	2.7	7.0	35.	12.	20.	38.	26.	41.
Oct - Nov	4	3.8	1.3	10.	9.0	8.4	5.	530.	410.
Dec - Jan	0	-		5.	31.	32.	5.	20.	17.

Table 6

Concentration of Heiberg Group (I) and (II) - V. cholerae, NAG
in Water Samples Taken in Meharon During Nov 76 - Jan 77

Sampling Point	n	$\bar{X}$	range
Tank edge	6	5.2	0 - 20.
Tank center - surface	6	4.3	0 - 24.
Tank center - interface	6	7.7	0 - 14.
Canal edge	6	14.5	0 - 80.
Canal center - surface	6	2.7	0 - 8.
Canal center - interface	6	0.15	0 - 1.

Ho : Group I + II organisms occur in the same concentration in
all 6 sampling points

Kruskal - Wallis test $H_c = 4.87$

$\chi^2_{0.05,5} = 11.07$ Ho is accepted.

Table 7

Concentration of Heiberg Group V V. cholerae, NAG in Water
Hyacinths, Sediment and Water Column Samples in
Meharon During Nov 76 - Jan 77

Source	n	No. Positive	$\bar{X}$	SD
Water	50	10	12.	15.
Hyacinth	25	4	250.	100.
Sediment	12	0	-	

$\bar{X}$ = mean CFU/g in positive samples

Table 8

Isolation of V. cholerae from Water Hyacinths and Paired
Water Samples Taken from Water Sources Associated
with Cholera Outbreaks During Dec 76 and Jan 77
(n = 152)

		Water	
		Negative	Positive
Plant	Negative	135	4
	Positive	9	4

Ho : Association of V. cholerae with plants is not greater
than that with surrounding water column.

By Fishers exact test - $P = 0.002$ Ho is rejected.

Table 9

Concentration of V. cholerae in Positive Water and Hyacinth Samples

Sources	No Positive by Enrichment	Direct Plating	Range of Cfu/g	Mann-Whitney Test Ranks
Water	7	1	0.16	7, 18 (7x)
Plant	7	6	20-4800	1, 3.5(2x), 4, 5.5(2x), 8, 11.5 (6x).

H_0 : Concentration of V. cholerae in plant samples is no greater than that in water samples.

Mann-Whitney Test $U^1 = 105$

$U_{0.01}(1), 8, 13 = 84$; H_0 is rejected

$P(U \geq 105) < .005$

These studies have provided basic information of importance to any family planning operation, and have been made possible by the quality of demographic information assembled by the Cholera Research Laboratory, and by the presence in these communities of CRL health workers. Such data should also be of use to the Bangladesh government.

Environmental Epidemiology

Environmental studies have expanded into new directions with the objective of determining the role of ecological factors, behavioral characteristics, and sociological determinants in the transmission of enteric pathogens and their persistence in the environment. The Committee heard six presentations, five dealing with studies completed or in progress, and one dealing with plans for a study in progress. A study of the relationship between water consumption and dysentery in Teknaf, a rural Bangladesh village, was designed to assess the relationship between the quantity of water used and the risk of developing clinical dysentery and/or laboratory confirmed shigellosis. The quantity of water carried to each family from a communal well was estimated in two communities in 1976 during a period of surveillance for diarrhea. There was an inverse relationship between the availability of water and the attack rates for clinical dysentery and shigellosis. An impressive reduction in both rates occurred when the amount of water increased from less than 20 to 30 or more liters per person per day, this in spite of the fact that the water was presumed to be contaminated. Presumably this improvement was due to the fact that larger quantities of water facilitated washing and cleaning of body surfaces and eating utensils - thus the term "water-washed" in referring to shigellosis and dysentery. This important study has demonstrated quantitatively, perhaps for the first time in the Asian subcontinent, the substantial impact of water availability on a water-washed disease.

Information concerning the comparability of the three study groups in this investigation was not available at the time of presentation. It will be important to validate these findings by demonstrating that the groups are comparable with regard to age stratification and socio-economic status. The investigators were also encouraged to pursue the opportunity available to them to compare attack rates not only among those who have water carried to their homes but also among those who have water near or inside their homes. If this can be done quantitatively, it may further demonstrate the value of water sources close to and in homes, and may also provide information on the optimum quantity of water necessary to give a maximum effort for control of a water-washed disease. Finally, the data showed considerable difference between the clinical dysentery attack rate (255/1,000/year) and the laboratory confirmed shigella attack rate (43/1,000/year) raising the possibility that etiological agent(s) other than shigellae might be operative.

A questionnaire study was designed to assess health consciousness, hygiene practices and attitudes in Teknaf to provide information that presumably would serve as a base-line and guide in health education and

in directing future sanitary improvements. It is uncertain how this information will be used. It would seem logical that the survey be repeated to assess the impact of an intervention such as intensive health education.

A two-year study of the efficiency of hand-pumped tubewells in the prevention of cholera and shigellosis has been conducted in rural Matlab. It was established that tubewell water was used for drinking purposes only, and that there were other sources of water for washing, cooking, etc. Users of tubewell water were not protected, for their attack rates were comparable and in some months significantly higher than rates among persons who used other sources of water. While this is a disappointment to some who had hoped for opposite findings, it is not surprising considering the fact that tubewell water does not serve as a substitute source but rather as an additive source of water. The message seems clear: transmission of these diseases will continue as long as people are exposed to contaminated water sources. The challenge is to find ways to motivate people to avoid these sources or to clean the sources.

A study of water and foods consumed by cholera patients and their family members was conducted to determine how transmission occurs in cholera. Family members of cases admitted to Matlab hospital, items of food, water for cooking and other uses, and environmental surfaces were cultured for ten days. Qualitative and quantitative assays were used to clarify the chain of transmission, and to determine whether there is a multiplication step between the contaminated source to the vehicle of transmission. A multiplication step could not be demonstrated. The study further showed that food did not appear to be responsible for transmission as only two of 1,511 food specimens were found contaminated with low numbers of *V. cholerae*. Evidence pointed to water as the major vehicle of transmission. Perhaps the most important finding was the low level of contamination in water, suggesting that low-inoculum exposure in cholera may be the rule rather than the exception. These water contamination findings are considered preliminary, as only 13 sites have been studied. The study should be continued along the same lines until a total of about 100 sites are available for analysis. If the completed study shows findings consistent with these preliminary data, this would heighten interest in those host factors which predispose to clinical disease.

A basic environmental study has been commenced at Meheran to characterize two types of water systems and to determine the biological cycles of micro-organisms, including *V. cholerae*. The water systems are a tank, which is a static system with marked layering, and a canal, a non-static system with no layering. An interesting preliminary finding was the apparent concentration of *V. cholerae*, probably by adherence, by the water hyacinth. The interaction of *V. cholerae* with water hyacinths and other plants will be studied in the laboratory. This is potentially a very valuable study, not only in revealing new knowledge of the life cycle of *V. cholerae* in the external environment, but also in providing new information of two types of water systems important to the citizens of Bangladesh. Drug resistance and plasmid analysis of human enteric organisms (obtained in this study) is also contemplated.

Plans were presented for a study of water use and defecation behavior. The study, now in its initial phase, has two complementary components. The first component consists of studies on traditional water use and defecation behavior and an analysis of factors which may influence any proposed interventions. The second component is the intervention step that will emerge from this analysis. The Committee liked these plans.

ADMINISTRATION: BACKGROUND AND TRANSITION

The Cholera Research Laboratory (CRL) was re-established in Dacca, Bangladesh with the signing of a bilateral Project Agreement between the Governments of Bangladesh and the United States on 15 May 1974. The Agreement provided for expansion into areas of biomedical research other than cholera and diarrhea, such as demographic studies and interaction between the malnutrition and infection. Although the project was to be funded by the two governments, provision was made to encourage other governments or organizations to contribute support. The Governments of Australia and Great Britain have responded to this opportunity by providing cash or materials and equipment needed by the Laboratory each year. These two countries had also contributed to the support of the CRL before the change in government in 1971.

The CRL remained intact during this change, although it functioned primarily as a service activity for Bangladesh, treating patients with diarrhea at its hospitals in Dacca and at the field station in Matlab Bazaar. Dr. George Curlin, an Epidemiologist from the Center for Disease Control in the U.S., served as Interim Director, and prepared the staff and field study area for resumption of research activities. Dr. Willard F. Verwey from the University of Texas Medical Branch at Galveston came to Dacca as the first Director of the reconstituted CRL. He served in this capacity from 1 June 1974, until he retired on 28 February 1977.

Dr. Verwey reorganized the CRL after his first year as Director into five new Divisions: Administrative, Clinical, Epidemiology, Laboratory and Maintenance. The staff currently consists of 535 Bangladeshi and seven expatriates. The expatriates are five scientists including the Director, a special assistant to the Director and a maintenance officer. Two of the scientists are epidemiologists provided by the Center for Disease Control, while the remaining expatriate employees are hired by contract. A biochemist from Britain will join the staff in the spring of 1977. The Johns Hopkins International Center for Medical Research is located in Dacca; its staff of two to four scientists conducts research of mutual interest, reimbursing CRL on a fee-for-service basis.

Dr. Wiley H. Mosley, formerly Chairman of the Department of Population Dynamics at Johns Hopkins University and formerly Chief of Epidemiology at CRL in the 1960's, became the new Director on 1 March 1977.

The present Project Agreement is due to expire on 30 September 1977. Negotiations are underway to extend the bilateral Agreement to September 1978, to allow time for converting the CRL into an independent

international institution with a broad base of support for more activity in several areas of medical research important to developing countries. The U.S. Agency for International Development (USAID) recognizes the unique resource of the CRL field population of 260,000 persons with census data dating back to 1965. No population of comparable size exists in a developing country where it is possible to do rate based studies. With the assistance of Drs. Verwey, Mosley and Lincoln Chen of the Ford Foundation, a five-year plan was developed and a prospectus drafted for developing such a new international institution. The concept has been accepted in principle by the Inter-ministerial Committee of the Government of Bangladesh and by USAID. The Ford Foundation has provided a grant to aid in the development of a charter under the laws of Bangladesh. The Governments of Australia and Great Britain are considering continuation of support. A final prospectus is being drafted to use in seeking other donor support. The USAID will continue as a major donor for several more years, but expects to reduce its percentage support as funds are available from other donors. The name of the CRL will be changed to reflect its expanded range of biomedical research; International Institute for Health, Nutrition and Population Research has been suggested. Plans for the transition seem to be progressing well.

CRL DIVISIONS

Administrative Division

The Administrative Division has made a number of improvements in the past year. Most of these center on the computerization of some records and development of a cost accounting system. This accounting system allows the charging of a fee-for-service to CRL protocols as well as to cooperating and non-staff protocols. There are still some defects in the system, and some protocols still do not have a cost figure.

The present staff is handling all activities well. Mr. Philip Weeks has been particularly helpful as Administrative Assistant to the Director, and has worked effectively with the administrative staff. His work deals mainly with AID and other activities outside the CRL, while the administrative staff is concerned with activities within CRL.

The Supply Branch has moved to renovated quarters in the storeroom. Most of the bulk storage was moved to a warehouse to make room for the staff. All items in supply have been indexed according to the NIH system, and the inventory placed in a computer. This should be a great improvement over the old card system. The usual delays in getting supplies from the U.S. still occur. Fortunately, there have been fewer problems in the last few months. Also, the ability to purchase in Singapore and other places has been a great help.

The CRL Library is making a real effort to be responsive to the needs of scientists in and around Dacca. It maintains close liaison with the National Library of Medicine, housed in another wing of the Institute of Public Health. The limited resources of each are coordinated to avoid unnecessary duplication of books and journals. The CRL has access to Medline literature searches through the National Library of Medicine in the United States. This cooperation is to be commended.

Clinical Division

Seven items of investigation were projected in the 1976 report of the Committee, and now call for some comment.

Studies on all aspects of shigellosis have been pursued. In particular, further studies of the leukemoid reaction and hemolysis have led to the formulation of a hypothesis regarding the pathogenesis of these complications, which will be tested by continued studies. The shigellosis research unit is functioning in as far as there is a separate 20-bed unit, with its own nursing and clinical medical staff, and record system. Studies on alternative drugs for ampicillin-resistant shigella have been concluded. Trimethoprim-Sulphamethoxazole has been found effective.

A comparative trial of Dacca and WHO solutions has been completed and results published. There appears to be little difference in efficacy between the solutions.

A comparison of sucrose and glucose in oral therapy has been completed and the resulting paper sent for publication. It was concluded that both sugars are equally effective in oral therapy.

The treatment of diarrhea by unsupervised oral therapy continues to be studied. Material now can be successfully packaged in glass bottles, and suitable orange flavoring has been developed.

Epidemiological studies continue in the Teknaf dysentery project. To obtain sex-specific attack rates, women have been employed in the field area to gather the necessary data. Data on socio-economic factors have been accumulated, but are not yet analyzed. Active bacteriological research continues, with coliform counts being done on water from ponds, tanks and wells. So far shigella species have been found only in human fecal samples. A comparative study of forms of intervention will commence early in March 1977. Three comparable study areas have been delineated, in all of which the baseline endemicity of shigellosis is now known.

Area A. In this area, an intensive health education campaign will be instituted, specially directed towards habits of defecation, particularly in children. The use of ash for hand cleansing after defecation will be promoted. There will be no other intervention.

Area B. Some tubewells exist in this area. The number will be increased to give one for each five or six families. The people will be encouraged to use this source of water only.

Area C. Tubewells will be sunk, giving one for each four or five houses. WHO-type latrines will be supplied, i.e. 15 ft. of concrete tubing with a water-seal squatting plate. The aim will be to supply at least one latrine per compound. The people will be encouraged to use the tubewells only, and will be instructed in the proper use of the latrines.

Evaluation of this comparative study, in terms of shigellosis incidence and general degree of use of facilities, will be continuous but a formal evaluation will be done in about a year's time.

A study of the effect of shigellosis on the growth of children is being carried out. Children of known age are being periodically weighed, both those who have had one or more attacks and those who have not. Preliminary results seem to indicate a long-term deficit in children who have suffered from shigellosis.

Considerable clinical research has been done on intestinal amebiasis, and incidence studies continue.

A study of the inter-relationships between nutritional status, cell-mediated immunity and infectious disease on the lines laid out in the 1976 report has been completed in the Matlab area. A paper is in the course of preparation.

With the expansion of interests of the CRL into wider fields of diarrheal disease, the Clinical Division has some fairly obvious deficiencies, of which three should have very early attention.

- (1) More clinical investigators. A gastroenterologist is an urgent requirement. A clinical nutritionist and a pediatrician should also be recruited.
- (2) Increased nursing expertise. It is suggested that suitably qualified nurses be sent for training to clinical research units overseas for periods of not less than six months. This would enable the senior nursing staff to take an important role in research within the CRL Clinical Division.
- (3) Increased ward space. The existing ward space is extremely crowded. Probably both the general ward unit and the special shigellosis unit should add more beds. Clinical research demands even more floor space.

Epidemiology Division

During the past year, epidemiologic activities have focused on the role of water in the transmission of cholera and shigellosis and, in a broader context, on the interrelation between man's environment and enteric diseases. The Committee supports this new direction, but emphasizes that the association of water or any other single environmental factor with a specific health problem is difficult because a number of variables -- cultural, educational, economic and others -- impact on the disease. As recognized by the staff, it will be increasingly important to identify these variables, and to obtain the collaboration of behavioral scientists, especially in the design of protocols.

Studies of cholera have given the CRL a broad and unique capability in cholera. As a result, expertise has been developed which is

applicable to other enteric diseases. The CRL was therefore in a good position to expand into studies of other acute enteric diseases, as it has done in recent years. Most members of the Committee felt that future infectious disease research activities should be restricted largely to the enteric diseases to exploit the CRL's unique capabilities. A minority of consultants felt that research activities, especially epidemiologic investigations, need not be so restricted if unusual study opportunities arise, provided there is full consultation with appropriate authorities and with medical institutions in Bangladesh that might have overlapping interests.

The Committee felt that it would be desirable to conduct some future field studies in geographic areas other than Matlab Thana, both urban and rural. An urban area in Dacca and one in the northern part of the country in the area near Rangpur may be particularly desirable, since these places have population and ecological features quite different from those found in current study areas.

Laboratory Division

The many investigative clinical and epidemiological projects emanating from the CRL have obtained good back-up support from the Laboratory Division. Many of the routine microbiological and biochemical tests used in large modern hospitals have been provided. These facilities are absolutely fundamental to any research and service program. For this reason they should be constantly modernized and their efficiency compared with that of similar services in other institutions. Isolation of bacteria, for example, is successful in only a portion of those cases from which one might expect positive results. This is true throughout the world, but performance at CRL should be regularly compared with that at other laboratories.

The provision of some immunological procedures should be planned on a more routine basis, and there is room for improvement in the variety of tests provided. The time has probably come to plan the establishment of a good immunology service laboratory.

Animal House

The CRL has been fortunate in recent years with having a good supply of clean, well-kept laboratory animals. The general standards in the animal house are good, and, with the exception of rabbits, the breeding supply is able to keep up with the demand.

CHOLERA VACCINE FIELD TRIALS

Dr. John Craig, Chairman of the U.S. Cholera Panel, reviewed for the members of the Committee and staff of CRL the options for efficacy trials of cholera immunogens that had been considered by the Panel. On the basis

of animal studies and tests in volunteers in the United States, it is now thought desirable to test the efficacy of a formalin inactivated, immuno-absorbed toxoid prepared by Burroughs Wellcome, and to compare this toxoid, which is made with a special aluminum hydroxide gel, with whole cell vaccine only, and with a combination of vaccine plus toxoid, the latter two preparations also to contain alumina gel. The inclusion of a Tet - Dip control group would require 100,000 subjects, or much of the accessible population in Matlab. Toxoid will have passed final testing for field use by early 1978.

Since whole cell vaccine prepared by Burroughs Wellcome has not been tested in the field, consideration had been given to a 1977 trial to compare the adjuvanted and non-adjuvanted forms of this product with the results obtained with Merck Sharp Dome whole cell vaccine in the 1960's. As previously detailed by Dr. George Curlin, such a trial would also require a large number of subjects in Matlab. Further, there are other constraints. The major one to be identified was the concern of the Bangladesh Medical Research Council (BMRC) as reported by Dr. Mjuibur Rahaman (a member of the BMRC and Acting Director of CRL) regarding the Council's responsibilities with reference to future field trials. The Committee was told that the BMRC wished for its scientists to be involved in the decision making process relative to the choice of cholera vaccines for testing and in the design and development of the protocol for a field trial. This would necessitate the participation of BMRC representatives in data review and planning, in discussions with vaccine producers and testers, etc. so that they would be thoroughly familiar with vaccine research and development before a protocol is submitted to the BMRC for its review. That these concerns were reasonable was recognized by the Committee and by CRL staff. The Director Designate then outlined several approaches to active and early participation of the BMRC and other Bangladeshi scientists. Given these concerns and those previously noted, it was agreed that there would be no field trial in 1977. The Committee felt that the question of the synergism of toxoid and whole cell vaccine is of such importance that the four-cell field trial projected for 1978 (described above) should be conducted.

This discussion highlighted an aspect of international health research which is being increasingly manifested in developing countries. Shifting attitudes regarding human experimentation combined with a growing nationalism that rejects any action that can be interpreted as the exploitation of the citizen of one country by investigators from another will demand the greatest of diplomacy and tact on the part of foreign investigators. That this is the case in Bangladesh was made clear recently in an article printed in the Bangladesh Times. The article was written by Dr. Zafarullah Chowdhury, a surgeon, who is Project Coordinator of the People's Health Center (formerly Bangladesh Hospital). Because of its relevance to the future of the CRL, a copy of the original paper submitted to the Times is included as Attachment No. 3

MEETING WITH DIRECTING COUNCIL

At its meeting with the Directing Council on the afternoon of February 25, the Committee learned with pleasure that at an Inter-ministerial meeting that morning approval had been given both for the appointment of Dr. Wiley H. Mosley as CRL Director, effective March 1, and to the proposal calling for the conversion of CRL into an International Institute. The proposal will now be forwarded to the top levels of government.

The Directing Council endorsed the trends in the CRL research program as listed in the Director's Report and as exemplified by the scientific data presented for the Committee's review. Reference was made to the apprehension that had been expressed concerning the extension of CRL research activities into the fields of population and nutrition, and to the need for coordinating work in these fields with that of the National Institute of Population Research and Training and the National Institute of Nutrition Research at Dacca University. It was agreed that duplication of effort and expense should be avoided, and acknowledged that these broad fields could absorb the talents of many investigators.

There then followed a useful discussion of vaccine field trials and of the appropriate role of the Bangladesh Medical Research Council. The Directing Council offered its support to the new Director in informing the BMRC of CRL activities, and in seeking its understanding and endorsement of the CRL research program.

COMMENTS

Recommendations regarding specific research projects have been included in the appropriate sections above. The Committee notes that many of the recommendations made in last year's report are still appropriate, and is pleased to observe that those relative to nutrition, population studies, training, and diversification of financial support have been reflected in the prospectus for the new International Institute.

The Committee again commends the CRL for its general plans for broadening its interdisciplinary activities to include a greater proportion of studies involving demography, nutrition, immunocompetence and population dynamics, particularly as these influence, and are influenced by, diarrheal disease morbidity and mortality in Bangladesh. The time is now at hand for specific and careful planning in these new areas.

While broadening the program of the CRL, however, the new Institute should not neglect the responsibilities of the Cholera Research Laboratory. The CRL has built a unique expertise and reputation in cholera studies. Surely all the problems of cholera pathophysiology and epidemiology have not been solved.

The Committee underscores its prior emphasis on the great value of the demographic data developed by the CRL. Basic demographic observations are both a logical outgrowth as well as an essential element of any field study. The critical base for any epidemiological study is establishment of the denominator, the population at risk. This inevitably requires a census to maintain an up-to-date population base. Hence registration of births, deaths, and migrations is required.

The impact of a specific disease process on the health and well-being of a population can be understood in the context of the overall level of morbidity and mortality in the population. Thus, in-depth analysis of mortality and morbidity in the population by age, sex, economic status, and other variables is required to define the critical risk factors. Disease processes, such as diarrhea, do not occur in populations as isolated phenomena. They are compounded by many factors such as malnutrition, other diseases in the populations, levels of health care available, traditional therapeutic practices, social and cultural practices relating to child care, etc. These factors, which must be understood if effective control measures are to be instituted, can be defined only by in-depth social-demographic studies. It is logical, then, as is proposed, to link research programs in enteric disease, nutrition, and population control.

Both descriptive and analytical studies of the trends and determinants of change in fertility draw in large measure on the same basic epidemiological data used to define patterns of morbidity and mortality. In considering interventions to alter fertility patterns, the CRL has established the important precedent of collaborating directly with the Family Planning Program of the Government of Bangladesh. As it must be, the Contraceptive Distribution Project is a joint CRL-GOB venture. The same is true of the Johns Hopkins Fertility Research Project which uses Matlab as a base. Clearly, the new Institute must integrate the research of this population project, just as it must integrate the diarrheal studies of the Johns Hopkins International Center for Medical Research.

With reference to the charter yet to be drafted for the proposed International Institute, the Committee expresses its conviction that the research program should continue to be reviewed by an outside, independent body. The Committee does not respond favorably to the suggestion that the Board of Directors function simultaneously as a Scientific Advisory Committee.

The Committee was pleased to learn that those planning the new International Institute are aware of the need to utilize an international scale of salaries for its scientists, regardless of national origin, as does the World Health Organization. How this will affect the support of scientists temporarily assigned from the U.S. and other countries remains to be determined.

Finally, the Committee wishes to comment on the review process per se and on the general conduct of its meeting. The Committee feels that the pressure of time experienced at this year's meeting validates the recommendations for scheduling made in last year's report. A minimum of nine days is needed to permit preparation of the final report for signature. Those who have traveled far should stay long enough to do a proper job. To make the best use of time, the Committee and its consultants should be split into groups for visits to different sites such as Matlab, Teknaf, and urban Dacca.

In this transition period, anticipated staff turnover and the need for long-range planning had an adverse effect on specific project planning. This, plus the apparent lack of coordination among projects, compromised the review process. The Committee asks that the Director and his staff consider the following:

- (1) There was a distinct tendency for the review sessions to become open seminars. This is a mistake. Meetings should be restricted to Committee members and consultants together with only those staff members who have material to present.
- (2) On a number of occasions, the standard of presentation was poor. One presentation was marred by colloquialisms totally out of place in a scientific paper. There is no excuse in a meeting of this kind for slides inserted upside down, etc. Sessions should be rigorously rehearsed.
- (3) Some of the hand-outs were marred by misspellings, mistyping, and poor English. They showed signs of very hurried preparation and some had clearly not been edited. Similarly, many slides had errors in spelling and displayed other evidence of lack of care in preparation.
- (4) There were far too many handouts, and these were distributed too late. It is totally impossible for the Committee members to read all of this material during the presentations. It should have been available to them before arrival or, at the very least, on arrival in Dacca.
- (5) The Committee spent most of its time reviewing work that had been done. It wishes to devote at least 50% of its time to consideration of proposals for new projects. Protocols of proposed projects should be available.
- (6) The time of the Committee should not be taken up by minutiae, e.g., small-scale drug trials, etc.

For the future, it is suggested that each major project be given a serial number. This will lead to better continuity from year to year. Each year those responsible for scientific review will then consider:

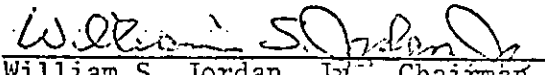
- (1) A list of projects completed, with a concluding assessment;
- (2) A list of continuing projects, with a status report;
- (3) A list of proposed new projects, with protocols.

To conclude, it is noted that the word "advisory" is included in the title of the present Committee. Clearly, the review process should be an educational experience for both the members of the Committee and the staff. The yearly visit of a group of interested scientists should offer an opportunity for valuable consultation as well as for critical review. As the research programs of the CRL and the Institute are broadened and diversified, the competence of the Committee and its consultants must be expanded by those responsible for their appointment.

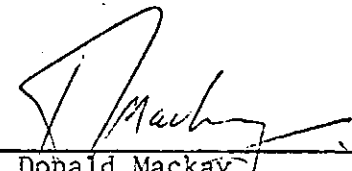
POSTSCRIPT

The Committee records with sadness the death during the past year of two former CRL Directors: Drs. Fred L. Soper and Robert A. Phillips.

Signed by the members of the Scientific Review and Technical Advisory Committee:


William S. Jordan, Jr., Chairman
United States Representative


M. A. Latif
Bangladesh Representative


Donald Mackay
United Kingdom Representative

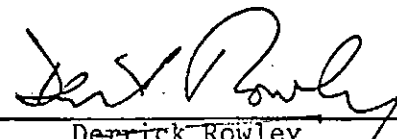
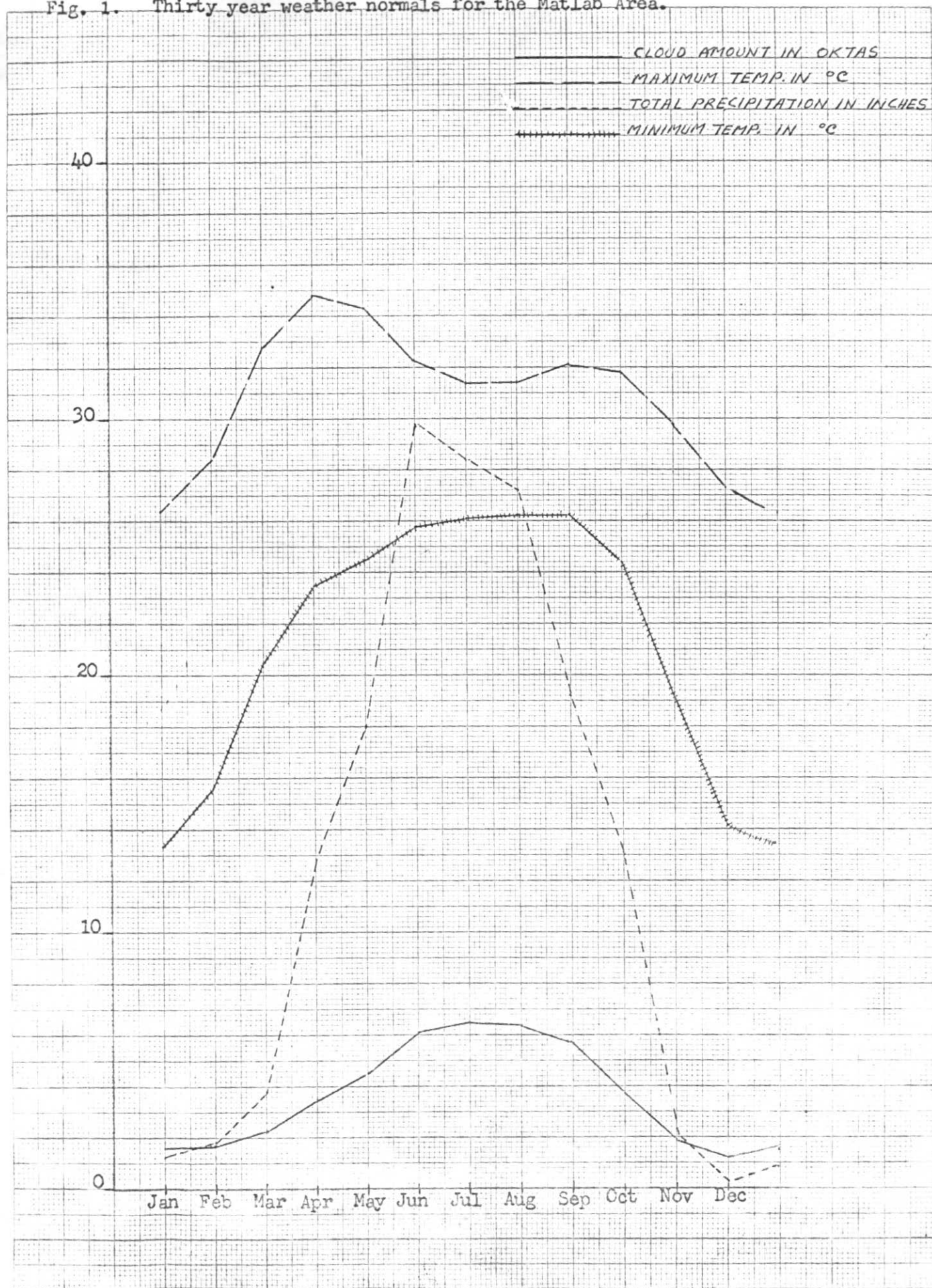

Derrick Rowley
Australian Representative

Fig. 1. Thirty year weather normals for the Matlab Area.



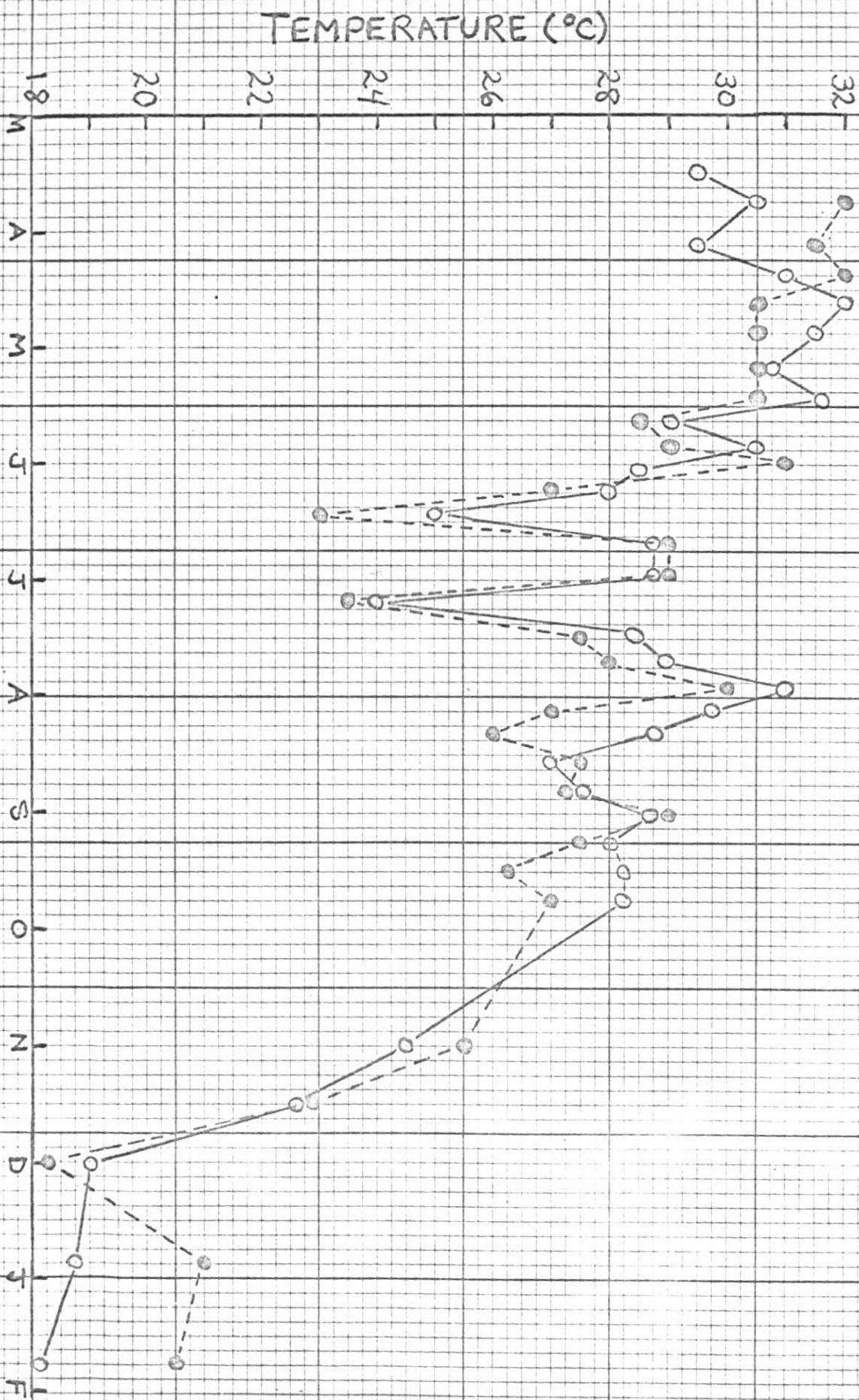


Fig. 2. Noon water temperature (60 cm. depth) at Neharon sampling sites from March 1976 to February 1977.
(O - Rank, ● - Canal)

Fig. 3. Dissolved Oxygen Tension depth profile at Meharon sampling sites on different dates. Profile taken at center of source.

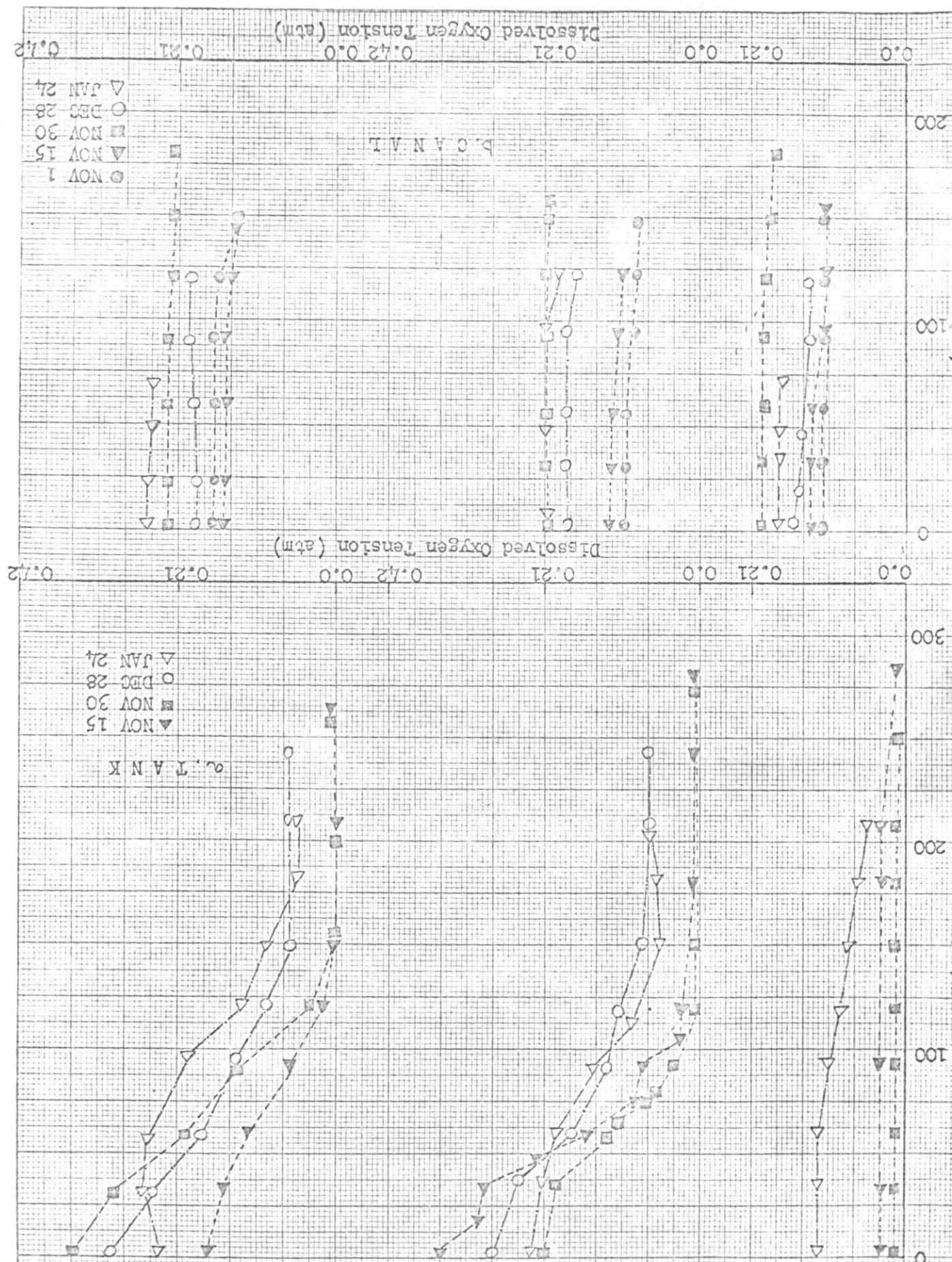


Fig. 4 pH depth profile at Neharon sampling sites on different dates. Profile taken at center of source (○ - Nov. 1, ▲ - Nov. 15, ■ - Nov. 30, ○ - Dec. 26, △ - Jan. 24)

