PAKISTAN-SEATO CHOLERA RESEARCH LABORATORY

TECHNICAL COMMITTEE MEETING

14-16, February, 1967.

PAKISTAN-SEATO CHOLERA RESEARCH LABORATORY

PROGRAM FOR MEETING OF TECHNICAL COMMITTEE
14-16 February, 1967.

TUESDAY, 14 FEBRUARY

0900-1000	Tour of Laboratory facilities.
1000-1045	Immuno-chemistry presentation
1045-1115	Coffee break
1115-1230	Immuno-chemistry presentation (continued)
1400-1430	Toxicology
1430-1530	Bacteriology
1530-1600	Tea break
1600-1630	Anthropology
1800-2000	Reception for Committee members at Director's home.

WEDNESDAY, 15 FEBRUARY

0900-1045	Clinical Investigation reports.
1045-1115	Coffee break
1115-1230	Discussion
1400-1530	Immunology
1530-1600	Tea for Committee members given by Staff Welfare Association.
1600-1700	Epidemiology

THURSDAY, 16 FEBRUARY

0900-1045	Section Chiefs' Presentations
1045-1115	Coffee break
1115-1230	Executive session
1400-1630	Executive session (Budget presentation)
1630-1700	Tea for Committee members given by CRL Ward Staff.

TECHNICAL COMMITTEE 1967

Chairman:

Dr. Colin MacLeod
Vice President for Medical Affairs
Commonwealth Fund,
New York.

Dr. J. W. Howie
Public Health Laboratory Service,
London.

Dr. A. N. Khan
Deputy Director General of
Health Services,
Government of Pakistan,

(representing Brigadier M.S. Haque).

CONSULTANTS:

Dr. Kenneth Goodner
Professor of Microbiology
Jefferson Medical College
Philadelphia, Pa.,

Dr. Alexander Langmuir
Chief of Epidemiology Branch
Communicable Disease Center
Atlanta, Georgia.

TECHNICAL COMMITTEE MEETING
14-16 February, 1967

SUMMARY OF DATA FOR PRESENTATION.

1. Summary of Clinical Research Section Activities.
2. Report on Clinical Laboratory.
3. Report on Bacteriology Section.
4. Report on Diagnostic Bacteriology.
5. Report on Epidemiology Section.
6. Summary of Ward Activities.
7. Administrative Section Report.
8. Report to the Cholera Advisory Committee (December 1966)
9. Report of the Clinical Investigation Committee (November 1966)
10. Protocols presented to Clinical Investigation Committee (November 1966).
11. PSCRL Publications, 1966.
12. Visitors to PSCRL during 1966-67.

1967 TECHNICAL COMMITTEE MEETING

STUDIES IN PROGRESS OR COMPLETED BY CLINICAL RESEARCH SECTION SINCE THE T.C. MEETING OF 1966.

1. Jejunal Biopsy Adenosine Triphosphatase in Cholera and Other Diarrheal Diseases (Drs. Hirschhorn, Rosenberg; submitted for publication)

2. Jejunal Biopsy Disaccharidases and Lactose Malabsorption in Cholera and Other Diarrheal Diseases (Dr. Hirschhorn, completed)

3. A Technique for the Measurement of Transmural Electric Potential in the Intact Human Intestine (Dr. Sachar, Mr. Saha, Completed)

4. Tetracycline Lavage Study (Drs. Northrup, Kinzie, Phillips, completed)

5. Antibiotic Treatment Study (Drs. Karchmer, Curlin, Hirschhorn, Completed)

6. The Effect of Hyperimmune plasma on the Clinical Course of Acute Cholera (Drs. Sachar, Hoque, Completed)

7. Electron Microscopic Study of Jejunal Biopsies in Cholera and other Diarrheal Diseases (Drs. Northrup, Abraham, in progress)

8. Intestinal Electrolyte and Water Fluxes and Transmucosal potential in Acute Cholera (Drs. Taylor anf Sachar, in progress)

9. Oral Glucose Solutions for Reducing Stool output in cholera (Drs. Hirschhorn, Taylor, Sachar, Northrup, Kinzie, Phillips, in progress)

10. Therapy of Acute Cholera with Bacteriophage (Drs. Monsur, Hirschhorn in progress)

11. Search for Carriers among Convalescent Cholera patients by Means of Cathartic Purgation (Drs. Mojid, Hirschhorn, in progress)

12. Fluorescent Tagging of Antibodies and Antigens in Jejunal Biopsies in Cholera (Drs. Northrup, Mosley, Crabbe, Mr. Salauddin, in progres

13. Pediatric Cholera: Study of water and Electrolyte Losses and Needs (Drs. Linneman, Rahaman, Hirschhorn, in progress)

14. Clinical Review of Effects of Cholera on pregnancy (Dr. Hirschhorn, in progress)

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TECHNICAL COMMITTEE MEETING
14-16 February, 1967

CLINICAL LABORATORY SECTION.
(Short Summary)

The Clinical Laboratory Section is gradually taking over all the chemistry done on patients in connection with the disease required either for treatment purpose or for study purpose. At present the laboratory is supporting lavage studies on gut segments on whole G.I. tract; effect of lavage with and without antibiotics and also oral Glucose and Electrolyte therapy. The laboratory is also doing Electrolyte chemistry on Admission Bloods and chemistry required on Emergency cases as and when required by the house physicians.

The laboratory also prepares and analyzes the lavage solutions used in the Clinical investigations.

Since December 1965, when cholera broke out in Epidemic form and hospital started having heavy load of patients every day, the clinical laboratory took up the production of I.V. Fluid used to rehydrate the patients and is continuing to make the fluid for home consumption both in CRL Ward and in Matlab Field Hospital.

Analyses now being performed:-

1. CO_2 (Van Slyke) - 3 machines operating
2. Specific Gravity - (Copper Sulfate Method)
3. Sodium and Potassium - (Baird Atomic Flame Photometer)
4. Chlorides. - (Aminco - Catlone Titrator).
5. Osmolarity - (Mechrolab Vapor Pressure Osmometer)
6. Glucose (Nelson Method).
7. PEG
8. D.S.P.
9. Creatinine (Dr. A.S. Hare's Method).

Proposed additional Proceaures.

1. Blood pH. (at present done in Clinical Research Lab.)
2. Ca, Mg & Tetracycline:-

   Flurometric Technique has been set up for above determinations and one about to be started for Study Purposes.

(more)

CLINICAL LABORATORY SECTION
(Short Summary)

3. Atomic Absorption Spectrophotometer:-

The equipment has arrived and is awaiting installation by the manufacturers. This will be used for determining all cations.

4. Parkin Elmer Spectrophotometer with record and flame attachment has arrived and is also awaiting installation.

5. Urea-Spectrophotometric Procedure.

The laboratory's main aim is to provide all support in Clinical Investigations and also to maintain standard on quality control.

Dr. Ruth S. Hare is particularly responsible for quality control; and this laboratory is running under her advice.

Production of I.V. Fluid.

Since December 3, 1966, the I.V. Fluid production started under Clinical Laboratories. The production and consumption figures are as under:

Production from December 3 to December 31,1966	= 9,331	bottles.
Production from January 1 to January 30,1967	= 5,241	
	14,572	
Consumption in the period above	=13,250	
Excess	= 1,322	

Syed Zafar Ahmad
Chief, Clinical Laboratory Sec.

PAKISTAN-SEATO CHOLERA RESEARCH LABORATORY

Technical Committee Meeting
14-16 February, 1967.

REPORT OF BACTERIOLOGY SECTION

The main function of the Bacteriology Section is the routine diagnostic work supporting the work of CRL Ward, Family Studies, Infant Study and other studies at Rayerbazar, Field Surveillance work of Matlab Vaccine trial area and other field studies taken up by different workers.

Over and above the mere supporting other people's work, the section is engaged in different research problems, allied to the field, done by the people of the section as well as other workers e.g. Dr. Hirschhorn's Purging Study, Dr. Northrup's antibiotic study etc.

Thinking of the major non cholera period of the year, it is n possible to hire as many people that are needed to do both researc and diagnostic work. As such we are to stop most of the research work during the heavy cholera season.

The total strength of personnel in the section is just upto its requirement. By the amalgamation of water Bacteriology Section with the main Bacteriology Laboratory we got 4 Sanitary Inspectors, One Lab Technician (all trained in sampling and plating for cholera only) and two Senior Research Assistants; one of them worked with phage and the other with diagnosis of vibrios from water. None of them had any experience with any other work such as isolation of members of the Enterobacteriacae and other pathogenic and nonpathogenic flora of the gut. As the water sampling and other studies are stopped, these people are given training in the routine work of the section.

With the establishment of the Matlab Bacteriology Field Laboratory one of our experienced Research Technicians has been posted there. Another Research Technician is working for the last eight months in Dr. Monsur's Lab. with phage. One of the Research Assistants has been taken by Dr. Northrup for working in the Histology Lab who will eventually be transferred there.

This year we had to support the Govt. of Pakistan for culturing the stool samples of the Hajis. By this time we have done approximately 4000 Hajis with another boat of 1400 people still to go by the end of February.

(more)

Attached herewith is the list of the Research work that are being undertaken by us since August 1966. Only one of them was done from January 1966 to May 1966. All others were started in August and September but we were to stop work on some of them due to the heavy pressure on diagnostic work during the Cholera season. But from mid January we have started work on all of them. At the time of handing over the charge of the Section Dr. Rizvi did not give me any papers on the research work done by him.

—

Md. Imdadul Huq
Chief, Bacteriology Section.

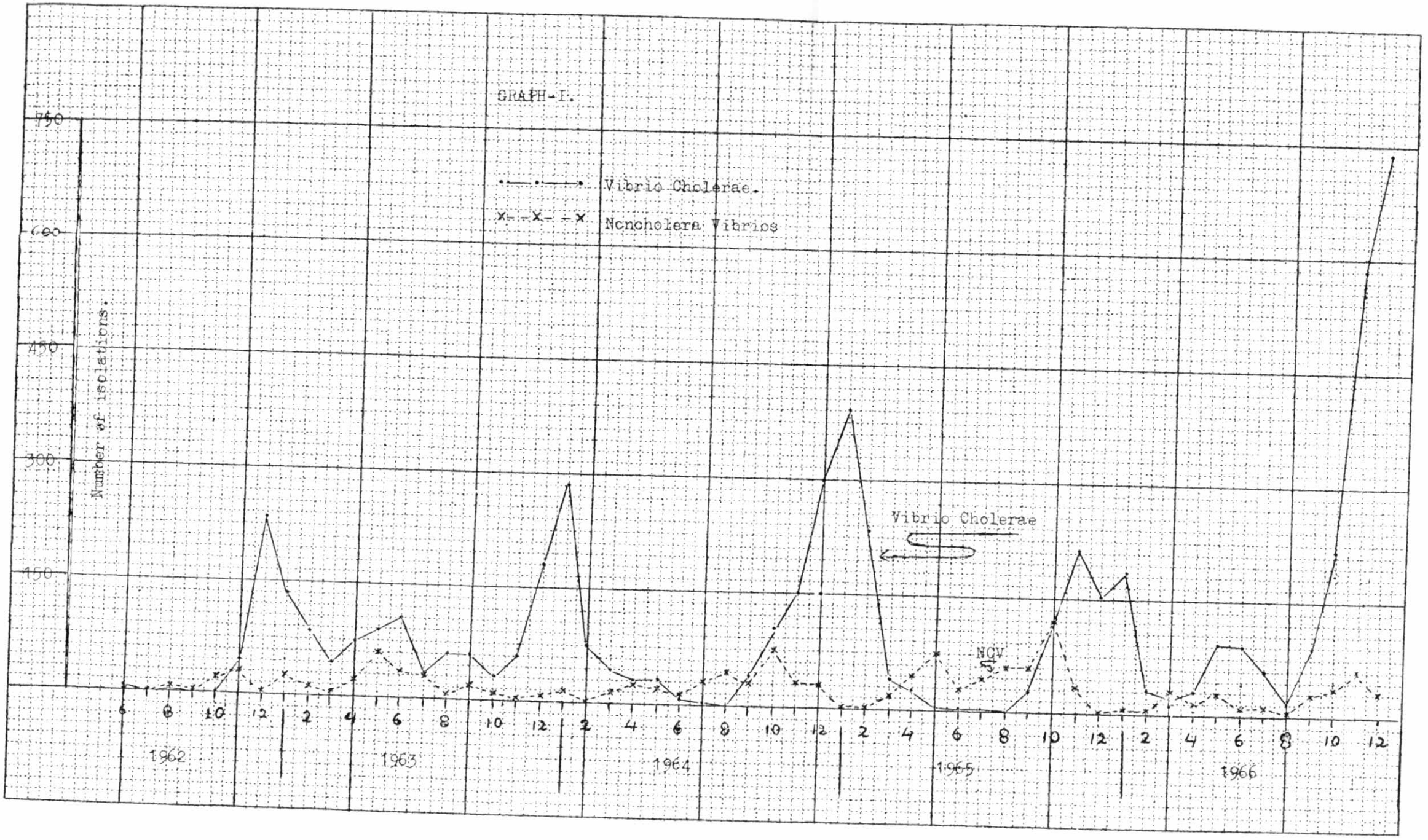
DIAGNOSTIC BACTERIOLOGY

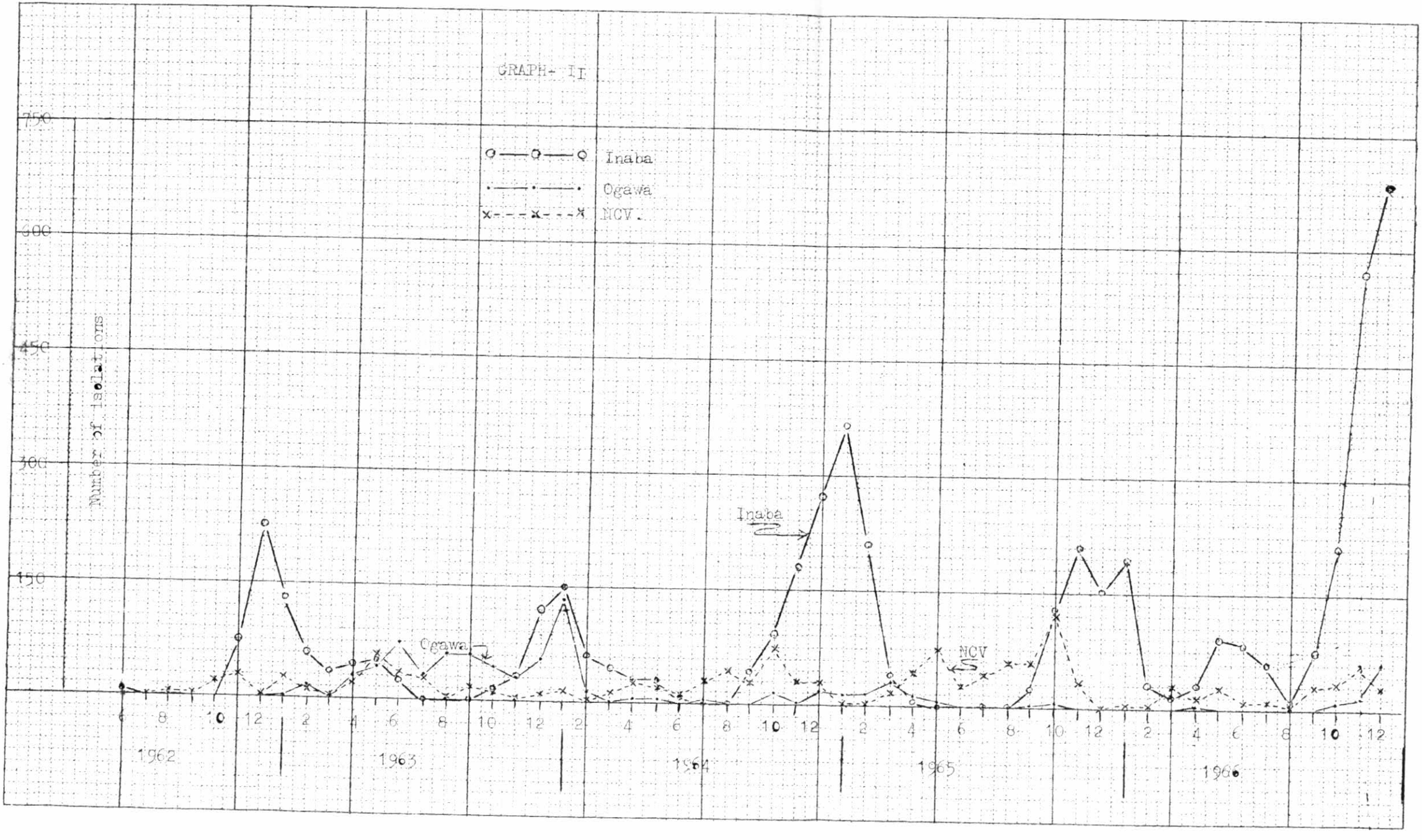
R. Rahman, F.K.B. Neogy, G. Kibryia, W.U. Ahmed.

Covering the period from October 1, 1965 to December 31, 1966 the diagnostic unit of the Bacteriology Laboratory received a total of 78,165 specimens for processing. Practically all of these are in the form of rectal swabs from patients of CRL Hospital and people under survillence by the Epidemiology Section. The following groups of pathogens were isolated.

Vibrio Cholera Inaba	5683
" " Ogawa	207
El Tor Inaba	3
El Tor Ogawa	8
Non Cholera Vibrios	592
Salmonella Group A	1
" " B	18
" " C	11
" " C	5
" " D	9
" " E	16
Salmonella like organisms	3
Shigella A	50
" B	268
" C	10
" D	32
Shigella like organisms	9

Like other years the incidence of El Tors were negligible in comparison to true Cholera cases. Number of isolation of Ogawa was also very low in comparison to Inaba. Fig. 1 shows the number of cases of true cholera vibrio and non cholera vibrios isolated in different months of the year since 1963. In fig. II number of cases of true cholera has been broken into Inaba and Ogawa serotypes.


GRAPH-I.
Vibrio Cholerae.
Ncncholera Vibrios
Number of isolations.
750
600
450
300
150
Vibrio Cholerae
NCV
5
6 8 10 12 2 4 6 8 10 12 2 4 6 8 10 12 2 4 6 8 10 12 2 4 6 8 10 12
1962
1963
1964
1965
1966


GRAPH- II
Inaba
Ogawa
NCV.
Number of isolations
750
600
450
300
150
Inaba
Ogawa
NCV
6 8 10 12 2 4 6 8 10 12 2 4 6 8 10 12 2 4 6 8 10 12 2 4 6 8 10 12
1962
1963
1964
1965
1966

Epidemiology Section
Epidemiology Presentations
15th February, 1967

1. Epidemiological Analysis of the Patterns of Cholera in the Matlab Vaccine Trial Area from 1963-1966.

2. Effect of Various Antibiotic Prophylaxis Schedules on the Infection Rate and Subsequent Cholera Experience in Family Contacts of Cholera Cases.

3. A Comparison of the Efficacy of Multiple Rectal Swabs vs. Purging in detecting carriers among Family Contacts of Cholera Cases.

4. Epidemiological Characteristics of Non-Vibrio Cholera.

5. A prospective Study of the Role of of Boatmen in the Introduction and Transmission of Cholera.

6. A prospective study of the Role of Migrants in the Introduction and Transmission of Cholera.

7. Estimation of the Level of Inapparent Infection in a Cholera Affected Village by Paired Serological Studies on the Entire Population.

8. Endemicity Characteristics of Cholera in Rural East Pakistan.

9. Prospective Observations of the Epidemiological Patterns of Cholera and Diarrhea in Rayer Bazar - 1965-1966.

10. Demographic Studies in the Matlab Vaccine Trial Area.

11. A Comparison of the Effectiveness of a High Dose Tetracycline Schedule with the Routine Schedule in Further Reducing the Clinical Course of Cholera.

Endemic Cholera in Rural East Pakistan

This data was collected in Matlab from November 1963 until June 1966. 703 cases of cholera were bacteriologically documented during this time. 476 of these cases occurred within our vaccine trial area for which an excellent census is available and where intensive surveillance was carried out.

Figure I	Cases by Month
Table I	Age Specific Attack Rate
Figure II	Age Specific Attack Rate
Figure III	Percentage Distribution of cases by age
Table II	Sex Specific Attack Rate
Figure IV	Sex Specific Attack Rate
Figure V	Hour of Onset
Table III	Percent Distribution by Age of Hospital and Field Cases
Figure VI	Percent Distribution by Age of Hospital and Field Cases
Table IV	Family Study Summary
Table V	Age Distribution of Asymptomatic Secondary Infection
Figure VII	Sex Distribution of Percentage of Asymptomatic Infections
Table VI	Vibriocidal Admission Titers
Figure VIII	Vibriocidal Admission Titers

Table I

Age Specific Attack Rate per 1000 population
for control plus non vaccinated members of PSCRL
Matlab Vaccine Trial Areas

Age Group	1963 - 1964			1964 - 1965			1965 - 1966		
	Cases	Population	Attack Rate/ 1000	Cases	Population	Attack Rate/ 1000	Cases	Population	Attack Rate/ 1000
Under 1	0	541	0	0	839	0	2	839	2.4
1 - 4	29	3483	8.3	67	5645	11.9	21	5645	3.7
5 - 9	18	3095	5.8	57	5676	10.0	11	5676	1.9
10 - 14	12	2293	5.2	17	4084	4.2	5	4084	1.2
15 - 19	0	1681	0	13	2768	4.7	6	2768	2.2
20 - 29	9	3149	2.9	23	5496	4.2	2	5496	0.4
Over 30	11	6412	1.7	26	11688	2.2	9	11688	0.8
Total	79	20654	3.8	203	36196	5.6	56	36196	1.6

Table II

Sex Specific Attack Rate per 1000 population for control plus non vaccinated members of PSCRL Matlab Vaccihe Trial Area - 1964-65 Cholera Season

Age Group	Male			Female		
	Cases	Population	Attack Rate/ 1000	Cases	Population	Attack Rate/ 1000
Under 1	0	422	0	0	417	0
1 - 4	40	2766	14.5	27	2873	9.4
5 - 9	32	2943	10.9	25	2733	9.2
10 - 14	11	2554	4.3	6	1533	3.9
15 - 19	4	1486	2.7	9	1282	7.0
20 - 29	7	2625	2.7	16	2871	5.6
Over 30	16	6774	2.4	10	4914	2.0
Total	110	19570	5.62	93	16623	5.6

Table III

Distribution by Age of All Confirmed Cholera Cases within the PSCRL Matlab Vaccine Trial Area - 1963-1966 (Vaccinated Cases Only)

Age Group	Hospitalized Cases		Field Cases	
	Number	Percent of Total	Number	Percent of Total
Under 1	0	0	3	2.6
1	6	4.2	17	14.8
2	10	6.9	17	14.8
3	17	11.8	17	14.8
4	10	6.9	15	13.0
5 - 9	62	43.1	25	21.7
10 - 14	14	9.7	9	7.8
15 - 19	8	5.6	3	2.6
20 - 29	11	7.6	4	3.5
30 - 49	3	2.1	3	2.6
Over 50	3	2.1	2	1.7
Total	144	100	115	99.9

Table VI

Mean Vibriocidal Inaba Titers
by Age PSCRL Matlab Hospital
Admissions 1965-1966

Age Group	Inaba Cholera		Culture Negative Gastroenteritis	
	Number of cases	Mean Titer	Number of cases	Mean Titer
Under 1	1	0	29	0
1 - 4	34	1.2	86	0.6
5 - 9	53	2.1	26	1.7
10 - 14	22	1.9	26	2.3
15 - 19	16	3.0	24	2.8
20 - 29	22	2.3[1]	72	3.5[1]
30 - 49	27	2.0[2]	107	3.6[2]
Over 50	11	3.5	85	3.6
Total	186	2.08	455	2.61

(1) Statistically Significant Difference (p = 0.05)

(2) Statistically Significant Difference (p = 0.01)

Table V

Distribution of Asymptomatic Secondary Infections by Age

Age Group	Male			Female		
	Number Positive	Number Cultured	Percent Positive	Number Positive	Number Cultured	Percent Positive
0 - 4	8	54	14.8%	15	80	18.8%
4 - 9	10	95	10.5	4	76	5.3
10 - 14	4	38	10.5	1	37	2.7
15 - 19	0	7	0	2	19	10.5
20 - 29	0	39	0	5	69	7.2
30 - 49	0	76	0	4	87	4.6
Over 50	1	39	2.6	1	48	2.1
Total	23	348	6.6%	32	416	7.7%

Distribution of Asymptomatic Secondary Infection by Age

Age Group	Both Sexes		
	Number Positive	Number Cultured	Percent Positive
0 - 4	23	134	17.2%
5 - 9	14	171	8.2
10 - 14	5	75	6.6
15 - 19	2	26	7.6
20 - 29	5	108	4.6
30 - 49	4	163	2.5
Over 50	2	87	2.3
	55	764	7.2%

Table IV

Family Studies

The family contacts of hospitalized cholera cases from within the vaccine trial area were followed. Vaccinated family members were studied. Daily rectal swabs were obtained for four consecutive days.

Number of families studied = 197

Number of family members studied = 836

Average family size (excluding index) = 4.2 members

Number of Individuals positive for V. Cholera = 86

Percent positive = 10.3%

Number of families infected = 58

Percent positive = 29.4%

Number of Symptomatic secondary cases = 31

Percent of positives who were symptomatic = 36.0%

Number of families with symptomatic secondary cases = 24

Percent of families with symptomatic secondary cases = 12.2%

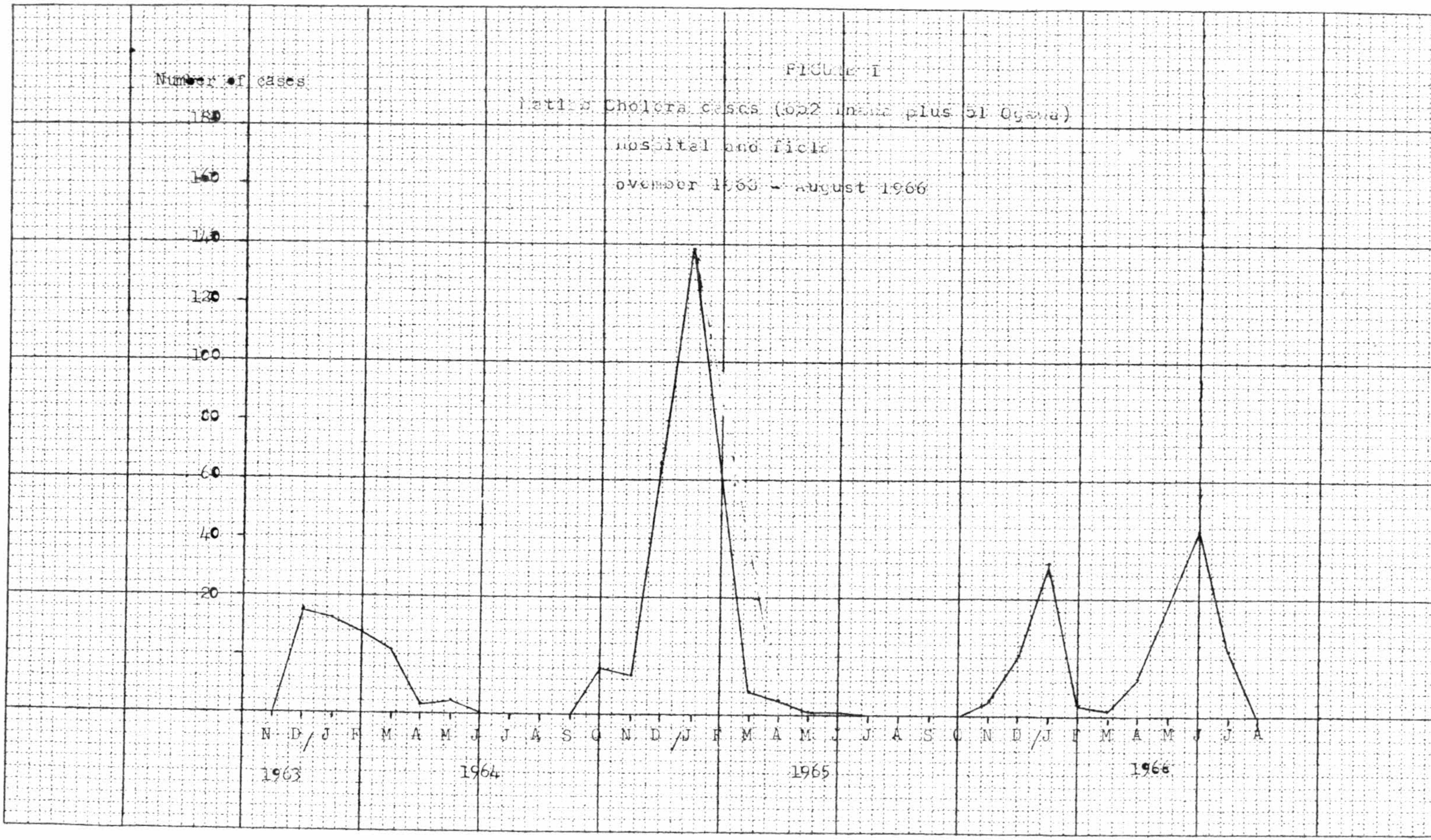


FIGURE I
Cholera cases plus 51 Ogawa)
hospital and field
ovember - August 1966
Number of cases
180
160
140
120
100
80
60
40
20
N D J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A
1963
1964
1965
1966

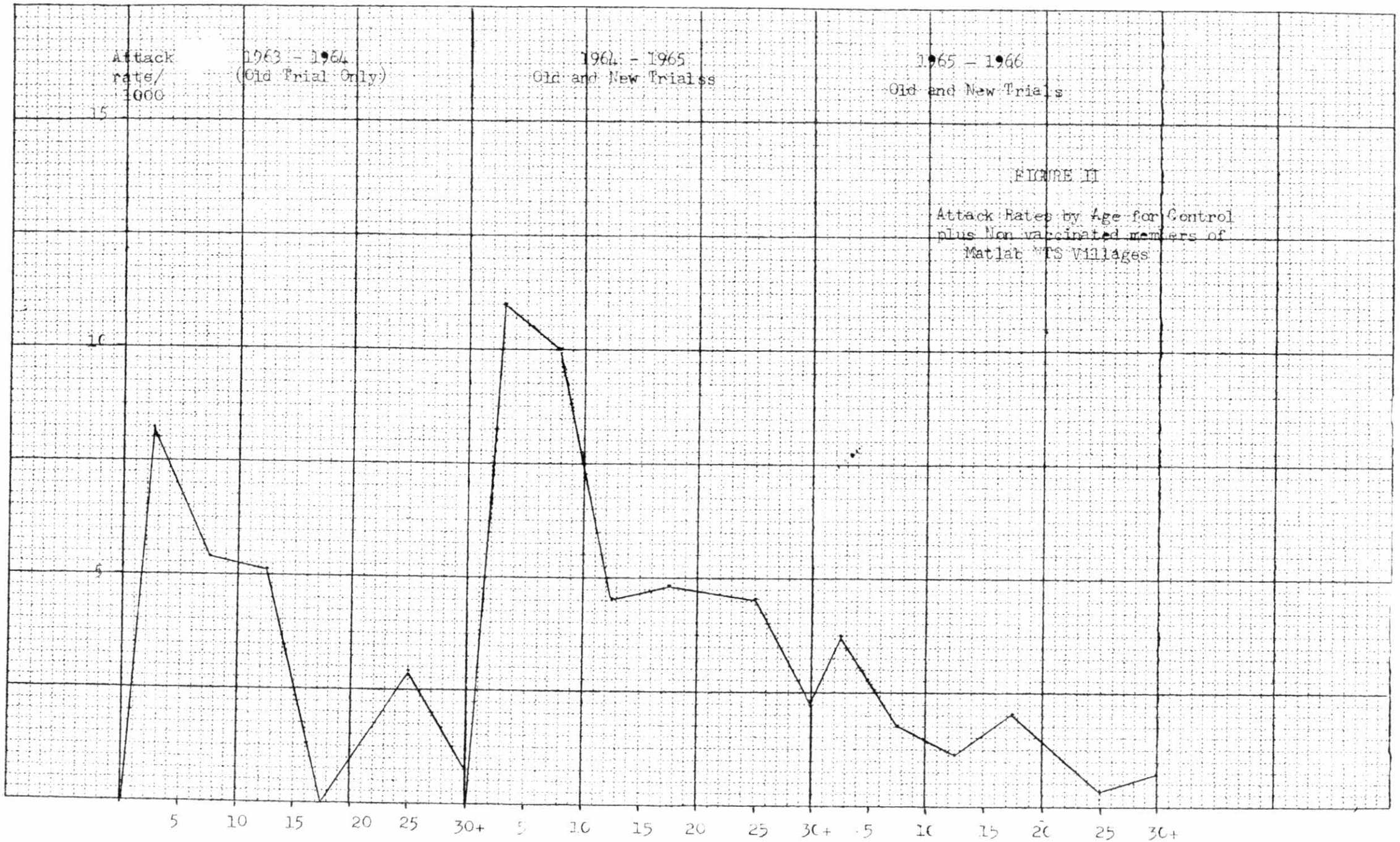
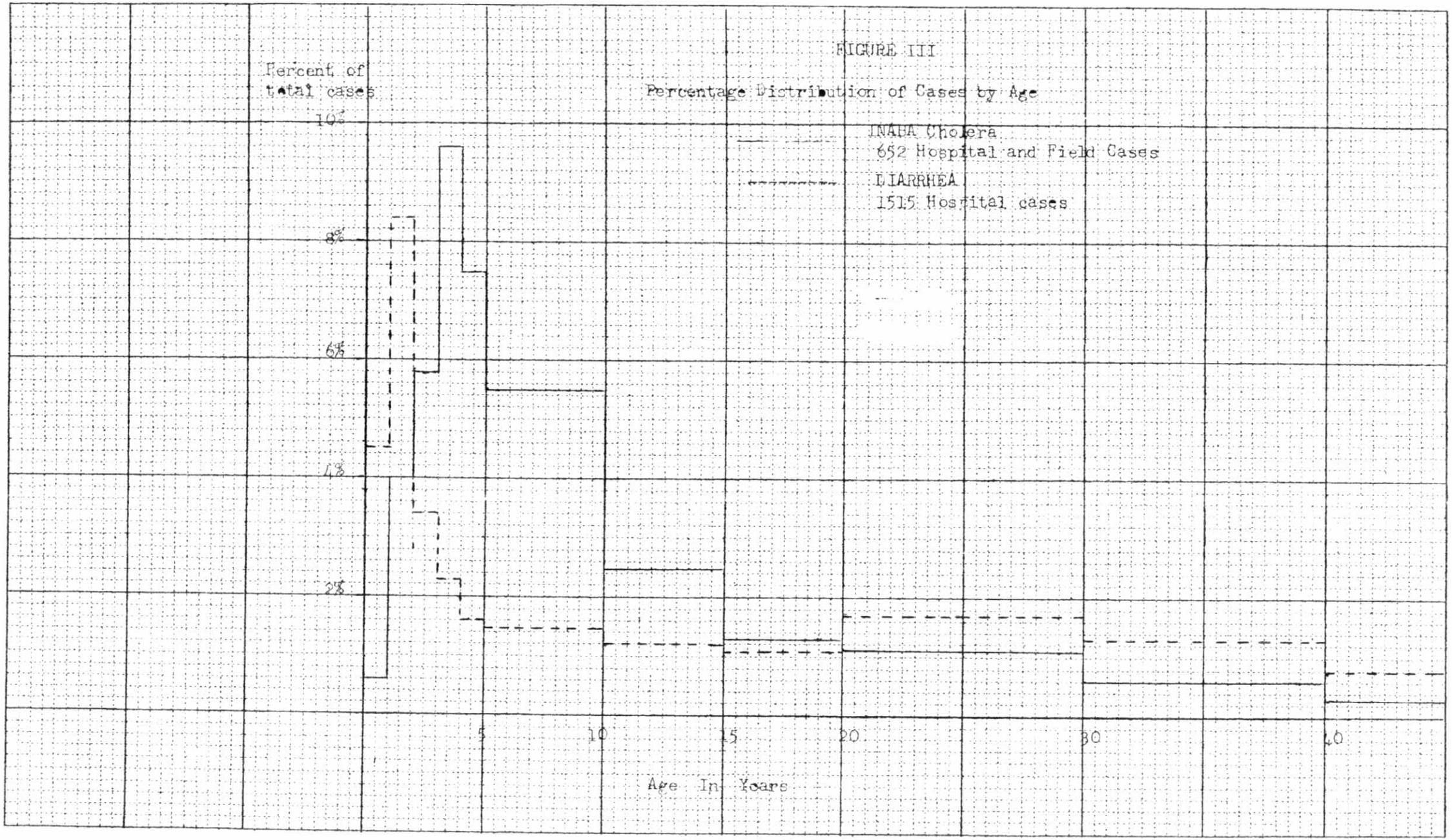


FIGURE II

Attack Rates by Age for Control plus Non vaccinated members of Matlab TS Villages

FIGURE III

Percentage Distribution of Cases by Age



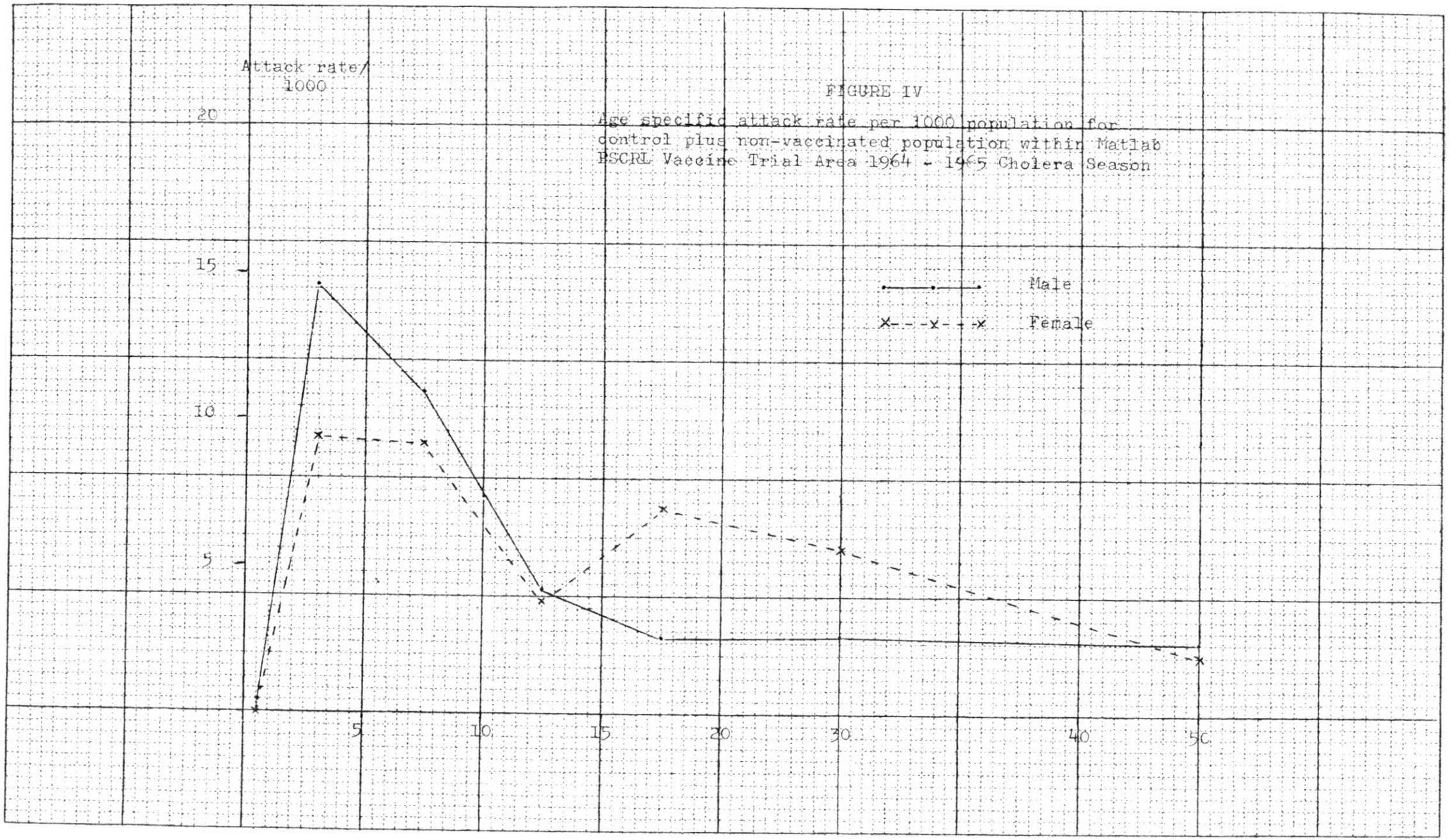


FIGURE IV

Age specific attack rate per 1000 population for control plus non-vaccinated population within Matlab ICDDR,B Vaccine Trial Area 1964 - 1965 Cholera Season

FIGURE V

Matlab Hospital 1963 - 1966
568 cholera and 1515 gastroenteritis
admissions by hour of onset

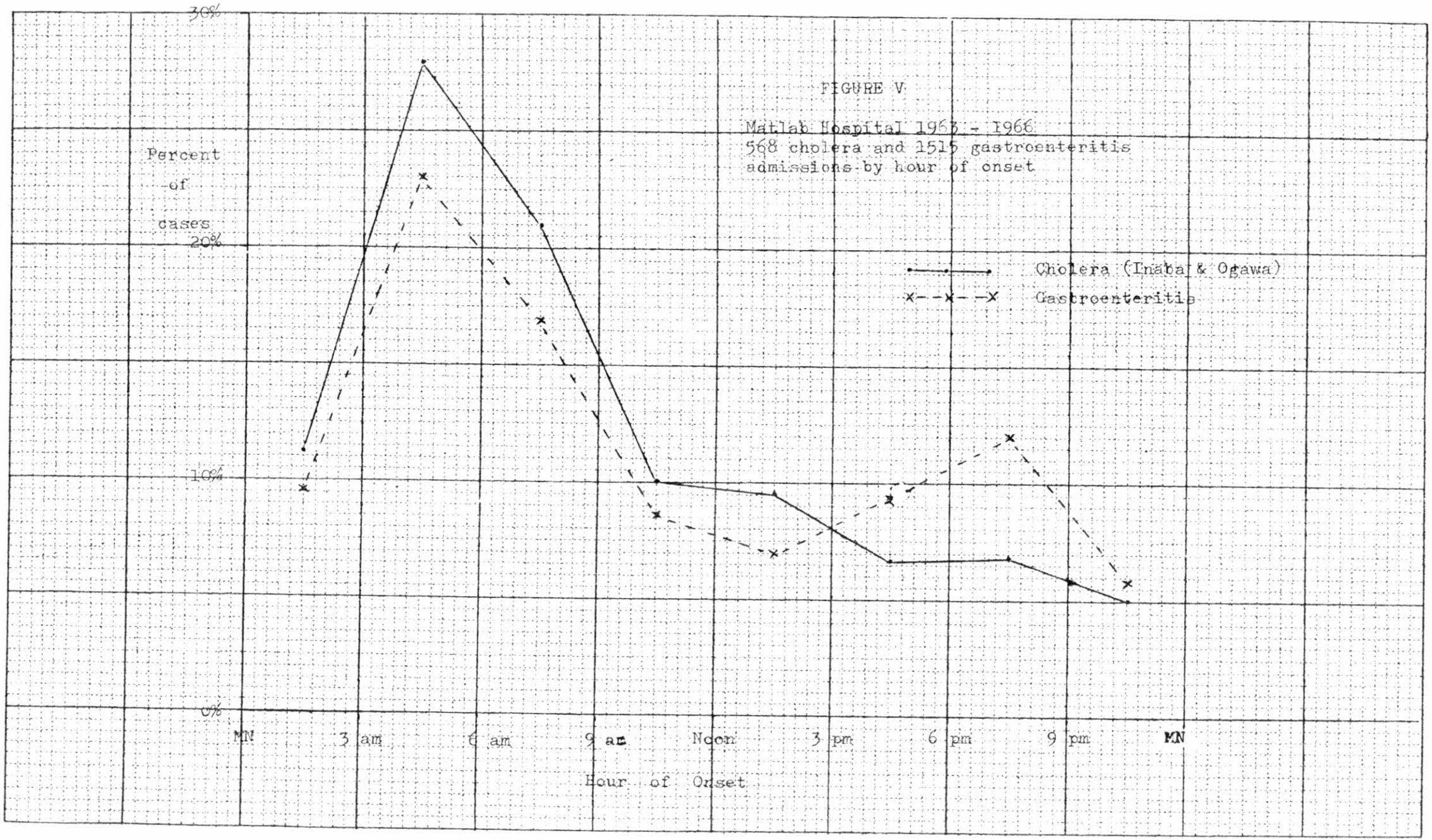
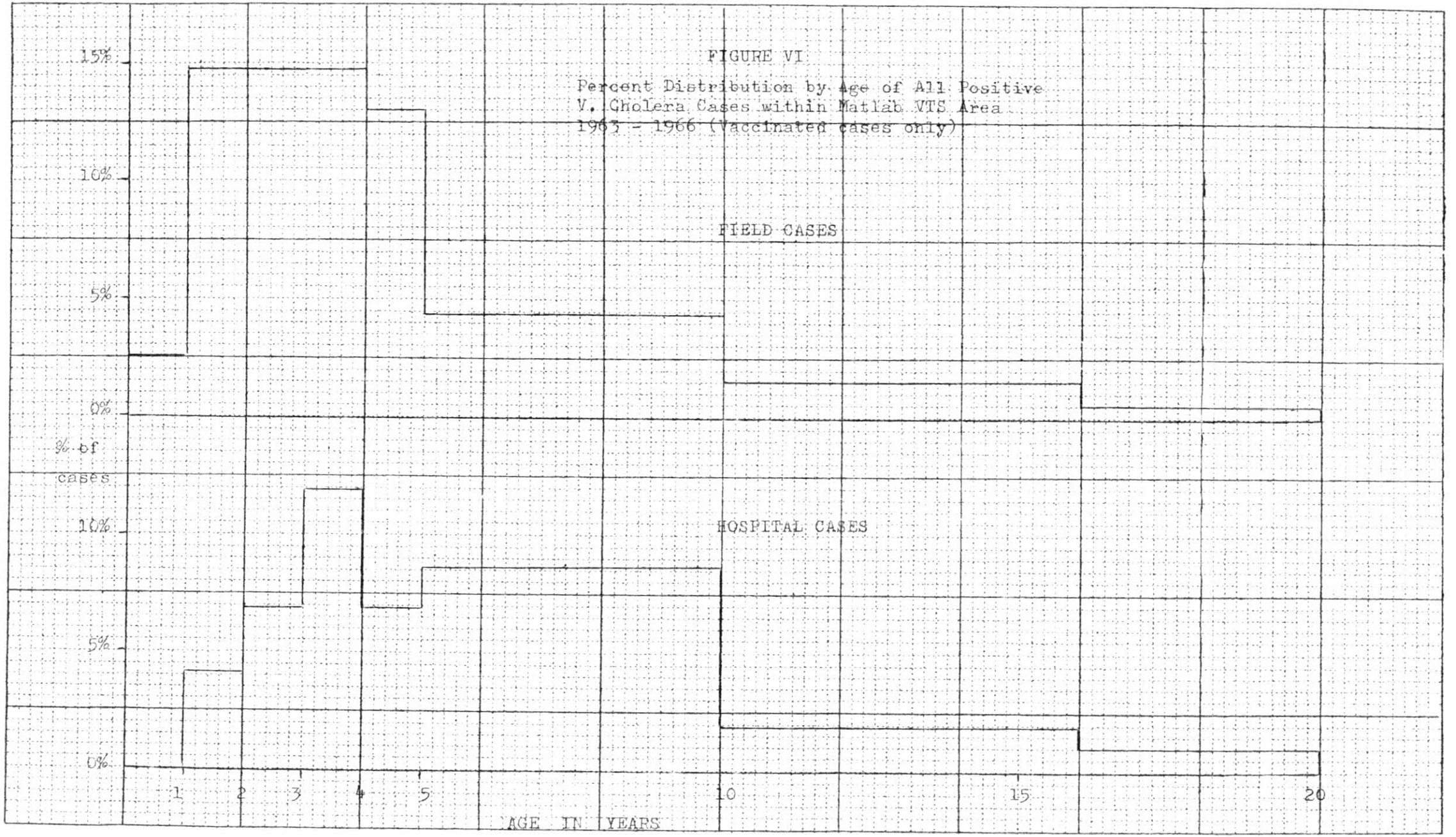


FIGURE VI

Percent Distribution by Age of All Positive
V. Cholera Cases within Matlab VTS Area
1963 - 1966 (Vaccinated cases only)



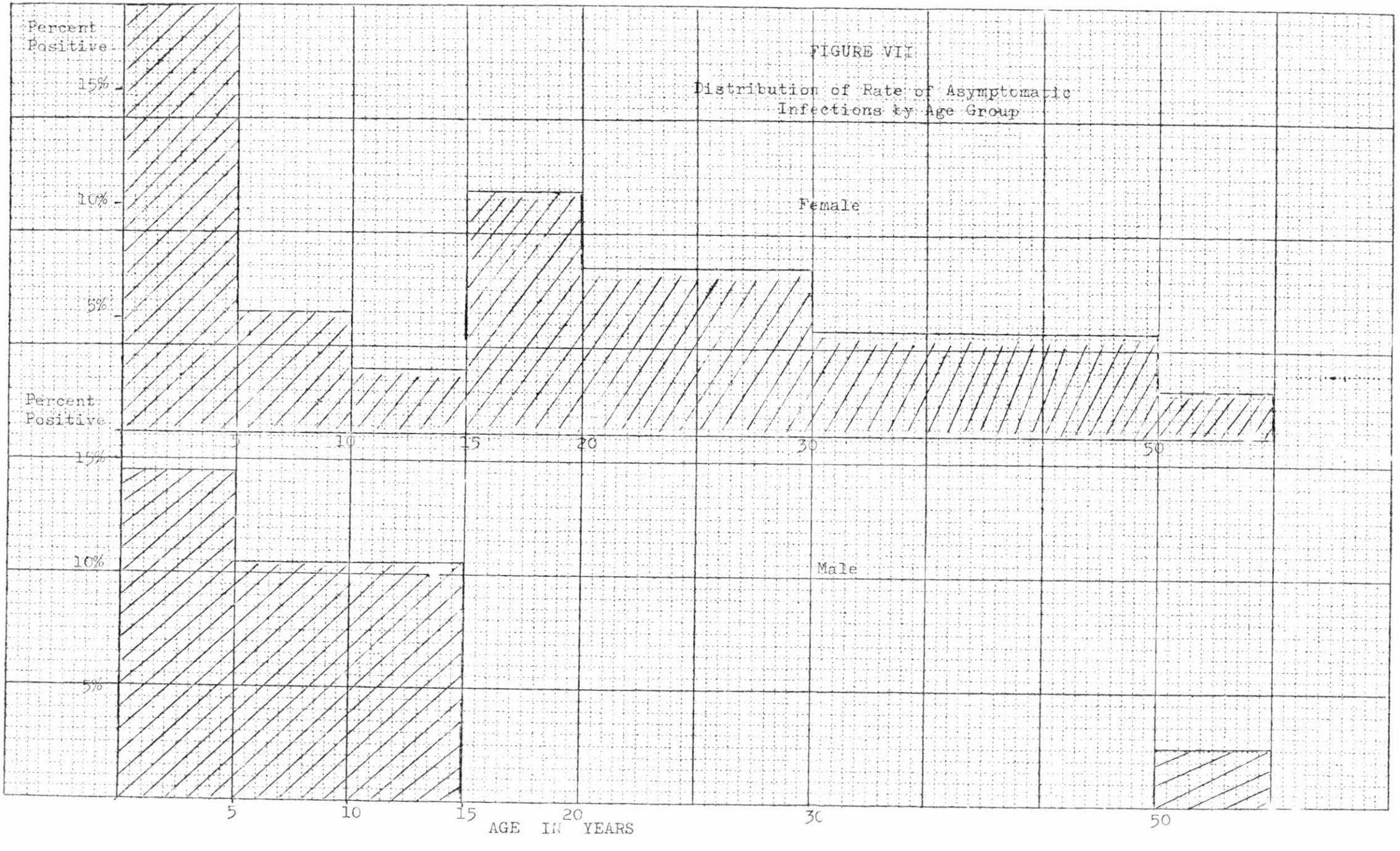
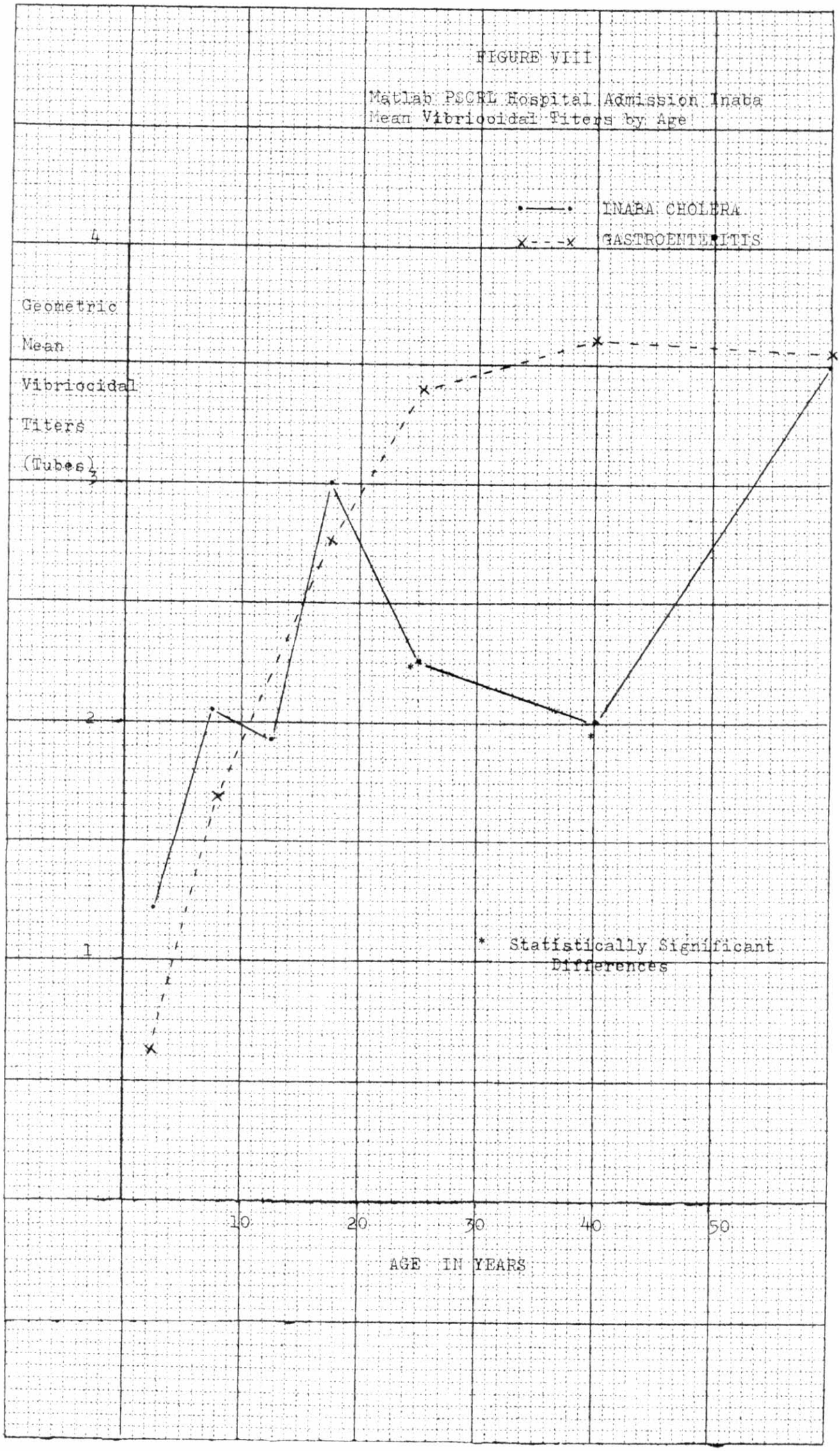


FIGURE VII
Distribution of Rate of Asymptomatic Infections by Age Group
Percent Positive
15%
10%
5%
Female
Percent Positive
15%
10%
5%
Male
5
10
15
20
30
50
AGE IN YEARS

FIGURE VIII

Matlab PSCRL Hospital Admission Inaba
Mean Vibriocidal Titers by Age



Effect of Various Antibiotic Prophylactic Schedules on the Infection Rate and Subsequent Cholera Experience in Family Contacts of Cholera Cases

Families were selected from index cases admitted to the PSCRL Dacca ward and assigned in rotation to the following groups:

A - Placebo in the same schedule as Group B.

B - Tetracycline 250 mg every 6 hrs. for 5 days.

C - Tetracycline one gram once daily for 5 days.

D - Tetracycline one gram once on the first day only.

For children under 10, the dose was half of that for adults. Rectal swabs were obtained daily for 10 days from each family member.

All Cases

	A	B	C	D
Number of Families	19	20	25	21
Number of positive members after day '1'	20	3	4	13

Cases with no Positive Members on Day '1'

	A	B	C	D
Number of Families	12	18	22	15
Number of positive members after day '1'	9	0	1	5

Field Case / Hospital case[1]	2	3	4	5	6	7	8	9	10
A Placebo	A 5-5	A 5-5	A 4-4	A31-4					
	A 7-9		A31-7	A31-7					
	A 8-4			A31-5	A31-5				
	A10-4				A10-4	A10-4	A10-4		
	A11-6	A13-5		A13-5		A13-5	A13-5	A10-7	
	A11-8	A12-9				A14-4	A14-4	A14-4	A14-4	A14-4
	A22-5				A18-3		A18-3		
	A22-7		A22-7		A27-5			A27-5	
	A22-8	A25-5	A25-5	A25-5	A27-3				
	A26-4	A26-4							
	A29-2		A29-2	A29-2	A29-2				
	A29-3								
	A29-6				A29-6				
B Tetracycline 250 mg q6h x 5 days	B20-4								
	B20-3								
	B26-6								
C Tetracycline 1 gm daily (single dose) for 5 days	C 8-8	C 3-3							
	C15-3								
	C16-4								
D Tetracycline 1 gm (single dose) on day '1'	D 7-7								
	D13-5				D 9-5				D 4-3
	D17-5				D14-5	D14-5	D14-5	D14-5	D14-5
	D18-2				D18-2		D18-2		
		D17-3					D18-4		
	D19-6	D19-6						D16-3	
	D20-7		D20-3						
	D22-2		D21-7					D23-7	
	D22-5								

Family Study

Purging of Family Contacts

Number of Families Studied: 12

Number of Individuals purged: 46

FS No.	Initial Rectal Swab	Purging Samples				Daily Rectal Swabs								
		I	II	III	IV	2	3	4	5	6	7	8	9	10
E 5-6	0	0	0	IN	IN	0	IN	IN	IN	IN	0	0	0	0
E 6-6	IN	IN	IN	IN	IN	IN	0	0	0	0	0	0	0	—
E 6-7	0	0	0	IN	—	0	0	0	0	0	0	0	0	—
E13-7	0	0	0	IN	IN	0	0	0	0	0	0	0	0	0
E 5-7	0	0	0	0	--	0	IN	IN	0	IN	0	0	0	0
E 7-5	0	0	0	0	0	0	IN	IN	IN	IN	IN	0	—	0
E10-6	0	0	—	—	—	IN	0	0	0	0	0	0	0	0
E 7-6	0	0	0	—	—	0	0	0	0	IN	0	0	—	0
E13-6	0	0	0	0	0	0	IN	--	IN	0	0	0	0	—

Total Positives - 9

Uncovered by Initial Rectal Swab - 1

Added by Purging - 3

Added by Daily Rectal Swabs - 5

0 = Negative
IN = Inaba
— = Not Examined

The Epidemiology of Non Vibrio Cholera

A- Seasonal Pattern:

Non vibrio cholera has a distinct seasonal pattern and is seen during the months of March, April, and May of each year. This differs from that of V. Cholera which is most prevalent during the winter months and also from the pattern of non specific gastroenteritis which is seen in East Pakistan, as elsewhere, during the months of July, August, and September. Although the entity we call "non-vibrio cholera" is much less frequent than non specific gastroenteritis, it is considerably more severe and much more likely to require hospitalization. Perusal of hospital records in Dacca, in Matlab, and in Dr. Mackay's Tea Garden population in Sylhet indicates that the peak of diarrhea admissions to the hospital is during the months of March and April. However, when we peruse out-patient records in Rayer Bazar, in Matlab, and in Sylhet we find the greatest number of cases to occur in July and August. To illustrate this, Figure 1 shows the seasonal patterns of hospital admissions of cholera and non cholera in Dacca. Figure 2 shows the distribution by month of hospitalized and non-hospitalized culture negative diarrhea cases occurring in one of our vaccine trial populations in Matlab.

B- Age and Sex:

Non vibrio cholera is almost exclusively a disease of adults whereas cholera has its highest attack rate in children. About three quarters of the cases we see in Dacca are in males. It is difficult to determine if this is a real difference or not because of the large number of males who come to Dacca during the spring months to obtain work. The 1961 census indicates that the population of Dacca city is 61% male.

C- Other Epidemiologic Data:

A prospective investigation of the cases of culture negative severe diarrhea was made during the months of March and April, 1966 and provided no indication of common source of infection or contact spread. The cases were typically in adult males with no history of contact with diarrhea. A spot map of the location of the homes of patients showed no clusters.

D- Clinical Data:

Although the disease in some cases, as the name indicates, may be as severe as cholera on presentation to the hospital most cases are not as severe as the average cholera case. In the few cases that on admission to the hospital are severely dehydrated and without pulse or blood pressure, the course is much shorter than that of cholera. Table 1 indicates the total volume of intravenous fluid required by cholera patients and non vibrio cholera patients presenting in circulatory collapse. Table 2 presents the duration of diarrhea. Neither group received antibiotics.

Table 1

Total Volume of Intravenous Fluid Administered to Non Antibiotic Treated Patients presenting in circulatory collapse

Volume Liters	Cholera		Non Vibrio Cholera	
	Number	Percent	Number	Percent
Less than 1	0	0	0	0
1.1 - 5.0	7	18.4	10	41.7
5.1 -10.0	6	15.8	13	54.2
10.1 -15.0	14	36.8	1	4.2
15.1-20.0	7	18.4	0	0
20.1-25.0	3	7.9	0	0
25.1-30.0	1	2.6	0	0
Total.....	38	100	24	100.1

Table 2

Duration of Diarrhea in Non Antibiotic Treated Patients presenting in circulatory collapse

Day	Cholera		Non Vibrio Cholera	
	Number	Percent	Number	Percent
0 - 1.9	0	0	8	33.3
2 - 3.9	3	7.9	12	50.0
4 - 5.9	8	21.1	2	8.3
6 - 7.9	20	52.6	2	8.3
Over 8	6	15.8	0	0
Unknown	1	2.6	0	0
Total.....	38	100	24	100

FIGURE I

Dacca PSCRL Admissions
By Month

July 1964 - June 1965

——— Cholera
----- No pathogen

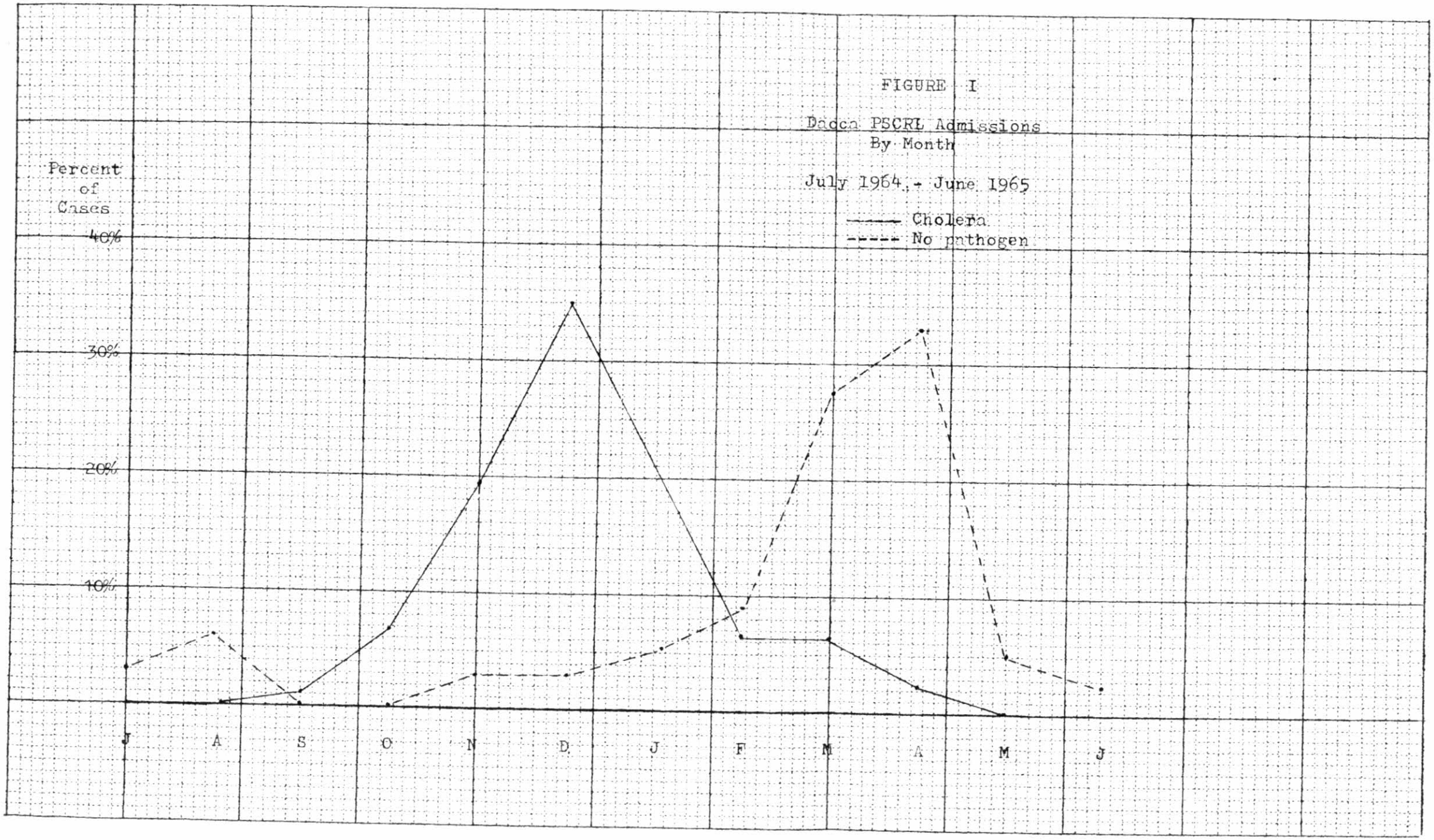


FIGURE II

Matlab AB Trial Population
June 1964 - 1965

Culture Negative Hospital and Field Diarrheas by month of onset.

_____ Field

------ Hospital

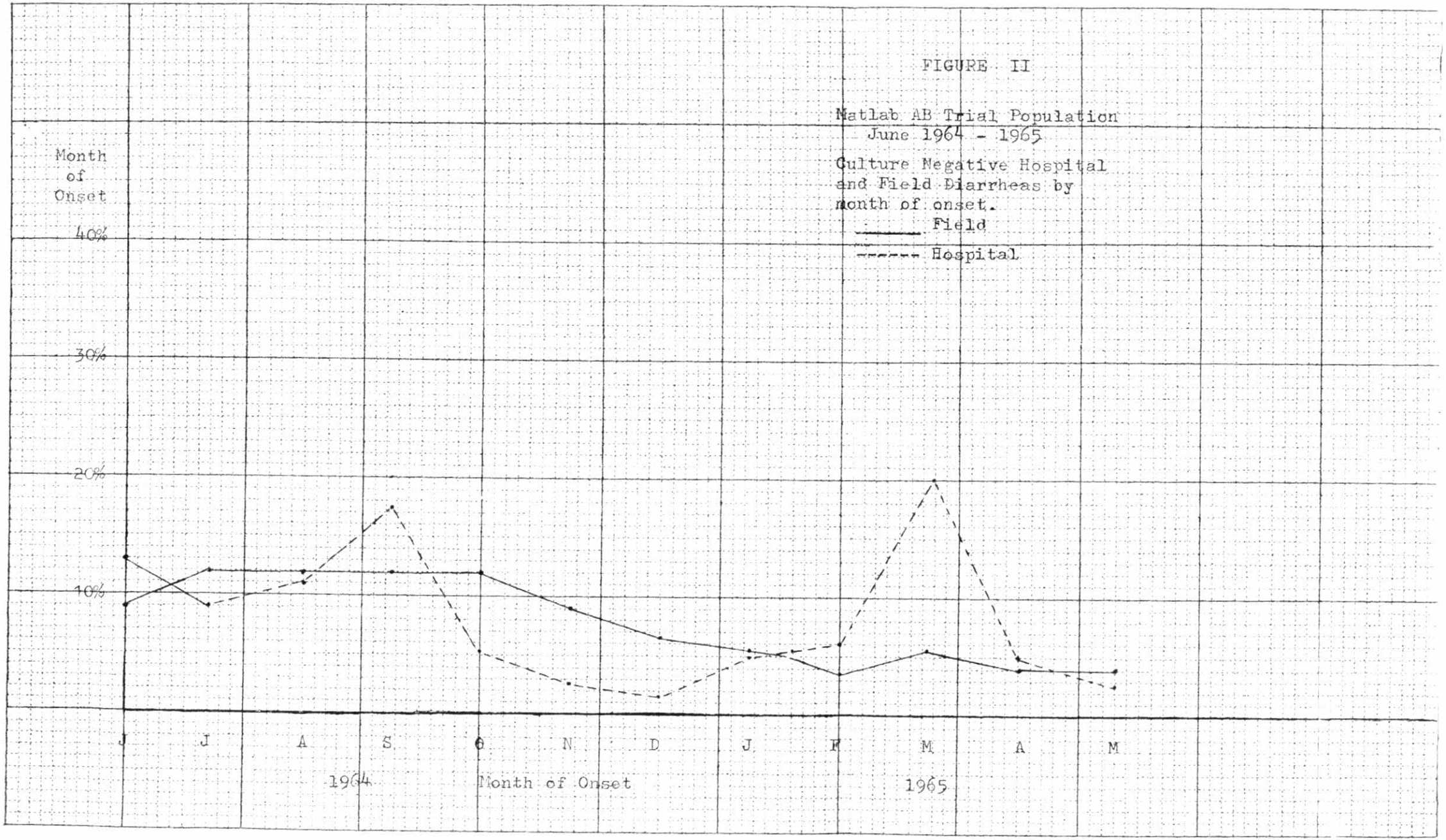


Table 1 a

Rayer Bazar Community Study, Oct. 1965 - Sept. 1966
Mortality Rate by Age and Sex

Age	Population*			Deaths			Rate/1000		
	M	F	Total	M	F	Total	M	F	Total
1	101	116	217	13	15	28	128.7	129.3	129
1	159	134	293	1	-	1	6.2	-	3.4
2	132	136	268	2	-	2	15.1	-	7.4
3	140	138	278	-	1	1	-	7.2	3.5
4	136	143	279	-	3	3	-	20.9	10.7
5 - 9	721	726	1447	1	1	2	1.3	1.3	1.3
10-19	837	781	1618	-	2	2	-	2.5	1.2
20-29	472	659	1131	-	1	1	-	1.5	.8
30+	1227	816	2043	6	7	13	4.8	8.5	6.3
Total	3925	3649	7574	23	30	53	5.8	8.2	6.9

* Adjusted Average, based on weekly correction for births, deaths and migrations.

Table 1 b

Births by Sex

	No.	Rate/1000 population
Male	128	
Female	133	
Total	261	34.5

Table 2

Rayer Bazar Community Study, Oct. 1965 - Sept. 1966
Diarrhea Rate by Age and Sex

Age Group	Population			Diarrhea			Rate/1000		
	M	F	Total	M	F	Total	M	F	Total
<1	101	116	217	208	174	382	2059	1500	1760
1	159	134	293	290	221	511	1823	1649	1744
2	132	136	268	213	155	368	1613	1139	1373
3	140	138	278	113	133	246	807	963	884
4	136	143	279	118	102	220	867	713	788
5 - 9	721	726	1447	360	287	647	499	395	447
10-19	837	781	1618	75	92	167	89	117	103
20-29	472	659	1131	16	88	104	33	133	91
30+	1227	816	2043	95	100	195	77	122	95
Total	3925	3649	7574	1488	1352	2840	379	370	374

Table 3

Rayer Bazar Community Study, Oct. 1965 - Sept. 1966
Shigella Case Rate by Age and Sex

Age Group	Cases			Rate/1000		
	M	F	Total	M	F	Total
<1	7	2	9	69.3	17.2	41.4
1	3	3	6	18.8	22.3	20.4
2	3	2	5	22.7	14.7	18.6
3	6	7	13	42.8	50.7	46.7
4	2	2	4	14.7	13.9	14.3
5 - 9	10	5	15	13.8	6.8	10.4
10-19	-	2	2	-	2.5	1.2
20-29	-	-	-	-	-	-
30+	-	-	-	-	-	-
Total	31	23	54	7.8	6.3	7.1

Table 4

Rayer Bazar Community Study Survey
for Pathogenic E. Coli - May 1966

Age Group	Diarrhea Cases			Asymptomatic Persons		
	Number Cultured	Positive No.	%	Number Cultured	Positive No.	%
1	15	2	13.3	46	7	15.2
1	42	4	9.5	34	-	-
2	32	1	3.1	30	-	-
3	22	-	-	23	1	4.3
4	22	2	9.	33	1	3.
5 - 9	62	4	6.4	102	1	.9
10-19	26	3	11.5	40	-	-
20-29	17	-	-	38	3	7.8
30+	64	6	9.3	37	-	-
Total	302	22	7.2	383	13	3.3

Table 5

Rayer Bazar Community Study
Cholera Cases and Rate/1000 by Age

Age Group	Population*	Cholera Cases				Rate/1000			
	Total	1964	1965	1966	Total	1964	1965	1966	Total
<1	217	2	1	1	4	9.2	4.6	4.6	18.4
1	293	-	-	2	2	-	-	7.2	7.2
2	268	1	-	2	3	3.7	-	7.4	11.2
3	278	3	1	3	7	10.8	3.6	10.8	25.1
4	279	2	2	1	5	7.1	7.1	3.5	17.9
5 - 9	1447	7	6	5	18	4.8	4.1	3.4	12.4
10-19	1618	4	2	5	11	2.4	1.2	3.1	6.8
20-29	1131	5	-	-	5	4.4	-	-	4.4
30+	2043	6	2	3	11	2.9	0.97	1.4	5.3
Total	7574	30	14	22	66	3.9	1.8	2.9	8.7

* Adjusted population for 1965-1966 study period.

Table 6

Zonal Incidence of Diarrhea and Cholera At Rayer Bazar

Name of Zones	Total Adjusted Population	Person with one or more Diarrhea	Rate of Diarrhea per/1000	No. of Cholera				
				1964	1965	1966	Total	Per/1000 Combined Rate
A	434	98	225.8	-	-	-	-	-
B	360	103	286.1	-	-	-	-	-
C	252	61	242.0	-	-	-	-	-
D	334	56	167.6	-	-	1	1	2.9
E	264	56	212.1	-	-	-	-	-
F	221	45	203.6	-	-	-	-	-
G	330	84	254.5	-	-	2	2	6.0
H	270	64	237.0	-	-	-	-	-
I	693	197	284.2	5	-	2	7	10.1
J	365	87	238.3	-	-	-	-	-
K	388	53	136.6	4	3	2	9	23.1
L	221	56	253.4	-	-	-	-	-
M	519	98	188.8	15	5	1	21	40.4
N	360	56	155.5	-	1	-	1	2.7
O	583	110	188.6	2	3	12	17	29.1
P	348	44	126.4	-	-	1	1	2.8
Q	909	171	188.1	-	2	1	3	3.3
R	595	96	161.3	-	-	-	-	-
S	125	22	176.0	-	-	-	-	-
T	240	42	175.0	-	-	-	-	-
Total 20	7811	1599		26	14	22	62	

Rayer Bazar Community Study -

Results of 6 daily rectal swab cultures in family contacts and neighborhood contacts of cholera cases, 1965-1966

A - Index Families

Number of Families	Total Members	Index Cases	Total Contacts	No. Cultured	No. Infected	% Infected
25	149	25	124	78	11	14.1

a - Includes 2 ^{o} cases

B - Contact Families

Number of Families	Total Members	No. Cultured	No. Infected
40	201	146	0

FIGURE I

Relation of Boatmen with the cases of the locality and the positive water culture

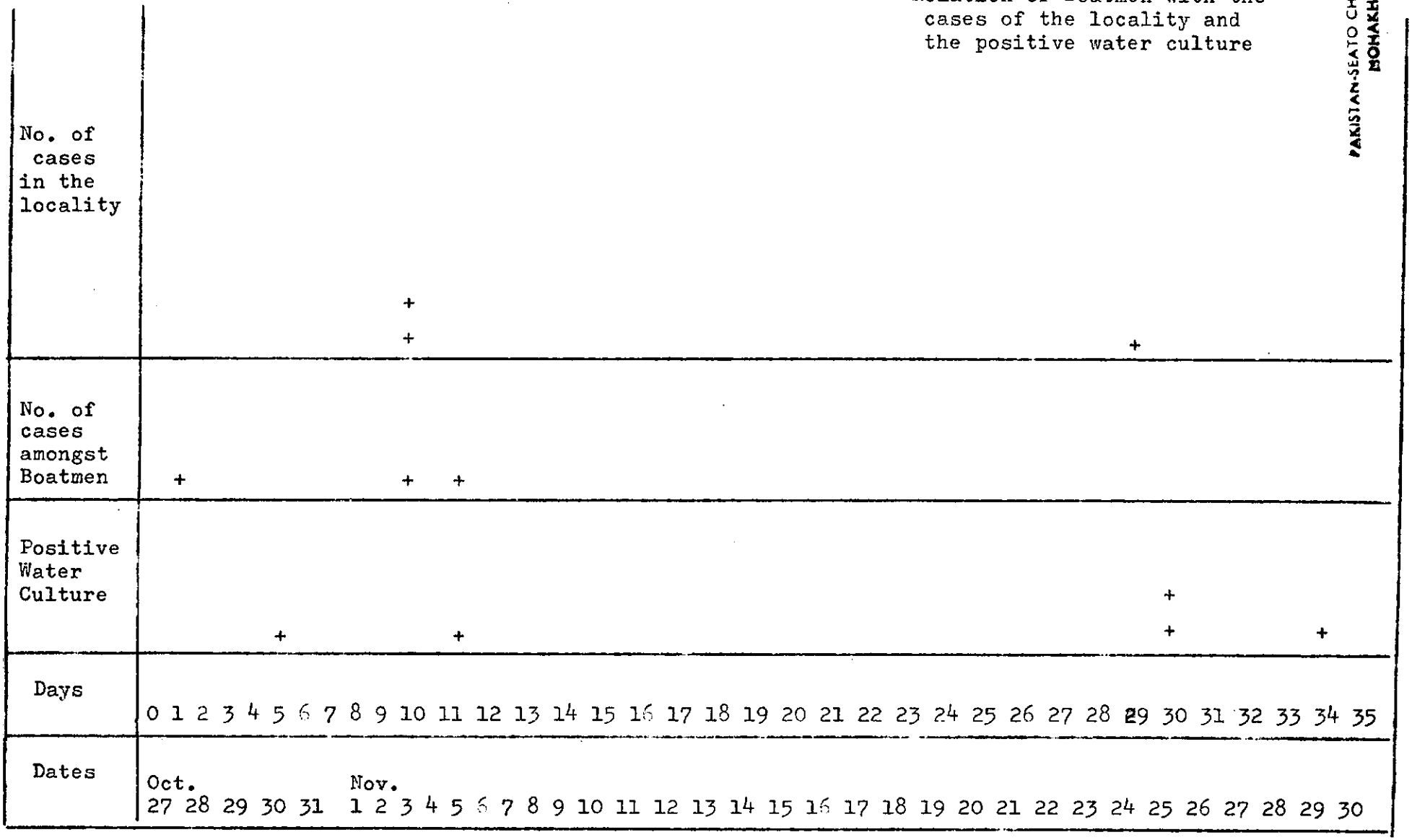


FIGURE II

Relation of Boatmen with the cases of the locality and the positive water culture

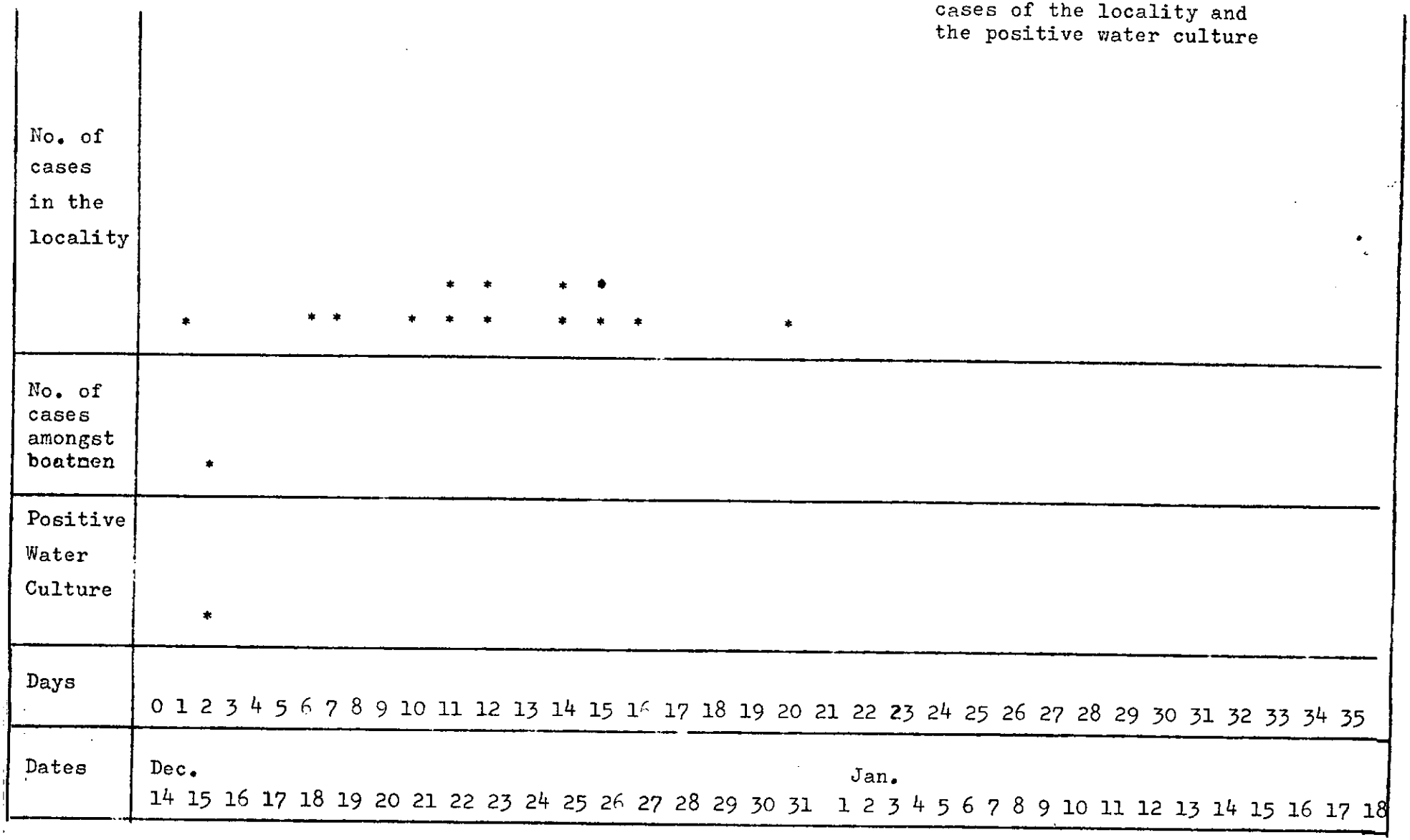


FIGURE III

Relation of Boatmen with the cases of the locality and the positive water culture

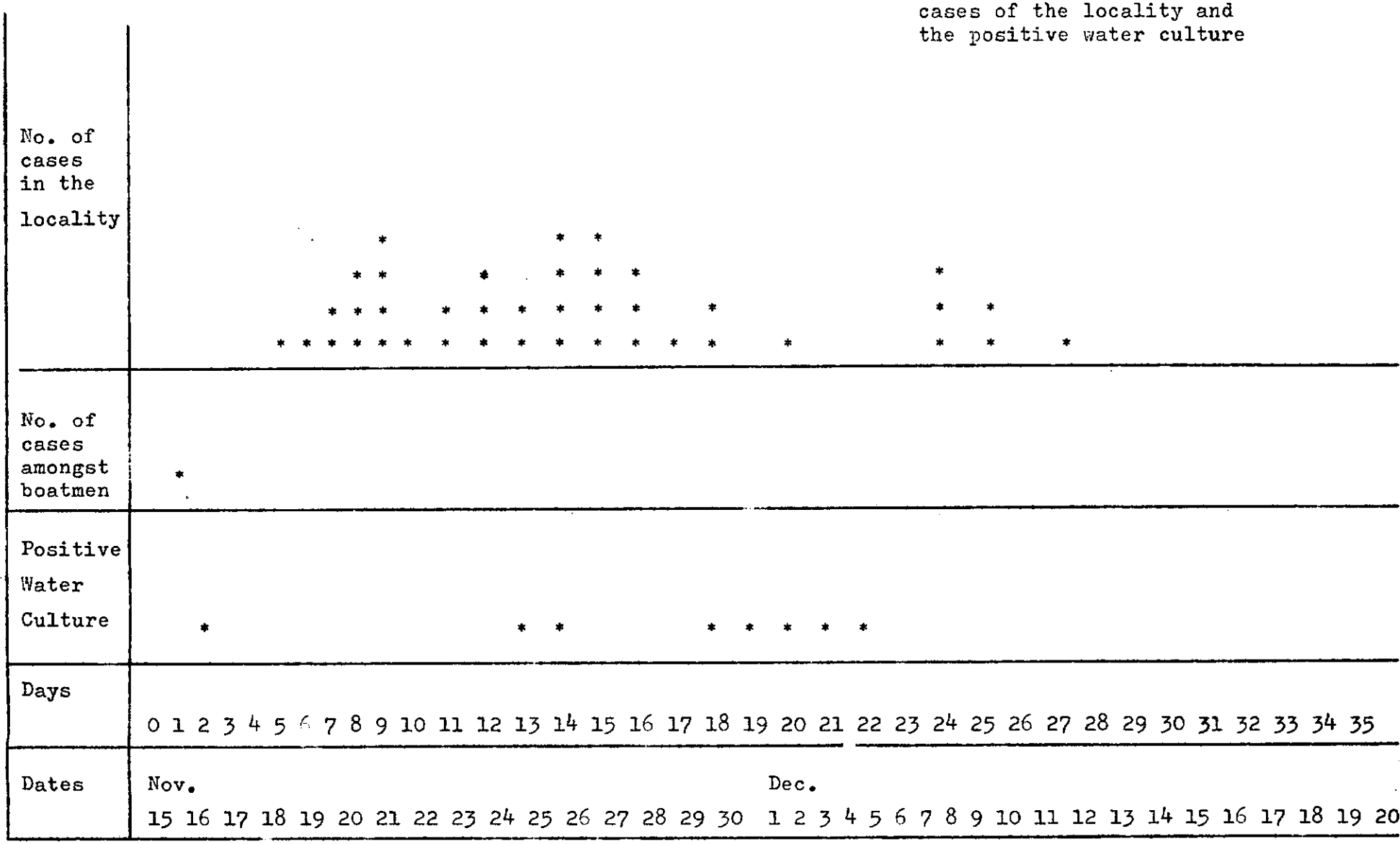
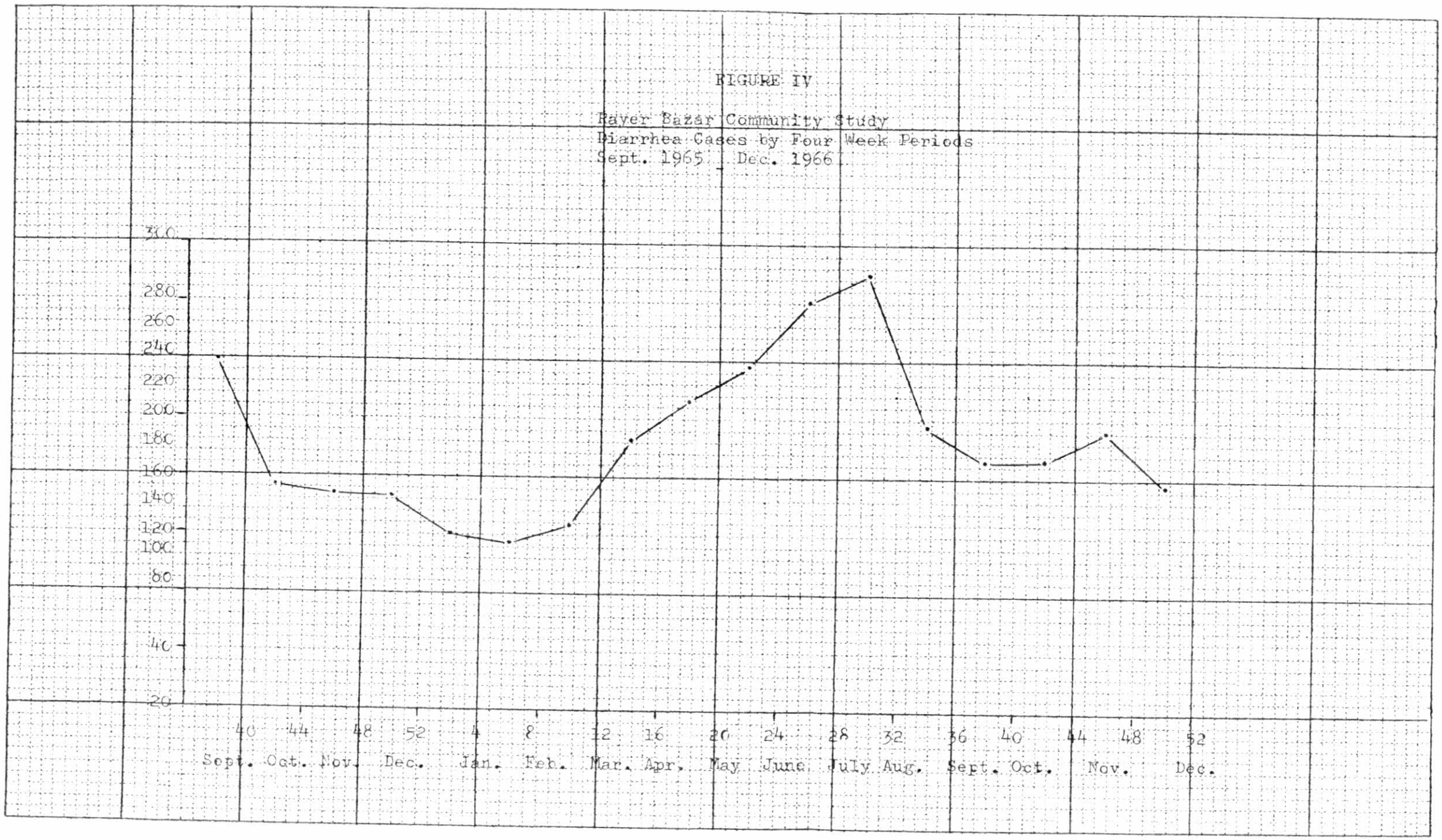


FIGURE IV

Rayer Bazar Community Study
Diarrhea Cases by Four Week Periods
Sept. 1965 - Dec. 1966



Prospective Study of the Role of Migrants in the Introduction and Transmission of Cholera

Two villages in Matlab Thana were selected. A rectal swab and travel history was obtained from all persons coming into the villages.

South Joypur (V70):

A predominantly Muslim agricultural village with a population of 497. A positive rectal swab was obtained from a visitor on 9 January 1967. There were no subsequent cases in the village.

Meharon (VB11):

A Hindu fishing village with a population of 1,608. No positive rectal swabs have been found among over 500 collected. Five cases of cholera have occurred in the village. The relationship of the number of swabs obtained from migrants to the cases within the village is shown in Figure I.

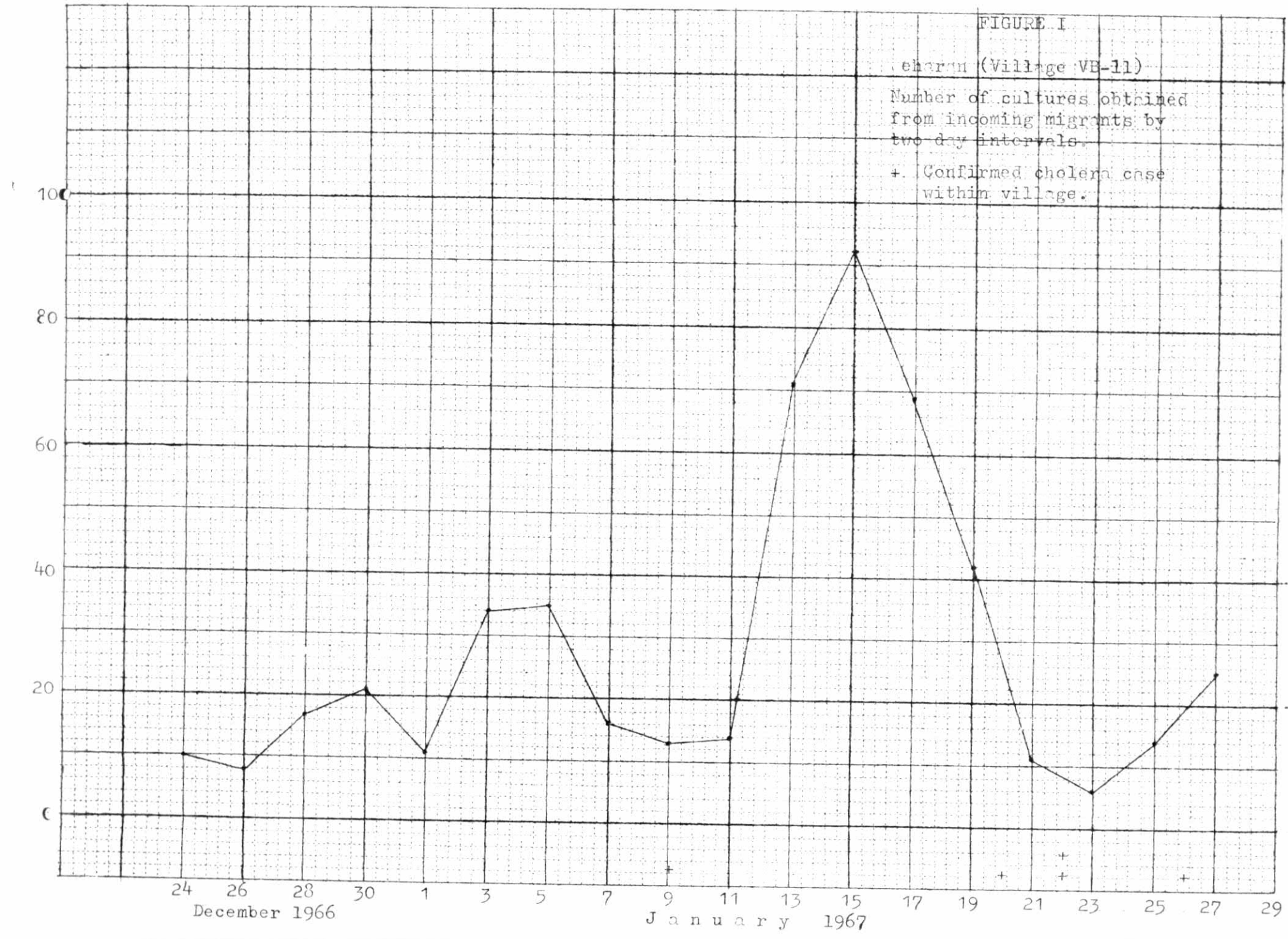


FIGURE I
Cheram (Village VB-11)
Number of cultures obtained from incoming migrants by two-day intervals.
+. Confirmed cholera case within village.
100
80
60
40
20
0
24 26 28 30 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29
December 1966
January 1967

Estimation of the Level of Inapparent Infection in a cholera affected village by paired serological studies on the entire population

Village Meharon (VB11) is a fishing village on the outskirts of our vaccine trial area with a population of 1,608.

In late November, 1966 serum specimens were collected from all of the village members present. Participation was better than 90% in all groups except adult males, many of whom were away from the village for the winter fishing season. In all 1,177 specimens were obtained.

Our field team is collecting a rectal swab from all cases of acute diarrhea occurring in the village. 5 cases of cholera have been bacteriologically documented to date.

A second serologic sample will be obtained soon. The number of titer rises seen in the paired sera will be compared to the number of bacteriologically documented cases to estimate the inapparent infection/case ratio.

Endemicity Characteristics of Cholera in Rural East Pakistan

Md. Fahimuddin, M.B.B.S., D.T.M.H.

The Pakistan-SEATO Cholera Research Laboratory may rightly be proud to have greatly advanced scientific knowledge in the pathogenesis and treatment of cholera in as much as the hospital mortality from acute cholera has been reduced to almost nil even though many young children and aged persons are included in the case load. In view of this achievement one wonders if there is any scope to totally eliminate cholera mortality by further research. Many gaps, however, exist in our knowledge of the epidemiology of cholera which baffle all our control and preventive measures as is evidenced by its recent invasions in countries such as the Philippines, Thailand, South Korea, Iran and Iraq which lie far away from the endemic foci. We do not know as yet the causes which precipitate its onset in man or the nature of changes in the environment which influence its secular or seasonal appearance or disappearance in a locality. We also do not know why Vibrio Cholerae becomes more aggressive in certain localities and during certain period of the year or what factors make some hosts more susceptible to the disease than others even though geo-physical or socio-economic conditions are similar. It is clear that for an outbreak or for endemicity of cholera, existence of the Vibrio or its introduction from outside is a necessity and the environmental factors of both the Agent and the Host must favour the interaction to produce the result. Environmental conditions in the Matlab Vaccine Trial area seem to be most favourable for growth and survival of the vibrio and to make a host susceptible to the disease throughout the year. Yet the disease prevails in Matlab only during certain seasons and sometime assumes an aggressive form though no significant changes are observed in the environment. Does Vibrio Cholerae exist then in some other form or in an unknown reservoir or is it introduced afresh from outside? To find an answer to this question an attempt was made during 1965-1966 cholera season to trace the origin of the disease as well as to follow its course in Matlab Vaccine Trial area where facilities for early bacteriological diagnosis are available. During the year of study there were two distinct outbreaks of cholera - one in the winter and the other in the summer with a clear period of quiescence between. The winter outbreak involved 21 villages with 80 attacks and the summer outbreak 12 villages with 38 attacks. 3 villages had both winter and summer outbreaks.

When the first case of the year was reported on the 11th November, 1965 after a break of 7 months and 23 days from the previous case; we were quite jubilant with prospect of getting a chance to study the behaviour of the seasonable cholera epidemic. It was a November afternoon. The 15 miles by road to Narayanganj, 40 miles over the treacherous Meghna and another 6 miles on foot or by peddle rickshaw did not deter us. When we ultimately reached the village, it was quite dark. It took another hour to find the house. The head of the family who is a daily labourer had gone to Goalundoghat in Faridpur district with his wife and two minor sons about a fortnight before to seek employment. Cholera was prevailing in the area at the time and when there was a death in a neighbour's house the family became panicky and left the place in a commercial launch on 6.11.65. Enroute the elder boy developed diarrhea and vomiting. On arrival at Chandpur next morning, the boy was admitted to Chandpur Hospital where saline was given. He improved a bit towards evening and returned with his family to their house in Digaldi (Village 31) the same night. A rectal swab of the patient was taken by the surveillance staff on 9th November, 1965. The other members of the family had no symptoms. Specimen from the other members as well as from the water sources were collected by us on 11.11.65. The other boy was positive; the other specimens were negative. We anxiously waited for secondary cases to develop but nothing happened.

The second case was reported from Durgapur (Village 35) about 12 miles upstream from the first village. The victim was a 2-year old child who had not been out of the village during the past few weeks. He had not had any contact with any known cholera case. The family used river water for drinking purposes. No other case developed in the family nor in the neighbourhood though the same source of the drinking water was used by all.

The third case was from Udamddi (Village A). He became sick on 30.11.65 while camping on the bank of the Meghna outside the VTS area using river water for drinking. He was brought home on 2.12.65 and admitted to the CRL hospital on 3.12.65. His younger sister developed cholera the next day and subsequently 14 more cases occurred in the village in 6 different families with no social or environmental link. The last case occurred on 10.12.65, 24 days after the immediate previous one and 40 days from the date of entry of the Index case. This was a 2-year old child of a family which had no prior cases.

Another interesting case occurred on 30.12.65 in Ludua (Village 36) which is separated from other affected villages by several miles and has no link by river or road with them.

The victim, a housewife who came to the village a month before she had apparently no contact with any other case nor did she use river or canal water for drinking or culinary purposes. The source of drinking water was a tank near her house which was also used by other families. Her contacts gave negative findings on 3 consecutive swabbings. Tank water gave negative results. Yet her 6-month old breast-fed child developed cholera on 13.12.65 and a neighbour's child on 15.1.66. None required hospitalization.

Subsequently cholera appeared in 18 more villages within the next 4 weeks, a total of 21 involved villages. Cholera patients from 10 of the villages came to the hospital before the surveillance staff could detect any case but mild cases of diarrhea were detected by them in the other 11 villages before cholera was suspected.

Of the 80 cases in 21 villages, 35 came to the hospital, 14 were identified as asymptomatic contacts, 2 were treated as out-patients and 29 were detected by the surveillance s staff as diarrhea needing no hospitalization. The last 3 groups contributed 56% of the total number of cases which would have been otherwise missed as asymptomatic or mild diarrhea. In 11 of the 21 villages there was only a single case. In the remaining 10 villages 45 families contributed 69 cases. Out of a total of 56 families 38 (67%) had only one case each, 23% - two cases, 7% - 3 cases and 2% - 4 cases each. Secondary cases seemed to have contracted infection simultaneously with the Index case. In only one instance there seemed to have been a 'person-to-person' infection.

Distribution of Cases by Age:

25 of the hospitalized cases (70%) were below the age of 10 years and 23 of the 29 field cases (80%) were children below the age of 10. Patients treated as out-patients and asymptomatic contactsshow similar distribution in age. Of the total of 80 cases, 61 (76%) were children.

Index Cases:

If the first victim in the village be taken as the village Index case, it will be seen that of the 21 village Index cases, 16 (16%) were children who did not move out of their village within the maximum incubation period and 5 adults, 3 males and 2 females. Two of the adults were fishermen and the third a boatman. Both of the females were housewives who did not leave the village during the 3 weeks before illness.

Of the 11 field Index cases, 10 (91%) were children and only one adult. Of the 10 children, only one contracted the disease outside and the other 9 seemed to be indigenous. The single adult, a boatman, became sick at Barisal. Index cases by family show that of the 56 Index cases 46 (82%) were children.

Summer Outbreak:

The summer outbreak followed the winter outbreak after a lapse of one month and 23 days. 12 villages were affected of which 3 had also had winter outbreaks. 4 villages (33%) had only one case each. Of a total of 38 cases, 28 were hospitalized, 5 were identified as asymptomatic contacts, 4 treated as out-patients and only one was detected by the surveillance staff. Of the 12 village Index cases, 5 were children below the age of 10 who had not moved out of their village during the previous 3 weeks and the remaining 7 were adults - 4 males and 3 females. Three adult males were fishing in the river at the time of attack and the other adult was living in a boat in the Meghna. Of the 3 adult females, one was attacked outside the trial area in her father's house in Kachua P.S. and the other two remained in their village. 30 families contributed 38 cases, 27 families one case each and 3 families more than one case. The families were widely separated and did not have any social or environmental contact. Family Index cases show that 20 out of 30 (66%) were children. All were apparently indigenous and no link could be established between any two of them. Adult male Index cases apparently got infected outside the trial area.

Result of Observation in Mehron and South Joypur:

Two villages - Mehron and South Joypur - were brought under similar study during the current season. Results of observation during the last two months show exactly the same pattern of the disease. All the 5 cases so far identified in Mehron belonged to 5 different families which had no social or environmental link except that water from one of the tanks but from different points was used by two of the cases. Four of them were children, all seemed to be indigenous and one adult - housewife who had visited a neighbouring village on the previous day which was also free from cholera at the time. In the other village, South Joypur, only one case was identified in a visitor. No secondary cases nor any asymptomatic contacts were detected.

Summary:

118 proven cases of cholera - 80 in 21 villages in winter and 38 in 12 villages in summer of 1965-66 were followed up in the Matlab VTS area. Three villages had both winter and summer outbreaks. No link between outbreaks in any two villages could be established. During the winter outbreak, 44% of the cases came to the hospital and the remaining 56% were detected as asymptomatic contacts or as mild diarrhea. Only one field case and five asymptomatic contacts were detected during the summer as against 32 hospital admissions.

85% of the affected families contributed one case each and 15% more than one case each. This compares with 14.1% of families in Dacca city with more than one case. Most of the secondary cases seemed to have contracted the disease at the same time as the Index case.

76% of all cases were children aged 10 and under. Index cases by village show a distribution of 75% children and 25% adults.

Field cases and those treated as out-patients show 91% of the Index cases were children.

Adult male Index cases were exogenous and 95% of the children Index cases were indigenous. No link could be established between any two index cases even though they belonged to the same village.

No link between Vibrio positive water source and a confirmed case could be established.

None of the patients used only tube well water. 33% of the cases reported to have unprotected tanks or ditch, 56% river or canal and 11% tube well and tank water. Tube well water in the area is unpalatable.

Conclusion:

The purpose of this presentation is to draw attention to the wide gaps that exist in our knowledge of the epidemiology of cholera which foils all measures to control the disease. Interruption of the disease will not be possible until the natural history of the Vibrio Cholerae is spelled out and clearly understood.

A Comparison of the Effectiveness of a High Dose Tetracycline Schedule with the Routine Schedule

Patients admitted to the Matlab hospital during December 1966 and January 1967 were assigned to groups on the basis of their hospital numbers:

Odd Numbers: Usual regimen, i.e., 250 mg of tetracycline every six hours for five days.

Even Numbers: High dose regimen, i.e., 1 gm of tetracycline on admission, then 500 mg every two hours for four doses, then 250 mg every six hours for five days.

For children weighing less than 15 kgs, the doses were half of the above. Only patients with bacteriologically confirmed cholera were included in the analysis.

All Cases

	Usual Dose	High Dose
Number of Cases	103	106
Mean intravenous fluid Requirement	244.9 cc/kg Body Weight	246.5 cc/kg Body Weight
Mean Duration of diarrhea	3.34 eight-hour periods	3.14 eight-hour periods

Adults, Pulseless on Admission

	Usual Dose	High Dose
Number of cases	30	26
Mean intravenous fluid requirement	336.9 cc/kg Body Weight	288.3 cc/kg Body Weight
Mean duration of diarrhea	4.53 eight-hour periods	4.42 eight-hour periods

None of the differences are statistically significant.

Epidemiology Section

Immunology Presentations
15th February, 1967

1. Field Testing of the Craig Skin Toxin as an Epidemiological Tool.

2. A study of the Relationship between an Individual's Reaction to Craig's Skin Toxin and his response to Infection with V. Cholera.

3. The Effect of 3 vaccine schedules on the serological response in 6 to 12 month old infants.

4. The effect of dosage size on serological response in adults and children.

5. A model for Vaccine Potency Testing in humans based on the serological response in 6 to 12 month old infants.

6. Serological studies in the American population in Dacca 6 months after their regular booster dose of cholera vaccine.

7. The design and preliminary results of the 1966-67 Vaccine Field Trial.

8. Retrospective analysis of the Relationship of Admission Antibody Titer to the subsequent clinical course of cholera.

9. Retrospective Analysis of the effect of previous cholera vaccination on the clinical course of cholera.

10. Prospective study of the effect of cholera vaccination on the clinical course of cholera.

11. A comparison of 10 daily rectal swabs vs. paired serological specimens in detecting carriers in family contacts of cholera cases.

Table 1

Vibriocidal Antibody Response in 6-12 month old infants receiving vaccine B.

Schedule A - 0.2 cc injected at week 0 and week 30

	Week							
	0*	1	2	3	4	5	30*	31
	OGAWA TITERS							
40,960								2
20,480								1
10,240								1
5120								3
2560								1
1280								2
640								3
320								3
160								1
80		2	1	1				
40		2	3	1	1	1	1	
20		2	3	4	4	3	1	
<20	20	14	13	14	15	17	15	
G.M.T.	0	15	16	14	12	12	11	2078

Table 2

	Week							
	0*	1*	2	3	4	5	30*	31
	INABA TITERS							
40,960								
20,480								1
10,240			1	1				1
5120								1
2560			1		1	1		2
1280			5	3	2			6
640			1	5	2	1		4
320			4	2	4	7		
160		2	4	5	2	6	3	3
80		1	1	2	4	1	2	
40		1	1		2	1	2	
20		5				1	4	
<20	20	10	1	1	1	1	5	
G.M.T.	0	19	383	345	201	178	31	1772

Table 2

Vibriocidal Antibody Response in 6-12 month old infants receiving Vaccine B.

Schedule B - 0.2 cc injected at week 0, week 1, and week 30

	Week							
	* 0	* 1	2	3	4	5	* 30	31
	OGAWA TITERS							
40,960								
20,480								
10,240								1
5120			1	1				
2560								2
1280			2	1	1	2		6
640			3	2	1			5
320		2	2	2	2	3	1	1
160		4	1	4	4	4		
80			3	3	3	4	3	
40		1	6	4	3	3	3	
20		1		1	2	1	3	
<20	20	12	1	1	2	2	6	
G.M.T.	0	29	160	138	86	103	27	1170

Table 1

	Week							
	0*	1	2	3	4	5	30*	31
	INABA TITERS							
40,960								
20,480								
10,240								2
5120								3
2560								4
1280								2
640			1					3
320		1		1				1
160							1	2
80		2	1	1	3	2	1	
40			4	2	2	4	3	
20		3	3	3	2	1		
<20	20	14	11	13	13	12	12	
G.M.T.	0	16	20	17	17	17	17	1564

Table 3

Vibriocidal Antibody Response in 6-12 month old infants receiving Vaccine B.

Schedule C - 0.2 cc injected at week0, week 4, and week 30

	Week							
	* 0	1	2	3	* 4	5	* 30	31
	OGAWA TITERS							
40,960								
20,480								
10,240						1		1
5120						2		3
2560						4	1	3
1280						4		3
640		1	1			6		2
320				1		2		•
160		1	1		1			1
80			1	1			1	
40			1	1	1	1	3	
20			1	2	1		4	
<20	20	17	15	14	14		8	
G.M.T.	0	14	17	15	13	1108	24	1470

Table 3

	Week						
	0*	1	2	3	4*	5	30*	31
	INABA TITERS							
40,960								
20,480								1
10,240						2		
5120						3		3
2560						5	1	4
1280						5		3
640		1				3		2
320		1	1			1		2
160		1	2	1			1	
80					1		1	
40		1	3	4	2	1	6	
20		1		1	4		2	
<20	20	15	14	13	13		6	
G.M.T.	0	19	19	16	15	1631	33	1847

Table 1

Vibriocidal Antibody Response in groups of 6-12 month old infants to 2 injections (0.2 ml/dose at 28 day interval) of different cholera vaccines

		Vaccines													
		O		I		B		J		P		X		L	
Titer	t	Og	In	Og	In	Og	In	Og	In	Og	In	Og	In	Og	In
40,960	12					1	1								
20,480	11					1	2								
10,240	10														
5120	9					1	3								
2560	8			1	3	3	4	3	2	2					
1280	7	1		2	3	4	2	3	4	1	1		2	1	
640	6	1		3	5	2	1	3	3	4	2	3			2
320	5	8	4	4	3	2	2	5	2	4	3	6	4	3	2
160	4	2	5	5	3			2	4	2	6	5	7	2	4
80	3	6	3	4	2	2	1	1	2	4	3	3	4	5	3
40	2		2			1	1			1	3	2	2	4	3
20	1		1					1	1	1	1			1	2
<20	0	1	4							1	1	1	1	2	2
	$\bar{t}$	4.16	2.84	4.84	5.68	6.71	7.53	5.56	5.28	4.50	3.70	4.05	3.90	3.00	3.06
Reciprocal G.M.T.[a]		179	72	286	513	1046	1848	472	388	226	130	166	149	80	83

a - Reciprocal geometric mean titer calculated from the formula = Reciprocal G.M.T. = $10 \times 2^{\bar{t}}$

Table 2

Relative Potency[a] of different cholera vaccines based on Vibriocidal Antibody Response in 6-12 month old infants.

	Vaccines													
	O		I		B		J		P		X		L	
	Og	In	Og	In	Og	In	Og	In	Og	In	Og	In	Og	In
Reference														
O	1.00	1.00	1.6	7.1	5.8	25.7	2.6	5.4	1.3	1.8	0.9	2.1	0.4	1.2
I	0.6	0.1	1.00	1.00	3.7	3.6	1.7	0.8	0.8	0.3	0.6	0.3	0.3	0.2
X	1.1	0.5	1.7	3.4	6.3	12.4	2.8	2.6	1.4	0.9	1.00	1.00	0.5	0.6

[a] Calculated from formula = G.M.T. test vaccine/G.M.T. reference vaccine

Table 3

Reproducibility of test system

Results of testing of Vaccine B in 6-12 month old infants in May, 1966 and November, 1966

Vaccine B

		May, 1966		November, 1966	
Titer	t	Og	In	Og	In
40,960	12			1	1
20,480	11			1	2
10,240	10	1	2		
5120	9	2	3	1	3
2560	8	4	5	3	4
1280	7	4	5	4	2
640	6	6	3	2	1
320	5	2	1	2	2
160	4				
80	3			2	1
40	2	1	1	1	1
20	1				
<20	0				
	$\bar{t}$	6.80	7.35	6.71	7.53
Reciprocal G.M.T.		1114	1631	1046	1848
Relative Potency		1.00	1.00	0.9	1.1

Statistical Analysis

Analysis of Variance Tables for differences between geometric mean titers ($\bar{t}$)

Ogawa Response

Source	d.f.	S.S.	M.S.	F
Between Means	6	148.56	24.76	6.90 $p < .001$
Error	124	444.98	3.59	
Total	130	593.54		

Least Significant Difference (LSD) between means at 95% confidence level (Fisher's Method):

$LSD = t_{.05}(124)\sqrt{2 \times 3.59/h}$ h = observation/mean

$= 1.274$

Vaccines

	B	J	I	P	O	X	L
$\bar{t}$	6.71	5.56	4.84	4.50	4.16	4.05	3.00

Vaccines underlined are significantly different from those not underlined.

Inaba Response

Between Means	6	303.27	50.55	13.70 $p < .001$
Error	124	457.43	3.64	
Total	130	760.70		

Least Significant Difference = 1.294

Vaccines

	B	I	J	X	P	L	O
$\bar{t}$	7.53	5.68	5.28	3.90	3.70	3.06	2.94

Vaccines underlined are significantly different from those not underlined.

Table 1

Distribution of In-patients and Out-patients (Confirmed Non-Cholera) according to their type of vaccine and Month of Diarrhea

Type of Vaccine	Oct. 1966	Nov. 1966	Dec. 1966	Jan. 1967	Total For 4 months	Percentage
OO	15	7	16	14	52	25.87
XO	16	10	5	18	49	24.38
XX	27	18	28	27	100	49.75
Subtotal	58	35	49	59	201	100.00
(-) O O (-) (-) (-)	5	3	4	3	15	
X (-) (-) X	6	4	5	3	18	
Subtotal	11	7	9	6	33	
Unassigned	91	47	26	31	195	
G.T.	160	89	84	96	429	

Table 2

Distribution of In-patients and Out-patinets (Confirmed Cholera) according to their type of vaccine and month of onset

Type of Vaccine	Oct. 1966	Nov. 1966	Dec. 1966	Jan. 1967	Total for 4 months	Effectiveness
OO	1	3	13	11	28	-
XO	-	2	3	7	12	57.2
XX	-	-	3	4	7	87.5
Subtotal	1	5	19	22	47	
(-) O O (-) (-) (-)	1	-	3	6	10	
X (-) (-) X	-	-	1	2	3	
Subtotal	1	-	4	8	13	
Unassigned	1	6	18	13	38	
	3	11	41	43	98	

The Relationship of Admission Antibody Titer to the subsequent Clinical Course of Cholera

It has been shown in Matlab that the cholera case rate is inversely proportional to the mean geometric vibriocidal antibody titer of the population. We have recently shown that the mean admission antibody titer of adults admitted to the Matlab hospital with cholera is significantly lower than the mean titer of adults admitted with non specific gastroenteritis. It is also lower than the antibody titer in the general population as shown by our serologic surveys.

Along this line, a retrospective analysis of 129 Dacca cholera admissions who had not received antibiotic treatment was carried out. An attempt was made to correlate antibody titer on admission with subsequent clinical course. Patients with the onset of symptoms more than 36 hours before admission were not included. The results indicate that although there is a slightly increased intravenous fluid requirement in the patients who have no titer on admission as compared to those who are admitted to the hospital with a measurable titer, there is a wide range in both groups and the difference between them is not significant, either statistically or practically. The difference amounted to only 68 cc of intravenous fluid per kg. of body weight. These results are summarized in Table 1.

Table 1

Clinical Course in Cholera Patients Not Treated with Antibiotics by Admission Antibody Titer, from PSCRL-Dacca Records 1964-65

Admission Vibriocidal Antibody Titer (tubes)	Number of cases	Average IV Fluid Requirement (cc/kg)	Duration of Diarrhea (days)
7 - 8 tubes	3	107 cc/kg	1.3 days
5 - 6 "	9	552 "	3.7 "
4 "	11	582 "	5.27 "
3 "	10	497 "	4.8 "
2 "	16	648 "	4.8 "
1 "	4	632 "	4.5 "
0 " (no titer)	76	618 "	5.1 "
All with titer 1 - 8 tubes	53	550 "	4.5 "

Retrospective Evaluation of the Effect of Previous Cholera Vaccination on the Clinical Course of Cholera

There have been conflicting reports in the literature as to the effect of prior cholera vaccination on the course of cholera in those patients who do get the disease.

We have reviewed the past three seasons' experience in Matlab and compared the clinical course of 27 vaccinated and 80 control patients who have been admitted to the Matlab hospital with cholera. All the patients in both of these groups have been treated with tetracycline and would be expected to have had modified courses. We looked at the percent change in body weight between admission and discharge as an indication of initial dehydration. We looked at the intravenous fluid requirement in cc per kg. during the course of hospitalization as well as the duration of diarrhea as measures of the course of the disease after admission. These factors indicated that the course was milder in the vaccinated population but with wide ranges. The differences are not statistically significant. This is summarized in Table 1.

Table 1

Clinical Course of Cholera in Vaccinated and Control Patients admitted to Matlab PSCRL Hospital 1963-1966 (All patients have been treated with antibiotics)

Vaccine Status	Number of Patients	Average Percent gain in body weight	Average IV Fluid Received (cc/kg)	Average Duration of Diarrhea (day)
Cholera Vaccine	27	6.9%	213 cc/kg	1.3 days
Control Vaccine	80	6.4%	256 cc/kg	1.8 days

Prospective Study of the Effect of Cholera Vaccination on the Clinical Course of Cholera

All patients 15 years of age or less from the Vaccine Trial area admitted to the Matlab hospital were included. Treatment was with intravenous fluid alone. If antibiotics were indicated for a concurrent illness, they were administered and the patient dropped from the study. Daily rectal swabs were examined for V. Cholera. Blood samples for serology were collected daily. The patients were hospitalized until 3 consecutive rectal swabs negative for V. Cholera were obtained.

	Vaccine Group		
	Control (00)	Single Dose (X0)	Double Dose (XX)
Number of Cases	17	8	5
Duration of diarrhea (days)	3.2	4.2	4.0
Duration of Bacteriologic positivity (days)	4.8	5.9	5.2
Total Intravenous Fluid Requirement (cc/kg body weight)	401.3 cc/kg*	549.4 cc/kg	842.2 cc/kg*
Mean Vibriocidal admission antibody titer:			
Ogawa	0.71*	2.13	2.00*
Inaba	2.24	2.00	3.60

* - Statistically Significant Difference ($p = 0.05$)

Family Study 1965-66

Comparison of Culture Results with Paired Serum Specimens in Determining Infection Rates in 79 Families

Table 1

Bacteriological Results and Classification of 522 members of 79 families - by age group

Age	Total Member	Index Case	Total Contacts	Culture Positive: Hospitalized 2° case	Field Diarrhea	Asymptomatic	Total	
				No.	No.	No.	No.	%
<1	24	—	24	7	1	3	11	45.8
1 - 4	89	27	62	7	2	11	20	32.2
5 - 9	114	23	91	9	1	13	23	25.3
10-14	64	7	57	3	2	4	9	15.8
15-29	101	13	88	4	1	2	7	8.0
30 +	130	9	121	1	1	5	7	5.8
Total	522	79	443	31	8	38	77	
		Percent	100	7.0	1.8	8.6	17.4	

Table 2

A Comparison of Serological and Bacteriological results in 108 hospitalized cases (index and secondary), all Bacteriologically positive

Age	Number	Positive Serology*	
		No.	%
<1	5	3	60
1 - 4	34	33	97
5 - 9	32	32	100
10-14	10	10	100
15-29	17	17	100
30 +	10	10	100
Total	108	105	97.2

* - Four-fold or greater rise in vibriocidal titer.

Table 3

A Comparison of Bacteriological and Serological results in 275 family contacts who had 5 or more daily rectal swabs, and paired serum

Age	Number Studied	Culture Positive							Culture Negative		
		Field Diarrhea		Asymptomatic		Total			Total	Serol. Pos.	
		No.	Serol. Pos.	No.	Serol. Pos.	No.	Serol. Pos. No.	%		No.	%
<1	11	1	0	2	1	3	1	33.3	8	1	12.5
1 - 4	43	2	2	8	8	10	10	100	33	4	12.1
5 - 9	61	-	-	10	7	10	7	70.0	51	3	5.9
10-14	38	2	2	4	3	6	5	83.6	32	4	12.5
15-29	48	1	1	3	3	4	4	100	44	1	2.3
30 +	74	1	0	3	1	4	1	25.0	70	2	2.9
Total	275	7	5	30	23	37	28	75.6	238	15	6.4

Table 4

A Comparison of Bacteriological and Serological results in 275 field contacts of cholera cases

	Culture Positive	Culture Negative	Total
Serology Positive	28	15	43
Serology Negative	9	223	232
Total	37	228	275

Number Positive by culture and/or serology = 52

Positive by culture only : 37/52 = 71.2%

Positive by serology only: 43/52 = 82.6%

PAKISTAN-SEATO CHOLERA RESEARCH LABORATORY
Technical Committee Meeting
14-16 February, 1967

SUMMARY OF WARD ACTIVITIES - 1ST JANUARY TO 31ST DECEMBER, 1966

A total of 2728 patients have been admitted to the ward from January to December, 1966 - 1487 Cholera - 10 deaths and 1241 Non-cholera with 19 deaths. See Tables 1 and 2 for breakdown.

Patients seen in the Outpatient Department numbered 985, of which 230 were admitted, 16 deaths were recorded. These were either brought dead or in a gasping condition and could not be rescusistated.

41 patients were transfered from the Ward to other hospitals.

35 patients with acute cholera were pregnant. Three cases were delivered in the ward, living male babies, and no complications. Four still births are recorded.

March, April and May were peak months for noncholera cases and a wide variety of gastroenterological cases were investigated and treated. The majority of infants and children admitted suffering from mixed syndromes of malnutrition and Kwashiorker-like states responded to good nursing care and appropriate diet.

In anticipation of a heavy season in patient load and number of Research projects planned, extra nursing staff were recruitied and the ward verandah extended to accommodate another 20-30 beds.

Severe outbreaks in Narayanganj, a town 16 miles away, and in Keraniganj, a village on the other side of the river from Dacca, made an early start to the 'season' in September. The admission rate steadily increased, 39 admissions in one day being the peak figure, the highest cencus was 147 patients (not including attendents, amounting to 100), I attendant per patient while patient is in acute stage is permitted, and one parent stays with a child patient for the duration of hospitalization. This arrangement relieves the nursing staff to some extent, though increases work for other departments, as each attendent is given three meals per day.

As the patient load far exceeded anticipation, shortage of chemicals for I.V. Fluids, needles, disposable I.V. sets etc.occurred and extra supplies had to be bought from local market and outside Pakistan at considerable cost.

More than 50% of the cholera admissions were below 10 years of age, and approx. 100 children were put on an Antibiotic study, which necessitated a longer stay in hospital. A tent and bamboo hut were erected to accommodate convalescent patients. From November, all patients not selected for 'Study' were given Tetracycline 250 mgs. 6 hourly for 5 days, under 15 Kgs. the dose was 125 mgs., and discharged immediately diarrhoea stopped. They were given Tetracycline to take at home

As the patient load increased, the demands on all staff working in the Laboratory were increased and routine investigations, such as stool, urine and blood examinations, were cut to a minimum.

From 1st January to 1st December, 1966, approx. 14000 litres of I.V. fluid were made in the Pharmacy, the staff working day and night to keep the pipe line open. In November, 3,686 litres were made. It was then decided to relieve the Pharmacy staff of this work and from December all I.V. fluids have been made in the Clinical Laboratory.

Without exception all ward staff have worked hard and with enthusiasim, particularly the nursing staff who responded to the challenge of coping with epidemic cholera and at the same time maintaining a high standard of nursing care, accurate recordings of intake and output, and assisting the Research Physicians, enabling the research work to be carried on.

T A B L E 1

	CHOLERA		NON-CHOLERA	
	Admissions	Death	Admissions	Deaths.
Children 10 yrs. and under.	795	9	472	12
Males over 10 years.	390	1	478	3
Females over 10 years.	302	nil	291	4
	1487	10	1241	19

Total admissions January-December 1966 - 2728
" " 1964-1965 - 1933
" " 1963-1964 - 865
" " 1962-1963 - 488

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T A B L E II

Month	Total Admissions	Cholera				Non-Cholera	
		Total	Inaba	Ogawa	Deaths	Total	Death.
1966.							
January	109	44	44	-	-	65	2
February	104	17	17	-	1	87	4
March	142	7	7	-	-	135	-
April	162	16	13	3	-	146	1
May	131	30	30	-	1	101	1
June	73	-	-	-	-	73	3
July	94	14	13	1	-	80	2
August	70	8	8	-	-	62	2
September	131	62	61	1	1	69	1
October	294	218	213	5	3	76	-
November	608	444	427	17	2	164	2
December	810	627	579	48	2	183	1
TOTAL:	2728	1487	1462	75	10	1241	19

T A B L E III

Total number of X-Ray Examinations 1966.

Total opaque medium (I.V.P., Barium meal, Barium Swallow & Cholecustohram)	=	33
Total others (chest, Abdomen, extremities, etc.)	=	1243
Total	=	1276

ADMINISTRATIVE SECTION REPORT

II. NEW BUILDINGS:

i) New Hospital at Matlab.

Plans for a new hospital to be constructed at Matlab were formulated during the period of the Directorship of Dr. Benenson. Land was acquired for this purpose but due to legal troubles, PSCRL did not take possession. Subsequently the Government of East Pakistan proposed to construct a Rural Health Centre at Matlab and PSCRL submitted plans to add a wing at CRL cost for use as a hospital, which was accepted. The wing cost to the Laboratory was Rs.83,541.00. The wing houses patients and an administrative office. The hospital was formally opened by Colonel S.A. Mallick, Director, Health Services, Government of East Pakistan on 31 July,1966.

ii) Status Report on Additional Wing for the PSCRL (Feb.10,1967)

Space has always been at a premium in this Laboratory, becoming especially acute during the current year with the expansion of ongoing programs and the inauguration of new ones. To illustrate the shortage of space, a large tent and bamboo house were erected and the garage was converted to shelter cholera patients during the current epidemic, because present ward space was originally designed only for 20 beds, Additional space is also required for new research programs and expansion of old ones. Proper storage of supplies and equipment is another acute problem for lack of adequate space.

Negotiations for an additional wing were started last year. The Government of East Pakistan has given its approval to build the additional wing subject to the approval of the Government Architect and Chief Engineer. Approximately one more month is required before final submission of the plans for the new wing is made to USAID, Dacca, for consideration and submission to AID/Washington with a request for funding.

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ADMINISTRATIVE SECTION

III. PREVENTIVE MAINTENANCE REPORT

Providing adequate supportive maintenance to the Laboratory has been a continuing problem. Such factors as inadequate maintenance and staffing, non-availability of spare parts, substantial time delays between requisition and acquisition of spare parts, andinadequate and unstable power supply, etc. have been a constant source of frustration for any attempts for improving maintenance. Due to these difficulties, efforts to establish a "Safety Code" or a "Preventive Maintenance Schedule" have been hampered in the past.

However, as a result of the visit of Mr. Charles Myers, Management Analysis Officer from NIH last summer, the Administrative Section was completely reorganized and included the establishment of a separate Equipment Maintenance Branch in an effort to provide a more systematic supportive maintenance schedule for the Laboratory.

Job descriptions are now available for all Maintenance Branch personnel. The Branch is responsible for the maintenance of all Electronic and electro-mechanical instruments, electrical appliances, compressors, electrifications, plumbing, carpentry and various other jobs. Preventive maintenance work, such as battery checking and charging, greasing and oiling, insulation test, periodical operation test of electro-mechanical, electrical equipments, etc., is also a responsibility of this Branch. Security personnel are regularly instructed on how to face emergencies after working hours. Regular maintenance personnel on "Call Duty" are also available.

The following preventive maintenance schedule work is carried out periodically i.e. Daily, weekly, monthly or as often necessary:

1) Laboratory Instrument:- Checking, meter setting, tuning, battery checking.

2) Generator, Compressors:- Operating checking, oiling and greasing, belt inspection and cut-out checks, etc.

3) Electric Power Panel/Switch Gear, etc:- Insulation test, Safety device operation, over-load switch, cleaning and switch operation.

4) Battery chargers:- Battery charging, cleaning, refitting and charging, short test and greasing, etc.

5) Operation test of thermo-switch, Safety valves of incubators, hot plates, distillation plants, autoclaves, incinerator, hot baths, etc.

(more)

Preventive Maintenance Report

6) Refrigerator, Air Conditioner and Freezer cleaning, greasing, oiling, checking of over-load and thermo controls, etc.

7) Centrifuges:- Operation check, cleaning, greasing and timer check.

8) Plumbing and Sanitation:- Checking of water and drain line, gas line, water connection and reserve tanks etc.

9) Building:- Checking of doors, windows, screens etc. and other alternation or repairing as requested.

10) Jet Guns:- Operational check, autoclaving and oiling etc.

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Electrical power supply report

(1) 500 KW diesel engine generator and accessories	$55,000.00
(2) 2 - 400 ampere transfer switches	6,000.00
(3) Construction of generator building	7,000.00
(4) Associated devices, wiring, etc.	7,000.00
Estimated Total	$75,000.00

2. Purchase equipment and construct a new substation, together with the installation of all wiring to the various sections of I.P.H. and CRL, as outlined on Drawings No. 4 and No. 5. This scheme would provide 2 - 1,000 KVA transformers for serving all of the main clinical center, including CRL. Under normal conditions, one transformer would probably serve the entire load; however, should it be desired, the load could be split between the two transformers. Interlock features would prevent paralleling the two transformers. Two 11,000 volt primary feeders would serve the substation with the possibility of selecting only one of the feeders at any time for carrying the load. New secondary feeders would serve the various wings of the clinical center and CRL. The estimated cost for accomplishing the above installation is as follows:

(1) 2 - 1,000 KVA transformers	$24,000.00
(2) Secondary switchboard	15,000.00
(3) High voltage switchgear	14,000.00
(4) Construction of substation building	12,000.00
(5) Secondary feeders	15,000.00
(6) Miscellaneous work (10%)	8,000.00
Estimated Total	$88,000.00

3. Purchase and install a package engine-driven 30 KW generator for standby service for the smallpox vaccine manufacturing - $ 5,000.00

4. Purchase and provide to CRL for replacement of the existing fuse panelboards four new panelboards complete with Code gauge steel cabinet, trim and hinged door with snap catch lock and two keys, all finished in gray enamel. The panelboard shall have 100 ampere main lugs only at the top and an insulated neutral bus at the bottom. The panelboard shall be constructed for 3 phase, 4 wire, 277Y/480 volts. The panelboards shall be equipped with bolted-on circuit breakers having A.I.C. of 10,000 and with the number of poles and trips as follows:

10 - 1P - 20A
3 - 1p - 30A
1 - 1p - 40A
2 - 2p - 20A
2 - 2p - 30A
2 - 1p - 15A

Estimated cost $ 1,200.00

5. Purchase and furnish as soon as possible to CRL a two-rate battery charger for maintaining the battery starting system for the existing 50 KVA engine-generator. The charger shall meet the following specifications:

   (1) 220 volt, 50 cycle input, 24 volt D.C. output
   (2) 6 ampere boost with mechanical equalizer timer
   (3) Trickle charge adjustable of either output for 2.17 volts per cell or for equalizer 2.33 volts per cell
   (4) Automatic line compensation
   (5) Automatic overload protection
   (6) Automatic D.C. voltage regulation
   (7) Silicon diode full wave rectifier
   (8) Automatic surge suppressors
   (9) Power failure relay with remote indicator connecting terminals
   (10) D.C. ammeter and voltmeter with 50 millivolt movement
   (11) Fused A.C. input and D.C. output

   The manufacture of such a charger is LaMarche Manufacturing Co., Model A-11. The distributor of this product is Air Products & Chemicals, Inc., 2900 - 52nd Avenue, Hyattsville, Maryland. The estimated cost is $265.00.

6. Install a continuous grounding conductor to all distribution equipment, including metal receways and armor of feeder cables in CRL. The size of grounding conductor should not be less than No. 4 AWG composed of 98 percent hard drawn copper. The grounding conductor should originate from a positive ground counterpoise. The incoming water main may be acceptable if adequate grounding result is available. The estimated cost is $400.00.

The complete report of Mr. Billie Myers was submitted to USAID, Dacca in January, 1967 for consideration and submission to AID/Washington along with USAID's final recommendations for the equipment necessary to provide CRL with a dependable source of regular and emergency electrical power for the present building and new wing as well as for the steam generator presently being installed, and the animal house.

ADMINISTRATIVE SECTION

V. STATUS REPORT ON STEAM GENERATOR.

The need for a steam generating plant as an alternative source of power to provide a dependable source of energy to operate the various equipment in the CRL has been a constant problem since the inception of the Laboratory. This problem has become increasingly acute with the expansion of ongoing research programs and the inauguration of new ones, with the consequential increased used of stills and autoclaves dependent solely on electrical power.

However, during the course of the past few months several developments occurred which hopefully will bring the CRL very close to the time when a steam generating plant will become fully operational and will be able to provide the PSCRL as well as the Institute of Public Health with a dependable source of demineralized water and steam for autoclaves, stills, and a hot water supply.

The CRL was fortunate enough to locate a brand-new Clayton Steam generator in the Institute of Public Health. This plant had been in storage for several years, but had never been used because it lacked a feed water system. The necessary accessories were ordered from Clayton by the CRL and have been received. A contract was negotiated in January,1967 with Chemical and Engineering Constructions, an engineering firm located in Dacca, to provide certain auxiliary components and to install the whole system. Therfore, in the near future, the CRL will hopefully be able to enjoy an adequate alternative source of power.

—

Kendrick Hare
Deputy Director.

The Laboratory, the wards and supporting personnel are functioning smoothly and efficiently when considered against the background of their environment.

The professional staff is now adequate in numbers; however it should be pointed out that the professional staff has doubled in the last few months and the facilities have not been adequate to match the administrative problems posed by such an increase in size.

Fortunately the laboratory was lucky in having such an expansion of trained staff because this cholera season is a "big" one. The attack rate in September was 2.7 times that in previous Septembers, and this attack rate has increased slightly over previous years in October and November. The ward capacity originally planned was for 20 beds. The admission rate in the last few days has been averaging 35 admissions per day. This past summer a large extension of the ward facilities was built on either side of the ward entrance. While this has nearly doubled the bed capacity it is still woefully inadequate under the present circumstances; therefore an army mess tent and two fifty-bed bamboo/jute shelters have been erected for convalescent patients. Should the epidemic reach its peak in January which is the usual peaking month, it is estimated that the admission rate will be about 70 or more patients per day.

Despite the above comments, the training fellows have been able to carry out their research work almost on schedule.

VACCINE EVALUATION STUDY

The number one mission of the laboratory, vaccine evaluation in the Matlab Bazar area, has progressed most efficiently under the direction of Dr. Mosely and his fellow workers from CDC. Dr. Mosley's study group which is under daily house surveillance has increased from 42,000 last year to 120,000 this year. The cholera season in Matlab Bazar lags behind that in Dacca and is only now commencing.

This year the vaccine being evaluated is a "standard" vaccine of specifications similar to that which is used throughout the world today in immunization. Whereas in the past the only area studied in the vaccine trials was attack rate since

all patients were given tetracycline on admission, this year the severity of the disease is also being studied and no tetracycline or other antibiotics are being given in the Matlab trial. Information will thus be available to determine if the standard vaccine produces any amelioration of the disease. Four randomized schedules are under study: the control is a tetanus diphtheria vaccine and the standard cholera vaccine is being given in one group without booster and in a second group with a booster a week after the vaccination and in a third group the booster is given one month after vaccination since the latter schedule theoretically should be more effective than a booster given after one week.

Despite the efficiency of the Matlab staff of some 270 persons, since the study group is three times the size of last year's and if the attack rate is three times last year's, personnel at the professional level will not be able to cope with the problem. In consequence it may be necessary to request additional temporary staffing from CDC or other Stateside facilities.

CLINICAL STUDIES IN DACCA

This year a major effort has been directed at bringing the chemical laboratories supporting the clinical investigation studies to the same degree of excellence which is characterized by the bacteriology and immunology laboratories developed under Dr. Benenson. Mrs.(Dr.) Hare, the wife of the Deputy Director, has had years of experience in developing quality control in biochemical laboratories. She has found, not to her surprise, that the technicians in the laboratory are as good as technicians she has had in stateside laboratories she has developed. Dr. Hare has improved methodology, instituted checks by recovery and provided the laboratory with meaningful results. Naturally the American fellows have responded appropriately and have brought a new zest and enthusiasm into their programs. When equipment and supplies that are on order finally arrive the laboratories will have an adequate excellence to conduct all the planned studies. At present this is not true.

The main emphasis in clinical investigation, utilizing the Dacca ward material, has been directed this year to elucidating the relationship between vibrio population in the intestinal tract and diarrhea.

Cholera subjects with a stool output of better than 400 ml an hour for any 8 hour period in the first 24 hours after admission are the study group. Patients

are selected for study on a randomized selection method. The basic project consists of lavaging the GI tract with an electrolyte solution having the composition of cholera stool, at a rate of 1 - 2 liters per hour. These studies have shown that one can wash out vibrios, decreasing the concentration by 1 - 3 logs over the 8 hour lavage period; but there is no decrease in stool output. When tetracycline is added to the lavage solution, which in this study is administered through a gastric tube at a constant rate infusion pump, a quite different pattern evolves. In the studies conducted so far, during the first hour the patient is given 1,000 mg. of tetracycline in the one liter lavage solution, and in the subsequent 7 hours of lavage the infusion solution has 250 mg per liter per hour. By the end of the second hour of lavage the stool has no detactable vibrio and this condition is maintained subsequently over the remaining 6 hours of the lavage period. There is no detactable change in the rate of stool output and the duration of stool output averages 30 hours from the start of lavage. However, there is usually evidence of vibrio by, colony count, which recurs 12-15 hours after cessation of lavage with tetracycline and continues until the diarrhea ceases, but the concentration of organisms never gets above 3 logs. It is proposed in future tetracycline studies to maintain the lavage until diarrhea ceases.

In other studies convalescent sera will be added to the lavage solution. Similar studies are planned with a buffered solution having a pH of about 4, the hydrogen ion concentration at which the vibrio does not proliferate.

It is also planned to add glucose to the lavage solution three hours after the onset of the tetracycline addition. Likewise urea as well as aspirin will be added since it is known that these substances, like glucose, enhance sodium transport in the kidney. Chelating agents will also be investigated after the gut is sterile; such agents were found to be ineffective when lavaging cholera patients who had not received antibiotics in the Saigon study.

In one study glucose has been added to the lavage solution. In this particular study the intestinal tract was lavaged at the rate of a liter an hour for two 8 hour periods, following which 55 mmoles of glucose was added for two 8 hour period, a 16 hour control period for a total of 80 hours. As has been found by NAMRU 2, addition of the glucose decreases stool output very appreciably, the

net rates dropping from 400 ml an hour to 200 ml an hour. Perhaps the most significant thing demonstrated by this study is that one can lavage the entire gastrointestinal tract for 80 hours with 80 liters of solution with no apparent untoward effects on the patient.

A considerable effort is being devoted to serological studies begun by Dr. Benenson and his staff; fluorescein antibody techniques are being developed and the monkey model of Dr. Benenson is being investigated again.

CONSULTANTS

Instead of bringing consultants to Dacca only for a three or four day visit at the time of the Technical Committee meeting, it is proposed to stagger these visits throughout the year and have visits to a week or longer. Individuals who have indicated their willingness to participate in this program this fiscal year are:-
Drs. Carl Moore, Paul Crabbe, Kenneth Goodner, A. Baird Hastings, Derrick Rowley, Andrew Abraham, Gustave Dammin, Alex Langmuir, Mrs. Cecilia Gomes, Randolph Eichner, Jack Craig, John Feeley and Bucky Greenough.

In addition, the laboratory has already had a large number of visitors for two to three days; Drs. Dorland Davis, James Mason, Colin MacLeod's Committee, George Whalen, Jim Fresh, Elvin Kabat and Howard Goodman.

CDC has also provided us with DRS. Curlin and Karchmer, EIS officers, and Mr. Wallace DeWitt, a bacteriologist. These will all be in residence for six to eight weeks.

MEMORANDUM

29 November 1966

To : Dr. John R. Seal
Chairman, Cholera Advisory Committee

From : R. A. Phillips, M.D.
Director

Subject : CLINICAL INVESTIGATION COMMITTEE; meeting of;
Pakistan-SEATO Cholera Research Laboratory; 26 November 1966.

Enclosures:-

1) Agenda

2) List of Committee Members

3) Recommendations of the Committee members in attendance.

This was the first meeting of the Committee to review the Laboratory's research proposals from the standpoint of the ethics and morals of human experimentation. Enclosure 1, 2 and 3 are forwarded for your information. The research proposals and the recommendations of the Committee members in attendance will be circulated to all members of the Committee and the results of this mail vote will be forwarded to you for appropriate action.

MEETING OF PSCRL CLINICAL INVESTIGATION COMMITTEE

Enclosure 2. Members of the Committee

* Dr. D. A. K. Black
Chairman of the Department of Medicine
University of Manchester
England

* Dr. Carl V. Moore
Chairman of the Department of Medicine
Washington University
St. Louis

Dr. Stanley Bradley
Chairman of the Department of Medicine
Columbia University
New York

Dr. Graham Bull
Director of Clinical Research Center
Medical Research Council
London

Dr. Nazir Ahmed
Dean
Institute of Hygiene and
Public Health
Lahore

* Lt. Col. S. M. H. Bokhari
Project Director
National Health Laboratories
Chaklala
West Pakistan

* Dr. A. K. Shamsuddin Ahmed
Professor of Medicine
Dacca University
Dacca

* - In attendance.

MEETING OF PSCRL CLINICAL INVESTIGATION COMMITTEE

... Enclosure 3.

* ITEM 1

(* numbering identical to that on enclosure 1 - 1 Agenda)

It was the feeling of the Committee in attendance that human experimentation in diseases such as cholera where the disease processes are still not completely known and in which a proven animal model is not available justified appropriate experiments delineated to discern the disease mechanisms to improve therapy and to enhance knowledge of means of preventing the disease.

It was pointed out to the Committee that an overwhelming majority of the patients were illiterate and if they were below the age of consent their parents were illiterate. This made it impossible to obtain informed consent for proposed studies in well over 99% of the patients. The Pakistani members of the Committee agreed that all patients or parents of patients would readily put a mark on a piece of paper acquiescing to experiments but they would have no appreciation of what might or might not be done. In consequence the Committee went on record to the effect that responsibility for experiments rested directly on the Director of the laboratory and his Deputy Director. The committee further went on record that the establishment of such a Committee who would be consulted from time to time on changes in experimental design would probably an acceptable legal device but it did not absolve the Directorship of the Laboratory from its responsibilities.

ITEM 2:-

The research protocols listed in this item on the agenda were reviewed by the Committee from the standpoint of "human experimentation" in its broadest aspects. No attention was paid in the formal Committee meeting to laboratory procedures.

The Director pointed out that it was the general policy of the laboratory to use only procedures which had been well documented by use in University hospitals throughout the world. The Director further informed the Committee that in instances in which a procedure whose safety had not been documented the Committee would be circularized with the proposal and a mail vote taken.

The Committee approved the procedures in experimental design in so far as the techniques involving human subjects, with the following observations:-

(Cont'd ... Enclo-3)

Protocol Number 22

"Demonstration of abnormal permeability in human cholera"

(Dr. Geral Keusch).

Because of the incidence of anaphylactic reactions in the more common instances of hypertensive reactions following the administration of iron dextran intravenously, the committee felt it would prefer not to give approval to a project in which iron dextran was to be given intravenously. It is recommended rather than a safer molecule be found which could be identified in the tissues, than iron dextran itself.

Protocol Number 15

"The effect of hyperimmune plasma on the clinical course of acute cholera".

(David B. Sachar, and Zahidul Hoque).

It was felt by the Committee that the passing of perimmune convalescent plasma from individual patients was a safe procedure since patients are to be given gamma globulin one week and one month following administration of the hyperimmune plasma.

The Director of the laboratory told the Committee that visitors to the laboratory the previous week, Dr. Elvin Kabat and Dr. Howard Goodman, W H O consultants in immunology, had suggested that an alternative procedure would be to use pooled convalescent sera which had been processed to remove the gamma globulin fraction. During the process any hepatitis virus would be destroyed. The Committee agreed that this would be a safe procedure but the Committee went on record as believing that the use of the whole convalescent sera should be studied and results compared with the specific administration of gamma globulin from convalescent sera.

PAKISTAN-SEATO CHOLERA RESEARCH LABORATORY

TECHNICAL COMMITTEE MEETING
14 - 16 February 1967.

Research Protocols submitted to Clinical Investigation Committee at its meeting on 26 November, 1966.

EPIDEMIOLOGY STUDIES:

1. Cholera Vaccine Field Trials. (W. H. Mosley, M.D.).
2. The Effect of Cholera Vaccination on the Course of Clinical Cholera. (W.M. McCormack, M.D., W.H. Mosley, M.D. & Dr.M.Rahman).
3. Serologic Survey for cholera antibody in an American population living abroad. (W.M. McCormack, M.D.).
4. A Comparison of the Serological Response of Cholera Vaccine in man with the Potency determined by the Mouse Protection Assay. (W.H. Mosley, M.D., in collaboration with Dr.J. Feeley & Dr. M. Pittman).
5. Serologic Studies with Cholera Vaccine. (Drs. W.M.McCormack, Ranjit K. Barui, Ansaruddin Ahmed & W.H. Mosley).
6. Immunochemical Studies of Antibodies in Cholera. (W.H. Mosley,MD Dr. Ansaruddin Ahmed, Mr. Samin Ahmed & Dr. Paul Crabbé).
7. Rayer Bazar Community Study. (W.H. Mosley, M.D.).
8. Rayer Bazar Infant Study. (W.H. Mosley, M.D.).

CLINICAL STUDIES:

9. Protocol for oral glucose and Electrolyte Therapy in Cholera. (Drs. N. Hirschhorn, Taylor, Sachar).
10. Intestinal Electrolyte and Water Fluxes and Transmucosal Potentials in acute cholera. (Dr. J.O. Taylor & Dr. D. Sachar).
11. A Technique for the Measurement of Transmural Electric Potential in the Intact human Intestine. (David B. Sachar,M.D. & J.R.Saha).
12. Tetracycline Lavage Study. (R. Northrup, M.D.).
13. Antibiotic Study. (Dr. A.W. Karchmer & Dr. George Curlin).
14. Cathartic Purgation of Convalescent Cholera Patients (Dr. N. Hirschhorn, Dr. Mojid Mollah & CRL Ward Staff).
15. The Effect of Hyperimmune Plasma on the Clinical Course of Acute Cholera (David B. Sachar,M.D. & Z. Hoque, M.B.B.S.).
16. Therapy of Acute Cholera with Bacteriophage. (Dr. K.A. Monsur & N. Hirschhorn,M.D.).
17. Bile Salts and Bile Pigments in Rice Water Stool. (Drs. Irvin H. Rosenberg & Norbert Hirschhorn.)

(more)

CLINICAL STUDIES: (Cont'd).

18. A Study of Intracellular and Extracellular Composition Changes During the Initial Therapy of Acute Cholera. (Drs. Northrup, J. Kinzie & M. Rahman).

19. Experimental Cholera in Monkeys. (Mr. Iqbalul Hasan).

20. Autoradiographical Study of Intestinal Permeability in Acute and Convalescent Cholera Patients. (Drs. J. Kinzie, R. Northrup & A. Abraham).

21. Suggested Protocol for the study of enteric biopsies from cholera patients with the electron microscope. (Dr.Andrew Abraham)

22. Demonstration of Abnormal Permeability in Human Cholera. (Dr. Gerald Keusch).

23. Report on Clinical Laboratory Section. (Mr. S. Z. Ahmad).

BACTERIOLOGY STUDIES:

24. Tetracycline action against V. cholerae in vitro. (R. Northrup, Mr. M. I. Huq & R. Rahman).

25. Relationship of Growth cycle of Vibrio Cholerae to production of " Skin toxin". (Mr. S.I. Hasan & M.I. Huq).

26. A Study of Resistance of V. Cholerae to Various Antimicrobial agents. (R. Rahman, P.K. B. Neogy, & M.I. Huq).

27. Comparison of the Division time of non Agglutinable vibrios with aeromonas, pseudomonas and commomonas and also with a standard vibrio culture. (R. Rahman & M.I. Huq).

28. Study on Cholera Phages. Phage typing of all the V. Cholerae isolated in the Lab. by using Routine Test Dilution method of Typing. (Mrs. A. Alam, Mr. M.I. Huq).

29. Study on Cholera Phages. Routine Survey for the detection of Cholera Phage from Patients with Vibrio and also non Vibrio diarrhoea. (Mrs. A. Alam & Mr. M.I. Huq).

30. Sensitivity patterns of the pathogenic members of the entero-bacteri acae group with special reference to salmonella, shigella and path. E. coli isolated. (P.K.B.Neogy & M.I.Huq).

31. Study of the Pathogenic Escherichta coli in children suffering from gastroenteritis in the study area Dacca City and Suburbs. (G. Kibriya, W.U. Ahmed & M.I. Huq).

32. Effectiveness of Enteropathogenic E. Coli in causing diarrhoea: Gut inflamation by loof technique in sucking rabits. (R. Rahman & M.I. Huq).

(more)

BACTERIOLOGY STUDIES: (Cont'd)

33. Staphylococcus enteritis: A survey of Staphylococcus in the stools of diarrhoea patients and normal persons. (G. Kibriya, W.U. Ahmed & M.I. Huq).

34. Effectiveness of using higher incubation temparature for selenite broth for the isolation of salmonella. (G. Kibria, W.U. Ahmed & M. I. Huq).

35. To compare the effectiveness of Japanese manufactured TCBS Media with TTGA (Monsurs) & GA (Goodner) Media used for routine isolation in CRL. (G. Kibryia, W.U. Ahmed & M.I. Huq).

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PROTOCOL

EPIDEMIOLOGY STUDIES.

P R O T O C O L

CHOLERA VACCINE FIELD TRIALS

W. M. Mosley, M.D.

1. RESEARCH PLAN

A. Introduction & Sepcific Aims

The CRL has conducted two cholera vaccine field trials in rural East Pakistan since 1963. These trials have utilized a commercially prepared phenolized cholera vaccine which had a very high potency based on mouse protection tests and a purified Ogawa antigen prepared by Drs. Watanabe & Verwey. These trials established the effectiveness of a single injection of the cholera vaccine in reducing the cholera case rate by 72-75% as compared to the controls. This effectiveness was maintained for over one year in the adult population, however, there was a considerable drop in the vaccine effectiveness in the children. The Ogawa purified antigen was virtually ineffective in children but produced some protection in adults. One observation in the trial was the fact that the cholera case rate in children was ten times higher than in adults and further that in the immunized children the cholera case rate remained much higher than in the unimmunized adults.

In September 1965 a serological survey was conducted in the vaccine trial population. This revealed that in the endemic area there was an increase in antibodies in the population with increasing age, and further, that the antibody titres in the population receiving cholera vaccine was significantly higher than in their corresponding controls. This was true even of the population that had received the cholera vaccine two years prior to the survey, however, in the population that received the purified Ogawa antigen the titres were not significantly higher than in the controls except in the oldest age group. There was a remarkable correlation between the cholera case rate in the population and the antibody titre as determined in the serological survey. Thus in the children with the highest case rate there was the lowest antibody titre. Likewise in the vaccinated children who had a higher case rate than the unvaccinated adults, the antibody titre was lower than in the adults.

The cholera vaccine trial, therefore, demonstrated the effectiveness of a parenteral cholera vaccine but at the same time has indicated that the protection provided is inadequate particularly for children who have the highest cholera case rate. The serological survey suggested that more intensive immunization of the children either by multiple injections or larger doses could raise the antibody level of the children to that of the adults, and presumably correspondingly provide them with a much greater degree of protection.

In this study a standard commercial cholera vaccine will be given to approximately 40,000 children between the age of 0-14 years in a double blind controlled trial in the Matlab Vaccine Field Trial Area. Effectiveness will be measured both by cholera case count and by serological survey. The specific aims will be:

1. To determine the level and duration of protection of a single dose vs. two doses of the cholera vaccine as compared to the control vaccine.

2. To determine if there is a correlation between the serological response induced by the vaccine and the level of protection provide .

At the end of the year a booster dose will be given to one-half of the group that received two injections of cholera vaccine the preceding year. During the following cholera season a further serological survey will be made. The specific aim of this procedure will be:

3. To measure any fall in protection that occurs in the groups that received one or two doses of cholera vaccine and determine if it correlates with a fall in the serological response.

4. To determine if a booster dose increases the level of protection as well as the serological response as compared to the population group that had received either a single injection or two injections of cholera vaccine one year previously.

The long term goals of the vaccine field trials are to provide a sound immunological foundation for the development of a good cholera vaccine. At present there is not a satisfactory laboratory model for preliminary evaluation of potential cholera vaccines. This study will provide data as to whether or not the serological response to the vaccine induced in man may be a good measure of the potential effectiveness of the vaccine in protecting against cholera.

B. Methods of Procedure

The cholera vaccine field trial will be a double blind controlled study conducted in the Matlab Bazar Vaccine Field Trial area and the design will be essentially similar to that of the two previous field trials.

The cholera vaccine will be a product prepared by a commerical U.S. manufacturer and will be potency tested and safety tested by the Division of Biological Standards of the National Institutes of Health. A dosage of 0.5 ml. will be given to all participants in the trial. The control vaccine will be either a Tetanus Toxoid or a Tetanus-Diphtheria Toxoid. The vaccine will be designated with a code letter.

The will be four vaccine groups in the trial; each group is expected to contain about 10,000 persons. The groups are:

Group One; two injections of the control vaccine,

Group Two; one injection of the cholera vaccine followed by one injection of the control vaccine,

Group Three; two injections of cholera vaccine,

Group Four; two injections of cholera vaccine and after one year a third injection of cholera vaccine.

The interval between the first two injections in each of the above groups will be approximately one month.

Assignment of the Vaccine: In order to include approximately 10,000 children in each vaccine group, or a total of 40,000 children the total population to be covered will be about 110,000 in approximately 100 villages. Prior to assignment of the vaccine the entire area to be included in the trial will be censused. Census books will be made up in triplicate for each village and each village will be mapped. Each individual will be assigned an individual census number and the entire census will be put on ICT punch cards for machine processing. Each family will be issued a census card indicating the census numbers of the family members.

The population to receive the vaccine will be stratified into the age groups: 0-4, 5-9, 10-14. The four vaccine groups listed above will be assigned within each of these age groups in strict alternation, and the assignments will be recorded in the village census book.

The vaccine will be administered by four teams with jet injectors. The teams will go village to village, locate the children and administer the designated vaccine and record the data in the census book. Vaccination of the entire area by these four teams will take approximately one month. After completion of the first round of vaccination the teams will then begin again and administer the second assigned dose.

Surveillance for cases: Surveillance for cholera cases will be maintained both by the Field Hospital in Matlab Bazar as well as by house to house surveillance by field assistants. The field hospital has been operated by the CRL for two and half years and has established an excellent reputation as a cholera treatment center (cholera mortality rate 0.5%). Over 90% of the severe cholera cases are expected to spontaneously seek treatment at this hospital. Because of the wide area covered by the vaccine field

trial with the most distant village being approximately 20 miles from the hospital, five speed boat ambulances will be stationed at various points along the major river and canals to provide rapid transportation to the hospital.

Surveillance in the field will be conducted by a daily house to house check for diarrheal disease by a lady field assistant who will usually be a resident of the village under observation. These lady field assistants will be supervised by field assistants who visit every village at least twice a week and will record all diarrheal illness on standard froms and take cultures from severe cases of diarrhea and see to it that all cases get proper treatment. The field Assistants will be supervised by sanitary inspectors who will provide weekly checks of all areas to insure that work is being properly performed.

Bacteriological Methods: A case of diarrhea will be classified cholera only if there is a positive bacteriological culture for V. cholera. All hospitalized cases will have rectal swab cultures which will be plated directly on Monsur's media and gelatin agar and also placed in bile peptone transport media and examined for cholera vibrios by the usual techniques established in this laboratory. The field assistants will obtain rectal swab cultures from all severe diarrheas which will be placed in bile peptone transport media for shipment to the laboratory where they will be examined for cholera vibrios.

Serological Studies: Prior to the administration of the first injection a random sample serological survey will be conducted on the vaccine trial population. 100 samples will be collected from each of the age groups 0-4, 5-9, and 10-14 in each of the four vaccine groups; in addition 200 samples will be collected from the adult population in the vaccine field trial area. During the peak of the cholera season, approximately three months after the second cholera vaccineinjection, a second serological survey will be conducted following the same design stated above. Approximately twelve months later, three months after the third cholera injection and again during the cholera season a third serological survey will be conducted. In addition to the serological surveys in the field, paired sera specimens will be collected from all hospitalized cases from the vaccine trial area.

The serological specimens from the field will be collected from finger prick blood into 50 lambda capillary pipets and diluted 1:10 in 0.45 ml. of saline, stored in a portable field icebox and shipped to the laboratory. Titres for agglutinating and vibriocidal antibodies will be determined by the microtitre techniques which have been developed at the CRL. A detailed description of the serological techniques will be in a separate protocol.

Analysis of the Data: All data obtained from the vaccine field trial will be put on ICT punch cards for processing by machine. The cholera case rates in each of the vaccine groups as compared to the

controls will indicate the efficacy of the various vaccine schedules outlines above. To determine the comparability of the vaccine groups the non-cholera diarrhea case rate for the various vaccine groups will also be analysed. In addition, the serological survey conducted prior to administration of vaccine will indicate the immunological comparability of the various vaccine groups.

The serological survey conducted three months after the second cholera injection will be analysed to determine the relative serological response elicited by the various vaccine schedules. In addition the cholera case rate will be correlated with the antibody titres in the population groups. In the second year, the serological survey three months after group four has received a booster injection will provide information on the persistence of antibody in the two groups which had received either one or two cholera injections one year prior to the survey and in addition would provide information on whether a booster dose provides a boost in the serological response. Further at this time the cholera case rate for the second year can be correlated with the antibody titres in the population.

The serological studies of the hospitalized cases will be analysed to determine whether or not the so-called vaccine failures, i.e., persons who received cholera vaccine but subsequently went on to develop cholera represent persons who serologically either had a very poor response to vaccine or whether there is no apparent relationship between antibody titre and risk of developing the disease.

C - Significance of this Reserach:

Both cholera vaccine field trials carried out by the CRL as well as the Philippine vaccine field trial conducted in 1964 have utilized only a single injection of cholera vaccine. In the Philippine trial there was a rapid and complete falloff in protection within the first 3-6 months after injection. Likewise in the Matlab trials there was a loss in protection in children with the second cholera epidemic approximately one year after injection though protection was sustained in adults. One explanation for this fall in protection is that the Philippine vaccine trial population as well as the children in the Matlab trial were receiving their first injection and therefore, had only a primary response whereas the adults in Matlab were receiving a booster and thus had a more sustained response. This hypothesis is supported by the serological survey conducted in the in the Matlab population in 1965.

The present protocol will analyze this question further by determining if a booster effect is obtained with two injections as compared to only a single injection and more importantly whether protection is more sustained with two injections are given initially as compared to a single injection. In addition it will also determine whether additional booster response is elicited by a third injection at the end of one year.

The cholera vaccine used in the two previous trials in Matlab was of unusually high potency and also had a higher toxicity than is presently acceptable. The present protocol will test a vaccine that is acceptable according to present U.S. standards. In the two trials already carried out using the potent cholera vaccine there was an excellent duplication of results with the potency of a single injection being estimated at 72 & 75% effectiveness as compared to a control. Thus, with this level of consistency being obtained in the field trials, it will be possible to indirectly compare protective effectiveness of the cholera vaccine to be used in this trial with the vaccine used in the previous field trials.

The most significant aspect of this study will be the comparison of the serological response induced by the vaccine with the level of protection. The 1965 serological survey has indicated an excellent correlation of the level of protection of the population with the level of antibody produced by the vaccine. This protocol will extend these observations by providing a one injection and a two injection group as compared to the control the first year and the same two groups plus a third group which has received an additional booster as compared to the control in the second year. If this study provides additional evidence indicating that antibody titre is a measure of protection then the serological response in man can be used both as a lab test in preliminary evaluation of cholera vaccines as well as in studying populations to determine their level of immunity to choler infection. This last observation would be exceedingly valuable in the control of epidemic cholera particularly in determining the susceptibility of a population and in evaluating the effectiveness of vaccine campaigns.

D- Facilities Available:

This trial will be conducted in the Matlab Vaccine Field Trial Area in Matlab Bazar Thana in Comilla District. This is the same area in which the two previous field trials have been conducted. In the past year the CRL has constructed a two-story brick hospital in Matlab Bazar for use as the treatment center as well as office space for the epidemiological field staff. Quarters for the physician have been obtained from the Govt. of East Pakistan. Nine outboard motorboats will be available to provide ambulance service from the more remote areas of the field trial as well as for support for the field surveillance activities. Communications between Dacca and field trial area will be facilitated by two jet boats as well as the launch "The Joseph E.Smadel". Supporting the field work will be the bacteriology labs, the serology labs and the statistics section of the CRL in Dacca.

SUPPORTING DATA

A- Previous Work done in this or Related Fields:

The CRL has already conducted two vaccine field trials in the Matlab Bazar area. The details of these two trials have been described in the

1964 and 65 CRL Technical Committee Reports. In the first trial 14,000 persons received either a cholera vaccine or a typhoid vaccine control. In this study the efficacy of cholera vaccine in protecting against cholera was demonstrated for the first time in a double blind control study. The following year the same cholera vaccine, a purified Ogawa antigen and a Tetanus Toxoid control were administered to additional 25,000 persons. This study confirmed the findings of the previous year's trial as regards the cholera vaccine and at the same time demonstrated that the purified Ogawa antigen had only a minimal protective effect.

During the past two and half years that the field trials have been in operation, there have been only three cholera deaths among the 39,000 persons in the trial giving a case fatality rate of less than 1%. Two of the deaths had received cholera vaccine. All three refused treatment.

In september 1965 a serological survey was conducted in these vaccinated populations. A random sample of 2,000 persons was selected for the survey. 1,575 persons were located and blood specimens were obtained from 1,572. This survey revealed first that in this endemic area there was an increase in antibody with increasing age. In addition the survey revealed that the population receiving the cholera vaccine had significantly higher titres than the population receiving the control vaccine and further, one of greatest interests there appeared to be a direct correlation between the level of antibody produced by the vaccine and the decrease in the cholera case rate. Of particular interest was the fact that the vaccinated children had a much lower antibody level than the unvaccinated adults and correspondingly their cholera case rate was ten times higher than unvaccinated adults. It was this observation that led to the design of the present protocol for determining whether multiple injections and a booster can raise the antibody titre of children to that of adults and correspondingly produce a marked reduction in the cholera case rate.

A pilot study for the present protocol was held in Ludua Village in Matlab Bazar Thana where the population was to receive a two injection schedule of cholera vaccine and a control vaccine one month apart and have a serological specimen taken prior to the first injection and six mohths after the second injection. This study was conducted in October 1965. Among the 1,193 persons available for this study 777 or 65% completed the schedule of both injections and the serological specimen. More details of this study are in the 1965 Technical Committee Report.

B- Personal Publications:

A present no publications are in the general medical literature in the areas discussed above. Preliminary papers are in the 1965 CRL Technical Committee Report with the following titles:

1. Vaccine Field Trial

2. Preliminary Analysis of the Serological Surveyfor Cholera Antibodies in the Matlab Vaccine Field Trial Area

3. Preliminary Report of the Effective Dosage & Immunization Schedules on the Antibody Response to a Proven Potent Cholera Vaccine; Ludua Vaccine Trial

3. BUDGET

The budgetary items include the following:

1. Personnel Services: this includes the salaries of the physician, two nurses, two aide-nurses, the ward boy, darwans and peon associated with the operation of the field hospital. For field surveillance; this includes the salaries of the two sociologists who act as field surveillance supervisor and deputy field surveillance supervisor, 9 sanitary inspectors, 45 field assistants and 72 lady home visitor. In addition this includes the salaries of 5 speed boats drivers, the barge care-taker, the four employees operating the launch, and for six months out of the year approximately 75-90 country boatmen.

2. Travelling and transportation of persons include the cost of gasoline, oil and diesel fuel for the operation of the speed boats and the launch to provide the regular communication services between the field trial area and the villages and Dacca.

3. Printing and reproduction costs include the cost of preparing standard forms for surveillance work and hospital records.

4. Other contractural services include the cost of repairs and maintenance on the building in addition to the cost of binding census book.

5. Supplies and materials include primarily the cost of medications for operation of the hospital and the cost of the paper for census books, etc.

6. Equipment includes primarily the cost of equipping the new hospital building which has just been completed with chairs, beds, tables, on so forth.

3. BUDGET (Cont'd):

7. Capital expense includes the cost of construction of the hospital.

8. Other major expenditures which are not included in the rupee fund are approximately $2,000 a year spent for intravenous fluids imported Switzerland, $5,000 for the cost of 8 jet injectors and $4,000 for the cost of three additional speed boats and seven additional motors to provide adequate coverage of the extended vaccine trial area.

STATEMENT OF EXPENDITURE INCURRED FOR V.T.S. MATLAB

Heads of Accounts	Actual Expenditure from July to April	Projected for F.Y- 1966
1. Personnel Services	Rs. 201,901.22	Rs. 252,000.00
2. Travel & Transportation of Persons 1/	111,018.64	126,000.00
3. Transportation of things	11.50	100.00
4. Rent, Communication and Utilities.	347.54	700.00
5. Printing & Reproduction	1,800.00	5,000.00
6. Other Contractual Services	8,510.30	12,000.00
7. Supplies & Materials	26,711.77	32,000.00
8. Equipment	5,330.00	7,000.00
	Rs. 355,630.97	Rs. 434,800.00
9. Capital Expense	77,905.00	83,844.00
TOTAL:	Rs. 433,535.97	Rs. 518,644.00

1/ Includes cost of 7,040 gallons of gasoline and 352 gallons of mobile oil @Rs.3.31 and @ Rs.8.71 consumed for operation of boats.

Also includes Rs.13,561.65 being the cost of diesel oil for operation of launch.

PROTOCOL

The Effect of Cholera Vaccination on the Course of Clinical Cholera

Dr. W.M. McCormack, Dr. W.H. Mosley
and Dr. Mizanur Rahman

1. RESEARCH PLAN

A- Specific Aims:

It has been shown that cholera vaccine can protect against cholera but that it is not 100% effective. The specific objective of this study will be to determine if the clinical course of cholera is modified in those vaccinated patients who become ill.

B- Methods of Procedure:

A cholera vaccine trial will be launched in the Matlab area in the fall of 1966. Cholera vaccine in different dosage schedules will be compared to a placebo (Diphtheria-Tetanus Toxoid). This trial will involve approximately 60,000 persons under the age of 15 years. Intensive surveillance will be carried out in the vaccine trial area and it is anticipated that the majority of cholera cases will be detected and referred for hospitalization and treatment at the field hospital in Matlab Bazar. The following modifications of the procedures, now in effect, at the Matlab-CRL hospital will be made in order to facilitate the study:

1. All VTS patients admitted will have rectal swabs for V. Cholera taken daily.

2. All VTS patients admitted will be treated with intravenous fluids only. This will be necessary because of the demonstrated modification of the clinical course of cholera by antibiotics. If, in the opinion of the physician-in-charge antibiotic therapy is indicated for medical reasons, the patient will be dropped from the study and appropriately treated.

3. A convalescent plasma protein sample will be obtained in addition to an acute sample.

INTERNAL ROUTING SLIP
PSCRL

From:

Date:_____

To :

Dr. Phillips
Dr. K. Hare
Dr. R. Hare
Dr. Mosley
Dr. Taylor
Dr. McCormack
Dr. Northrup
Dr. Kinzie
Dr. Martin
Dr. Fahimuddin
Dr. Rahaman
Dr. K.M.S. Aziz
Mr. Imdadul Huq
Dr. Nalin

Dr. R. Cash
Mr. Zafar Ahmad
Mr. P. Talmon
Mr. N. Nabi
Mr. Tucker
Mr. A.H. Choudhury
Mr. N.A. Husain
Mr. Md. Shahabuddin
Miss Marian Smith
Miss Diana Bowen
Miss Dorothy Torrance
Mrs. Parvin Molla
Mr. A.F. Choudhuri
Supply Management Branch.

☐ Infor and return: ☐ Infor and **retain:** ☐ Infor and return to Library.

☐ Request comments: ☐ Action: ☐ Make - copies:

☐ Order: ☐ Prepare reply:

4. Patients, who are bacteriologically positive for V. Cholera, will be kept in hospital until 3 consecutive negative cultures have been obtained. A feedback system will be instituted so that results of the bacteriologic examination of rectal swabs will be available to the physician at Matlab.

5. A serum sample will be obtained daily from each bacteriologically positive patient for 10 days.

The following criteria will be used to compare the clinical course of those patients who have received cholera vaccine and the clinical course of those patients with cholera who have received the control vaccine:

1. Decrease in plasma protein from admission to discharge.

2. Percent gain in body weight between admission and discharge.

3. Pulse on admission.

4. Blood pressure on admission.

5. Total stool volume in cc per kg.

6. Total intravenous fluid requirement in cc per kg.

7. Day of last positive culture for V. Cholera.

8. Day of end of diarrhea.

9. Admission and discharge antibody titer.

C- Significance of this Research:

Until such time as a vaccine for cholera can be developed that is truly 100% effective, it will be valuable to know what effect, if any, vaccination will have upon the clinical course of cholera.

D- Facilities Available:

The 1966 Pakistan-SEATO Cholera Research Laboratory Field Trial will be conducted in 134 villages in Matlab Thana. This trial will involve about 60,000 persons under the age of 15 years. Two doses a month apart will be given to each participant. There will be four groups:

a. Tetanus-Diphtheria Toxoid (control).

b. One dose of cholera vaccine followed by a dose of control vaccine.

c. Two doses of cholera vaccine.

d. Two doses of cholera vaccine followed by a third dose of cholera vaccine in a year.

Vaccination will be carried out in the fall of 1966 and will be completed before the beginning of the cholera season. Intensive surveillance, which will include a daily visit to each vaccinated family, will be carried out in all the villages. All patients with severe diarrhea will be referred to the field hospital in Matlab Bazar. Less severely ill patients who are not referred to the hospital, who have both diarrhea and vomiting, will have a rectal swab taken which will be cultured for cholera vibrios. The field hospital in Matlab Bazar is situated in a wing at the newly constructed rural health center. In charge of the hospital is a M.B.B.S. physician who has had several years of experience in the Epidemiology and Clinical Research Sections at CRL. The record of the Matlab hospital in the treatment of cholera is comparable to that of the parent hospital in Dacca. A code has been devised (Clinical-Epidemiological Code 000) which is routinely completed on all patients admitted to the hospital. This code reports in a manner suitable for machine-processing both epidemiological and clinical information. These code sheets are prepared by a specially-trained sociologist and checked by a physician.

2. SUPPORTING DATA

A- Previous Work done on this project:

It has been conclusively shown in this and other laboratories that the clinical course of cholera can be dramatically modified by the administration of tetracycline and other antibiotic agents. In reviewing the experience of the first three years in the vaccine field trial in Matlab, we have found that in all vaccinated and control groups about the same percentage of patients with positive cultures for V. cholera, required hospitalization, i.e., about 53%. However, an analysis of the hospital course of the 31 vaccinated patients compared with the 82 control vaccine patients, who were admitted to the Matlab PSCRL hospital with clinical cholera, has disclosed a somewhat milder clinical course in the vaccinated group. Although the differenes are not statistically significant, the vaccinated group had less total stool volume, less total I.V. fluid requirement and a shorter duration of diarrhea. All patients in both groups were treated with antibiotics and for this reason, all would be expected to have had a modified course.

B- Personal Publications:

None

C- Results Obtained by Others:

El-Ramli (1948), in a clinical study of cholera cases during the 1947-48 epidemic in Egypt, found that the mortality rate in unvaccinated patients was 17.3% whereas the mortality rate in patients who have received cholera vaccine, at least 7 days before the onset of their illness, was only 7.6%. Shousha (1948) noted mortality rates in different fever hospitals in Egypt and found that the fatality rate was 26.5% and 42.9% in the vaccinated and non-vaccinated groups respectively. In the joint Philippines-Japan W.H.O. Cholera El Tor Research Project conducted in the Republic of the Philippines from June to December, 1964 a comparison of symptoms among confirmed cholera cases showed no difference in the vaccine and the control groups. They concluded that vaccination does not alter the symptoms or the course of cholera once the individual has developed the disease. In this study a comparison of the duration of diarrhea was also done. This showed no difference between the control and the vaccinated group although there is a definite trend for the course to be longer in the controls.

D- Justification of Budget:

The majority of the personnel and equipment necessary for this study are already provided for in the vaccine field trial and in the Matlab hospital. Processing of the data would require approximately one month's time by one of our key-punch operators. No permanent equipment will be needed. It will be necessary to provide additional intravenous fluid as the lack of antibiotic treatment required by this protocol will result in an increased intravenous fluid requirement or approximately five liters per patient. The increased burden placed on the CRL hospital staff at Matlab may necessitate the payment of overtime or the hiring of extra nursing personnel. The increased load in the bacteriology section, due to the introduction of daily culturing, will also have to be considered.

3. BUDGET

A- Personnel:

1.	Key-punch operator (1) for 1 month	Rs 500.00
2.	Extra nursing personnel (1) for 3 months	Rs 500.00

B- Permanent Equipment:

None

C- Expendable Equipment:

1.	Intravenous fluid - 1,000 liters @ Rs 3.5 per liter	Rs 3,500.00
2.	Food for 200 patients for 4 additional hospital days	Rs 2,400.00
3.	Miscellaneous expendable equipment approximately	Rs 500.00

D- Travel and Transportation:

No new expenses

Total..... Rs 7,400.00

PROTOCOL

Serologic survey for cholera antibody in an American population living abroad

Dr. W. McCormack

1. RESEARCH PLAN:

A. Specific objective: The specific objective of this study is to determine the effect of repeated vaccination with standard cholera vaccine on the antibody titer of American individuals presently residing in East Pakistan.

B. Method of procedure: Each American employed by the Government of the United States in an official capacity in East Pakistan is under the care of the Health Unit at the American Consulate in Dacca. International regulations require that cholera re-vaccination be given every six months. The injections are given at the Consulate Health Unit by the Consulate Nurse under the supervision of the Consulate Physician. The International record for vaccination and immunization must be presented at the time of inoculation.

Each American presenting at the Health Unit for the six monthly cholera booster will be asked to participate in this study. Those who agree will have a finger prick sample of blood collected into a 50 lamda capiliary pipet and discharged into 0.45 cc of saline in a sterile vial. This sera will be refrigerated until it can be transported to the serology laboratory at the Pakistan -SEATO Cholera Research Laboratory. At the laboratory, the serum saline supernate will be separated from the cells and frozen. The supernate will be maintained in a frozen state until tested. Titers will be determined for vibriocidal, agglutinating and toxin neutralizing cholera antibodies by the methods already in use by the laboratory.

At the time of the serum collection, a questionaire will be compled for each study participant using the information available on the international certificate. In addition to the name, age, and sex of the individual, the date of each previous cholera inoculation will be recorded. The completed forms will be forwarded to the statistic unit of the Epidemiology section at PSCRL. The following information will be coded onto ICT cards for mechanical processing: Age, sex, total number of cholera injection received, interval since last cholera injection, vibriocidal titer to Inaba, vibriocidal titer to Ogawa, Agglutinating titer to Inaba, Agglutinating titer to Ogawa, and toxin neutralizing antibody titer.

C. Significance of the research: It is a long range aim of the Epidemiology Section at the PSCRL to re-evaluate the effectiveness of the presently used cholera vaccine dose and dosages schdule i.e., a vaccine containing 8,000 million organisms per cc. and which is given initially in two doses of 0.5 cc and 1.0 cc at a 7-28 day interval with booster doses of 0.5 cc six monthly thereafter. There is evidence from our endemic cholera area in Matlab Bazar that there is an association between circulating antibody titer and protection from cholera. If this is indeed the case, it will

(more)

be of a considerable importance to develop a cholera vaccination dose and dosages schedule that will produce the highest and most prolonged antibody titers.

D. Facilities available: The Health Unit at the United States Consulate General is at present staffed by a physician and a nurse. At any given time, there are approximately 300 "official" Americans in East Pakistan. The turn over rate is about 50% per year. Many have been in the Foreign Service for a long period of time and have received repeated cholera inoculations. All are required by International regulation to receive a cholera re-inoculation every six months. It is responsibility of the Consulate Health Unit to provide this inoculation. It is a routine already established to send a reminding notice to each individual every six months. The information required for the completion of the questionaire will be obtained from the International certificate which must be presented. In most cases this form could be completed by the study participants themselves. A messenger routinely calls daily at the Consulate Health Unit to bring specimen from the Health Unit to the PSCRL.

The Serology Section at the PSCRL is equipped to routinely process large numbers of samples for vibriocidal, agglutinating, and toxin neutralizing antibodies. The number of samples involved in this study can be easily handled. The statistics unit has been the personnel and equipment to code and process the information.

2. SUPPORTING DATA:

A. Previous worked done on this project: While there have been many studies on the immediate effect on cholera vaccination on antibody titers, no studies have come to our attention where the relationship between the number of previous cholera injections and antibody titer have been evaluated.

B. Personal publications: None

C. Results obtained by others: No studies on the same type by other have come to our attention.

D. Justification of the budget: It is estimated that in the course of six months, about 250 individuals will agree to participate in this study. The budget is calculated for this number of specimen. Results obtained during this period will be analyzed. The cholera inoculation program at the Health Unit is already in existence. A messenger already carries specimens on a daily basis from the consulate Health Unit to the PSCRL. The Serology Laboratory will require about one week to process 250 specimen which will involve the full time of two Technician for this period. Coding and machine processing of the data by the statistics unit should require no more than one week's time by one operator.

BUDGET

A. Personnel

1. Serology Technician (2) for one week	Rs 250.00
2. Key punch operator (1) for one week	Rs 125.00

B. Permanent Equipment

None.

C. Expendable Equipment

1. Steri-sharp Lancets (250)	Rs 15.00
2. Capillary tubes (250)	Rs 40.00
3. Serum vials (250)	Rs 75.00
4. ICT cards	Rs 50.00

D. Travel and Transportation

No additional cost.

Total:	Rs 555.00

PROTOCOL

A Comparison of the Serological Response of Cholera Vaccine in Man with the Potency determined by the Mouse Protection Assay

Dr. W.H. Mosley in collaboration with Dr. J. Feeley & Dr. M. Pittman

1. RESEARCH PLAN

A- Introduction & Specific Aims:

The presently accepted potency test for cholera vaccines is the mouse protection test. This test consists in immunizing groups of mice with graded doses of the cholera vaccine under study and then challenging the mice by the intraperitoneal inoculation of a standard dose of cholera vibrios of either the Ogawa or the Inaba serotype suspended in mucin. The potency is calculated from the number of surviving mice after a given interval of time and is expressed in terms of the potency relative to a reference vaccine. There are several theoretical reasons why this test may not be an appropriate expression of potency for a cholera vaccine in man. The most obvious is that this test measures a mouse's resistance to an intraperitoneal challenge of the organism while in man the infection is limited to the intestinal lumen. In addition, there has been recent evidence based on studies with the purified Ogawa antigen prepared by Dr. Verwey that a vaccine which may show a high level of protection against only the homologous serotype in the mouse may actually cross protect against the heterologous serotype in man. (In addition, serological studies with this vaccine have shown that man develops an antibody response against both the homologous and the heterologous serotype and that the protective efficacy in man apparently correlated with the serological response induced by the vaccine.

This present study is being carried out in coordination with Dr. Margaret Pittman of the Division of Biological Standards, NIH and will be an evaluation of the immunological response in infants to different cholera vaccines. Specifically groups of infants will be given 7 different cholera vaccines to determine:

1. if there are detectable differences in the antibody response of infants to different lots and different types of cholera vaccines.
2. to determine if these differences correlate with the vaccine potency as determined by the mouse protection test, and

3. to determine if the differences in serological response correlate with the vaccine potency based on field trial studies in man (for those vaccines used in vaccine field trials).

B- Methods of Procedure:

The following vaccines will be used:

Vaccine B - this is the cholera vaccine used in the 1963 and 1964 field trials in Matlab. This is Lilly vaccine, Lot 6117-798226, a whole cell cholera vaccine containing both Inaba and Ogawa organisms.

Vaccine L - this is a whole cell cholera vaccine, Lederle, Lot 1976-81B. This represents a low potency cholera vaccine originally sent to CRL for field testing.

Vaccine X - this is a whole cell cholera vaccine. Lilly, Lot 9GP04 which is presently being used in the 1965 cholera vaccine field trial in Matlab.

Vaccine J - this is a lot of bivalent whole cell vaccine produced by the Walter Reed Army Institute of Research. It is prepared with a concentration of bacteria three times higher than in standard and it is a lyophilized vaccine. It was used in the W.H.O. sponsored field trial of cholera vaccines in Calcutta, India in 1965.

Vaccine P - this is a whole cell bivalent cholera vaccine prepared by the Institutes of Public Health in Dacca, East Pakistan.

Vaccine O - this is a monovalent Ogawa vaccine which is prepared for an NIH reference standard. This is a dried vaccine.

Vaccine I - this is a monovalent Inaba vaccine also prepared as an NIH reference standard and is also a dried vaccine.

Procedure:

The study will be carried out in 6-12 months old infants in the Sylhet Tea Gardens. None of these infants have received any other cholera vaccine or had any known exposure to cholera. 140 infants will be used for this study; there will be 20 infants in each vaccine group. The vaccines will be assigned to these infants in a random manner. Two injections of 0.2 ml of each of the vaccines are to be given with syringe and needle at

an interval of 28 days. A serological specimen will be obtained prior to the first injection and 7 days after the second injection. Serum specimens will be obtained from finger tip blood. 50 microliters of blood will be obtained in a calibrated capillary tube and diluted 1:10 in 0.45 ml of saline in a screw-capped vial. These specimens will be refrigerated during shipment to the laboratory where the cells will be separated and the specimens will be frozen until titrations are performed. Agglutinating and vibriocidal antibody titers will be performed on the serum pairs using the micro-techniques developed in this laboratory. Geometric mean titers will be calculated and the relative potencies will be related to the serological response induced by the NIH reference vaccines.

Dr. John Feeley, Laboratory of Biological Products, DBS has assayed the mouse protective potency of each lot of vaccine. The human antibody response to the vaccine will then be compared with the mouse protective potency to determine if there is a direct correlation between human antibody response and laboratory assay.

C- Significance of this Research:

This study is a small phase of a broad study to evaluate the protective potency of cholera vaccines in man. The cholera vaccine field trials conducted by the CRL have demonstrated the protective efficacy of a whole cell cholera vaccine and have shown that some protection could be induced by a purified Ogawa antigen. Serological surveys as a part of these trials have demonstrated that there was a direct correlation between the antibody response induced by these vaccines in man and the protective potency. This suggested that the antibody response in man to a vaccine may be a useful measure of the protective potency of a vaccine.

At the present time the primary measure of vaccine potency is the mouse protection test. Recent work by Dr. Verwey using the purified Ogawa antigen has indicated that there is no relationship between the mouse protective potency of a vaccine and the serological response induced by this vaccine in man. Since the mouse protective test is the present-day accepted standard for evaluating cholera vaccine potency, it becomes important to know what, if any, relationship there is between this test and man's antibody response to the vaccine so that the value of this test may be put in its proper perspective. This study in conjunction with the field trials may lead to a laboratory test for vaccine potency that best measures the potential effectiveness of the vaccine in man.

D- Facilities Available:

The field studies required in this study will

of Dr. Donald Mackay, the Medical Director. All antibody titrations will be performed in the CRL serology laboratory. The mouse potency test will be done at NIH by Dr. John Feeley.

E- Facilities Required:

There are no special facilities required other than what are listed above as already available.

2. SUPPORTING DATA:

A serological survey conducted as a part of the cholera vaccine field trial last year revealed a direct correlation between the protective efficacy of the cholera vaccine in man and the vibriocidal antibody response. This data has been given in a separate report. In April, 1966 preliminary studies were conducted on the serological response of infants to cholera vaccines. 120 infants were studied utilizing 4 different cholera vaccine schedules and 3 different cholera vaccines. This study revealed that a single injection of a high potency whole cell cholera vaccine produced a negligible serological response in these infants. Two injections of this vaccine at an interval of one week produced a marked secondary response with the mean vibriocidal titer of approximately 1:300, however, two injections of the same vaccine at an interval of 28 days produced a better secondary response with a peak mean titer of approximately 1:1200. Based on these preliminary observations, the schedule of two injections at a 28 days interval has been used in this present [illegible] to evaluate differences between vaccines.

PROTOCOL

Serologic Studies with Cholera Vaccine

Dr. Ansaruddin Ahmed, Dr. W. Mosley

I RESEARCH PLAN

A. Specific Aims

It is the purpose of this study to investigate serologic response to and side effects from various dosage routes and dosage schedules of cholera vaccine, specifically:

a. To determine the dose response curve within the tolerable dosage range.

b. To determine the effect of cholera vaccine given by the intradermal route on antibody response.

c. To determine the effect of varying the size of each dose in a two dose schedule.

B. Method of Procedure

The study will be conducted in the Balisera Medical Department in South Sylhet. Two large groups of patients will be utilized. The first group (Group A) will consist of young-children who have never received cholera vaccine and who have never been exposed to V.Cholera. The second group (Group B) will be a group of adults who have a long history of cholera vaccinations but who have not been exposed to clinical cholera. Two vaccines will be used: Vaccine X is a commercial U.S. cholera vaccine. Vaccine O, the control vaccine, is a commercial U.S. Diphtheria-Tetanus Toxoid. Each person in the study will receive two doses of vaccine. The doses will be given 28 days apart. All doses will be given with a disposable tuberculin syringe and a disposable 25 gauge needle. The two large groups will each be broken down into 7 smaller groups as outlined in Table 1. Each of the small groups will contain 15 individuals. Subjects will be assigned to each group on a random alternation basis.

50 Lambda of capillary blood will be obtained before the first injection (day 0), before the second injection (day 28) and two weeks after the second injection (day 42).

In order to determine the reactions which occur following the vaccination, each patient will be seen by a physician daily for 7 days. At this time, the temperature will be recorded, the condition of the vaccine site will be examined and the patient (or the patient's parents) will be asked about constitutional symptoms. This information will be recorded in a special form which is attached.

C. Significance of the Research

In reviewing the literature on the development of cholera vaccine, it becomes apparent that there is little concrete scientific evidence for the currently recommended vaccine dosage and dosage schedule. It is a long-term goal of the Epedemiology Section to

more clearly define the response to cholera vaccine in man in various dosages, dosage schedules and routes of administration.

D. Facilities Available

The study will be carried out in the Tea Estates under the Balisera Medical Department in South Sylhet. This is an area which has been virtually free of cholera for the last 20 years. All persons residing in this area fall under a comprehensive medical program. Cholera vaccination is administered every year as are other routine immunizations. Accurate records are kept. It is anticipated that we will have the full cooperation of the medical department as well as the management of the tea estates. The cooperation of the population involved in our past studies in this area has been outstanding and we anticipate that it will continue.

Samples will be collected by physicians on our own staff with the aid of the area medical officers in the tea gardens. The clinical observations during the week following the vaccination will also be made by the CRL physicians.

Facilities are available at the CRL for the machine-processing of the data obtained during this study as well as for the analysis for vibriocidal and agglutinating antibodies of the serum specimens.

II SUPPORTING DATA

A. Previous Work done on this project

A previous study conducted in the tea gardens has shown the clear superiority of the antibody response to two doses of cholera vaccine given 28 days apart as compared to two doses given 7 days apart and to a single dose. The current project will be an extension of these findings.

B. Personal Publication

None.

C. Results Obtained by Others

International regulations recommend that cholera vaccine for adults be given in a concentration of 8,000 million organisms per cc in tow doses of 0.5 and 1.0 cc 7 days apart. If only one dose can be given, 1.0 cc is recommended. Revaccination is recommended every six to twelve months. Almost all authorities exclude children under the age of one year and agree that the dose of cholera vaccine should be reduced for older children.

The older papers recommend cholera vaccine given in doses based on a milligram of culture mass per milliliter. The various doses recommended are based on antibody response in either animals or man or on apparent vaccine efficacy.

Dzen and Yu (1936) in guinea pig experiments demonstrated that 2,000 million organisms per cc was inadequate. They recommended that the concentration be raised to at least 4,000 or preferably 8,000 million organisms per cc.

Robertson and Pollitzer (1939) introduced a vaccine with the vibrio count of 6,000 million organisms per cc. They administered 1 cc and did not note any side effects. It is their clinical impression that this vaccine was more effective than vaccine containing 2,000 million organisms per cc.

D. Justification of the Budget

It will be necessary to maintain a physician in the tea gardens for the duration of this study which will be about two months. This will require that he be provided with per diem as well as transportation. The usual equipment necessary for the administration of vaccine and the collection of serologic samples will be needed. Processing of the serologic samples and the statistical analysis of the resultant data will require time on the part of the sections involved.

BUDGET

A. Personnel

1. Per diem for one physician for 2 months.	Rs 600.00
2. One key punch operator for 1 week.	Rs 125.00
3. One serology technician for 2 weeks.	Rs 250.00

B. Permanent Equipment

None

C. Expendable Equipment

1. 700 disposable tuberculin syringes.	Rs 225.00
2. 700 disposable 25 gauge needles.	Rs 200.00
3. Miscellaneous supplies, (spirit, cotton, balloons, micropipets, lancets, vials, etc.)	Rs 500.00

D. Travel and Transportation

Two round-trip tickets to Sylhet	Rs 150.00
TOTAL	Rs 2050.00

TABLE 1

Group A

Non-Immunes (Approx. 1 year olds with no prior vaccination)

Sub-Group	First Dose (Day 0)	Second Dose (Day 28)
A	0.1 "X"	0.1 "X"
B	0.5 "X"	0.5 "X"
C	1.0 "X"	1.0 "X"
D	0.1 "X"	1.0 "X"
E	1.0 "X"	0.1 "X"
F	0.1 ID "X"	0.1 ID "X"
G	0.5 "O"	0.5 "O"

Group B

Immunes (Adults)

H	0.1 "X"	0.1 "X"
I	0.5 "X"	0.5 "X"
J	1.0 "X"	1.0 "X"
K	0.1 "X"	1.0 "X"
L	1.0 "X"	0.1 "X"
M	0.1 ID "X"	0.1 ID "X"
N	0.5 "O"	0.5 "O"

Cholera Vaccine = Vaccine "X"

Control Vaccine = Vaccine "O"

NAME: _____ AGE: _____ NUMBER _____

ADDRESS: _____

First Serology (Day 0) _____ First Injection (Day 0) _____

Second Serology (Day 28) _____ Second Injection (Day 28) _____

Third Serology (Day 42) _____

Days After Injection

	1	2	3	4	5	6	7
Temperature							
Swelling (mm)							
Induration (mm)							
Tenderness							
Constitutional Symptoms							
Others							

PROTOCOL

Immunochemical Studies of Antibodies in Cholera

Dr. W. H. Mosley, Dr. Ansaruddin Ahmed,
Mr. Samin Ahmed in collaboration with
Dr. Paul Crabbe

1. RESEARCH PLAN

A. Introduction & Specific Aims:

As a part of a broad-base study on immunity in cholera, the antibodies produced in man against cholera antigens will be studied utilizing immunochemical techniques. By these procedures, we hope to define the immune response in man that develops with cholera infection. This will involve both a study of the serum antibodies as well as antibodies produced in the intestinal secretions. In addition, studies will be carried out on the immune response of man to cholera vaccine to determine how this compares to the response produced by natural infection. In addition, studies are being conducted on the characteristics of the "natural" antibody found in the majority of East Pakistani adults and on the transplacental transfer of cholera antibodies.

These studies are based on the fact that in man the immunoglobulins are a heterogeneous group of proteins which have distinct physical, chemical, and biological characteristics. The three major classes of immunoglobulins are: The gamma M, or 19S or macroglobulins which are characterized by their high molecular weight, averaging 900,000. Antibodies against the somatic O antigens of gram-negative bacteria are characteristically found in the IgM class and frequently IgM is the first antibody to a pear in the immune response. The gamma G or 7S immunoglobulin has a molecular weight of approximately 150,000. It is characteristically seen in the secondary antibody response to a variety of antigenic stimuli, many antitoxic antibodies have been demonstrated in this class of immunoglobulins, and it is transferred across the placenta. The third class is the IgA or Gamma A globulin. The most interesting characteristic of this immunoglobulin is that it is present in high concentrations in secretions of the saliva, colostrum and intestines.

B. Methods of Procedure:

The following techniques will be utilized to study the immune response to cholera antigens:

a. Fractionation with 2-mercaptoethanol:

2-mercaptoethanol at a final concentration of 0.1 molar has been shown to selectively break the disulfide bonds of the gamma M immunoglobulins. Subsequent reaggregation is prevented by alkalization with iodoacetamide. The gamma G globulins are resistant to this treatment. This procedure will be carried out on serial blood

specimens obtained from cholera patients to follow the sequence of gamma M and gamma G antibody production. This procedure will also be utilized to determine the nature of the "natural" vibriocidal and agglutinating antibody found in the adult East Pakistani population, and in the study of transplacental transfer of cholera antibodies.

b. Separation of immunoglobulins by column chromatography:

Column chromatography using Sephadex G-200 and DEAE-Cellulose to separate the various classes of immunoglobulins will be utilized in the study of serial specimens from cholera patients as well as isolating the immunoglobulins from intestinal secretions.

c. Density gradient centrifugation:

Ultracentrifugation of the immunoglobulins found in cholera in sucrose density gradients will be carried out to define more accurately the characteristics of the antibody.

d. Additional techniques to be utilized in characterizing the immune response to cholera antigens will be:

Double diffusion studies in agar according to the method of Ouchterlony and immunoelectrophoresis.

These procedures will be applied to the following types of specimens:

a. Serial blood specimens obtained from cholera patients during the course of their illness and convalescence.

b. The intestinal secretions of cholera patients during their acute illness and convalescence.

c. Serial blood specimens and intestinal secretions obtained from individuals following their primary and booster injections with cholera vaccine.

d. Normal East Pakistani adults who have a high level of circulating cholera antibodies.

e. Serum specimens from chronic carriers of V. Cholera.

f. Cord blood obtained at the time of delivery of a new born to study the transplacentally transferred anti-cholera antibodies.

The antibodies will be assayed using the vibriocidal and agglutinating microtiter test developed in this laboratory. For detection of very low titer antibody, such as is found in cord blood, a modification of the routine vibriocidal technique has been developed which involves utilization of only 10^2 instead of 10^7 organisms which greatly increases the sensitivity of the test. The toxin neutralizing antibody will be determined according to the procedures developed by

Dr. John Craig. In addition, assays for antibody which may bind antigen but with no agglutinating, vibriocidal or precipitating activity will be carried out using either immunofluorescent techniques or the Farr technique incorporating radio-active labeled antigen followed by the selective precipitation of the antigen-antibody complex with NH_4SO_4.

C- Significance of this Research:

At this time very little is known about the role of immunity in cholera. The recent vaccine field trials have established conclusively that a whole cell cholera vaccine administered parenterally can protect against cholera, further that the level of protection seems to be correlated with the level of vibriocidal antibody induced by the vaccine. Since the injected vaccine produces primarily a circulating antibody, this raises a fundamental question in immunology as to how this circulating antibody can protect against an intestinal infection. The serological survey suggested that the vibriocidal antibody was correlated with the protective efficacy of vaccine. However, recent studies in our laboratory have indicated that the vibriocidal antibody is primarily a gamma M or 19S immunoglobulin and since it is known that this globulin remains primarily within the vascular compartment, it seems unlikely that this per se is the protective antibody. Recent studies have suggested that gamma A globulin may be selectively excreted into intestinal secretions and thus it would be important to know what role this immunoglobulin plays in cholera, and more specifically, whether or not vaccination elicits an immune response in the gamma A fraction.

In these studies, if it is possible to determine specifically what immunoglobulin is related to protection against cholera and how this protection acts, this will provide information not only on the fundamental question of immunity to intestinal infections but also on the development of cholera vaccines. Thus, it may be possible to relate the protective efficacy of the vaccine to the production of certain immunoglobulins. If this is the case, then further vaccine development could lead towards preparing antigens which specifically stimulated this particular antibody response.

D- Facilities Available:

All of these studies will be carried out at the cholera laboratory and all the necessary equipment is available including fraction collectors, spectrophotometer, immunoelectrophoresis equipment and an ultracentrifuge. The clinical material will be supplied from the cases in the cholera wards and the studies on cholera vaccine will be carried out in coordination with Dr. Mackay at the Sylhet Tea Gardens and in the Matlab vaccine field trial area.

E- Facilities required:

Expendable materials such as specific antisera, chromatographic resins, rabbits, agar, I^{131} and various reagents.

F- Organization Framework:

These studies are being carried out in the Clinical Research Section under the guidance of Dr. W. H. Mosley. Dr. Ansaruddin Ahmed is the research physician supervising these studies and other staff involved are: Mr. Samin Ahmed, senior research assistant, Mr. Manik Paul, research assistant, and Mrs. Pashi and Mr. Gomez, technicians.

In January, 1967 Dr. Paul Crabbe will be joining the program for 5 weeks in a collaborative project with particular reference to defining the immunoglobulins in intestinal secretions.

2. OWN WORK DONE ON THIS PROJECT:

To date the following studies have been completed:

(Utilizing 2-mercaptoethanol fractionation it has been established that the majority of the vibriocidal and agglutinating antibody found in the normal adult East Pakistani population is a gamma M globulin. It has also been established that while only the small amount of the vibriocidal and agglutinating antibody is in the 7S fraction, studies of cord blood of new born infants have demonstrated that this antibody does cross the placenta.)

In a study of serial serum specimens taken from acute cholera patients, utilizing 2-mercaptoethanol treatment it has been established that initial vibriocidal and agglutinating antibody response is a gamma M globulin which reaches its peak titer by the 7th day of the disease and this is followed by a gamma G response which reaches its peak titer by the 10th day.

Toxin neutralizing antibody has been shown both by 2-mercaptoethanol treatment as well as by Sephadex column chromatography to be entirely a 7S immunoglobulin.)

Serial blood specimens have been studied on several patients by Sephadex chromatography and this has confirmed the observation that the initial antibody response is almost entirely a gamma M globulin, while in convalescence a relatively large proportion of the antibody is 7S. The identification of the immunoglobulin classes isolated by the Sephadex gel filtration has been established both by Ouchterlony double diffusion studies as well as by immunoelectrophoresis using specific anti-gamma G, antigamma A and anti-gamma M antisers.

Preliminary fractionations have been carried out on the DEAE-Cellulose and the studies are continuing to better characterize the role of gamma A globulin in the immune response to cholera.

Preliminary studies of intestinal secretions from acute and convalescent cholera patients utilizing the vibriocidal and agglutinating test procedures have demonstrated a high titer bacteriolytic activity (antibody?) in the acute specimens and low titer activity in the convalescent. In addition, gamma A globulin has been demonstrated in acute cholera stool by Ouchterlony double diffusion procedures utilizing specific antisera.

PROTOCOL

RAYER BAZAR COMMUNITY STUDY

W. M. MOSLEY, M.D.

1. RESEARCH PLAN:

A. Specific Aims: The Rayer Bazar Community Study consists of prospective observations on the patterns of Cholera, diarrhea as well as births, deaths and migrations in approximately 1300 families in a suburban low economic community. The aims are:

1. To define the pattern of spread of cholera with particular emphasis on the relative role of inapparent infections, contact spread, and water in the transmission of the disease.

2. To define the pattern of acute diarrheal illness on a community wide basis and to study the relationships between factors contributing to acute diarrheal disease and to cholera.

3. To define the social and demographic character of the community for the purposes of maintaining an up to date population record for the cholera and diarrhea studies as well as to see how such factors as population mobility relate to the introduction and dissemination of cholera infections.

4. To maintain a defined community which will be available for the incorporation of epidemiological field studies relative to enteric diseases. The prospective study of newborn infants, which is presented in a separate protocol, is one of the additional studies that has been incorporated into this community.

B. Method of Procedure: The entire community was censused during August 1965. In preparation for the census the community was divided into 20 areas designated alphabetically A-T. Each household was identified by the area letter, given a serial number and located on a detailed map. At the time of census information was collected regarding the names, age, sex, relationship, occupation and smallpox and cholera vaccine history of each person. In addition, information on the structure of the house, number of rooms, economic status and sources of water was also collected.

B. Method of Procedure (contd.)

Upon completion of the census, the data for each individual was coded on ICT punch cards for analysis. In addition, each family was issued a 5"x7" card giving the household number, the location, the name, age, sex, and individual number of each member. These cards are used by the field teams and clinic personnel in identifying each individual in the community for coding purposes.

A centrally located clinic has been established. This clinic is staffed by a physician and an aid nurse and is open daily for the treatment of diarrhea cases. The patients are encouraged to bring their census cards at the time they seek treatment so that they may be correctly identified for recording relevant data and also for ease in location if followup is necessary.

Field surveillance is carried out by two teams, each consisting of a sanitary inspector and a female field assistant, who visit each house in the community once weekly. If a diarrhea case is reported a rectal swab is collected and information regarding the individual's census number, age, sex, date and time of onset of illness and date culture taken as well as a check list of symptoms is recorded on a standard form. The individual is then treated in the field with a kaolin-bismuth mixture or referred to the field clinic or the hospital as appropriate. The field teams also record all births, deaths and migrations on standard forms in order to maintain the census up to date.

Beginning in September 1965 surveillance for inapparent cholera infections was begun by collection of rectal swabs from all children below the age of six in every tenth household each week. The households are selected each week with the same terminal digit of the census number as the calendar week of surveillance. This method of selection insures cross sectional coverage of the entire community each week and coverage of every household with children every ten weeks.

Routine water collections for bacteriology are made three times weekly from the tanks and various points along the canal which are used by large segments of the population.

When a cholera infection is identified, all members of the affected household as well as all members in the adjacent households are cultured daily for five days and water collections from the water sources used by the infected household are examined daily for five days. In addition detailed investigation is conducted to determine the possible source and routes of spread of the infection.

Bacteriological studies are done as follows: specimens are collected by the field personnel with a tellurite impregnated swab

which is placed in bile peptone transport media and subsequently plated on Monsur's media after six hours incitation and examined only for vibrios. Specimens from the field clinic are collected both with a tellurite swab which is plated directly on Monsur's media and then placed in bile peptone transport media, and with a plain swab which is streaked directly on McConkey's and SS media and placed in selenite broth. Clinic specimens are examined for vibrios, shigella, salmonella, and an enteropathogenic E.coli.

C. Significance of This Research: Although cholera has been endemic in East Pakistan for generations, almost the entire body of knowledge on the disease in this area has been based primarily on death reports usually coming from untrained persons or from the hospital experience. With the availability of bacteriological support at the CRL, it is now possible to define much more accurately the epidemiological patterns of cholera. Preliminary analysis of the CRL Hospital data demonstrated that cholera had a sharp seasonal pattern while diarrheal disease occured throughout the year. The hospital experience, however, gives no indication of what is occuring in the community at large particularly with regard to inapparent infections and mild cases of cholera. Only by examining the community in its entirety through regular surveillance such as has been established in Mayer Bazar, will it be possible to determine whether the organism is completely absent from the community between epidemics or whether it is maintained within the community in human carriers or in the environment with a low level of transmission and perhaps producing only mild cases between epidemics. If it can be established that the vibrio cholera organism is entirely absent from the community between the annual epidemics, this information may prove of extreme value in the ultimate control of the disease.

While it is well known water plays an important part in cholera transmission, it is not clear whether this is the only means for the spread of infection particularly in the endemic areas. Again by studying the entire community with particular reference to the water and environmental conditions as well as to the degree of contact between individuals, it will be possible to define whether or not such additonal factors as contact spread directly from individual to individual are important in cholera epidemiology. This problem can be approached only by bacteriological examination of the healthy people in the community as well as those with illness since, as has been shown with other infectious diseases, e.g. poliomyelitis, a high level of inapparent infection due to contact spread can be significant factor in the epidemiological pattern. Again, knowledge of the level of the inapparent infection in a community with cholera cases is of fundamental importance in devising ultimate control programs.

Since cholera is a diarrheal disease and is associated with such factors as poor sanitation and poor nutrition that are common with all thediarrheal diseases, it can only be studied in the perspective of the general diarrheal experience of the community. Only by examining every diarrheal disease that occurs in the community throughout the year will it be possible to determine whether cholera is being maintained in the community as a mild infection. In addition by studying cholera in the light of the overall diarrheal experience of the community, it may be possible to determine why cholera apparently enters and then completely disappears from the community whereas other diarrheal diseases are endemic throughout the year. For example, this study will provide information whether the seasonal pattern of cholera is related to seasonal changes in the susceptibility of the host to diarrheal illness, seasonal changes in the environment or water, or to seasonal fluctuations in the population due to migrations or births.

D. Facilities Available: A centrally located building has been loaned for the use of Pakistan-SEATO CRL from the community. This building has been completely renovated by the laboratory and is the center for our clinic activities in the field. Supporting the field work is the bacteriology laboratory of the CRL and the hospital facilities. In addition, for the handling of the data, the statistical unit of the epidemiology section at the laboratory including key-punch operators and date processing machines are available.

2. SUPPORTING DATE:

A. Previous Work done on this Project: A preliminary report of the Rayer Bazar Community Study summarizing the data available from September through December 1965 has been included in the 1965 CRL Technical Committee Report. To summarize briefly the results of that report: The census at the time of the initiation of the study was 6,440 persons and 1105 households. During the first sixteen weeks of study there were 50 births and 13 deaths in the community; the majority of the deaths were in the age group under five. In the sixteen weeks period a total of over 1200 cases of acute diarrhea were seen by both the field teams and in the clinic. Ten of these cases were due to Vibrio cholera; 9 of the 10 occuring within the three weeks period; eighteen of the cases were due to Shigella. 1262 asymptomatic children who were examined for cholera over the seme sixteen weeks period were bacteriologically negative. Further investigation revealed that all of the cholera cases were apparently related to a canal which was confirmed as infected with Vibrio cholera. The cultures of the community as well as the neighbourhood contacts of the cholera cases revealed no evidence of contact spread of the infection. From January through April 1966 there had been no further cholera cases in the community although diarrheas are continuing to occur with regularity.

B. Results Obtained by Others: Prospective observations of entire communities in an endemic cholera area with bacteriological confirmation of cases have not been conducted before. Dr. John Craig studied a series of 244 families in Rayer Bazar during 1964 and 1965. The results of these studies are in the 1964, 1965 CRL Technical Committee Report. No Cholera cases developed in the families under study during his period of observation. However, since that was a family study and not a community study the results obtained are not comparable to the type of data to be obtained under the present protocol.

C. Justification of Budget: The personnel services include Dr. Moslemuddin Khan who is supervisor of this project and also the physician in the field clinic. There are two teams for house to house visiting in the field each consisting of one sanitary inspector and one lady health assistant. In addition, one of the lady health assistants acts as nurse in the clinic assisting Dr. Khan.

Travel and transportation costs are the costs of taking the field personnel daily to and from Rayer Bazar. The majority of the costs for contractual services and equipment is that money spent on remodeling and furnishing the building, and will not be an annual expense. Sixty percent (60%) of cost of supplies and materials include medication costs, and forty percent (40%) the cost of forms and punch cards for coding and analyzing date.

STATEMENT OF EXPENDITURE INCURRED FOR RAYER BAZAR FAMILY STUDY DURING THE F.Y. 1965-66

Heads of Accounts	Actual Expenditure from July to April	Projected for F.Y.66
1. Personnel Services	Rs. 41,158.22	Rs. 51,200.00
2. Travel & Transportation of persons	Rs. 8,184.00	Rs. 9,500.00
3. Transportation of things	-	-
4. Rent, Communication & Utilities	Rs. 36.70	Rs. 50.00
5. Printing & Reproduction	-	-
6. Other Contractual Services.	Rs. 2,890.08	Rs. 3,950.00
7. Supplies & Materials	Rs. 7,215.96	Rs. 10,000.00
8. Equipment	Rs. 1,053.00	Rs. 1,200.00
TOTAL....	Rs. 60,537.96	Rs. 75,900.00

PROTOCOL

RAYER BAZAR INFANT STUDY

Dr. W. H. Mosley

1. RESEARCH PLAN

A. Specific Aims: The Rayer Bazar Infant study consists of the prospective followup of newborn infants in the Rayer Bazar Community at weekly intervals. Serial rectal swabs culture and stool specimens will be obtained to determine the rate of acquisition of intestinal pathogens, both bacterial and parasitic, and the patterns of morbidity due to these pathogens will be studied. At 6 months of age, the infants will be started on cholera vaccines in various dosages and dosages schedules and the serological response to the vaccine will be studied to determine the best immunization procedure in infants. In addition, the general patterns of meternal and child care, the patterns of disease in the child, and the growth curve will be studied.

In addition to the above special studies, this program will also involve to some degree preventive care for the mother and infant. The infants will be given DPT vaccine and smallpox vaccine and general observations will be made on the acceptance by this population of a preventive medical care program. These observations will include a study of the cultural and social factors influencing the population's behaviour.

B. Methods of procedure: All infants born in the Rayer Bazar study area will be selected for study. Notification of newborns is presently being made by the sanitary inspectors on their weekly diarrhea morbidity rounds. Notifications are generally made one to ten days following the infant's birth. Past experience indicates that there will be an average of 3-4 newborns per week.

The initial visit to the infant will be made in the home by a physician and a nurse as soon following notification as possible. Information regarding the mother's pregnancy history, history of delivery and her present health will be obtained on a standard form. The new born will be weighed and examined and this information will be recorded on a standard form. A rectal swab culture will be obtained from the infant on the initial examination.

Subsequent followup of the infant will be at weekly intervals for the first six months and monthly thereafter. After the initial visits in the home, the mother will be encouraged to bring her child to the clinic for the regular weekly examinations. At this time the weight will be recorded and a rectal swab culture will be obtained. Any illness that the infant has will be noted and treated as necessary. Diarrheal illness requiring hospitalization will be hospitalized at the CRL hospital. Other acute illness requiring hospitalization or extensive treatment will be referred to the Dacca Medical College Hospital.

(more)

The bacteriological methods are as follows: two rectal swabs will be obtained from each infant at weekly intervals. A tellurite impregnated swab will be placed in bile peptone transport media and examined for vibrios. A plain rectal swab will be streaked directly on McConkey's and SS Media and then placed in Selenite broth. These cultures will be examined for shigella, enteropathogenic E. coli and salmonella.

A stool specimen will be obtained at monthly intervals and examined while fresh for the presence of parasites and ova. In addition, a small portion of each specimen will be preserved in merthiolate-iodine-formalin for future study as indicated.

Beginning at the age of six month, the infants will be given cholera vaccine. Various dosage schedules will be studied to determine which provides the best serological response.

C. Significance of this Research: It is well recognized that acute diarrheal diseases are the major killers particularly in infants and young children in underdeveloped or developing countries. Extensive studies of the diarrhea problem have been carried out in a variety of countries in the world particularly in Central America, South America and Africa. Information on the magnitude of the problem in East Pakistan based on the observations at the CRL hospital in Dacca as well as observations in the vaccine field trial area in Matlab, indicate acute diarrheal disease due to agents other than Vibrio cholerae are by far the major cause of morbidity and mortality in the community. Thus even if cholera were eliminated, it would have relatively little impact on the overall diarrhea morbidity and mortality figures for the country as a whole. Yet detailed studies on the role that different pathogenic bacteria and intestinal parasites play has not been defined in East Pakistan.

In reference to cholera, a complete understanding of cholera can be available only in the perspective of the general diarrheal disease problem within the community. Of particular interest in this respect are two features of cholera which must be contrasted with acute diarrheal disease: first, cholera has a distinct seasonal pattern, and second, cholera is extremely infrequent in infants. The prospective study of infant diarrhea may shed some light on the reasons for these distinctive features in cholera.

The general observations on the acceptance by the community of this type of study, particularly of this preventive health program in children will be extremely valuable in considering the ultimate control of cholera. As more effective cholera vaccines are developed, some systematic program will have to be devised to regularly immunize all children. This study will provide information both on what will be the best schedule for cholera immunization in children and in addition, it will also provide information on what is the best approach to families in East Pakistan in getting them to accept regular preventive health care services.

D. Facilities Available: This study will be conducted in the Rayer Bazar Community which is already under regular house to house surveillance for births as well as mortality, migration and diarrheal illnesses. The clinic building used for the regular Rayer Bazar Study will be available for the followup of the infants. Bacterioligical and serological support will come from the facilties at the CRL. In the event that the infant needs hospitalization for diarrheal disease, this will be provided at the CRL.

II. SUPPORTING DATA:

A. Previous Work Done on this project:- This project was initiated in March 1966. In the first ten weeks of study 45 newborns have been enrolled in the study. Review of the data available to date reveals that one infant four days old required hospitalization for shigellosis and at least five infants have been shown to be carriers of enteropathogenic E. coli although none have required any treatment or have had any associated symptomatology.

B. Results Obtained by Others: Similar studies have been done by a variety of investigators in other parts of the world, notably the studies by Gordon with INCAP in Central America. In general these studies have revealed that pathogenic bacteria account for only around 20% - 30% of all acute diarrheal illness. Research for viruses by Sabin in South America revealed that only an additional 20% - 30% were due to these agents. Specific information on this problem in East Pakistan is not available.

C. Justification of Budget: The salaries include Dr. Shamsa Ahmed, the physician in charge of this project and Mrs. Binapani Sau, the nurse supervisor. There are two lady field assistants; one to assist each of the above named investigators. The cost of medication include multivitamin preparations and a variety of medications necessary to treat the various illnesses seen in the infants.

STATEMENT OF EXPENDITURE AT RAYAR BAZAR INFANT STUDIES GROUP

		Expense from July - April30	Projected for F.Y.1966
1.	Personal Services	Rs.4,744.51	Rs.9,600.00
2.	Travel & Transportation of Persons	500,00	600.00
3.	Transportation of things	-	-
4.	Rent Communication & utilities	-	-
5.	Printing & Re-production	-	-
6.	Other contractual services	-	-
7.	Supplies & materials	1,309.98	1,500.00
8.	Equipment	355.00	500.00
		Rs.6,909.49	Rs. 12,800.00

PROTOCOL

CLINICAL STUDIES

PROTOCOL FOR ORAL GLUCOSE AND ELECTROLYTE THERAPY IN CHOLERA

Drs. Hirschhorn, Taylor, Sachar

Introduction and Aims

It is known that the transports of glucose and sodium from the gut lumen to the blood stream are mutually dependent. Glucose requires the presence of sodium ion for both its passive entry into cells (phlorizin sensitive step) as well as its transport against a concentration gradient (oubain sensitive step); active sodium transport across the intestinal mucosa requires the presence of glucose (10-20 mM/L appears to give the maximal effect).

Work in the Philippine Islands showed that oral glucose can stimulate the next movement of sodium ions and water from the gut lumen to the plasma. Bicarbonate, chloride and potassium ions alsomoved in the same direction. The failure to cause a net movement of water intothe plasma in those experiments may have been due to the extreme tonicity of the oral solution containing glucose and electrolytes.

Studies at CRL in acute cholera patients showed that a 1% glucose solution (55.6 mM/L) in an isotonic electrolyte solution can cause a shift of sodium and water from the lumen to the plasma as well as an increase in the negative electrical potential in the lumen.

We intend to give an oral solution containing electrolytes and glucose in an attempt to reverse the flow of water and electrolytes in acute cholera; that is, to stop the diarrhea. The concentration of glucose to be used will be chosen on the presumption than an effect on absorption is desired along a large segment of the small intestine and that this concentration should be high enough to be effective in the face of dilution in gut fluids, yet not too high to have significant osmotic effect. This concentration is not yet known.

In addition to the measurement of stool output as a measure of effectiveness of therapy, a non-absorbable marker, polyethylene glycol (PEG) will be added to each liter of infusion fluid; the changes in concentration of this marker will reveal shifts of fluid and electrolytes over the entire gut (net flux).

Method and Procedures

An actively purging adult will be selected for study (generally one who has purged more than 500 cc/hour in the first full eight hour period in the hospital). The patient will be properly hydrated and brought into electrolyte balance in the usual way (this will require 2-4 hours after admission). Only patients who have put out urine before the start of the glucose lavage will be studied. A heparinized 23 gauge scalp vein needle will be placed in the radial artery.

There will be five study periods, each 16 hours long. The first, third and fifth periods will be control periods; the second and fourth periods will be study periods. A warmed isotonic solution composed of five grams of sodium chloride, four grams of sodium bicarbonate and one half

gram of potassium chloride (5/4½) with 1% PEG will be lavaged into the stomach at the rate of one liter per hour (by infusion pump) in the control periods. In the study periods ten grams of glucose per liter will be added. (This concentration of glucose may be changed as results of the preceding study are known).

Arterial blood samples for pH, electrolyte concentrations, glucose, plasma protein, specific gravity of plasma and hematocrit will be taken initially and after every four hours. Stool samples for measurement of electrolytes, glucose, pH and PEG will be taken every four hours. Quantitative vibrio counts will be done once in each study period. Urines will also be measured four hourly for electrolyte concentration. Intake and output will be followed four hourly; the patient will be weighed every eight hours. Vital signs will be taken every six hours. The principal investigator and a nurse will be present throughout the study.

The need for intravenous fluids will be maintained by the usual criteria of pulse volume, urine output, measurements of plasma specific gravity plus electrolytes plasma. One patient only will be studied fortnightly. Four to six patients may be sufficient to provide specific information.

Significance of Study

If a patient with cholera can be treated safely with an oral electrolyte solution by addition of glucose, this could profoundly affect the treatment of cholera in rural areas where intravenous fluids are scarce and forbiddingly dear and where treatment centers are hours away.

Facilities Available

The Clinical Research, Clinical Laboratories and Bacteriology Section are currently performing all analyses needed in this study. Care of the patient will be attended to by the principal investigator.

Facilities Required

No unusual or expensive new facilities are needed. Three chemistry, and three bacteriology technicians and three nurses per day will be needed for this project (eight hour shifts).

Organizational Framework - See above.

Work Already Done in this Area

In studies in cholera patients with multilumen tubes and a non-absorbable marker, the infusion of a 1% glucose solution in 5/4/½ has caused a shift of the net fluxes of sodium ion and water from the lumen to the plasma in small intestinal segments. The optimum concentration of glucose at the site of action is about 16 mM/L.

INTESTINAL ELECTROLYTE AND WATER FLUXES AND TRANSMUCOSAL POTENTIALS IN ACUTE CHOLERA

Dr. J. O. Taylor & Dr. D. B. Sachar

I. RESEARCH PLAN:

A. Introduction and Specific Aims:

It has been shown by Greenough and confirmed by us that the occlusion of the intestinal lumen just distal to the ligament of Treitz with the balloon of a Miller-Abbot tube markedly decreases and sometimes stops the output of cholera stool in an actively purging patient, and at the same time volumes of fluid equal to the previous volume of cholera stool can be aspirated from above the occluding balloon. This observation suggests the possibility that the major volume of the cholera stool may be contributed by the duodenum or possibly by the pancreas and liver through the ampulla of Vater. It has also been shown that the concentration of electrolytes in the aspirate from the duodenum is much closer to the concentrations of electrolytes in plasma than to those in the cholera stool. This suggests that the larger amounts of potassium and bicarbonate found in the cholera stool are added below the ligament of Treitz.

Phillips and associates found in Manila that oral glucose reduced the volume of stool output in active cholera patients. In vitro studies have shown that glucose both enhances the active transport of sodium and causes a marked change in potential difference across the membranes in which this enhancement of sodium transport is taking place.

A potent new tool to study the movement of water and electrolytes into and out of the gut lumen was introduced by Fordtrand with the development of intestinal perfusion techniques using non-absorbable markers. These techniques depend on measuring the change in concentration of a non-absorbable marker over a study segment defined by two collection ports on a multilumen tube within the intestinal lumen. By infusing a solution containing the marker at a constant rate, it is possible to calculate the movement of water into or out of the study segment. Knowing the amount of water entering or leaving the study segment and the change in electrolyte concentrations across the study segment, it is then possible to calculate the net fluxes of the various electrolytes into or out of the study segment.

A method of measuring the electrical potential across the gut mucosa is now available (see the attached description of this method).

The specific aims of this study are to use these methods of intestinal perfusion and transmucosal electrical potential measurement:

1. to study the contributions of the various levels of the gut to the volume and electrolyte composition of the cholera stool and to compare this to their function in convalescence,
2. to study the electrical potentials of the various levels of the gut in active cholera and to compare these to the potentials of the same levels in convalescence.

3. to study the effect of glucose on the net fluxes of water and electrolytes in the various levels of the gut in acute cholera and to compare this with the effect of glucose on these fluxes in convalescence.

4. to study the effect of glucose on electrical potentials in the various levels of the gut in acute cholera and to compare these with the effects of glucose in the same levels in convalescence.

It is planned that in the near future isotopes such as Na^{22}, Na^{24}, and tritium may be used in the study to determine the unidirectional as well as the net fluxes of sodium and water at each of these levels.

B. Method of Procedures:

1. Intestinal perfusion techniques: A specially constructed multilumen polyvinyl tube is prepared for each study patient. The tube consists of four lumens with an infusion port, a 10 cm mixing segment, a proximal collection port, a 20 cm study segment, distal collection port, and finally an inflatable balloon (see Fig.1).

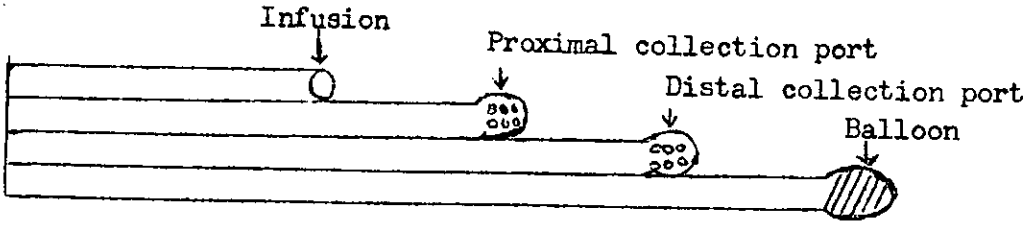

FIG.1

Two cc of mercury are placed in the balloon and the tube is swallowed by an actively purging cholera patient. The patient is placed on his right side, and as soon as the mercury bag has passed the pylorus, it is inflated with 35 cc of water and the balloon tube is clamped. When the tube has moved into the desired position, an X-ray is taken to verify the location of the tube. The balloon is deflated and samples of the intestinal juice at the proximal and distal collection parts are taken and analyzed for electrolyte composition. An infusion solution of the same electrolyte composition is prepared, and to this solution are added three non-absorbable intestinal markers: polyethylene glycol (PEG) of average molecular weight 4000, sulphobromophthalein (BSP), and $Cr^{51}Cl_3$. The PEG is determined by the turbidometric method of Hyden. The BSP is measured by colorometric methods. Chromium 51 is measured by a gamma type well counter.

The solution is infused at a fixed rate using a constant infusion pump. After an equilibration period of one hour, a bolus of another marker, such as FSP, is given rapidly through the infusion tube. Multiple collections are then made from both ports, a dye dilution curve is drawn, and the transit times to each port and across the segment are calculated. With the segment in steady state, collections are begun from the first port at a constant known rate (e.g. 1 ml/min). After an interval equal to the mean transit time across the segment, collections are begun from the second port. Samples are collected from each port for an hour and triplicate

determinations of each parameter being measured are done on samples from the pooled collections. The following are determined: PEG, BSP, Cr51, glucose, sodium, potassium, chloride, osmolarity, and pH. Transmucosal electrical potentials are measured following the collection of the samples.

Then 1% glucose is added to the solution being infused and the same study is repeated. Following this, a solution containing no glucose is again infused and the study is again repeated.

At intervals during the study, blood samples are drawn to monitor hematocrit, plasma protein, plasma specific gravity, plasma electrolytes, and osmolarity. The patient is weighed at the beginning and end of the study. The position of the study tube is again verified by X-ray at the end of the study.

At the end of the acute study the study tube is removed. After ten to twelve days, when the patient has made a full clinical recovery, the entire study is repeated. The tube is again placed in the same position as during the acute study, the composition of the infusion fluid is kept the same, and an attempt is made to adjust the infusion rate so that the rate of flow entering the study segment is also the same as it was during the acute study.

Water and electrolyte fluxes and mean electrical potentials are calculated for each of the study periods.

It is planned that studies will be done across the duodenum, the proximal small bowel, distal small bowel, and colon. It is hoped that by doing enough studies at each level of the bowel, it will be possible to determine the average contribution of each level of the bowel to the volume and composition of the cholera stool and to correlate these results with the electrical potential results from the same levels.

C. Significance of this Research:

The information obtained from this study will, hopefully, be of value in the elucidation of the pathophysiology of cholera. Boththe anatomic location of the defect in the gut in cholera and the type of alteration of fluxes of electrolytes and water that occur in acute cholera will be of value in attempting to understand the mechanism of production of stool in cholera. The changes in electrical potential in cholera will give additional clues to the mechanisms of ion movement involved. The effect of glucose in stimulating net absorbtion of water and sodium in cholera and in changing the electrical potentials across the gut give further indication of which transport pathways are defective and which are intact in cholera.

The determination of the magnitude and anatomic location of the enhancement of net sodium and water absorbtion by glucose has therapeutic implications in the eventual development of an oral therapy of cholera with glucose-containing solutions.

D. & E. Facilities Available and Facilities Required

All of the facilities required for the above study are now available at the Pakistan-SEATO Cholera Research Laboratory. There is an ample supply of acutely ill cholera patients. There is a clinical chemistry laboratory which is able to determine electrolytes by the most modern methods (sodium and potassium by flame photometry on a Baird Atomic Flame Photometer, Chlorides on a Cotlove Chloridometer, Co_2s on a VanSlyke apparatus). PEGs and BSPs are done on a Unicam Spectrophotometer, Cr^{51} on a Picker well type gamma counter. Polyvinyl tubing and tetrahydrofuran solvent are available. Two Bowman type constant infusion pumps are available. A radiometer potentiometer is now set up for these studies.

Na^{22} is on order from the U.K. and negotiations are under way for the procurement of Na^{24} from either Rawalpindi or Bangkok. No specific arrangements have yet been undertaken for the acquisition or measurement of tritium.

F. Organization Framework

The measurement of fluxes is the chief responsibility of Dr. Taylor; the measurement of electrical potentials is the chief responsibility of Dr. Sachar. There is close cooperation in the meshing of these measurements and in the design of the overall study. The chemical determinations are supervised by Dr. Ruth Hare. The flux calculations are done by a qualified statistician, Mr. Alauddin Chowdhury, under the supervision of Dr. Taylor.

II PRELIMINARY RESULTS AND PROGRESS REPORT

At the time of writing this report (November 25, 1966), we have done 12 studies: 6 trans-intestinal studies (3 acute and 3 convalescent), and 6 single segment studies (3 acute jejunal studies, 2 convalescent jejunal studies, and 1 acute jejunal study). The data from these studies has not yet been completely analyzed but it is possible to make certain tentative conclusions.

Only three acute single segment studies of the jejunum have been fully analyzed at this time. The results from these three studies are all very similar. Without glucose in the infusion solution there is a loss of fluid and electrolytes into the 20 cm study segment in the jejunum. When 1% glucose is added to the infusion solution, there is a significant net flux of water and electrolytes out of the lumen. This change in flux is accompanied by a marked increase in negative potential. When glucose is stopped, both the potential and the fluxes revert to the pre-glucose state. No other firm conclusions can be made from the data presently in hand.

The major problem so far has been in obtaining a truly steady state as defined by reproducibility of measurements of marker concentration during the study period. Dr. George Whalen has made some very valuable suggestions for improving those measurements and getting a better approximation of true steady state. His suggestions have been incorporated into this protocol.

PRELIMINARY RESEARCH REPORT

A Technique for the Measurement of Transmural Electric Potential in the Intact Human Intestine

David B. Sachar, M.D. and Jnan Ranjan Saha, M.Sc.

November 1966

Introduction

The nature of ion transport across a biological membrane may be reflected by an electrical potential gradient generated across that membrane. For this reason, there has been considerable interest in the measurement of transmural electrical potential differences in various species of intestine. Using this technique in animal intestinal loops in vivo, and in diverse preparations of animal and human intestine in vitro, a number of intestigators have demonstrated that the small intestine maintains a slight but consistent transmural electrical potential gradient, mucosal surface generally negative with respect to serosal surface. This potential difference is thought by most workers to be attributable chiefly to the active transport of positively charged sodium ions from the mucosal to the serosal side of the intestine.

It has also been shown in a number of different preparations that actively transported hexoses and amino acids, when added to the mucosal medium, enhance this potential gradient, possibly by stimulation of the sodium pumping mechanism. This enhancement is produced by any actively transported sugar or amino acid, regardless of whether or not it is metabolized by intestinal epithelium. Sugars and amino acids that are not actively transported, however, invariably fail to elicit this response.

Such measurements of transmural electrical potential have greatly advanced our understanding of the mechanisms of ion transport, but to our knowledge they have never been carried out in the intact human intestine for the purpose of studying ion transport. We have therefore devised a system for the quantitative determination of the transmural electrical potential in the human intestinal tract, and we have measured the electrical responses to a variety of experimental conditions. *

Methods

The mucosal electrode consists simply of a saline-filled polyvinyl tube introduced into the intestinal lumen. It is connected through a saline reservoir and a thin polyethylene agar-saline bridge to a calomel electrode in a beaker of 3.8 M KCl.

The choice of an appropriate site for the "serosal" electrode is crucial to any quantitative measurement of the transmural potential. Pursuing the suggestion of other investigators, we have confirmed that the intestinal serosa, peritoneal cavity, subcutaneous tissue, and intravascular space are all consistently equipotential with each other, and with the lightly abraded skin of the forearm. Consequently, we have used the abraded forearm skin as a stable, convenient, neutral site for the "serosal," or reference electrode.

An agar-saline polyethylene bridge, taped under saline-soaked cotton to the abraded skin, leads directly to another calomel electrode in 3.8 M KCl. The calomel

*Since this work was undertaken, we have learned that very similar studies are being done by K. H. Soergel et al in Milwaukee. We are presently in active collaboration with G. E. Whelen of his group.

electrodes, in turn, lead to a high-input impedance compensating-type potentiometer (Radiometer pH Meter 4, Radiometer Co., Copenhagen). A 0.2 microfarad capacitor is connected across the electrodes just before they enter the potentiometer, in order to eliminate AC interference, to dampen out endogenous high-frequency fluctuations within the subject, and to promote the undisturbed measurement of baseline DC potentials within the system.

The subject must be meticulously insulated from ground, and metal connections should be eliminated from any part of the system where they could generate artefactual junction potentials. Electrode potential differences across the entire system are invariably less than 1 mV., and usually less than 0.2 mV. The system is represented schematically in Figure 1.

Experimental subjects were healthy volunteers, all young adult male Bengalis who had made full clinical recoveries from attacks of mild gastroenteritis.

Results

Measurements of intestinal potentials by this technique in our human subjects have confirmed most of the findings of other investigators' animal and in vitro studies. The small intestine maintains slight but steady transmucosal potentials usually in the neighborhood of zero, but occasionally in the order of a few millivolts. The sign and magnitude of the potential vary somewhat from subject to subject and from day to day. Perfusion of the lumen with physiologic electrolyte solutions at rates up to 1500 ml. per hour does not seem substantially to alter this potential.

At all levels of the small intestine, however, there is a dramatic and immediate increase in the potential difference upon the addition of an actively transported sugar to the perfusing solution. The lumen becomes markedly more negative with respect to the serosa, with the potential gradient often increasing more than 10 mV. This effect occurs with actively transported sugars such as glucose, which is metabolized by the intestinal epithelium, and galactose, which is not; but it is not seen with non-actively transported sugars such as fructose (which is also metabolized), mannose, or xylose.

Moreover, there is a close and consistent relationship between the concentration of the sugar in the perfusing solution and the magnitude of the increase in potential difference. This correlation is reflected in Figure 2, which shows the results of a representative experiment in the distal ileum.

Figure 3 shows a similar response to galactose in the jejunum, this time plotting electric potential response against concentration of galactose infused (10 centimeters proximal to measuring point).*

This potentiating effect is rapidly reversible and repeatedly demonstrable, as shown in Figure 4A, which depicts a representative experiment in the distal ileum, with infusion 20 cm. proximal to the measuring site. Figure 4B shows an even more prompt response with the infusion 10 cm. proximal to the measuring port.**

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* Note that since Figure 3 plots potential response against infusion concentration instead of against luminal concentration, it cannot be quantitatively compared to Figure 2.

** Note that in Figure 4B the responses to galactose and glucose <u>can</u> be quantitatively compared, and that they are quite similar.

Together with most other investigators, we believe that this enhancement of electrical potential by actively transported sugars represents a stimulation of active sodium transport. Electrical potential, however, as distinguished from short-circuit current, cannot differentiate between active and passive ion transport, and it should therefore be possible with our technique to demonstrate diffusion potentials across the intestinal wall (assuming, as current theory holds, that the intestinal epithelium allows across itself a faster movement of cations than of anions).

For example, infusion of a hypotonic solution into the lumen should create a concentration gradient favoring the influx of sodium (faster than the influx of chloride). This would allow the development of a positive potential in the lumen. As figure 5A shows from a representative experiment in the ileum, this is precisely what occurs.

Similarly, adding to the lumen a non-absorbable solute such as mannitol should create an osmotic gradient which, with the influx of water, will also produce a concentration gradient favoring the influx of sodium. This too would allow the development of a positive potential in the lumen. Figure 5B demonstrates that this is indeed what occurs.

It is of note that the responses to water and mannitol are very much the same, even though the directions of bulk water flow are presumably opposite to each other in the two experiments. This suggests that the observed electric potential changes are independent of fluid movement or "solvent drag". On the other hand, we have by no means attempted to prove that the electrical potential is primarily attributable to the movement of sodium ions; we have merely tried to show that our measuring system is capable of demonstrating certain basic phenomena in common with other systems using animal and in vitro preparations.

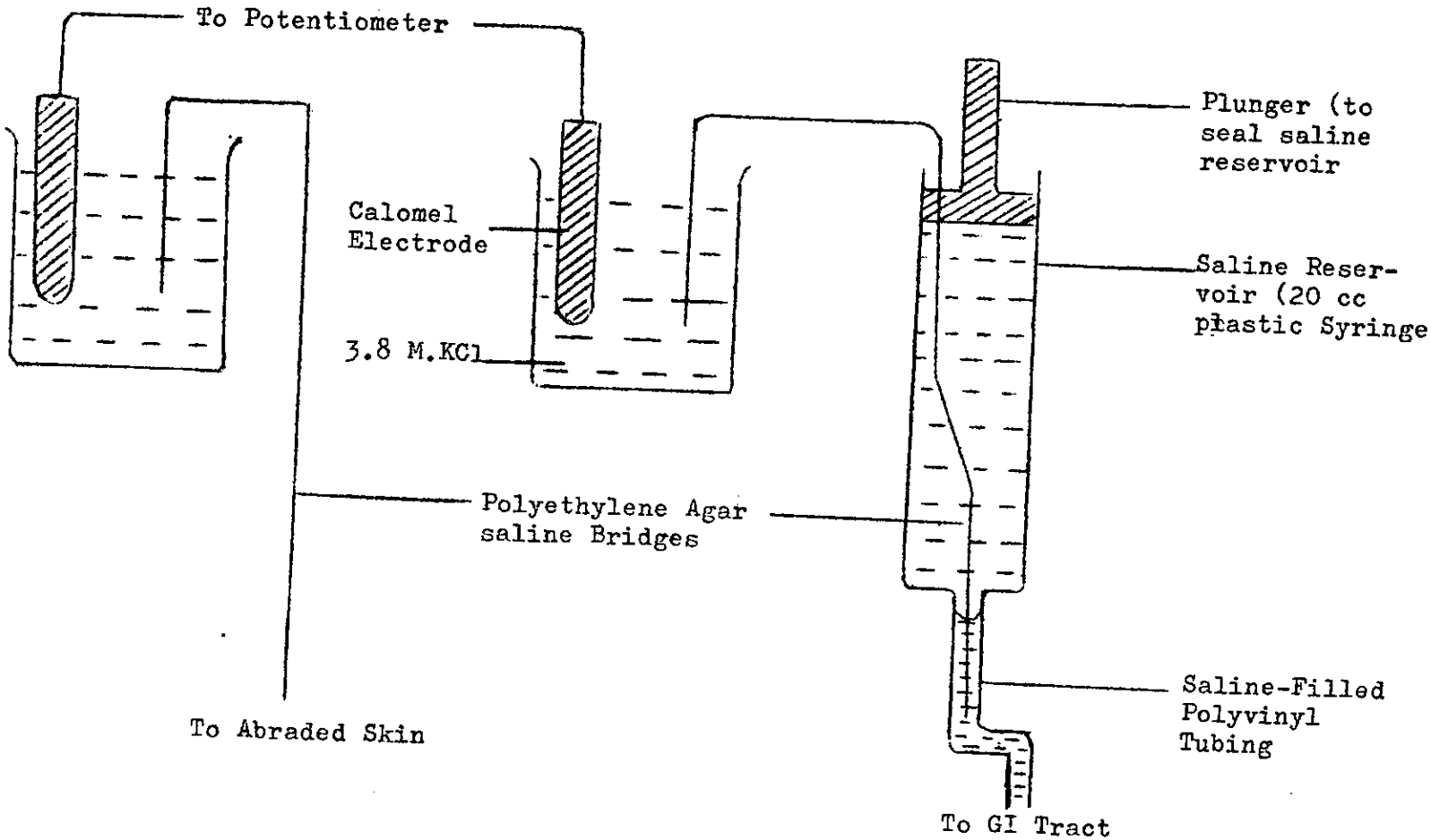
Summary and Conclusions

We have described a technique for the measurement of transmural electric potential difference in the intact human intestine, and have hypothesized but not proved that this potential reflects primarily the movement of sodium ions across the intestinal wall. With this technique, we have demonstrated that the infusion of actively transported sugars markedly increases the electrical negativity of the intestinal lumen, possibly by stimulation of the sodium pumping mechanism, and independently of their osmatic effects.

The applications of a technique such as we have described could be numerous and diverse. Specifically, we plan in the future to make measurements of electrical potential responses to various other sugars and amino acids singly and in combination. This may provide at least some indirect information concerning the carrier systems for these substances in the human intestine. At present, we are assisting Dr. Taylor in a study utilizing multiple-lumen tubes and non-absorbable markers, attempting to correlate directly the effects of glucose in enhancing the electrical potential with its effects in stimulating sodium absorption across segments of intestine. Of particular relevance to the interests of this Laboratory, we are making these measurements of electrical potentials, and of net water and electrolyte fluxes, and their response to glucose, in cholera patients during the acute phase of their disease and again in convalescence. The design and results of these studies will be the subject of a separate report.

FIG.1

SCHEMATIC DIAGRAM OF
MEASURING SYSTEM



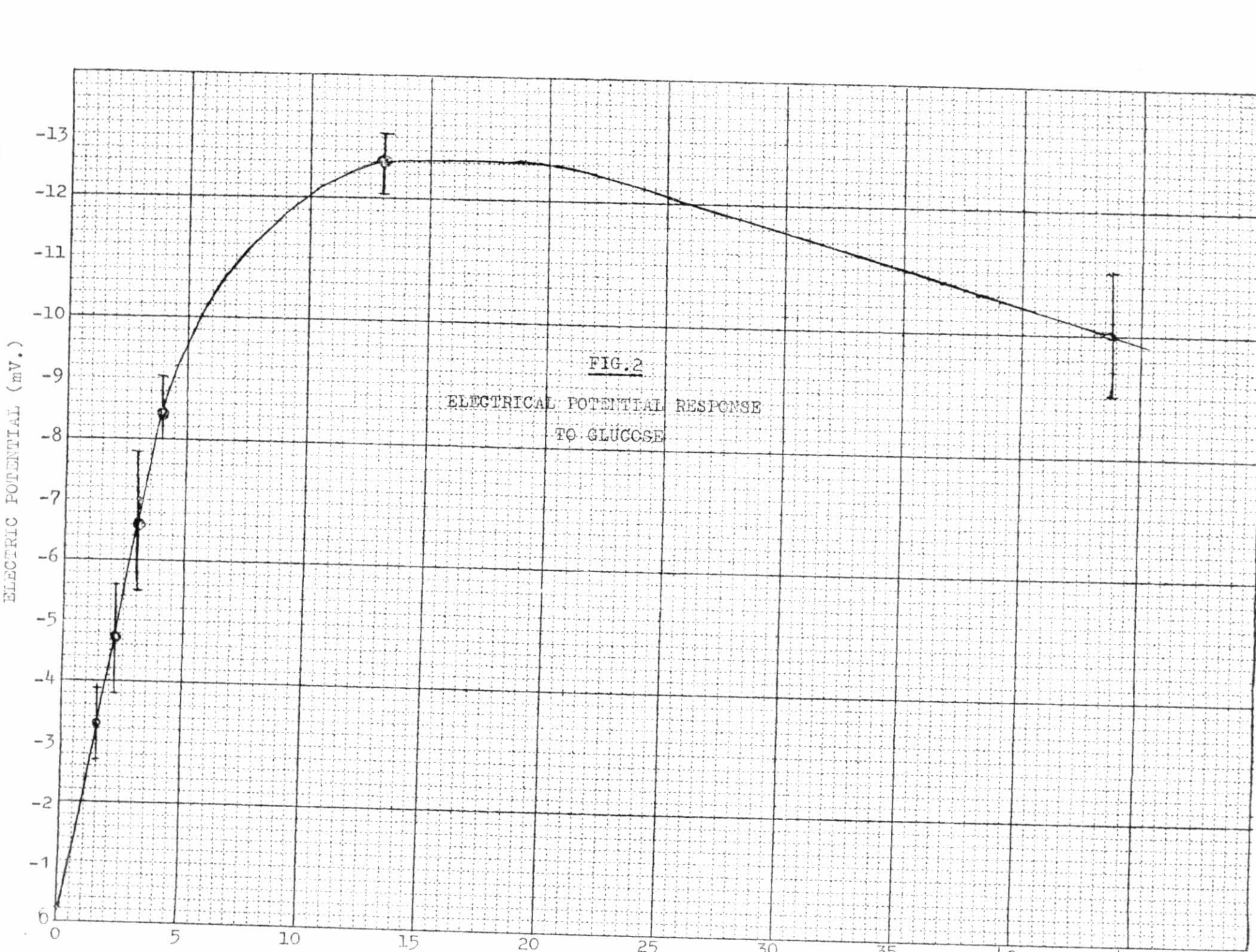


FIG. 2

ELECTRICAL POTENTIAL RESPONSE TO GLUCOSE

FIG. 3

ELECTRICAL POTENTIAL RESPONSE
TO GALACTOSE

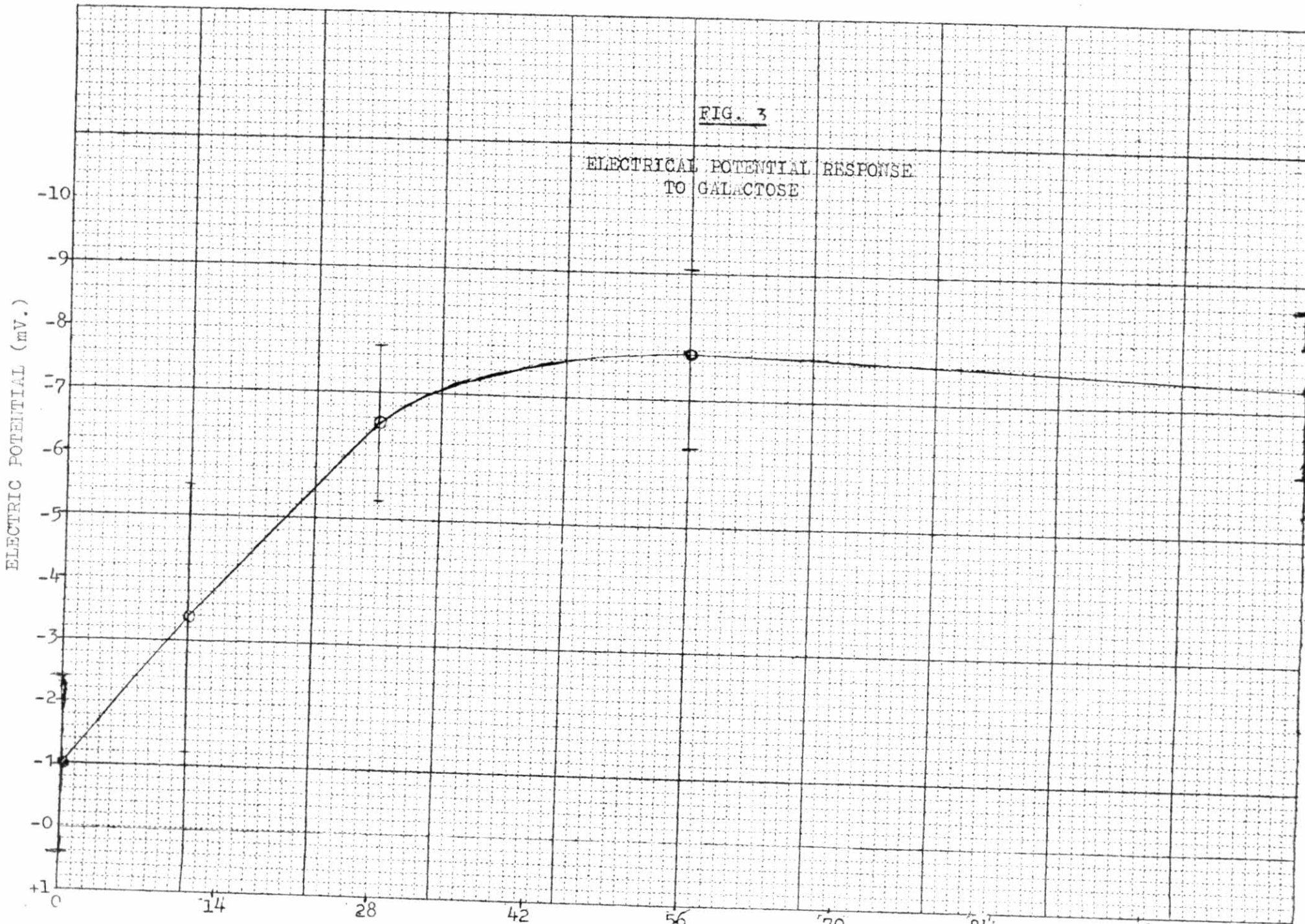
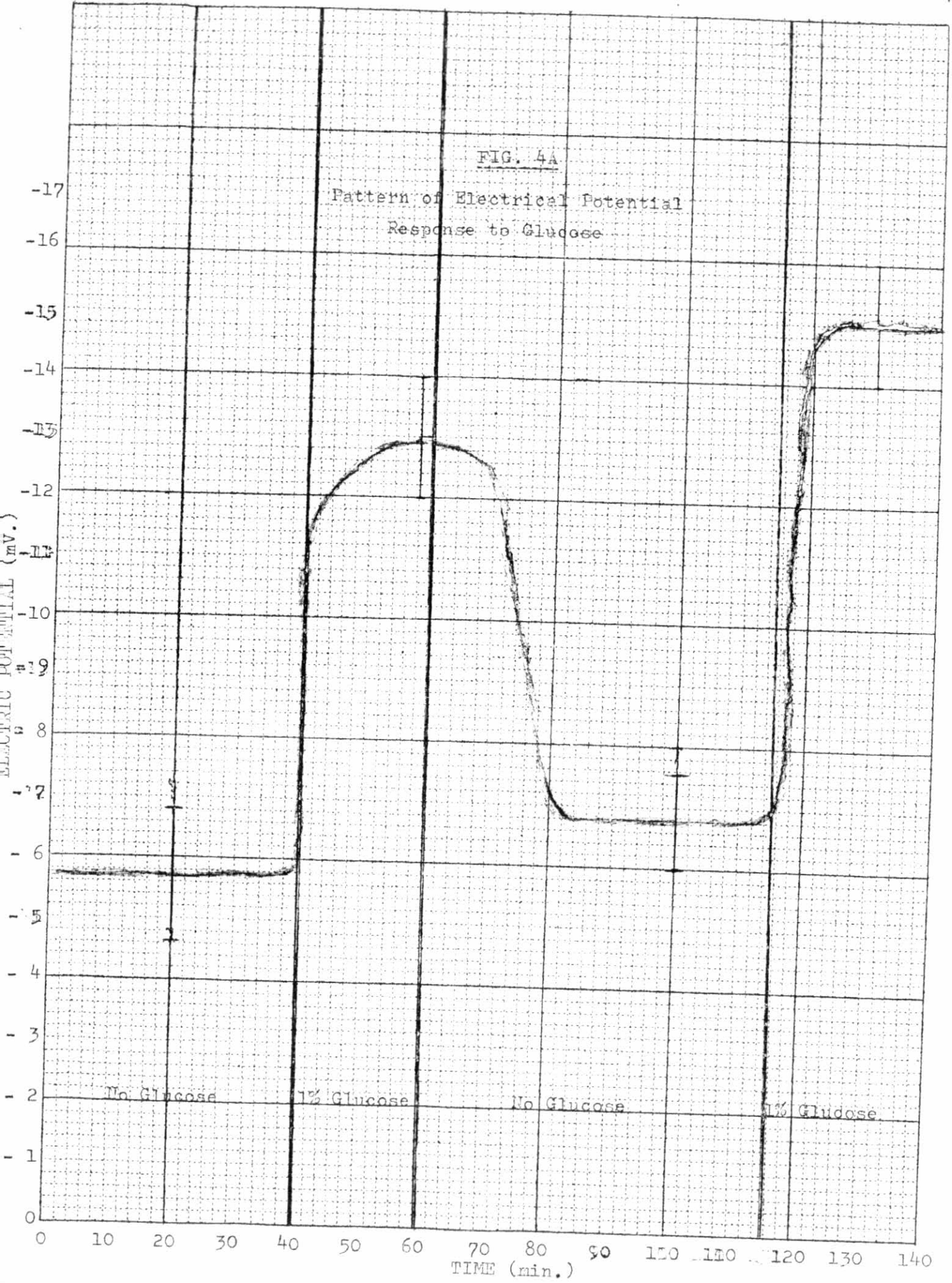


FIG. 4A

Pattern of Electrical Potential Response to Glucose



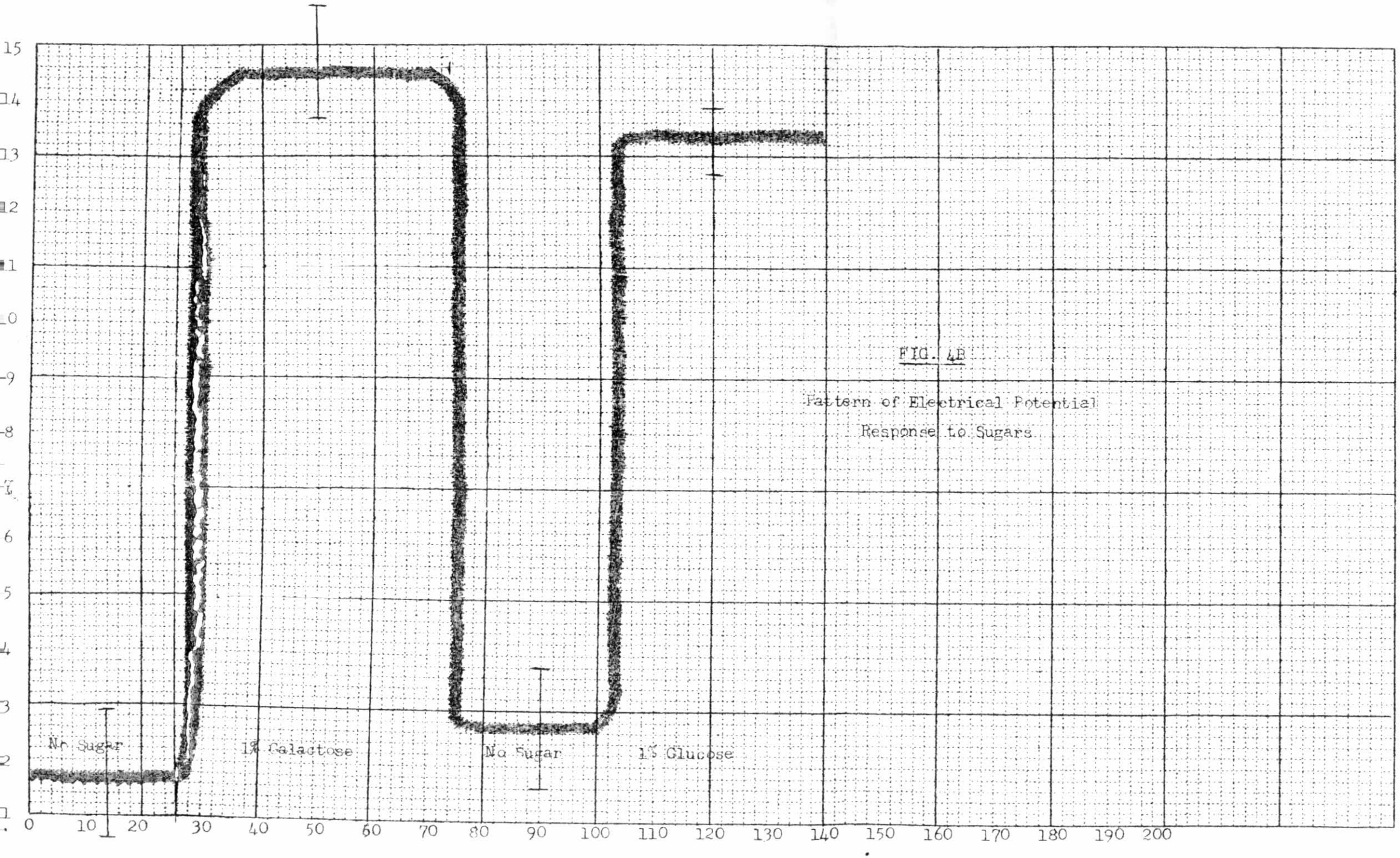


FIG. 4B

Pattern of Electrical Potential Response to Sugars

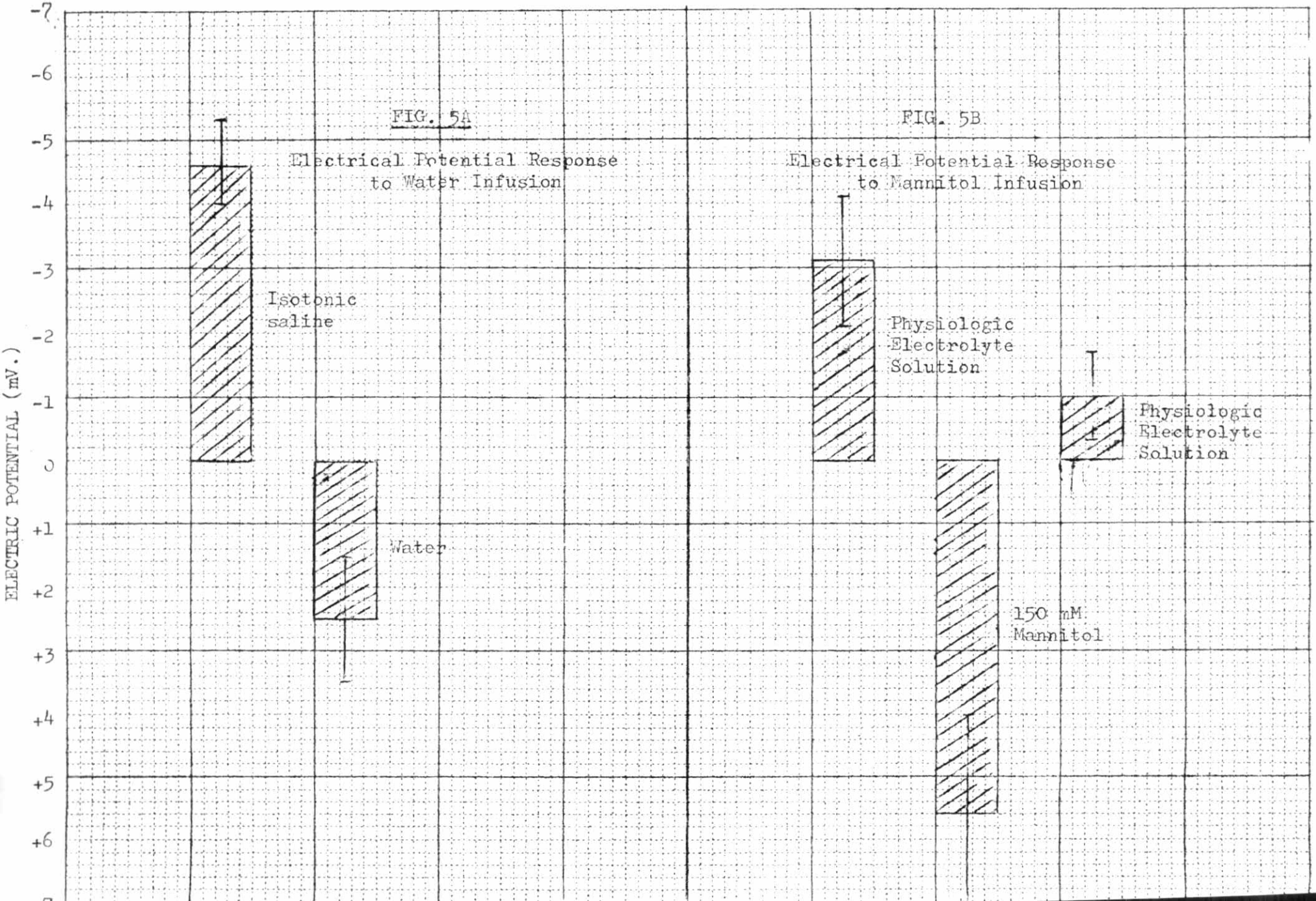


FIG. 5A
Electrical Potential Response to Water Infusion
Isotonic saline
Water
FIG. 5B
Electrical Potential Response to Mannitol Infusion
Physiologic Electrolyte Solution
150 mM Mannitol
Physiologic Electrolyte Solution
ELECTRIC POTENTIAL (mV.)
-7
-6
-5
-4
-3
-2
-1
0
+1
+2
+3
+4
+5
+6

TETRACYCLINE LAVAGE STUDY

R. Northrup, J. Kinzie, R.A. Phillips.

Tetracycline has become well accepted in the treatment of cholera. (1,2,3). It has been demonstrated to reduce significantly the duration and amount of vibrio and stool excretion. Lindenbaum (3) studying patients from the time of admission using various dosages and dosage schedules, found a mean duration of diarrhea in his treated group to be 5.9 8-hour periods, and a mean total stool output of 9.0 liters. His control group in contrast had a mean duration of diarrhea of 12.0 8-hour periods, and a mean total stool output of 21.5 L. Perhaps most important, however, was that his average duration of positive culture following the start of treatment was 2.7 days, slightly longer than the duration of diarrhea. Thus, the bacteria and the diarrhea disappeared together. What, then, might happen of the bacteria could be eliminated completely at a given point of time early in the course of the disease: would the diarrhea stop at the same point?

Other work in progress suggested that the diarrhea would probaly not stop immediately (Craig, pers commun). This work, involving the intraintestinal administration of the supernatant from a culture of vibrio cholerae (Craig's skin toxin, Finklestein choleragen) to a laboratory animal, shows that the diarrhea produced in this way persists for some hours after the toxin is given.

We, then, proposed to investigate this point by administering an agent sufficiently powerful to reduce the vibrio excretion to nothing practically immediately, and then observe the subsequent course of diarrhea. Preliminary in vitro studies with tetracycline were encouraging; a concentration of over 250 ug/ml could reduce a vibrio count of 10^7 to 10^8 organisms (taken immediately after the addition of the antibiotic to the culture) to zero within an hour. Administration of this agent in a large amount of fluid might theoretically enhance its effectiveness, by stretching the gut and exposing more surface to the antibiotic, by delivering the drug to those organisms deeper in the crypts, and by removing dead or immobilized organisms or toxins at a more rapid rate. Patients excreting over 400 cc/hour of stool could be expected to put out practically all of an electrolyte solution with the composition of cholera stool administered orally. Hence we designed our study along these lines.

STUDY DESIGN:

Patients with active vibrio cholera infection were selected for the study. An average output of over 400 cc stool during a nursing period of 8 hours (over 3200 cc during that period) allowed admission to the study, although the overall average stool output during the total time prior to the study may have been in fact less than that amount. Patients were studied generally within 24 hours of admission although one patient was begun after 40 hours in the hospital. A series of random number was prepared for twenty patients and this was used in allocating patients t-

a tetracycline or non-tetracycline lavaged group. Following admission to the study, a control quantitative vibrio count was obtained, and control samples of blood and stool were collected for other determinations. A naso-gastric tube was then passed into the stomach, and a liter of electrolyte solution with an approximate composition of Na 138 mEq/L, K 14mEq/L, Cl 100 mEq/L, and HCO3 52 mEq/L (CRL standard IV solution 5-4-1) was infused within the next hour. During the following hour, in the treated group, 1000 mg tetracycline was added to the next liter of infusate, while in the untreated group the plain electrolyte solution was continued both at a rate of one liter per hour of lavage, 1000cc electrolyte solution with 250 mg tetracycline was infused in the treated group; this was subsequently continued at the rate of 1 liter per hour (group A). Thus a total of 8 hours of lavage and 2500 mg tetracycline in 8 liters of electrolyte solution were administered. The non-tetracycline (group B)received the same amount of lavage but without the drug. Hourly vibrio counts were determined during the study period and weights, bloods, and stools were collected and analyzed frequently during the study to ensure that the patient was maintained in electrolyte balance. Net stool production (total hourly stool minus hourly infusion) was determined during the study, and continuous intravenous therapy with standard CRL 5-4-1 electrolyte solution was given to replace net losses. Tetracycline content of stool, blood, and urine was determined both during and after the study period.

Following the study period, the patient was maintained on oral fluids only for at least 12 hours, following which he was allowed a soft diet (milk, bananas, and bread) until stool stopped. Two-hourly stool output was determined until the cessation of diarrhea, which was defined as the last spontaneously passed liquid stool prior to the first soft or former stool. The quantitative stool vibrio count was also followed at frequent intervals after the cessation of lavage. Serologic antibody response (vibriocidal and agglutinating) was followed in some patients until cessation of stooling.

Laboratory methods were standard except for the vibrio determinations and tetracycline concentration. In performing the quantitative vibrio count, the anus and peri-anal area were cleansed with a disinfectant ("Dettol"), dried thoroughly, and a sterile rectal catheter inserted into the rectum. A 5-10 cc sample of stool was collected through this catheter into a sterile test tube, and rushed to the bacteriology laboratory. There it was immediately diluted in five 10-fold steps in alkaline peptone broth, and 0.1 cc of each dilution plated on each of two plates of Monsur's media. These plates and all tubes of broth used for the dilutions were incubated for 24 hours (or as long as necessary to produce good growth), and the plates were then read. Doubtful colonies were confirmed with type-specific serum by agglutination. Any samples which appeared to be completely negative on the direct plating were then analyzed further. In this the broth dilution tubes were streaked onto fresh plates and incubated overnight as well as a sample from each tube served under the phasecontrast microscope for typical V. cholerae-motility.

Tetracycline concentrations were determined using a biological system, with a culture of *B.* serum as a reference standard. Serial dilutions of the sample to be tested (plasma, urine, or centrigued and micropore-filtered stool) were incubated overnight with a loopful of culture of the reference organism, and the cultures then observed for growth. The inhibitory point was then determined from a reference tetracycline dilution and test.

Other details of the study procedure are as follows: stool samples for electrolyte determination were collected under oil from the rectal catheter previously mentioned. Venous blood samples were collected for hematocrit, plasma protein by both optical density and specific gravity (copper sulfate method of Phillips) methods, Na, K, Cl, and HCO_3 determination (under oil), and tetracycline level. The samples were collected either from a freely flowing brachial venipuncture without the use of a tourniquet, or from a femoral venipuncture. Stool production was measured both by volume and weight furing the lavage period, and by volume alone afterwards, using a 2000 cc graduated column. Other than the hourly or at times half hourly vibrio count stool collections, determinations were performed at the beginning of the study, and after 2,5, & 8 hours of lavage.

Following the initial few patients, after which the effects of lavage alone became relatively obvious, other patients whose stool output qualified them for admission to the study were given an infusion of tetracycline into the stomach in the same dosage-time pattern over a seven hour period, but in a total volume of only one liter of 5-4-1 (group C). Other patients have been given the standard clinical regimen of 250 mg tetracycline every 6 hours until stool stopped. Ancillary laboratory determinations were not done on groups C&D .only stooling pattern was observed.

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TETRACYCLINE LAVAGE STUDY

Northrup & Kinzie and
R.A. Phillips.

Group	Treatment Regimen
A	2500 mg of tetracycline with 8 liters lavage
B	No tetracycline with 8 liter lavage
C	2500 mg tetracycline with 1 liter lavage followed by 250 mg 6 hourly
D	250 mg - No lavage.

Mean hours for Diarrhea to stop

A	34.6 (7)
B	92.0 (4)
C	40.1 (11)
D	39.3 (18)

Bracketed figures indicate number of patients in each group.

B is significantly different from A,C, & D at 1% level of significance. A C D are of same group.

Mean (total) stool vol.(net) during and after study to end of diarrhea

A	5,969.7 (7)
B	26,642.0 (4)
C	9,924.0 (11)
D	10,385.0 (18)

B is significantly different from A, C, & D, at 1% level of significance and A, C, & D are of same group.

PAKISTAN-SEATO CHOLERA RESEARCH LAB.
MOHAKHALI. DACCA.

Hourly stool rate of 7 patients** A Group

A.

Patient #	Pre Study	by 8 hour periods				by 12 hour periods	
		1st Period	2nd Period	3rd Period	4th Period	1st Period	2nd Period
4030	495	62	197	266	112	167	175
4028	323	-1	518	188	125	180	292
4078	555	13	379	155	46	202	162
4377	448	373	230	125	106	355	130
4341	311	207	262	85	45	248	175
4477	495	280	149	55	13	244	76
4438	409	223	404	193	108	364	184
Mean	*482	165	306	152	79	251	171

**Given 2500 mg. tetracycline 8 hourly with 8 liter lavage.
*Weighed mean.

B.

**Patient #	Pre Study	by 8 hour periods				by 12 hour periods	
		1st	2nd	3rd	4th	1st	2nd
4097	520	321	428	544	467	306	611
4145	484	556	447	531	371	555	451
4161	720	308	286	190	175	333	190
4638	425	185	236	180	229	213	226
	*546	342	349	361	311	352	369

*No tetracycline only 8 liter lavage.

C.

No.of Patients		1st	2nd	3rd	4th
11	473	336	331	217	130

S.E. of Means. 63.97

D.

No.of Patients		1st	2nd	3rd	4th
18	503	399	322	228	164

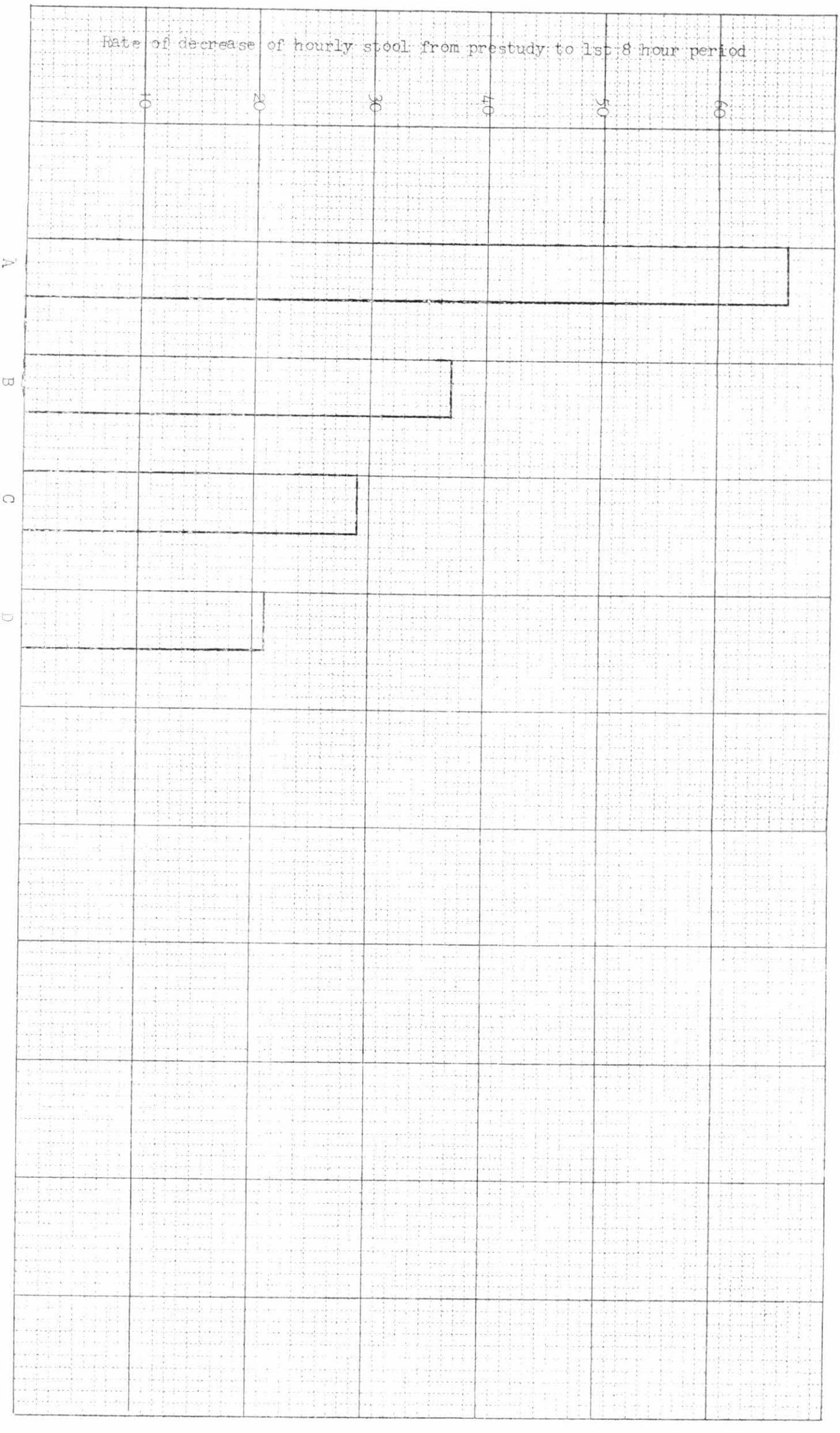
Absolute difference between rates of Pre-study and 1st Period of percentage. 1st and 2nd Period

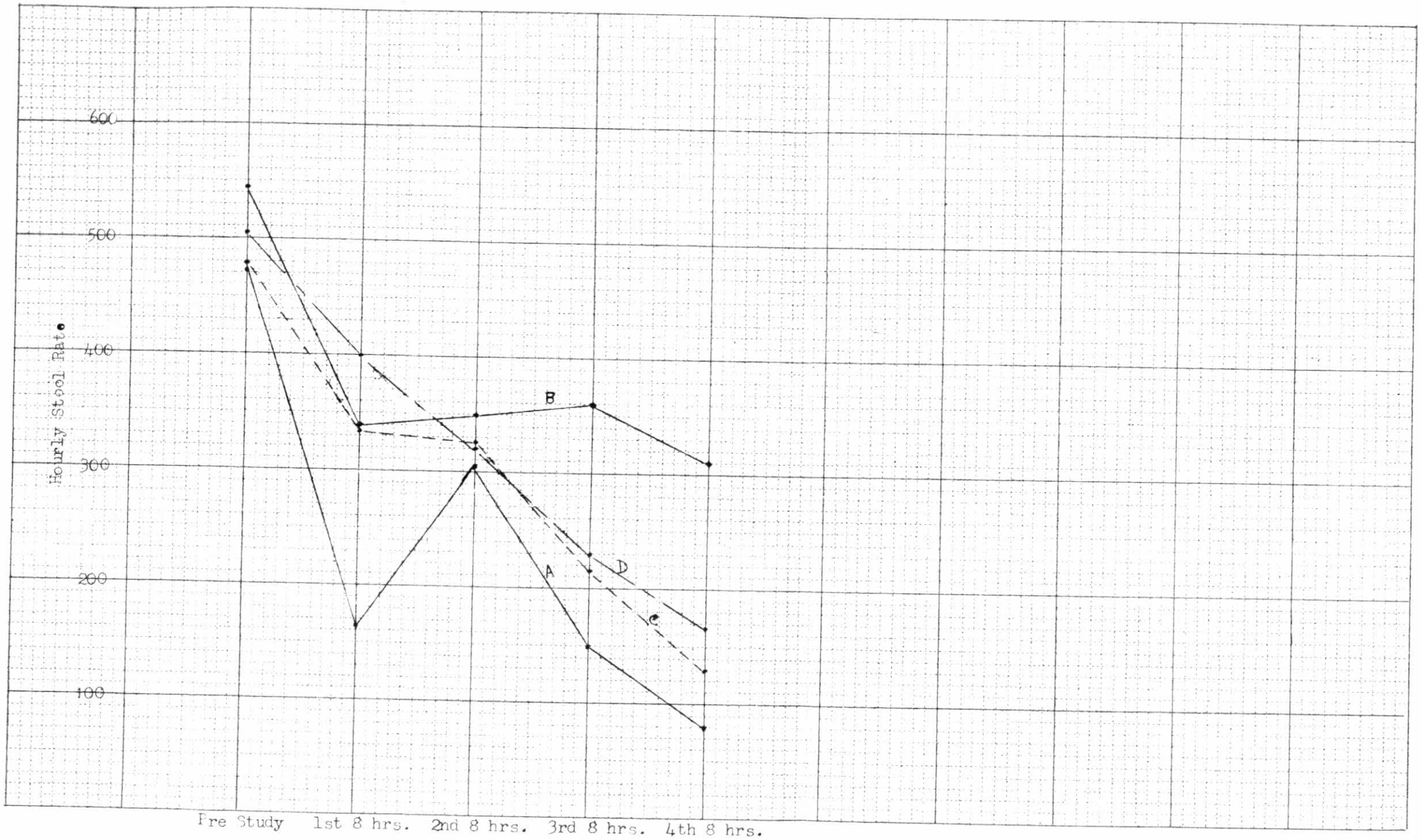
A	317	66%	+141
B	204	37%	+ 7
C	137	29%	- 5
D	104	21%	- 77

HOURLY STOOL RATE

Group	Pre-study	1st 8 hrs.	2nd 8 hrs.	3rd 8 hrs.	4th 8 hrs.
A	——	——	——	——	
B	——	——			
C	——	——		——	
D	——	——		——	

	Pre-Study	1st 12 hrs.	2nd 12 hrs.
A	——	——	
B	——	——	


Rate of decrease of hourly stool from prestudy to 1st 8 hour period
10
20
30
40
50
60
A
B
C
D


600
500
400
300
200
100
Hourly Stool Rate
B
A
D
C
Pre Study
1st 8 hrs.
2nd 8 hrs.
3rd 8 hrs.
4th 8 hrs.

PROTOCOL FOR ANTIBIOTIC STUDY

DR. A. W. KARCHMER AND DR. GEORGE CURLIN

INTRODUCTION

Following the introduction of the concepts of continuous measurement of stool volume and the isotonic replacement of stool fluid and electrolyte losses, the mortality rate in acute cholera has been markedly reduced to less than one percent in both children and adults. Although antibiotic therapy has not further reduced cholera mortality, the treatment of acute cholera with tetracycline and chloramphenicol has reduced the duration of diarrhea, the duration of shedding vibrios, and the intravenous fluid requirement. In addition to reducing the cost per patient of each cholera admission, antibiotic therapy has made the treatment of cholera more practical by reducing the duration of close nursing supervision required and the length of hospitalization necessary.

The effect of antibiotics on the epidemiologic aspects of cholera is less clear. Although clinical trials have shown tetracycline and chloramphenicol to be effective in reducing the length of time V. Cholera can be recovered from the stool, reversion to positive stool cultures after one or more days of negative cultures has occurred in a significant percentage of several dosage schedules. Furthermore, purging following presumed adequate antibiotic therapy and clinical recovery from cholera of biotype EI Tor resulted in the recovery of that organism. This has suggested the presense of carrier states of potential epidemiologic importance.

Oral tetracycline, in doses of 125 mgm. every six hours for patients fifteen kilograms or less and 250 mgm. six hourly for patients weighing more than fifteen kilograms, is considered the drug of choice and is administered from three to five days. Prior studies at the CRL have suggested, although not established, that duration of therapy rather than magnitude of dose may be important in the elimination of bacteriologic relapse in cholera.

Furazolidone, a poorly absorbed nitrofuran derivative, has been shown to be as effective in vitro against classical V. cholera as an equimolar amount of tetracycline. Furthermore, in limited, uncontrolled trials it has been used effectively to reduce diarrhea morbidity in acute cholera. According to The Medical Letter, adverse side effects have been uncommon and minor in nature when furazolidone has been used to treat other acute bacterial diarrheas.

Furazolidone belongs to a family af antibacterial agents to which resistance has not been observed to develop. Consequently, if shown to be safe and effective in the treatment of cholera it will provide an alternate therapeutic agent to which resistance is less likely to develop.

It is proposed to study the effectiveness of furazolidone in cholera at doses of 5 mgm/Kg/day - the currently recommended dose for enteric infections - and an equimolar dose of tetracycline, 10 mgm/Kg/day. These regimens will be compared to a control group of patients, who will receive the same treatment but without the benefit of any antibiotics, and a group of patients receiving tetrocycline in doses at mgm every six hours, the present CRL schedule for patients weighing less than fifteen kilograms. Each of the schedules will be evaluated for effectiveness in reducing diarrhea morbidity, intravenous fluid requirements, the rate of elimination of vibrios from the stool, and the ability to prevent bacteriologic relapse.

EXPERIMENTAL DESIGN

Study Patients:

All children who are able to communicate with the nurses, with an admission weight between 8 and 16 kilograms admitted to the CRL hospital with a presumptive diagnosis of cholera will be included in the study and allocated to one of four therapeutic protocols. Patients in whom the diagnosis of cholera cannot be bacteriologically established will be removed from the study. Beaause concurrent studies are utilizing the majority of adult cholera patients admitted to CRL, this study will be limited to children.

Allocation to a therapeutic protocol will be determined by the day of admission of each patient. Each day will be designated for the application of a specific therapeutic regimen thus forming a daily cohort. Consequently, all admissions qualifying for the study according to the above criteria will be treated with the designated therapeutic regimen for that day.

All therapy excluding antibiotics will be determined by the ward staff according to present procedure. Only antibiotic therapy will be determined by admission into this study.

1. Control Group:

No antibiotic therapy is to be given.

2. High Dose Tetracycline Group:

Each patient will receive 125 mgm of tetracycline p.o.q. 6h.

3. Low Dose Tetracycline Group:

Each patient will receive approximately 10 mgm/kg/day of tetracycline. This total dose will be divided into four oral doses.

A. Patients weighing 8-12 kg will receive 25 mgm tetracycline p.o.q. 6h.

B. Patients weighing 12-16 kg. will receive 35 mgm tetracycline p.o.q.6h.

4. Furazolidone Group:

Patients will receive approximately 5 mgm/kg/day of furazolidone divided into four doses, to be administered orally.

A. Patients weighing 8-12 kg will receive 12.5 mgm p.o.q.6h.

B. Patients weighing 12-16 kg will receive 17.5 mgm p.o.q.6h.

Each patient will be assigned to one of the above groups according to day of admission and to a sub-group where indicated according to admission weight.

Medication should be begun as soon after admission as practical and continued according to the standard 6 hourly schedule for twenty-eight consecutive six hour periods.

Any questions regarding study patients should be addressed to Dr. Curlin or Dr. Karchmer, one or both of whom will see each study patient at least once daily. Persistent vomiting or nausea seemingly out of proportion to the diagnosed illness should be promptly reported to the above physicians. Likewise, any rash occurring following admission should be reported.

Observations and Laboratory Data to be collected:

Observations on Admission:

1. Age, sex, time of admission by day, month, hour.

2. Onset of symptoms in day, month, hour of the day.

3. Clinical history of the presenting illness including symptoms, past history of cholera, past history of cholera vaccination.

4. Physical signs of dehydration including pulse rate and quality, blood pressure, skin turgor, eye condition, and presence of "washer woman hands" and state of consciousness.

5. Admission weight in kilograms and height

6. Laboratory tests:

Hematocrit, plasma protein concentration, whole blood and plasma specific gravity, serum for vibrio agglutinating and cidal antibodies.

7. Bacteriologic data:

rectal swab for direct plating and enrichment broth.
Observations during initial 7 days of hospitalization:

1. Daily rectal swabs for direct plating and enrichment broth.

2. Volume of stool on an eight hourly basis until diarrhea cases.

3. Character of stool including the first solid stool and any relapse into liquid stools following solid stools. Observations are to be made on eight hourly shift basis.

4. Intravenous fluid requirement for each eight hour period.

5. Eight hourly urine output.

6. Daily weight in kilograms.

Observations during second 7 days period of hospitalization:

1. Daily rectal swabs for direct plating and enrichment broth.

2. 9 days after the onset of symptoms early convalescent sera will be secured for vibrios agglutinating and cidal antibodies. Hematocrit, plasma protein concentration, and whole blood and plasma specific gravity will be determined.

3. On the 10th, 12th, and 14th days after admission (following the daily rectal swabbing) each patient will undergo a $MgSO_4$ purge. Liquid stool specimens will be collected for direct and enrichment broth culture for vibrio cholera

Additional Studies:

A. Selected patients will be studied for antibiotic absorption during active cholera. During the second 24 hours of hospitalization, the following collections will be made on these patients:

a) serum for creatinine

b) serum for antibiotic level

c) 24 hr.urine for antibiotic concentration and creatinine concentration.

These collections will be repeated during the final 24 hours of antibiotic therapy.

Assays for antibiotic levels will be set up in the clinical laboratory at a later date. Specimens will be frozen until the assays are available. Furthermore, Eaton Laboratories has offered to perform the assays for furazolidone on split samples of specimens being analyzed at CRL.

B. From each daily group (cohort) one positive stool culture shall be randomly selected and those vibrio organisms examined quantitatively for sensitivity to tetracycline and furazolidone. The final positive culture for each of the patients who relapses or has positive culture longer than 3 days shall likewise be examined for antibiotic sensitivity.

PROTOCOL

Cathartic Purgation of Convalescent Cholera Patients.

by:
Dr. N. Hirschhorn, Dr. Mojid Mollah and CRL Ward Staff.

Purpose:

The existence of a prolonged carrier state in cholera has often been postulated to play a role in the dissemination of the disease. While positive stool cultures are known to exist in many convalescent patients from one to two weeks after onset of illness, prolonged positivity is a rare occurrence although its incidence is not known.

It may be reasonably suspected that in both the convalescent and prolonged carrier states the vibrio organisms reside in the upper small intestine and possibly in the gall bladder. To test this hypothesis Gangarosa (1965, unpublished) gave magnesium sulphate by mouth to convalescent cholera patients with negative stools and showed that the stools turned positive in 8 out of 38 patients from 2 to 6 weeks after onset of illness. Three patients had received no antibiotics. In one patient tested, the bile was positive after magnesium sulphate.

A similar, also unpublished, study by Wallace in Calcutta (1965) showed no positivity in 25 convalescent patients after administration of cholecystokinin.

We propose to perform this study in Dacca utilising castor oil, or magnesium sulphate which are both cholegogues and produce diarrhea.

Material and Methods:

Patients: adults and children convalescent from cholera, willing to cooperate will be chosen. Both antibiotic and non-antibiotic treated patients will be used.

Procedure:

After three days of negative direct and enrichment cultures obtained by rectal swab and stool culture the patient will be admitted to the study. A Crosby-Kugler capsule without the knife block or dome will be passed into the third portion of the duodenum (confirmed by x-ray) and a pre-purgative aspirate taken for culture. Then 50 cc (200 cc in children) of caster oil will be administered by mouth or stomach tube and aspirations of bile will be made every 15 minutes for two hours and cultured. As stool are passed the last portion of each stool will be taken for culture as will a rectal swab.

Bacteriology of purged stool and jejunal aspirate:

1. Homogenize last portion of bowel movement
2. Streak stool heavily on GA, TTGA
3. Plate 0.1 ml portions of the stool and also of 10^{-2}, 10^{-4}, 10^{-6}, and 10^{-8} dilutions in T_1N_1 for actual vibrio count.
4. Make stool (1ml) enrichments in 100 ml alkaline peptone water and 100 ml bile peptone. After suitable incubations streak on GA, TTGA
5. Plate 0.1 ml portions and also 10^{-2}, 10^{-4}, 10^{-6} dilutions of the jejunal aspirate on GA and TTGA.
6. Make aspirate (1 ml) enrichments in 100 ml alkaline peptone water and 100 ml bile peptone. Streak on GA and TTGA after suitable incubation.
7. Several experiments will be done to detect any inhibitory effect of castor oil and rinocileic acid on vibrio growth.

The experiment will be repeated at appropriate intervals in patients whose cultures turn positive.

One nurse, one staff doctor, one attending doctor, and the bacteriology section will be required to staff the project.

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One nurse, one staff doctor, one attending doctor, and the bacteriology section will be required to staff the project.

PROTOCOL

The Effect of Hyperimmune Plasma on the Clinical Course of Acute Cholera

David B. Sachar, M.D. and Z. Hoque, M. B. B. S.

November 1966

Introduction

It has been demonstrated that circulating vibriocidal and agglutinating antibodies are correlated with a significant level of immunity against infection by Vibrio Cholerae, but it is still unknown whether or not antibodies play any role in promoting clinical recovery from the disease once it has been contracted. To be sure, circulating antibodies first appear about the fourth or fifth day of the disease, at roughly the time that the diarrhea ceases, but the fact remains that this apparent relationship might be purely coincidental.

Information concerning the role of antibody in modifying the clinical course of cholera is being sought at this Laboratory by Dr. McCormack, who is comparing the severity of the disease in vaccinated and unvaccinated patients. In a more direct effort to establish the role of antibody in promoting recovery from the disease, we have administered hyperimmune cholera convalescent plasma to a number of acute cholera patients, and have carefully observed the subsequent clinical course.

Methods

Hyperimmune Plasma Collection. 450 cc of whole blood are collected under sterile conditions in citrated blood packs (Fenwal) from healthy volunteer cholera convalescent patients of known blood group and type, with negative VDRL's. The collections are made on approximately the 10th day from onset of illness. The red blood cells settle by sedimentation in a refrigerator overnight, the plasma is aseptically separated into a plasma pack (Fenwal), and the packed cells are immediately returned to the donor. The plasma is frozen at -60°C, and vibriocidal, agglutinating, and toxin neutralizing antibodies are measured on a small aliquot.

Selection of Patients for Study. Any patient qualifies for study who purges at least 3.2 liters in either of the first two 8-hour periods following admission, provided that onset was less than 48 hours previously and that no antibiotics had been administered. Once a patient is selected for study, a randomized sealed envelope determines whether he will be a plasma recipient or a control patient. (Selection of intravenous or intraintestinal route of administration of plasma, however, depended upon the

interests of the Laboratory at the particular time of study).

Intravenous Plasma Recipients. Blood is drawn for grouping, typing, crossmatching, and pre-infusion antibody levels. Stool is collected by rectal catheter for quantitative vibrio count, and an aliquot is frozen for measurements of antibody and toxin levels (these latter techniques are currently being developed by Dr. K.M.A. Aziz).

Compatible plasma of the highest available antibody titer is selected, and a Coombs crossmatch is performed in order to be absolute certain of complete compatibility. After several small aliquots have been moved for antibody testing and for long-term storage, the bulk of the plasma (200-250 cc) is administered rapidly intravenously (over 5-10 minutes) to the recipient.

A blood sample is drawn for antibody titer 5 minutes after completion of the transfusion, and blood and stool are collected 4 hours later and again daily for one week. The clinical course is also followed closely. At the end of week, all the blood samples are measured for antibody titers in a single serologic protocol. The stools are processed as mentioned above.

The recipient receives 10 cc of gamma globulin one week and again one month after transfusion, in order to help minimize any risk of viral hepatitis.

Intra-intestinal Plasma Recipients. These patients are handled the same way as the intravenous recipients, except that they receive the plasma, diluted to 1000 cc in 5-4-1 solution, in an 8-hour drip through an intraduodenal tube. Grouping and crossmatching are not performed, but gamma globulin is administered exactly as to the intravenous recipients. (Gel diffusion tests kindly performed by Dr. A. U. Ahmed have confirmed that plasma immunoglobulins can be recovered immunologically intact from the stools of the intraintestinal recipients).

Control Patients. These patients receive no hyperimmune therapy at all, but their stool and blood specimens are processed and their clinical course followed just as though they had been recipients.

Results

To date, 19 patients have been studied. 7 intravenous recipients, 2 intra-intestinal recipients, and 9 controls. Collection and analysis of data are not yet sufficiently complete to be sure whether or not antibody has played any role in clinical recovery from the disease, but it does not seem so far that the clinical course of the plasma recipients has been significantly different from that of the control patients. Comparisons have not yet been corrected, however, for the variable effectiveness of each plasma in raising the antibody levels of the recipient. Data analysis will be completed within a few weeks.

Future Plans

The decision regarding continued study of intravenous plasma will depend upon the results of the pending data analysis. Any further intra-intestinal studies should be based upon a minimum of 36-48 hours of plasma infusion, since with an 8-hour infusion even a dramatic antitoxic effect would probably be marked completely by rapid manufacture of new toxin. Supplies of hyperimmune human convalescent plasma are too limited to permit this approach to the study at present, but Dr. Kabat and Dr. Goodman have recently suggested that hyperimmune antitoxic horse serum could possibly be produced for this purpose. The specific question of whether or not intraluminal antibody is capable of exerting a clinically significant antitoxic effect has obvious implications for future developments in the therapy and prophylaxis against cholera.

Protocol

Therapy of Acute Cholera with Bacteriophage

by:

Dr. K. A. Monsur and Dr. N. Hirschhorn

Current therapy of acute cholera using adequate amounts of intravenous water and electrolytes has decreased mortality to under 1%. Therefore after replacement of initial and continuing fluid losses evaluation of any other therapy must be based on the principal features of the disease, namely total stool volume and duration of diarrhoea. At this writing, tetracycline, one gram a day for three days in divided doses, is the most effective therapy of cholera reducing the mean total stool output from 27 liters in controls to 13 liters, and the duration of diarrhea from 4.5 days in controls to 2.2 days. It would be of great therapeutic and scientific interest to discover and agent that will stop diarrhea sooner and reduce stool volume more than tetracycline.

Bacteriophage could be such an agent when the ratio of phage particles to organism is between 100:1 or 1000:1 a rapid lysis of organisms within a few minutes is seen to take place (Monsur). Lysis of all vibrios in the gut could stop diarrhea quickly. A number of practical problem must be overcome, however.

First, to assure rapid lysis of all vibrios the titer of phage should be two to three logs higher than that of vibrios. In preliminary experiments we have found that vibrio counts may be as high as 10^9 organisms/ml in jejunal aspirates. Therefore the titer of the phage should reach 10^{12} particles/ml. There should also be a mixture of types of phages to reduce considerably the possibility of survival of resistant vibrios.

Second, the gut volume of a cholera patient in the acute state has been estimated by Phillips to average 1123 cc (range 570-1690 cc). Therefore the total volume of phage given at one time should range from two to twenty cc.

Third, since the mean transit time from stomach to rectum in the acute cholera patient has been calculated to be 30.3 minutes (range 14.9 to 54.7 minutes), it may be desirable to provide a second dose of phage ten minutes after the first or give a constant intestinal lavage of high titer phage.

Previous studies on bacteriophage therapy have both claimed success and admitted failure. All studies based results on mortality rates and all were on patients receiving inadequate water and electrolyte therapy. The clinical details of treated and untreated patients where not presented in most studies. The titers of bacteriophage used were not given and measurements of the stool vibrio count were usually not done. We anticipate that our proposed study will correct these defects.

(more)

Therapy of Acute Cholera with Bacteriophage.

Materials and Methods:

To achieve a concentration of phage of 10^{12} particles/ml an ultracentrifuge is required. (The full list of equipment is supplied on a separate sheet). These will have to be purchased and in operating condition before the next epidemic season ten months away.

Partients acutely ill with cholera will be admitted to the study. Treated and untreated cases will be chosen by the same experimental design governing the antibiotic trials of 1964-65 and 1965-66. Phage will be administered directly through a gastric tube. Vibriophage and cholera vibrio titers will be measured in the stool of these patients before and after therapy. Intravenous fluid therapy will be given to all patients. The effectiveness of phage therapy will be determined by the reduction of stool output, duration of diarrhea, and titer count of the vibrio organisms in the stool.

The clinical aspects of the program will be managed by Dr. Hirschhorn and the CRL staff; the preparation of phage will be performed by Dr. Monsur and his staff; the bacteriological and vibriological work will be done both the bacteriological section of CRL and in Dr. Monsur's department.

PROTOCOL

Bile Salts and Bile Pigments in Rice Water Stool

Drs. Irvin H. Rosenberg & Norbert Hirschhorn.

Introduction and Aims:

The effect of cholera on the liver and biliary system and their role in the pathogenesis of cholera has never been clearly defined. Clinicians have often commented on the appearance of green pigment in the stool of cholera patients as a herald of cessation of diarrhea, while the acholic appearance of rice water stool has been variously attributed to spasm of the bile ducts or Spincter of Oddi.

It has also been suggested from this laboratory that the liver, by hypersecretion, can contribute to the copious rice water stool of cholera.

Bile salts are either conjugated (taurcholate, glycoholate) or unconjugated (cholate, deoxycholate). The unconjugated bile salts are created by action of lower gut flora on the conjugated salts. V. Cholerae is not known to deconjugate bile salts. Normally these deconjugated bile salts are reabsorbed in the ileum and reexcreted in the duodenum. In the malsbsorption of bile salts, these salts would be present in the stool but not in duodenal drainage.

During acute cholera, in which the gut is largely populated with V. Cholerae, and where the rice water stool is clear and copious, the appearance of unconjugated bile salts in the stool, especially at the time of color change, may signal the return of activity of other microorganisms and diminution of diarrhea. Failure to find unconjugated bile salts in the duodenal drainage at the time of their presence in the stool would be another measure of malabsorption in cholera diarrhea. The presence or absence of conjugated bile salts and bile pigment in the duodenal aspirate, before and after stimulation by a fatty meal may give information on the relative contribution of the liver to the makeup of the rice water stool.

Method and Procedure:

A patient who is purging clear rice watery stool, vigoriously, will swallow a double lumen tube with a mercury balloon, a distal samping pot in the duodenum and a proximal infusion port in the stomach. Daily, during diarrhea, a 100 cc sample of duodenal content will be aspirated for measure of bile pigment (by the diazo method of Malloy and Evelyn), for quantitative aerobic and anaerobic counts (vibrios and others), and then taken to dryness for measure of bile salts. The latter will be done by Dr. Rosenberg at the Thorndike Memorial Laboratory, Boston, by thin-layer and a gas chromatography. A sample of stool will be taken at the same time and treated similarly. In some patients a fatty meal will be given into the stomach after the initial duodenum aspiration and a duodenal aspirate taken fifteen minutes later. Any changes in color will be noted.

Significance of Study:

We hope to secure further information on the possible role of the liver and biliary system in the pathogenesis of cholera, the absorptive processes in cholera diarrhea; in addition the historical question of the cause of acholic stools may be answered.

Facilities Available:

All facilities of the Clinical Research and Bacteriology Sections are available for the study. One bacteriology and one chemistry technician will be needed. Dr. Rosenberg plans a trip to Dacca early next year and can transport specimens to Boston.

Facilities Required:

No new or unusual facilities are required. About 4-6 patients will be studied.

Organization Framework:

See above.

Work Already Done in This Area:

In this laboratory, BSP given intravenously has been recovered (about 60%) in the stool of patients with acute cholera. Studies with a Miller-Abbot tube have suggested that a considerable amount of fluid in cholera comes from above the Ligament of Treitz. Studies here have also shown widespread malabsorption in cholera and other diarrheas.

PROTOCOL

A Study of Intracellular and Extracellular Composition Changes During the Initial Therapy of Acute Cholera

R. Northrup, J. Kinzie, M. Rahman

The acute rehydration of the severely dehydrated and acidotic cholera patient represnts the extremely rapid correction of an imbalance which has taken some time to develop. Both the time course and the agents used in this rehydration have been disputed over the years, with some investigators favoring isotonic saline and isotonic bicarbonate solution in various combinations, some favoring isotonic saline and hypertonic bicarbonate solutions, some favoring or not favoring the addition of potassium to the regimen. Early workers in the field of cholera also feared the consequence of too rapid rehydration, the most dangerous complication from this being the development of pulmonary edema. At this laboratory we have arrived at a single solution for the routine treatment of the cholera patient, an isotonic electrolyte solution containing 5 grams of sodium chloride, four grams of sodium bicarbonate, and one gram of potassium chloride per liter of H_2O. This study is designed to assess the effect of the rapid infusion of this fluid on the cation, anion, and gas composition of the extracellular and intracellular fluid and expiratory air.

The condition of the cholera patient on admission to the hospital makes any delay in the institution of therapy of more than a very few minutes dangerous. For this reason obtaining a muscle specimen for evaluation of intracellular electrolytes by the usual surgical methods is impossible. The usual chemical analysis of tissue specimens, however, requires a piece of tissue of a size obtainable only by those surgical methods. Accordingly, we plan to use a needle biopsy technique for obtaining the specimen, and analyze it by neutron activation analysis, all according to the methods of Bergastrom (Stockholm, 1962). An accuracy can be obtained in this technique equal to that of chemical analysis.

STUDY DESIGN

Adult patients in a pulseless condition on admission to the hospital ward will be utilized for the study. At the time a study is desired, all three investigators will wait on the ward for the arrival of a suitable patient. On arrival the patient will be rushed to the study room, where one investigator will obtain a blood specimen from the femoral artery, another will obtain a needle muscle biopsy from the outer thigh muscle according to the method of Bergstrom, and the third investigator will begin the rapid intravenous infusion of 5-4-1 solution. At the same time, a member of the ward staff will obtain vital signs and assess the patient clinically. As soon as the IV is running well, that investigator will apply a respiratory gas collecting apparatus (Douglas bag) to the nose and mouth of the patient, and collect a specimen of expired air for subsequent analysis. In the meantime, the investigator who has performed the arterial puncture

will perform the pH at the bed-side, and then distribute the remainder of the specimen partially clotted under oil, and partially in heparin. The investigator who obtained the muscle biopsy will have to rush with it to the electro-balance (Cohn), where using deionized instruments he will divide it into two portions, suspend each on a platinum wire hook and weigh each on the balance at 20 second intervals, determining also by means of stop-watch the total elapsed time from the instant the biopsy was obtained (this procedure enables the exact wet weight of the specimen to be calculated).

During this time, two liters of 5-4-1 will be rapidly infused. Following this, a second arterial blood, muscle, and expiratory gas specimen will be obtained vital signs and clinical re-evaluation performed and the rehydration continued. A third group of simultaneous specimens will be obtained during convalescence. It is to be stressed that the patient will be constantly observed by one of the investigators during the acute rehydration for any signs of clinical degeneration or untoward complication.

Hematocrit, plasma protein, pH, sodium, potassium, chloride, and bicarbonate determinations will be performed on the arterial blood specimens using standard techniques. Expiratory gas analyses will be performed for pCO_2 and PO_2 in the usual manner, using a LLoyd-Haldane gas analysis apparatus familier to one of the investigators (M.R.) on loan from Dacca Medical College. Ventilatory volume will also be measured. Neutron activation analysis will be performed according to the method of Bergstrom in collaboration with the Pakistan Institute of Science and Technology at Rawalpindi, West Pakistan, utilizing their reactor and coounting set-up. The method will be checked at a later date by subsequent analysis of the same muscle specimens using a U.S. reactor.

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PROTOCOL

EXPERIMENTAL CHOLERA IN MONKEYS

Iqbalul Hasan

1. RESEARCH PLAN

A. Introduction & Specific Aims: In view of the fact that the three existing cholera models differ considerably in experimental manipulations and also possibly from human cholera, experiments were started in this laboratory in 1963 to induce cholera in monkeys with simple procedures of oral challenge. Although some monkeys showed clinical symptoms of cholera, the disease could not be induced with any degree of prediction. Recent developments on the culture conditions required for the production of skin reactive toxic extracellular products of Vibrio Cholerae stimulated interest in some investigators to use the same growth conditions for the preparation of challenge material for experimental cholera in laboratory animals. Large numbers of logarithemically growing Vibrio Cholerae cultures in Syncase broth were used by Doctor Carpenter and his co-workers to induce cholera in adult Mongrel dogs. Using this material they were able to produce cholera in 40% of the dogs challenged orally. During the past three weeks a pilot study was done in this laboratory on ten monkeys using the same culture conditions and experimental manipulations. A disease resembling cholera was seen in all monkeys thus challenged with 40% mortality. The electrolyte determination and plasma specific gravity data is not sufficient enough for analysis. An extended study is necessary to define this disease. The main objective of this study will be to establish a cholera model in Rhesus monkeys with all patho-physiological and immunologic changes seen in human cholera.

B. Methods of Procedure: Adult 2-4 kg Rhesus monkeys of both sexes will be fasted for 48 hours. On the day of challenge a gastric tube will be inserted into the stomach and 50 ml. of 6% sodium bicarbonate solution will be instilled. Immediately afterthis, 100 ml of five hour aerated Syncase broth culture will be given by the same tube followed by 100 ml of fresh Syncase broth. All challenged monkeys will be seated in the chair and the urinary bladder will be catheterized to avoid contamination ofurine with stool. Stool volumes will be measured to the tenth of a ml. but electrolytes will be determined only on the stools collected by rectal catheters. A blood sample will be collected before challenge and after the administration of sodium bicarbonate. Electrolytes, specific gravity, vibriocidal and agglutinating antibody titers will be determined to establish any previous unintentional exposure to Vibrio Cholerae antigens. A third sample will be taken after twenty four hours. Sodium, potassium, chloride, carbon dioxide, whole blood and plasma specific gravity and hematocrit will be determined on all blood samples. Cultures for Vibrio Cholerae will be taken until three negative rectal swabs are obtained. Severity of dehydration will be estimated by plasma specific gravity change and the replacement of the losses will be initiated by infusion. After complete rehydration the monkeys will be sacrificed. All segments of the gastrointestinal tract will be cultured for cholera vibrios and tissues will be fixed in zenker's for histology and electron microscopy.

C. Significance of this Research: Rhesus monkeys are available in the Sunderban areas of East Pakistan and are less expensive than any other place where facilities to work on cholera are available. Pilot study on ten monkeys showed somewhat higher infection rate than those reported in Mongrel dogs. It is possible that under proper reconditioning and with modified culture conditions this model would be superior to any other existing cholera models.

Because of the comparatively smaller size of monkeys, infection with toxic products of Vibrio Cholerae would require less amount of purified materials than adult dogs. Long-termed study on pathophysiology and immunology of cholera would be possible to do on monkeys and the results could be more meaningful than any other laboratory animal.

D. Facilities Available: Cholera Research Laboratory has facilities for keeping monkeys for this study. Cages are available to house at least 20 monkeys at a time. One monkey chair is available at the present time on which animal could be seated for study period. Bacteriology and immunology support is available. Blood chemistry support is expected from Clinical Laboratories of CRL. The laboratory has two trained veterinarians for help in handling and care of the animals.

E. Facilities Required: The animal quarters of CRL have been used for housing both uninoculated monkeys and other laboratory animals. There are possibilities that under these conditions some animals would be unintentionally exposed to vibrio antigens. Some arrangement should be made to keep the infective material in isolated room and proper sterilization should be done.

F. Organization Framework: One monkey will be challenged at one time. Blood and stool specimens will be supplied to Clinical Laboratory in proper washed tubes for electrolytes and the Chief of the Section will be notified for possible alterations in the schedule if required. Bacteriology will be done in the Clinical Section since there is no space available in Bacteriology Section for this work. Serology will be done in the routine serology section.

2. WORK DONE ON THE PROJECT

1. CRL Technical Committee Reports 1964

2. CRL Technical Committee Report 1965

3. Honolulu Cholera Research Symposium, 1965 (Personal Publication).

PROTOCOL

Autoradiographical Study of Intestinal Permeability in Acute and Convalescent Cholera Patients

Dr. J. Kinzie, Dr. R. Northrup and Dr. A. Abraham

Recent investigations intothe properties of certain "toxins" associated with V. Cholera have pointed to an abnormal permeability in the intestinal capillary bed as a factor in the origin of the fluid stool in cholera. This altered permeability particularly suspected in the rabbit skin response the infant bunny diarrhea, and in the rabbit ileal loop preparation. Additionally, biopsy specimens from acute cholera patients show dilated, distended lacteals, and it has been suggested that the lymphatics are incapable of handling the amount of fluid extravasated across a leaky capillary membrane. If such increased permeability to electrolyte fluid is present, one would expect an increase in permeability to other molecules as well. We wish to evaluate the magnitude of intestinal capillary permeability to albumin in cholera patients during their acute disease and in convalescence by means of autoradiography.

All patients studied will be adults, either non-lactating, non-pregnant females or males. Each patient will receive a blocking dose of potassium iodide intravenously on the day prior to study, and for several days thereafter. After swallowing a Crosby biopsy capsule, each patient will be given 100 microcuries of I^{131} labeled human albumin intravenously. After allowing 45 minutes for equilibration with the body albumin pool, a jejunal biopsy will be obtained. A repeat biopsy after a repeat dose of tagged albumin will be taken during convalescence, six or seven days after cessation of the diarrhea.

The resulting tissue specimens will be rapidly fixed, cut and placed on microscope slides. A thin layer of photographic emulsion will be spread over the slide, which will be shielded, placed in a refrigerator for eight days, then developed and stained. A qualitative (possibly semi-quantitative) measure of an albumin leak will be made by examination of the slide under light microscopy and counting the number of "grains" representating I^{131} disintergrations in the interstitial space and in the central capillary of the villous.

Suggested protocol for the study of enteric biopsies from cholera patients with the electron microscope.

by :

Dr. Andrew Abraham.

Purpose of the study: To investigate the possible ultrastructural alterations of enteric mucosa by the electrone microscope in the acute and convalescent phases of cholera, which might provide a better understanding of the mechanism of the disease. In the animal model, using cholera challenged guine pig, major changes were noted in the mucosal capillaries, the interstitial tissue and the cytoplasmic vesicles of the epithelial cells. (Drs.G.J.Dammin, A.S.Benenson, D.Feldman, S.B.Formal, and E.Sprintz: Clinical and Histopathologic Correlations in Acute Diarrheal Disease. Proceedings of the Cholera Research Symposium, Honolulu, Hawai, January 24-29, 1965.)

Selection of Patients: Although the study of biopsies from a single patient might provide the necessary information, the feasible number of patients, selected by the clinical staff is considered as about twelve patients. The collection of biopsies from control normal adults is to be considered, althoug the same patient in the convalescent phase may serve as his own control.

Technical considerations: The timing of biopsies, correlated with the progression of the disease is suggested as 16 hours, 2 days, 5 days and convalescence. The biopsies are to be taken by the Crosby capsule from the upper jejunum, from adult patients only. The recovered biopsy specimen will be fixed with glutaraldehyde and osmium tetroxide and embedded in Epon 812 in Dacca according to the accepted methods of electron microscopy. If possible, one half of the specimen will be saved for elective embedding or processing after glutaraldehyde fixation and will be stored in the cold in a sucrose-buffer solution. The biopsy specimens will be worked up in the elctron microscope laboratory of the Peter Bent Brigham Hospital, Boston, Massachusetts.

PROTOCOL

Demonstration of Abnormal Permeability in Human Cholera

Dr. Gerald Keusch
SEATO Medical Research Laboratory

Evidence has been accumulating that cholera involves vascular damage with an increase in permeability of the villus vessels. The nature of this evidence includes both morphologic demonstrations of capillary damage[1,2], and measurements of intestinal fluxes indicating excessive exsorption and normal insorption of fluids[3]. Permeability factors have been found in human cholera stool[4], and have been isolated from vibrios grown under controlled conditions[5]. This latter material, called choleragen, produces a cholera syndrome in the infant rabbit, the human, and causes marked enterosorption in the ligated ileal loop in the adult rabbit. We have recently demonstrated an increase in permeability of the villus vessel of the choleraic infant rabbit, utilizing the technique of "vascular labeling". Following intravenous injection of a colloidal marker, in this case ion-dextran (Imferon, Lakeside Laboratories), topographic examination of the tissue for the presence of the marker is carried out. Previous studies of the histamine injury have demonstrated separation of endethelial cells and passage of the marker between them where it is retained by the basement membrane, thereby identifying the site of the leak. This is important because the site of the leak varies with different eliciting stimuli, and may yield pathogenetic clues. It is therefore proposed to study human cholera in a similar manner.

Plan: Patients at the SEATO Cholera Research Laboratory with acute cholera, acute non-choleraic diarrhea, and convalescent subjects will be selected. In the acutely ill subjects, water and electrolytes will be administered until satisfactory clinical improvement has occurred. At this time a Crosby-Kugler capsule will be passed into the jejunum. Intravenous therapy will be continued. When the capsule is in place, an infusion of ion-dextran equivalent to 12.5 mgm/Kg body weight(14.5 cc Imferon for a 50 Kg patient) will be given over 1 hour. This dose is based upon our experience in the rabbit model, and may be revised according to the findings. At the end of the infusion the biopsy will be taken and the capsule removed. The tissue will be fixed in formalin, stained for ion by the Gomori technique, and examined to pographically for the presence of ion. A small piece of tissue will be embedded for electron microscopy, if possible. The formalin fixed tissue can later be imbedded in paraffin and sectioned.

Significance: The clear demonstration of a permeability defect in vivo in the human with cholera will help clarify the pathophysiology of the disease, and perhaps other diarrheal syndromes as well. It is our contention that cholera is a classic inflammatory response in the villus vessel, and visible proof of this will help focus attention and bring the nature of the lesion into the fold of a well studied, though only partially understood, mechanism. Therapeutic benefits may then follow rational exploration

of the lesion.

Risk: There is a small but definite risk to biopsy with the Crosby capsule. Many cholera patients have previously been biopsied and there appears to be no undue risk in this disease. The principle investigator is experienced with the procedure and has performed over 200 biopsies in the past year without complication. Intravenous ion-dextran is a clinically tested and accepted procedure for rapid correction of ion-deficiency anemia, particularly in England. While the compound has been shown to cause sarcomas in rats after i.m. injection of very high doses, this does not appear to be of concern for the human. As with many drugs, some patients experience transient systemic symptoms and fatal anaphylaxis has been reported following intravenous ion-dextran. It is our feeling that such an unfortunate outcome is very unlikely, however we propose to maintain constant medical vigilance for any complication during the period of study and be fully prepared to take immediate therapeutic measures. In addition Dr. Phillips has informed us that no customs or practices leading to excessive ion storage exist in the population to be studied which might be aggravated by parenteral ion. The converse situation is more likely to be encountered. The patients are not exposed to dextran in other forms, and there appears to be little chance that they will be. We feel that the scientific information to be derived from this study of a small number of patients will be of significance and that the risks are not sufficient to be a contraindication. It is our opinion that the best insurance for a successful outcome is full preparation for and knowledge of the possible complications.

REPORT ON CLINICAL LABORATORY SECTION

S. Z. Ahmad

This Laboratory has been recently reorganised as a clinical research laboratory. Its function is to provide laboratory support for clinical investigators and also to provide necessary chemical analysis on patients hospitalized at CRL.

I was appointed Section Chief in this area before my return to Dacca and took over on September 26. Drs. Kendrick and Ruth Hare arrived at the same time as I and have been assisting with the reorganisation of the laboratory. Techniques are being standardised, revised and set up in an attempt to provide the most precise information for the various studies.

There are eleven technicians working in this section of whom six have Masters or Bachelors degree in Science, and four are undergraduates. There are four glasswashers who have been instructed in the proper washing techniques. It is planned to add one more technicians so that a larger volume of work can be done, it is also planned to add a man with some education and background to supervise the glassware washers and explain procedures to them. In fact a person has already been selected for this appointment.

At present the laboratory is supporting Lavage Studies on gut segments; long term lavage of the whole G I tract; effects of lavage with and without Antibiotics; and a comparison of two antibiotics in treating children with cholera. We are also collecting admission blood electrolyte values on a few patients a day and doing any necessary emergency determinations requested by the house physicians. The laboratory also prepares and analyzes lavage solutions used in the clinical investigations.

Analyses now being performed:-

1. Specific gravity of whole blood and plasma

   Copper Sulfate Method (R.A. Phillips, J. Biol. Chem.,1950,83,305)

2. CO_2 content of serum, GI contents, stool, lavage solutions and urine. All material collected with as little as possible exposure to air and delivered under mineral oil. Two Van Slyke machines are now in constant use and a third is expected soon. Attachments for the micro modifications are also available.

3. Sodium and Potassium analyses are done on the same materials mentioned above and also on sweat samples. The Baird Atomic Flame Photometer (KY-2) [illegible] method is used.

4. Chlorides analyses are done on samples similar to those listed for CO_2 analysis. The Aminco-Cotlone Titrator at medium rate is used.

5. Osmotic pressure is determined on any fresh sample or on samples which have been stored after freezing. The Mechrolab Vapor Pressure Osmometer is used. Although the manufacturers mistakably sent a non-aqueous probe, we had fortunately an aqueous probe with us brought by Drs. Hare from U.S.

6. Glucose analyses are done on specimens stored with fluoride or on those which are precipitated immediately after collection. Two methods are in use; the enzymatic "Glucostat" method (Washko & Rice, J. Clin. Chem., 1961, 7, 542) and the Nelson modification of Somogyi (J. Biol. Chem. 1944, 153, 375). The latter method is now being used in most cases; it offers the advantages of greater precision and lack of fluoride interference.

7. Polyethylene Glycol (PEG) is used as an indicator of water movement in the gut. Samples are stool, lavage solutions, and GI contents. The method of Hyden with some modification (Ref: The Annals of Royal Agriculture College of Sweeden) is used. This is a turbidimetric analyses and is unsatisfactory in many ways. Attempts to improve it are in prospect. Optical density is measured in a Unicam Spectrophotometer.

8. Bromosulfo-phthalein (BSP) is also used as an indicator of water movement in the gut and also in measuring transit time and gut volume. Simple dilution of samples with alkali and measurements of optical density in any spectrophotometer is required. We are comparing values obtained for BSP with those for PEG.

9. Creatinine analyses on plasma, serum and urine Samples are done as an estimate of renal function or check on urinecollection. The non-specific Folin & Wu method (J. Biol. Chem. 1919, 38, 81) is now being used. It is planned to set up a specific method soon (R. S. Hare, Proc. Soc. Exptl. Biol. & Med. 1950, 74, 148).

10. Arsenic determination on urine and other biological fluids are done occasionally. Both Reinsch & Gutzeit determinations are done for a semi-quantitative analysis.

Proposed additional procedures.

1. Blood pH:- A Corning pH meter with thermostated microelectrode has been ordered. It is planned to measure arterial blood pH at or near the bedside with this instrument in order to avoid delay between collection and analysis. (Blood pH is now measured in the Clinical Research Section some distance from the ward.)

2. Calcium, Magnesium and Tetracyclines:-

It is planned to set up Fluorimetric techniques to measure these. At present no laboratory is measuring Ca & Mg. The bacteriology section is doing bio-assay measurements of tetracyclines. The fluorimeter is operational and as soon as suitable filters and an additional technician are available, these techniques will be set up.

3. Whenever a clinical investigator requests analysis for additional materials every effort will be made to set up techniques. It would be most desirable to have a versatile Spectrophotometer such as Beckman Model B and also a research Spectrophotometer with recorder when new colorimetric procedures are being tried.

4. Atomic Absorption Spectrophotometer:- This has been ordered but will probably not arrive untill this season is over. It is planned to analyse for the Cations with this instrument.

Summary :-

In the opinion of Dr. R. S. Hare who is incharge of quality control the quality of technical help in this laboratory is excellent. The major difficulties lie in glassware washing and supply, additional instruments and utilities.

So far this season the laboratory has been able to cope with several studies as well as hospital load. Future expansion will improve this laboratorys sup ort for investigations.

PROTOCOL

BACTERIOLOGY STUDIES

PROTOCOL

TETRACYCLINE ACTION AGAINST V.CHOLERAE IN VITRO

R. NORTHRUP, M.I.HUQ, A. RAHMAN

Preliminary studies of the in vitro effect of tetracycline on V. cholerae were performed as an adjunct to planned clinical lavage. Although the minimal inhibitory concentration of tetracycline has previously been shown to be 0.625 ug/ml, we wished to find a concentration of the antibiotic which would produce nearly immediate sterilization of a culture.

Accordingly an overnight culture of v. cholerae, at a vibrio concentration of between 10^7 and 10^8 colonies/cc, was incubated with tetracycline in concentration of from 10 ug/ml to 500 ug/ml. It was found first that the original count could be recovered immediately after addition of the antibiotic, demonstrating that the agent did not appear to prevent recovery of the organisms in the method used. Second it was found that with higher concentrations of tetracycline, the vibrio count was more rapidly decreased, such that at 500 ug tetracycline/ml culture, apparent sterilization of the culture was obtained within an hour of the time of addition of the drug. This was true even when millipore filtering of the culture was performed at the same time as samples were taken, in an attempt to further reduce the antibiotic concentration. We did find however, that overnight culture of the higher dilutions of the culture samples would at times show growth of the organism after overnight incubation, demonstrating that complete killing had not occured. Further investigation into this aspect is planned for the clinical off season.

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Protocol

Relationship of Growth cycle of Vibrio Cholerae to production of " Skin toxin".

by :
S.I.Hasan & M.I.Huq.

The interest in the toxic products of V. Cholerae has been recently renewed by demonstration of a skin reactive factor in the stool filtrates of cholera patients and in sterile culture filtrates of V. cholerae. The growth conditions required for the enhanced production of this factor are a topic of current interest. Selection of strains, growth medium and incubation temperatures have been shown to be important variables for elaboration of this toxin. In our hands the production of this toxin under conditions thought to be optimal by other workers has been giving erratic results. A complete definition of each variable is required for consistent results. This study is directed towards this objective. In addition the relative ability of serial isolates from cholera patients will be studied for a correlation between loss of toxigenicity and recovery from clinical cholera.

Methods: Syncase broth will be used for all experiments. Tissue culture rack will be used for slow shaking of the cultures. A number of tubes will be setup for each time interval. At each time period a set of four tubes will be withdrawn from the incubator and pooled. From this pool a total vibrio count will be done by serial dilutions. The remaining broth culture will be filtered immediately through millipore filter and kept under refrigeration. This material will be used for titration of skin toxin. A total length of 48 hours will be studied for optimal length of incubation. Toxin will be titrated in rabbit or guinea pig skin. Neutralization tests will be done only on the filtrates which have sufficiently higher titers of toxin. End point will be determined for each strain and compared.

PROTOCOL

A STUDY OF RESISTANCE OF V. CHOLERAE TO VARIOUS ANTIMICROBIAL AGENTS

R. RAHMAN, P. K. B. NEOGY, AND M. I. HUQ

1. RESEARCH PLAN

A. Introduction & Specific Aims: In spite of the fact that the sensitivity of Vibrio cholerae to various antimicrobial drugs was known for many years, the use of these drugs in the treatment of cholera is a recent development. Controlled studies in Dacca and Calcutta have clearly indicated the usefulness of antibiotics in the treatment of this disease. A large number of patients with cholera are now being treated with antibiotics. With the increasing use of the antimicrobial agents in the treatment of infectious diseases, the organisms resistant to various drugs are also increasing. The laboratory is faced with the task of supporting the treatment with sensitivity tests to these agents which is an essential adjunct to rational therapy. At this point it should be indicated that streptomycin resistant strains of Vibrio cholerae have been isolated and employed in the study of experimental cholera. Further, Vibrio cholera biotype el tor is resistant to polymyxin B. This property is now used for differential test. With these facts in mind one can no longer neglect the possibility of finding strains resistant to antibiotics in a given epidemic. The purpose of this study is to see whether strains resistant to various antibiotics are present in this endemic area. The possibility of inducing such mutants in the laboratory by various methods will also be studied. Such mutants will be employed in the study of the dynamics of the uptake of antibiotics by Vibrio cholerae.

B. Methods of Procedure:

a) Mutational Origin of Resistance: Mutants resistant to a given drug are present in a large population of cells independently of the exposure to the drug. If such cells are plated out on a medium containing drugs, the resistant mutants are selected out from the more susceptible organisms. Using this technique stool samples from cholera patients will be screened for such mutants. Also, stock culture strains will be looked for such mutation.

b) Nongenetic Origin of Resistance (Adaptation): The containing enverionment acts not only as a selective agent but also as a direct and necessary stimulus to gradual development of resistance of the entire microbial population. Possibility of inducing such resistance will be explored by growing V. cholerae in medium containing low concentration of drugs with long incubation at lower temperatures.

c) Resistance induced by Mutagenic agents: Attempts will also be made to increase the frequency of such mutation by exposure of large numbers of exponentially growing cells of V. cholerae to ultraviolet light. Selective medium will be used to isolated resistant mutants.

C. Significance of this Research: Drug resistant mutants of V. cholerae will be useful in the study of the fate of the drugs in the bacterial cells. In some cases it has been found that the action of antimicrobial drugs was related to the accumulation of the drugs in the bacterial cells. Differences were noted in the resistant and susceptible organisms. Dynamics of the uptake of antibiotics by V. cholerae will be studied.

COMPARISON OF THE DIVISION TIME OF NON AGGLUTINABLE VIBRIOS WITH AEROMONAS, PSEUDOMONAS AND COMMOMONAS AND ALSO WITH A STANDARD VIBRIO CULTURE.

by :

R.Rahman & M.I.Huq.

In the past the isolation and characterisation of the Non cholera vibrios faced quite a lot of problems due to the absence of detailed sophisticated techniques. Many a time our isolates specially of Heiberg Group IV to VI has been termed as not vibrios by expert survey. The oxidation fermentation test of Hugh & Leifson(1953) was found to be very helpful in recognising strains of Pseudomonas and commomonas which resembles NCV's and anaerogenic strains of Aeromonas also posed a problem as we did not have any test except oxidation of gluconates. Preliminary studies done in this lab suggests that the vibrios has much shorter division time than Pseudomonas & Aeromonas, isolated in this lab; though there is very little difference between the vibrios, irrespective of their agglutinable property. Dr.Feeley also suggested that Lysine, Arginine and ornithine decarboxylase tests are of great value in differentiation of NCV's and anaerogenic strains of Aeromonas.

Materials & Methods: We have already brought the standard strains of Pseudomonas, Aeromonas and Commomonas from NCIB, London and the Amino acids have been ordered and will be reaching us shortly.

For the study of the division time, organisms will be grown in plain T_1N_1 broth (Trypticas 1% and sod. Chloride 1%) at $37^{\circ}C$ and bacterial counts made at different time interval. From the graph by plotting Bacterial count against time the division time is calculated.

For the test for Decarboxylase activity Mollers Method (1955) has been suggested by Dr.Feely. To the basal media 1% L-Lysine dihydrochloride, 1% L-ornithine dihydrochloride and also 1% L-arginine monohydrochloride added separately in three batches to test for the decarboxylase activity of the cultures separately.

This study will be started as soon as the amino acids reaches and also the work load decreases.

STUDY ON CHOLERA PHAGES.

Phage typing of all the V.Cholerae isolated in the Lab. by using Routine Test Dilution method of Typing.

by :
(Mrs) A.Alam, M.I.Huq.

Phage Typing (Mukherjee etal 1960) has been accepted as a good criteria to differentiate between strains of vibrio cholera.

Results of the phage typing of about 800 strains of V.Cholerae isolated in the lab in 1965 showed that except for 4 or 5 strains all are not attacked by Phage Group 2 and as such fall in type 2 of Mukherjee's Phage typing. In that typing method each phage was given in Neat to 10^{-3} dilution. No attention was given to the routine test dilution of the specific phage in which it attacks the standard strain and compare this with the strains typed.

To find out subtypes within the types described by Mukherjee, all the strain of the V.cholerae isolated in 1966 are typed by Routine test dilution method.

Materials & Method:

Routine test dilution of a particular phage is first ascertained by using standard V.cholerae strain 154 Using this routine test dilution, the vibrio cholerae strains are typed and results read as IRTD, 10 RTD, 100 RTD and so on. In all the cases lawns of cultures are made onto T_1N_1 (Trypticase 1% and Sod. Chloride 1%) Agar with a 3-4 hours young culture.

Preliminary results have shown that the routine test dilution method is of some help in distinguishing strains isolated from different places and as such seems to have some importance from Epidemiological point of view.

STUDY ON CHOLERA PHAGES.

Routine Survey for the detection of Cholera Phage from Patients with Vibrio and also non vibrio diarrhoea.

by:

(Mrs.) A. Alam & M.I. Huq.

It has been suggested by different workers that the stool of patients or normal individuals sometimes contains phage. Dr. Burrows (personal communication) also reported the presence of phage in 10-12% of cases of cholera. An occassional survey for the last few years also has shown that the presence of phage in patients admitted to the Hospital is seasonal in incidence.

In order to find out the pattern of phage isolation at different times of the year and also its relationship to the cholera positive cases in the sarrounding we have started a routine survey of all the positive patients as well as some of the negative patients during the course of the disease. Stool of some normal healthy individuals will also be tried as controls.

Materials & Methods: Routine stool samples or Rectal swabs collected in BP enrichment media for Bacteriological culture are used for isolation of phage. 0.5cc chloroform is added to each of the enrichment bottles after it has been streaked for routine diagnostic work. The bottles are shaken and kept at room temperature overnight. Next morning 5 cc medium taken out and placed in a test tube, and kept at 37°C for 1 hour with occasional shaking to drive away the chloroform. The broth is then dropped into a lawn of culture 154 (Mukherjee's universal strain) and incubated after drying. Next morning plates are read for the presence of phage lysis.

The phage thus isolated are typed against Mukherjee's 5 typing strains. (154, 508, 528, 542, Bg29).

It has been found that about 20-25% of the cases contains lysogenic phages. Results of typing show that 70-80% of the phages isolated are of Mukherjee's Group I, while the rest are of group IV. No new types have been found.

PAKISTAN-SEATO CHOLERA RESEARCH LAB.
MOHAKHALI, DACCA.

PROTOCOL

SENSITIVITY PATTERNS OF THE PATHOGENIC MEMBERS OF THE ENTERO-BACTERIACAE GROUP WITH SPECIAL REFERENCE TO SALMONELLA, SHIGELLA AND PATH. E. COLI ISOLATED.

F. K. B. NEOGY AND M.I. HUQ

It is a known fact that, like staphylococcus, many of the members of the Enterobacteriacae family acquire resistance to specific antibiotic which is used widely making a good deal of problem to physicians. Not much information is available in this part of the world on the sensitivity patterns of the members of the Enterobacteriacae group which even here is the cause of many common diseases like dysentery, fever, etc. Dysentery due to Shigella and infantile diarrhoea due to Each. coli are prevalent in this part of the world ; although salmonella cases are not that frequent.

In order to find out the sensitivity pattern of the organisms to different antibiotics all the isolates of shigella and salmonella of these lab. from 1963 to date and also the Path. E. coli isolated in 1966 along with some Klebsiella were tested with Eight antibiotics by multiple paper disc method.

Materials & Methods - From the pure growth on the MacConkey plate a colony is picked up and suspended into a little broth to give visible turbidity (10^5 org/ml). One loopful of the suspension is streaked all over a blood agar plate (D.S.T. Agar base, oxoid code No.CM261 + 7% Blood) and the multidisk containing the antibiotics plate placed over the streaked media. All the plates were incubated overnight and readings taken

Multidisk used	-	U.S. pat code No.	11-160A
contains		Erythromycin	2 mcg
		Tetracycline	5 mcg
		Neomycin	5 mcg
		Albamycin	5 mcg
		Lyncomycin	2 mcg
		Chloromycetin	5 mcg
		Penicillin	2 units
		Dihydrostreptomycin	2 mcg

Preliminary reports with 20 salmonellas & about 80 shigella isolates shows that Both salmonella and shigellas are uniformly resistant to Albamycin, Erythromycin, Penicillin & Lyncomycin. Out of the 100 cultures only 6 have been found to be Streptomycin sensitive. All isolates are sensitive to novobiocin but 3 Tetracycline resistant & 2 chloramphenicol resistant strains have been found. Further work follows.

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PROTOCOL

STUDY OF THE PATHOGENIC ESCHERICHTA COLI IN CHILDREN SUFFERING FROM GASTROENTERITIS IN THE STUDY AREA DACCA CITY AND SUBURBS.

G. KIBRIYA, W.U. AHMED AND M.I.HUQ

During the study period (January 1966 to May 1966) Stool samples or Rectal Swabs were obtained from 917 diarrhoea patients from Hospitals & Clinics. In addition 509 normal healthy adults and children without any history of diarrhoea were also cultured.

This survey indicated that Enteropathogenic E. coli are significant in diarrhoeal diseases of children. 12.4% of the sick children under 1 year showed Pathogenic E. coli against 3.5% in normal children under 1 year.

Serotype 0127:B8 was the most frequently found strain both in patients and in symptomless individuals. Serotype 0111:B4 which are common in other parts of the world were encountered rarely.

This study also illustrated the correlation between age and susceptibility to infection with Enteropath. E. coli; the younger the individual; more susceptible he is.

This study will be continued again from January 1967.

PROTOCOL

EFFECTIVENESS OF ENTEROPATHOGENIC E. COLI IN CAUSING DIARRHOEA: GUT INFLAMATION BY LOOP TECHNIQUE IN SUCKLING RABITS.

R. RAHMAN AND M. I. HUQ

Taylor (1959) reports the dilatation of the ligated loop of rabbit intestine due to Entero path. E. coli. Combiescu et al (1957) have reported death caused in 1 day old rabbits when they were inoculated intranasally with Enteropath. E. coli.

To confirm the above report as well as to find out the effectiveness of the E.E.C. in causing dilatation of the ligated loop and also diarrhoea in suckling rabbits; experiments have so far been done with two pairs of suckling rabbits but the rabbits did not produce diarrhoea although it passed Path. E. coli in the stool. Further work in this line will follow.

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PROTOCOL

STOPHYLOCOCCUS ENTERITIS: A SURVEY OF STAPHYLOCOCCUS IN THE STOOLS OF DIARRHOEA PATIENTS AND NORMAL PERSON.

G. KIBRIYA, W.U. AHMED AND M.I.HUQ.

It is being recognised to an increasing extent that the staphylococcus can be incriminated as a possible pathogen in some cases of gastroenteritis.

Capocaccia etal (1963) studied the possible role of the staphylococcus in causing enterocolitis and concluded that only 9% of their diarrhoeas are due to Staph.

Chan and Lucas (1964) found that 38.2% of their cases of diarrhoea were due to the staphylococcus.

In order to find out the prevalence of staphylococcus enteritis in non vibrio diarrhoea patients as well as in normal individuals, a survey has been undertaken starting September 1966.

Preliminary reports suggest that about 15-17% of the diarrhoea cases may be due to staphylococcus, as no other pathogenic organisms have been isolated from them.

Methods & Procedure - Stool samples or Rectal Swabs were collected and streaked on to Blood agar plates and enrichments done in cooked meat broth with 10% sodium chloride. Suspected colonies were tested for staph and also coagulase tests done. Some of the isolates were coagulase positive and some turned to be negative.

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EFFECTIVENESS OF USING HIGHER INCUBATION TEMPARATURE FOR SELENITE BROTH FOR THE ISOLATION OF SALMONELLA

by :

G.Kibryia, W.U.Ahmed, M.I.Huq

For the last 2 years though the number of Shigella isolates were as high as expected the number of salmonella isolated were very few. MacConkey and S.S. Agar "Difco" are used for plating and Selenite broth at 37°C are used for enrichment in our routine diagnostic procedure.

Bacto selenite broth at an incubation temp. of 37°C has been recommended as an enrichment medium in the isolation of Salmonella group from faeces, urine and infected tissues.

The lower number of isolates of salmonella from patients with diarrhoea and fever and also other allied cases has tempted us to follow other fancied tests for its isolation.

In the booklet on the Reports of Salmonella cases Georgala & Bothroyed published some of their findings where they have reported that selenite F broth inoculated with meat samples was more selective for Salmonella when incubated at 43°C in a water bath instead of 37°C air incubation.

For this method two plain rectal swabs are taken from each new admissions instead of one and each one goes to selenite broth. One of them incubated at 37°C and the other at 43°C in a water bath. Plates are done after 24 & 48 hrs. incubation onto MacConkey and SS Agar and results read.

Preliminary reports from about 350 patients shows that of these 3 were positive in both the method and one in 37°C only and the other in 43°C only.

TO COMPARE THE EFFECTIVENESS OF JAPANESE MANUFACTURED TCBS MEDIA WITH TTGA(MONSURS) & GA (GOODNER) MEDIA USED FOR ROUTINE ISOLATION IN CRL.

by :

G.Kibryia, W.U.Ahmed & M.I.Huq.

It has reported by Dr.Gangorosa from his experience in Iran that TCBS Agar 'Eiken'* is a very effective media for the field isolation of V.Cholera El Tor biotype. Other workers in the CDC also supports the views expressed by Dr.Gangarosa. The media has the advantage over all others in that it does not require any autoclaving; simple low temprature cooking is enough for melting and subsequent pouring.

No reports have yet been published about the effectiveness of TCBS in the islation of true cholera and it has not been tried in any comparative field and hospital study.

In our Lab we use Gelatin Agar (Goodner) and TTGA (Monsur's) for the isolation of Vibrios. Both the media are prepared here from their ingredients and also requires autoclaving. For the latter reason it is not possible to prepare them in the Field Hospital where there is no electricity or other facilities.

In order to find out the effectiveness of TCBS "Eiken" media in the isolation of True cholera and also to compare the effectiveness with our Gelatin Agar and TTGA, a comparative study has been undertaken starting October 1966. The TCBS media for the purpose has been obtained by Dr.MacCormack & Dr.Mosley from CDC.

* TCBS Agar "Eiken"

Manufactured by NIHONEIYO KAGAKU CO.LTD.
TOKYO, JAPAN.

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PSCRL PUBLICATIONS 1966.

1.	The Treatment of Cholera-1965 The Method of the Pakistan-SEATO Cholera Research Laboratory.	Alam, A.K.M. Jamiul, M.B.B.S., Haque, Zahedul, M.B.B.S. Ally, Mrs. K.M., Akbar, Mrs. Rezia, M.B.B.S., Firoz, Azad, Hirschhorn, N., M.D. and others. East Pakistan Medical Journal Vol. X No.2 April, 1966.
2.	Cholera In Children.	Rosenberg, I.H., M.D. Greenough, W.B.III, M.D. Lindenbaum, J., M.D. Gordon, R.S., M.D. American Journal of Clinical Nutrition. (In Press).
3.	Hypoglycemia in Children with Acute Diarrhea.	Hirschhorn, N., M.D. Lindenbaum, John, M.D. Greenough, W.B.III, M.D. Mahmood Alam, S. Lancet 2:128-132, 1966.
4.	Cholera in the Perspective of 1966.	Phillips, R.A., M.D. Annals of Internal Medicine, Vol. 65, No.5, November, 1966.
5.	Malabsorption and Jejunitis in American Peace Corps Volunteers in Pakistan.	Lindenbaum, John, M.D. Kent, Thomas H., M.D. Sprinz, Helmuth, M.D. Annals of Internal Medicine, Vol.65, No.6, Dec. 1966.
6.	Antibiotic Therapy of Cholera.	Lindenbaum, John. M.D. Greenough, W.B.III, M.D. Islam, M.R., M.B.B.S. Bulletin of the W.H.O. (In Press)
7.	Phage Resistance in Vibrio Cholerae.	Rizvi, S., Ph.D., Benenson, Abram S., M.D. Bull. of W.H.O. (In Press).
8.	Nutritional Studies in Cholera.	Rosenberg, I.H., M.D. Greenough, W.B. III, M.D. Lindenbaum, J., M.D. Gordon, R.S., M.D. American Journal of Clinical Nutrition. (In Press).

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PSCRL PUBLICATIONS 1966.

9.	Severe Acidosis as a Cause of Pulmonary Edema and Death.	Greenough, W.B., III, M.D. Gordon, R.S., M.D. Lindenbaum, M.D. Hirschhorn, M.D. In Press.
10.	Studies of Intestinal Biopsy Adenosine Triphophatase in Cholera and other diarreal diseases.	Hirschhorn, N., M.D. Rosenberg, I.N., M.D. In Press.
11.	The Role of Carriers in the Intrafamilial Spread of Cholera.	Khan, A.Q. In Press.
12.	Hemodynamic Effect of Dehydration and Metabolic Acidosis in Asiatic Cholera.	Harvey, R.M., Enson, Y., Lowis, M.L., Greenough, W.B., Ally, K.M. and Pamo, R.A. In Press.

TECHNICAL COMMITTEE MEETING
14-16 February, 1967.

VISITORS TO PSCRL

I: OVERSEAS VISITORS ATTENDING PSCRL MEETINGS.

1. Directing Council Meeting, March 1966.

Dr. Ralph Knutti, National Heart Institute, N.I.H.
Brigadier M.S. Haque, Director General of Health Services, Govt. of Pakistan.
Dr. David A. Wraight, Deputy Secretary General, SEATO Secretariate, Bangkok.
Dr. David Mackenzie, U.K. Representative.
Dr. W. Boynton, US/AID, Lahore.

2. Clinical Investigation Committee, November 1966.

Professor Douglas A.K. Black, Manchester University, England.
Dr. Carl V. Moore - Washington University, St. Louis.
Lt. Colonel S.M.H. Bokhari, National Health Laboratories, Islamabad.

II: RESEARCH WORKERS AND ADVISORS.

February 1966: Dr. J. Fresh, NAMRU-2; Taipei; to review pathology.

February 1966: Lt. Gary Brown, NAMRU-2; Taipei; to review administrative functions.

February 1966: Lt. J. Baucomb, NAMRU-2, Taipei; to review maintenance functions.

July 1966: Dr. William Greenough, National Heart Institute, NIH; to advise on setting up of laboratory procedures.

July 1966: Dr. Randolph Eichner, C.D.C. Atlanta; Work with John Craig on skin tests.

July/August 1966: Mr. Charles Myers, Management Consultant, NIAID, NIH; TDY, Executive Officer.

August 1966: Dr. Abram S. Benenson, ex-Director, PSCRL; review of clinical studies.

August 1966: Mr. Billie Myers, Chief of Electrical Engineering Division, NIH; to review electrical requirements.

August 1966: Dr. John F. Craig, State University, N.Y.; Skin test program in epidemiology section.

August 1966: Dr. John Feeley, Div. of Biologics Standards, NIH; review of bacteriological and serological procedures.

August 1966: Dr. Michael Clark, NAMRU-2, Taipei; Virus isolations.

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VISITORS TO PSCRL

October 1966: Dr. G. Curlin, C.D.C., Atlanta; Epidemiology studies.

October 1966: Dr. A. Karchmer, C.D.C., Atlanta; Epidemiology studies.

November 1966: Dr. George Whalen, Marquette University, U.S., to discuss fluid and electrolyte transport.

November 1966: Dr. Andrew Abraham, AFEB, Harvard Med. School; Fluorescein tagged microscopy.

November/ December 1966: Mr. Wallace de Witt; C.D.C., Atlanta; to set up field bacteriology laboratory at Matlab.

December 1966: Dr. Howard Goodman & Dr. Elvin Kabat; W.H.O. sponsored tour; review of immunology.

January 1967: Dr. Larry Griffith, NAMRU-2, Taipei; Pediatric cholera studies.

January 1967: Dr. C. Campbell, School of Tropical Medicine, University of Sydney; Study on treatment of cholera.

January 1967: Dr. Nathaniel Pierce, Johns Hopkins Center, Calcutta; Review of current Clinical studies.

January 1967: Dr. John Banwell, Johns Hopkins Center, Calcutta; Review of current Clinical Studies.

January 1967: Dr. Paul Crabbe, Louvain University, Belgium, Immunology study.

January 1967: Dr. C. Linneman, C.D.C., Atlanta; Epidemiology studies.

January 1967: Dr. T. Vernon, C.D.C., Atlanta; Epidemiology studies.

III: TRAINING VISITS.

April 1966: Mrs. C. Gomez and Miss D. Gaetos, Public Health Service, Philippines, SEATO sponsored training tour in bacteriology.

July 1966: Dr. Anthony C.H. Chan, Infectious Diseases Hospital, Hong Kong, W.H.O. sponsored study tour in infectious diseases.

December 1966: Twenty W.H.O. doctors for regional training course in epidemiology and treatment of cholera, sponsored by W.H.O.

Jan./Feb.1967: Twelve Turkish and eight Iranian doctors on CENTO sponsored training course in epidemiology and treatment of cholera.

IV: INSTITUTIONAL REVIEW VISITS:

1. Horing Committee:

Dr. Colin MacLeod; Deputy Director, Office of science and Technology, Washington.

Dr. Milo D. Leavitt; Deputy Assistant Secretary for Health, Education and Scientific Affairs, U.S. Public Health Service.

Dr. Theodore Woodward; Professor of Medicine, University of Maryland.

Dr. Harold Margulies; Association of American Medical Colleges.

Dr. Herman Chinn; Scientific Attache, American Embassy, Teheran. (non-member).

May 1966: Dr. Randall L. Thompson; NIH Science representative. American Embassy, India. To review PL-480 Medical Research Projects.

June 1966: General Jesus Vargas; Secretary General of SEATO.

July 1966: Dr. P.W. Dill-Russell; Deputy Medical Advisor, Ministry of Overseas Development, U.K.

September 1966: Dr. Dorland Davis, Director, NIAID, NIH.

October 1966: Dr. James Mason; C.D.C., Atlanta; General Laboratory Advisor. To follow up on Horng Committee visit.

January 1967: Mr. Herbert Spielman; SEATO Affairs, American Embassy, Bangkok.

January 1967: Mr. Anthony Curnow; Public Relations Department, SEATO Secretariat, Bangkok. To review PSCRL activities for SEATO publications.

February 1967: Dr. Howard Minners; Special Assistant to Chief, Office of OIR, NIH. Discussions with OIR staff.

V: MISCELLANEOUS VISITS SPONSORED BY OTHER INSTITUTIONS.

February 1967: Dr. B.N. Simpkin, General Manager, Smith Kline & French, West Pakistan.

March 1966: Dr. A.H. Taba, Regional Director, W.H.O., E. Mediterranean Region.

March 1966: Dr. Charles S. Davidson, Thorndike Memorial Hospital, Boston.

May 1966: Dr. B.V. Bekker, W.H.O. Consultant to East Pakistan.

May 1966: Dr. Watanabe, Bacterial Disease Unit, W.H.O., Geneva.

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V. (cont'd)

June 1966: Dr. Zia Alam Zadeh. Deputy Director, Health Corps Organization, Iran.

July 1966: Dr. Jose Navarro, Chief, Division of Personnel Training, Health Dept., Manila.

August 1966: Dr. Md. Abdul Aziz, Pakistan Medical Research Council, Lahore . To discuss possible research project at PSCRL.

September 1966: Dr. M.S. Dizon. Medical Consultant, NRIFP, Ford Foundation, Karachi.

September 1966: Dr. B.J. Harlow. Searle Pharma Co., U.K.

October 1966: Dr. Ahmed Darai, Director General of Technical Training, Govt. of Iran.

November 1966: Dr. Madison Cawein, Norwich Pharma, New York.

November 1966: Dr. Ibrahim Hamidi, Dean, Medical College, Monsul, Iraq.

November 1966: Professor Mansour, Dean, Medical Faculty, Khartoun.

January 1967: Dr. Irvine Rosenberg, Thorndike Memoraial Hospital, N.Y.

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PAKISTAN-SEATO CHOLERA RESEARCH LAB.
MOHAKHALI, DACCA.
MEDICAL RESEARCH LIBRARY

HISTORICAL BACKGROUND

The library was created in 1962, and was housed in a small room, about 400 sq. ft. During its early stage of development, the concentration was on subscribing to the most essential periodicals in biological sciences, chemistry, biochemistry, epidemiology, pathology, tropical and general clinical medicine, public health, gastroenterology, clinical laboratory methods, laboratory animal care, etc. Together with subscribing to new periodicals, a sizeable collection of backfiles of important journals was acquired as a fundamental need. This included the most valuable reference tools, such as Index Medicus, Biological Abstracts, Excerpta Medica, and other items such as encyclopedias.

In the absence of any other staff in the library, the librarian was assigned, in addition to routine duties, such extra duties as labeling and stamping of books and periodicals, carrying periodicals to research staff and collecting them back. However, despite the multiplicity of duties, there was still opportunity to make certain improvements in library procedures, such as the introduction of subject-wise card catalogue, shelf-list card system and the subsequent regular system of stock-taking. Lists of the currently subscribed periodicals, lists of the complete backfules of bound journals, and regular monthly lists of "Books Recently Added..." were compiled and circulated throughout the country's medical libraries as well as to the universities and other research institutions, physicians, teachers of medicine, etc. Moreover, the book-collection was completely reclassified according to Barnard's scheme for medical libraries - a system particularly suited for a highly specialized collection of books on medicine and medical research. Similarly, the work of sub-dividing the reprint collection has been taken in hand. In order to mark

the inauguration of the first training course on cholera (through W.H.O.) the library arranged an exhibition of books, periodicals and reprints on cholera, which was used liberally by the delegates.

In March 1966, after an agreement between the Directors of Pakistan-SEATO Cholera Research Laboratory, and Institute of Public Health, the libraries of the two bodies were combined. The entire responsibility of planning the details of redecoration, equipment spacing, transfer of volumes and other materials, re-arrangement in the new premises (about 1400 sq. ft.) was entrusted to the librarian, Mrs. Molla. One library attendant was appointed in this month. Within a month, the new library greeted the readers with separate sections of periodicals displays, a conference room, a general reading room, a wide space in the main hall for seminars, a separate room for typing and processing of books and periodicals, facility for inquiries through a telephone in the library, and numerous other devices like air-coolers, fire-extinguishers, etc.

Later on in May 1966, an assistant librarian was appointed. At the same time, the new collection was brought together, accessioned separately in the record books of each individual owning bodies, yet catalogued and shelved together for open access. Within a very short time, many medical men from all over Pakistan visited the library, and many requests were made to us for permission of loan facilities. Subsequently, the loan facilities are being extended to the students of Institute of Post-Graduate Medicine, staff of Institute of Para-Medical Training, Malaria Institute, Institute of Chest Diseases and Hospital, and such others. A course of talks on 'How to make the best use of the medical research library' given by Mrs. Molla, was inaugurated in September, 1966.

REFERENCE SERVICES

Index Medicus, Biological Abstracts, Excerpta Medica etc. are gaining popularity, as each investigator learns about references and reviews contained therein by constant consultation to these reference works. A sizeable collection of reprints of cholera and other infectious diseases is available for reference. Inquiries from outside are answered on the telephone or by letters when necessary. Nearly four hundred items were borrowed on interlibrary loan and obtained as photocopy this year. Research staff keep the librarian informed of current projects according to their needs for reference material. Current issues of nearly 170 scientific and medical periodicals are routed automatically to the senior research staff. Instead of lending periodicals-volumes, photocopies of articles are sent to users of the library on request. Availability of scientific and medical periodicals backfiles anywhere in Dacca can be ascertained now through the publication "Union catalogue of scientific periodicals in Dacca." This facility allows personal inspection of journals in the libraries owning them, because interlibrary-loan scheme is non-existent. However, photocopies would be available at a small charge whenever required.

ROUTINE PROCEDURES

Records of periodicals subscriptions, book orders, book issues, interlibrary loans, etc. are maintained by the librarian. Periodicals are routed, and circulation check kept by the assistant who prepares the photocopies on request. Cataloguing books, preparing journals for binding, and other processes are allotted to the assistant librarian. Besides answering queries of investigators, making literature searches, the harmonious co-ordination between the activities of two institutes is maintained by the librarian, be it regarding acquisition of books, or regarding conferences, seminars, or information

dissemination. Today, the library, with its staff of two full time librarians, two part-time assistants and one attendant serves a large clientele of nearly 800 readers regularly from various laboratories both within CRL, Institute of Public Health, and from the medical and scientific communities at large.

STATISTICS OF LIBRARY ACQUISITIONS IN RETROSPECT

Year	Books Cumulative Total	Bound Journals Cumulative Total	Periodicals Subscribed	Inter-Lib.Loans Per Year	Reprints Cumulative Total
1962	380	250	-	140	200
1963	626	624	63	185	500
1964	852	738	70	203	976
1965	1010	970	80	225	1448
1966	1585 CRL 600 IPH 2185	1715	146	352	2600 CRL 120 IPH 2720

A graphical illustration of this is attached.

AREA OF THE LIBRARY:

Main Hall	34 x 30 =	1020 sq.ft.
Conf.room	21 x 12 =	252 " "
Catalog	21 x 7 =	147 " "
Total Area	=	1419 sq.ft.

FURNITURE & EQUIPMENT :

29 Book Cases
5 Display units for periodicals
4 Fire extinguishers
5 Staff tables
4 Air-coolers
1 Telephone
3 Reading Tables
3 Filing Cabinets
3 Catalogue card cabinets
40 Chairs
1 Conf. Table

SUGGESTIONS FOR IMPROVEMENTS

a) Space for conferences, seminars, displays, reading, book-processing, and shelving is critically short. As a result, reading or reference work in the main hall is constantly disturbed due to shortage of space. Moreover, about 70 volumes and about 100 reprints and pamphlets are added to the library stock every month, thereby occupying 3/4 of a new book-case and a sizeable part of the filing cabinets. The present shelf-space will allow for only three months of additional volumes, after which a state of emergency will exist. Further space for stacks is thus urgently needed.

b) Book-orders from both Pakistan-SEATO Cholera Research Laboratory and Institute of Public Health are being dealt with independently without consultation as to the likely duplication which might arise. In order to avoid such waste and to coordinate all the library functions and policies of the two institutions, it would be advisable to form a library committee comprised of senior officials of both the institutions, and the librarian

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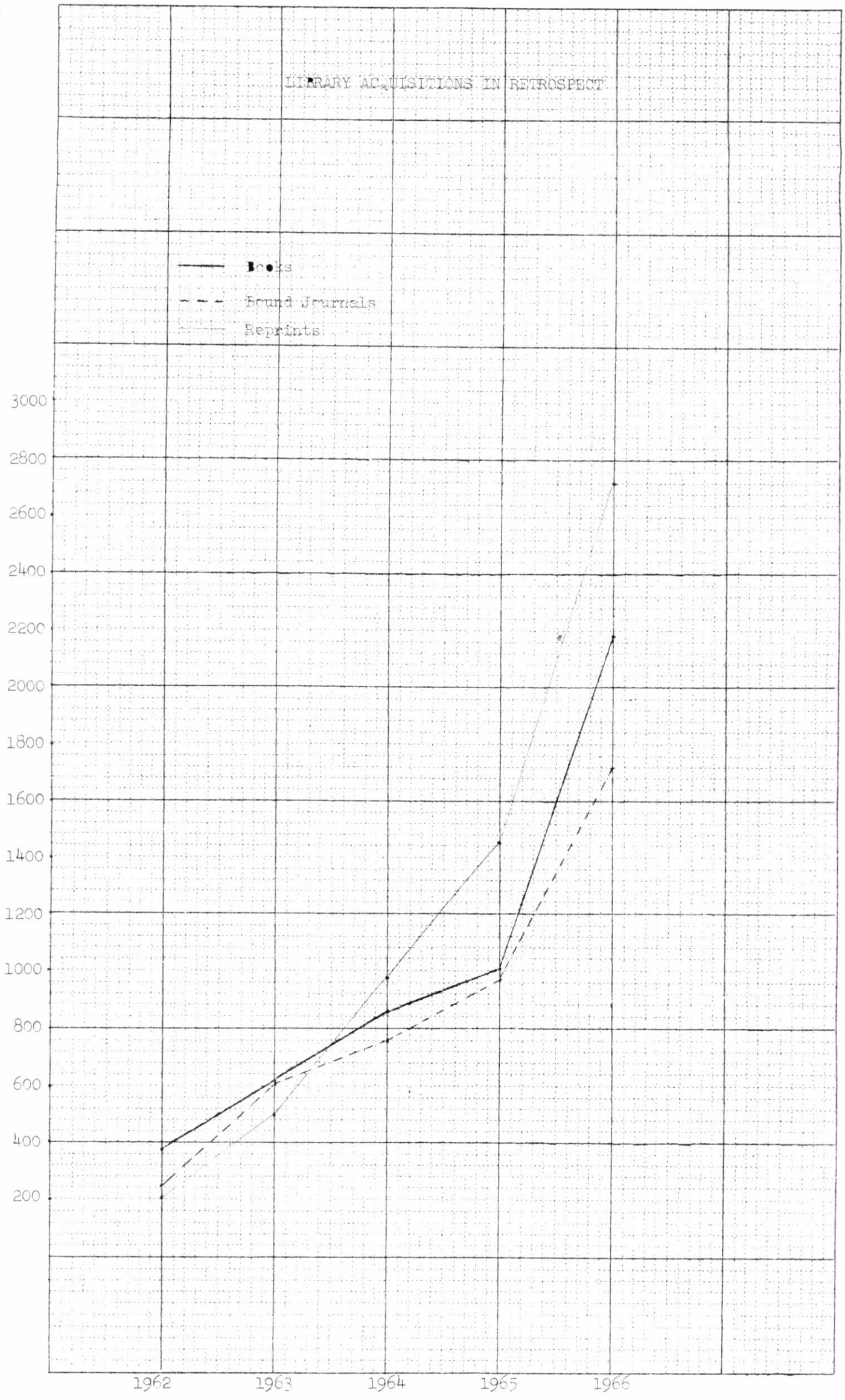
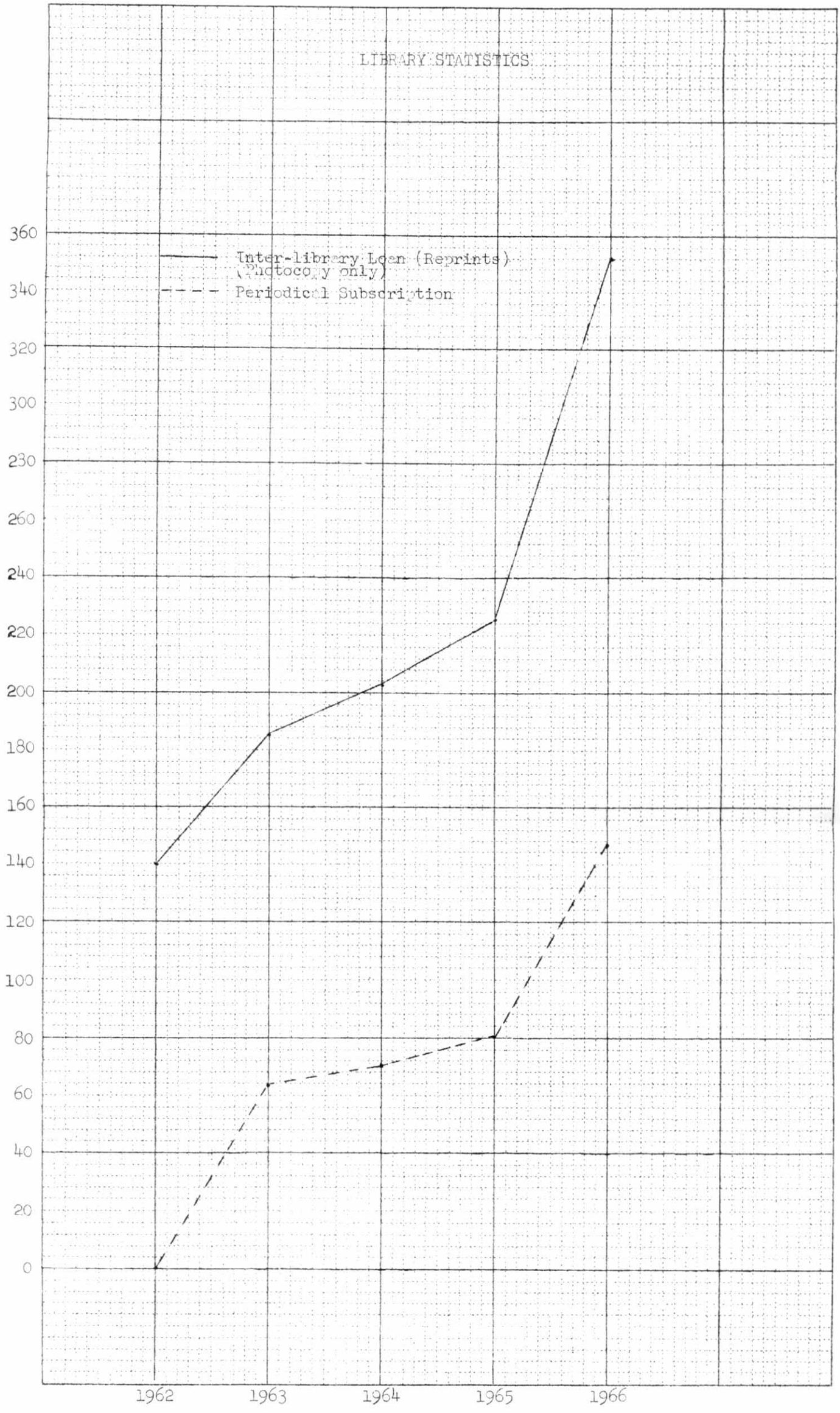

LIBRARY ACQUISITIONS IN RETROSPECT
Books
Bound Journals
Reprints
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
1962
1963
1964
1965
1966


LIBRARY STATISTICS
Inter-library Loan (Reprints)
(Photocopy only)
Periodical Subscription
360
340
320
300
230
260
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180
160
140
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1962
1963
1964
1965
1966

AGREEMENT FOR COMBINING THE TWO LIBRARIES OF

Pakistan-SEATO Cholera Research Laboratory

&

Institute of Public Health

For the mutual interest and mutual benefit of the Pakistan-SEATO Cholera Research Laboratory and Institute of Public Health, to provide better library facilities, we hereby agree to the following:

1. The library materials, e.g. books, periodicals, pamphlets, reports, reprints, and such others will be jointly shelved and jointly displayed so as to be available to the maximum number of library users.

2. The conference room of the library and the reading hall would be used as and when required by the staff of either of the institutions, with prior scheduling by the librarian.

3. General maintenance of the library will be the responsibility of Pakistan-SEATO Cholera Research Laboratory, whereas other items, e.g. stationery, furniture would be contributed by both the institutions.

4. Book-acquisition registers will be maintained separately by each of the institutions.

5. The property of each organization will bear the name of its relevant owner e.g. books, furniture, equipment etc.

6. The librarian would be responsible for the smooth co-ordination and management of the combined library.

7. The directors of both the institutions would finalize staff increases, staff duty-schedules, etc. as and when need would arise.

Signed
Dr. K. A. Monsur 7.2.1967
Director
Institute of Public Health
Mohakhali, Dacca-12

Signed
Patrick G. Talmon 7.2.1967
Executive Officer
Pakistan-SEATO CRL
Mohakhali, Dacca-12

PAKISTAN-SEATO CHOLERA RESEARCH LABORATORY

REPORT OF TECHNICAL COMMITTEE

February 14 - 16, 1967.

The Technical Committee met on February 14 to 16. It was assisted in its review by Dr. Kenneth Goodner and Dr. Alexander Langmuir who acted as consultants. The Committee appreciates greatly their contributions. The Technical Committee wishes also to thank Dr. Robert A. Phillips and his staff for their co-operation in this review, and for their generous hospitality.

In this report we shall deal with recommendations relating to the general operations of the Laboratory as well as recommendations that pertain to the Section.

The Committee first wishes to express its appreciation for the remarkable progress that has been made during the past year. This progress is exemplified by the quality and scope of investigations which have been accomplished or are under way, and by other indicators. For example, during the recent cholera season (September,1966 through January,1967) the Laboratory treated 2,225 patients within its meagre hospital resources, with a mortality rate of 0.9%. This admission rate presents a four-fold increase over that anticipated. These statistics are remarkable in themselves but are made more so when it is realised that the supplies of commercially prepared intravenous fluids were grossly inadequate and that the Laboratory was forced to produce 12,000 liters of therapeutic fluids under conditions that would ordinarily be considered as almost totally inadequate. Inadequacies included an undependable power supply, inadequate source of good quality distilled water, a staff untrained in the preparation of fluids for intravenous use, inadequate facilities for washing and sterilisation of glassware and solutions, and no facilities for testing for pyrogenicity. In addition there was gross overcrowding of facilities for patients. The dedication of the whole staff- both laboratory and clinical - was the ingredient that produced such remarkable results under circumstances so adverse.

The Technical Committee is much impressed with the morale of the whole staff. Part of this can be explained by the common "baptism of fire" they had in caring for such a large number of patients during this major outbreak of cholera. While this may be a factor we are more inclined to attribute the high morale to intelligent and imaginative leadership.

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Because of the magnitude of the clinical responsibilities, we believe it is imperative to move as rapidly as possible toward the construction of the new laboratory hospital wing presently under consideration. This will provide 40,000 sq.ft. of space of which 10,000 sq. ft. will be used for patient care. We do not believe it prudent to go through repeated cholera seasons under the present inadequate circumstances, and urge that all steps be taken to construct the new facilities in the shortest possible time.

The Technical Committee wishes to record its admiration for the efforts of the medical and nursing staffs for their remarkable accomplishments during the past cholera season.

Despite the very large patient load the staff was able to continue its investigations. These will not be reviewed in this report except in the broadest terms.

GENERAL RECOMMENDATIONS

1. Epidemic Assistance and Training for Cholera Control.

During the last year the Laboratory has responded to requests from WHO and from CENTO to provide indoctrination and training in cholera control. In addition the Laboratory responded to a request from the Philippines for assistance in the study of an outbreak of cholera.

The Technical Committee believes these to be important functions for the Laboratory to perform and recommends that its staff be augmented as necessary to meet these legitimate demands.

2. Graduate Education and Training.

The Laboratory has reached a high degree of intellectual and technical competence in the study and control of cholera and related diseases. Advantage should be taken of it as a place to train students from Pakistan and other countries. We believe that this competence should be recognised and that less emphasis should be placed on sending students abroad for graduate experience. The desirability of this function for the Laboratory emphasizes the need constantly to upgrade its professional competence and its scientific equipment.

Provision of more senior research personnel on a long-term basis will greatly enhance the Laboratory's potential for research and training. The Technical Committee recommends that provision be made to accomplish this end.

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We are also concerned that the equipment and supplies of the Laboratory should be of first quality so that students who come to it can be trained under the best circumstances. We regret that insufficient attention has been paid to these requirements in the past.

3. The need for a long-term commitment.

The Pakistan-SEATO Cholera Research Laboratory has existed on a short term basis since its founding. The time has come to re-evaluate this concept in the light of the accomplishments of this and other laboratories and in the light of requirements for control and treatment of cholera. Elsewhere in this report projections are made for the need for a ten-year commitment toward the development of an effective vaccine which will give enduring immunity. The Technical Committee recommends that planning be based on a ten-year projection with the option of an additional five to ten years, subject to annual review by the Technical Committee.

We are of the firm opinion that long-term projection of the Laboratory's program is essential to its scientific health and particularly to its ability to recruit and retain high quality scientific personnel.

4. Competence in Bacteriology and Immunology.

The Technical Committee is much concerned with the inadequacies in equipment and supplies necessary for adequate bacteriological diagnosis of cholera and related diseases and for research. Since this competence is central to all other activities of the Laboratory, the Technical Committee recommends that steps be taken immediately to remedy the defects.

5. Animal Facilities.

The need for experimental animals is steadily expanding. The use of small animals (mice, guinea pigs and rabbits) in assay of toxic vibrio products represents a large part of the need. Future projects of developing an animal model of cholera and of investigating the organ or systemic effect of vibrio products will require colonies of dogs and monkeys. These experiments can best be done in Dacca because of the rich supply of fresh stool, blood, urine and intestinal fluids during all stages of the clinical disease and because the investigator can collect and process the materials he studies. The animal house and its facilities are unfit for use. The creation of the proper environment for experimental and breeding colonies will necessitate the provision of electric power, sewage disposal, ventilation, air conditioning, cages, food supplies and a suite of experimental rooms with appropriate equipment and instruments.

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The building is of solid and sound construction but its design will make animal care, especially isolation, difficult. The Technical Committee recommends that the animal facilities be improved at an early date.

6. Balance in Programs of Clinical Investigation; Pediatric Problems.

Since more than 50% of the admissions to Pakistan-SEATO Cholera Research Laboratory are in the pediatric age, this institution should provide special facilities for the study of the cholera problems peculiar to the infant and child. These problems include extreme acidosis, hypoglycemia, osmotic and electrolyte aberrations, the remarkable immunity of the infant (under 1 year of age), neurological manifestations and renal insufficiency. The study would require a pediatric ward and study room, special balances, bassinets, pediatric beds and a microchemical laboratory. Moderate modifications of the bacteriological and immunological procedures will accommodate the pediatric study. However, more personnel will be required.

The Technical Committee recommends that these needs be met.

7. Time of Meeting of the Technical Committee and the Directing Council.

The Technical Committee recommends that in future the meetings of the Technical Committee be held in late November or early December and that the Directing Council meet in January. We have two reasons for this recommendation: (a) so that the visits will coincide with the cholera season; (b) so that the meeting can have a useful relationship to budgetary plans for the ensuing fiscal year.

8. Budget for Fiscal Year 1968.

The Technical Committee reviewed the budget for fiscal year 1968 and approves it in the light of projected needs. The Technical Committee, however, is concerned that inadequate provision has been made for renovating, expanding and refurbishing the Section on Bacteriology and the animal quarters. The Technical Committee recommends that a supplementary appropriation be sought to improve these essential functions.

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RECOMMENDATIONS ON BACTERIOLOGY LABORATORY

The laboratories were not designed for the enormous load now existing. The load is likely to increase in the future. A considerable renovation is imperative and the Technical Committee strongly recommends that this be done.

1. Autoclaves.

These are badly located and in poor operating condition. Capacity of present equipment is not adequate for single shift operation and the turnover time is very poor. A proper installation should involve a reliable steam generator capable of sustained 60 pounds pressure and a 2" line to be bled directly to autoclave. New installations should consider anticipated load character, i.e. intravenous fluids, media, glassware, etc.

2. Sterilising Ovens.

These are of inadequate capacity and tend to be overloaded. There is a distinct possibility that effective time temperature requirements are not fulfilled and that this may be an important factor in contamination of media and glassware.

3. Media Preparation Facilities.

Plate pouring situation is very poor. There is great waste because of contaminated materials. Large cold room badly needed for storage of media and specimens.

4. Glasswashing Facilities.

Presently seriously inadequate and improper. There is no supply of hot water for washing or for rinsing. One suspects that detergent and/or soap is to be found in considerable residue on each piece of glassware.

5. Physical Arrangement.

Space could be better utilised. The open gallery should be more fully employed. All sterilisers should be moved out and given shielding against the monsoon.

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6. Glassware Problem.

There is an evident attempt to cut costs by repeated utilisation of plastic laboratory materials such as Petri dishes. This matter is entirely unsatisfactory. Re-use should be discontinued. Glass Petri dishes are in short supply. The breakage rate is probably high under present conditions. A good investment would be a large laboratory glass-washing machine (such as "Better-Bilt").

7. Costs.

A proper refurbishing of the bacteriologic lay-out would require about $80,000. In addition the present power source is not dependable. Proper laboratory operation demands reliable electric power.

IMMUNOCHEMISTRY

It is known that increasing age is associated with a rise in serum antibody titers to various cholera antigens and a corresponding fall in the incidence and case mortality rate. It is important to determine which of the antibody fractions (immunoglobulins) is related to protection because the answer affects the whole problem of vaccine production.

The 7S or IgG antibodies represent 75% of the toral immunoglobulins found in the serum of cholera patients. The 19S or IgM antibodies likewise tend to remain within the circulation. The 7-13S, or IgA antibodies are essentially secreted, local antibodies. They tend to be found in the intestinal tract in cholera. In a variety of studies, using sera from affected patients and from normal healthy contacts, from meternal and cord blood, and from day-to-day studies of two cholera patients, it has been postulated that in the bloodsteam the 7S antibody corresponds best with protection against cholera.

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Studies carried out in collaboration with guest investigator Dr. Paul Crabbe' of Louvain, indicate, however, the possible significance of 7-13S (IgA) antibody in the local intestinal reaction in a patient suffering from cholera from whom biopsy material was collected and studied by immunofluorescent methods. These observations on plasma cells in intestinal villi and their IgA antibody are well worth pursuing. In order to carry out these important studies adequate photomicrographic apparatus should be made available at PSCRL.

EPIDEMIOLOGY SECTION

1. Organisation.

In December,1966 a third medical epidemiologist reported for duty at the Laboratory thus completing the basic professional staff specified in the recommendations of the Technical Committee Report for 1966. Also, as recommended, short term assignments were arranged in collaboration with CDC, USPHS for five medical epidemiologists and one bacteriologist. These men contributed effectively to the conduct of specific research projects and provided needed assistance in the epidemic of October-December,1966. This practice should be continued as one solution to the variable demands of the PSCRL for professional personnel.

The present professional staff of the Epidemiology Section is technically competent and adequate in number to conduct the present heavy program commitments including large scale field trials of cholera vaccines, the development and evaluation of serological tests for diagnosis and immunity and limited but intensive epidemiological field studies of cholera as it occurs and spreads in selected study populations.

To meet the needs for epidemic assistance, additional staff, including medical epidemiologists, bacteriologists, a biostatistician-demographer and an immunochemist will be necessary if the Laboratory is to fulfil its broad missions of:

1. developing practical immunizing agents that can provide long lasting immunity;

2. determining the sources of infection, mode of spread and other essential factors in the occurrence of both endemic and epidemic cholera, and

3. providing epidemic aid, epidemiological consultation and demonstrating effective measures for the practical control of cholera in the SEATO nations.

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2. Immunology.

A major achievement of 1966 has been the demonstration of the effectiveness of a standard lot of cholera vaccine of average potency. Two doses administered at an interval of 28 days to children aged three months to 14 years in Matlab Bazar conferred 87% protection against clinical cholera during the epidemic which occurred one to three months following inoculations. Previous successful vaccine trials had been carried out with a vaccine of exceptionally high potency.

A comperative study of antibody response (agglutinins and vibriocidins) in young Pakistani children was made at the Balisera Tea Garden with results indicating that a representative lot of Pakistani-produced vaccine was comparable to the American - produced vaccine used in the successful 1966 field tests. A number of other vaccines were also compared for their antigenicity in humans and in turn will be compared by standard mouse tests used in routine vaccine production. These are major steps toward the production of uniformly affective and standardised vaccines for worldwise use.

A serious limitation of present vaccines is the apparent short duration of protection. Vibriocidal antibody titers decline to a low level within six months to one year, suggesting a concurrent reduction of protection. The present vaccines cannot be considered adequate for practical public health programs other than emergency epidemic measures or in those limited populations to whom repeated inoculations can be given.

It is recommended that the PSCRL plan future vaccine evaluations according to the following schedule:

Fiscal Year 1967-69 :	continue present study into second and third years to test duration of protection from the 2-dose schedule.
Fiscal Year 1969-72 :	initiate large-scale test with purified antigens, depending on availability.
Fiscal Year 1972-75 :	initiate large-scale test with the toxoid expected to be available by that time alone and in combination with purified somatic antigens.
Fiscal Year 1975-78 :	initiate large-scale tests with live, attenuated vaccines, both orally and parenterally.

The conduct of large-scale field trials should be planned on a basis of a minimum of three years because of the variation in the attack rate from year to year. It must be emphasised that East Pakistan is an endemic area for classical cholera. Vaccines found to provide protection here must also be tested in other areas where

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cholera has been absent for some years but now threatens and where various biotypes are occurring. Thus, a period of at least ten years and perhaps longer must be projected before an adequate practical vaccine having a long duration of effectiveness can be anticipated.

Concurrently with vaccine field trials, a variety of serological studies should be conducted within the vaccine test areas and in other selected population groups in East Pakistan and elsewhere to test the antigenic responses of new candidate vaccines, to develop new and more practical immunological tests, specially those dealing with antitoxic immunity, and to sharpen the effectiveness of these tools for epidemiological investigations. Of particular importance is the development of a test or tests measuring immunity to infection and disease. Such tests would greatly facilitate the screening and evaluation of new vaccines without primary dependence upon expensive and often protracted field trials.

3. Field Epidemiology.

The intensive field observations which have been conducted concurrently with the vaccine field trials have elucidated many factors or importance in the spread of cholera. In Rayer Bazar cholera recurs annually in two small areas characterised by greatest crowding, maximum mobility of the population, and most accessibility to the canals where contamination of water by boatmen has been repeatedly demonstrated. Significant spread to the more stable parts of the community has not occurred. In Sylhet a sharply localised water-borne epidemic was discovered during other field studies. In Matlab Bazar the cases continue to occur in sporadic fashion without evident source and often with little spread to family or community contacts . It is apparent that the factors determining the spread of the cholera vibrio are still obscure. Even in crowded and poorly sanitated populations direct person to person spread is difficult to demonstrate. Further detailed and more sophisticated field epidemiological studies should be pursued as one of the major functions of the PSCRL.

The additon of an anthropologist to the staff is a wise development since a detailed knowledge of the customs and mores of the people is essential to the interpretation of epidemiological findings and in determining the appropriate steps that can be taken in control procedures. The elucidation of the determinants of the occurrence of cholera may, in the long run, be more significant for the effective control of the disease than the development of an adequate vaccine.

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CLINICAL INVESTIGATION PROGRAM

This year, a broad and comprehensive study of gastrointestinal physiology was instituted under the guidance of the Director, the Deputy Director and Mrs. Hare. The study was designed so that experiments conducted during the acute phase of the disease would be duplicated on the patient during convalescence; thus each patient would serve as his own control.

The studies were designed around the lavage procedure with the introduction of an electrolyte solution having the composition of cholera stool into the stomach, the duodenum or lower portions of the GI tract with collection at lower points of the GI tract including the collection at the anus. The solutions contained a non-absorbable marker so that volume changes over the section of the intestine under study could be determined.

In addition to the non-absorbable marker technique, the use of the bolus technique for measurement of gut volume was also employed and it is planned in the future to determine bi-directional fluxes with the use of appropriate isotopes.

Emphasis this year has been on the relationship of the vibrio and its products to the rate of stool production over the entire GI tract and over segments. The studies included rapid lavage at the rate of two to three liters an hour over the entire GI tract and proportionate rates over study segments. Having found that lavage itself did not have any appreciable effect on rate of stooling, various potentially therapeutic agents were studied. These included tetracycline, bacteriophage, convalescent sera and glucose. Studies in the future will include urea, electrolyte solution having the pH of 5 or less, cultures of lactobacillus, dinitrophenol, ouabain,etc.

Depending on results of studies, the electrolyte composition of the lavage solution will be varied as appropriate. The studies were designed to assess agents which could decrease gut lumen volume and increase gut lumen volume.

Projected studies include an attempt to assess mesenteric blood flow. One of the objects of the studies is to determine the relative importance of the flow of water and electrolytes from plasma to gut lumen, and the flow of water and electrolytes from gut lumen to plasma.

It was also proposed to investigate electrical potential changes in the intestine and to that end Dr. Sachar spent six weeks in the Laboratory of Ussing in Copenhagen.

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So far the effort in this program must be considered as a time of development of techniques so that meaningful results can be obtained, and in many studies the chemical analyses have not been completed. However, certain results have been obtained which are worthy of comment.

For the first time, potentials have been measured in man and it has been found that with the addition of glucose to the lavage solution there is a reversal of electrical potential and a marked negativity. This coincides with in vitro studies reported in the literature.

A good agreement has been obtained by three methods for determination of gut volume - Cr51, BSP and T-1824. The administration of glucose by stomach tube has repeatedly demonstrated that the stool output can be greatly diminished.

Following the administration of tetracycline to the lavage solution, within six hours stool was free of vibrio as determined by culture techniques. However, the diarrhea did not abate until an additional 18 to 24 hours had elapsed, indicating that a continuous supply of vibrio products is not necessary for maintenance of the disease state.

Results with convalescent sera and bacteriophage were tantalizing; in both there seemed to be a suggestion of an effect on vibrio counts and stool volume but the studies are too few and too irregular to draw any conclusions.

Balanced studies of pediatric cholera have begun, to ascertain if pediatric cholera due to the classical vibrio is the same as pediatric cholera due to the El Tor variant.

The Technical Committee is greatly impressed with the originality and high quality of the Clinical Investigation Program and its importance to an understanding of the pathogenesis of cholera as well as to elucidation of gastrointestinal physiology.

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APPENDIX 1

GENERAL OBSERVATIONS

1. Clinical Investigation Committee.

The Technical Committee notes with approbation that the Clinical Investigation Committee has been appointed and has met during the past year. The Clinical Investigation Committee has reviewed programs involving human experimentation and has provided important guidance to Pakistan-SEATO Cholera Research Laboratory.

2. Guest Investigators.

Several guest investigators have been appointed during the past year. The Technical Committee approves heartily of this step. At the same time, the Technical Committee wishes to point out that the relation of guest investigators to the Laboratory Sections and to the administration has not been defined adequately. We are concerned, for example, that direction has not been given to the Section Chiefs in Bacteriology and Clinical Chemistry to seek the advice of guest investigators in these sections.

3. Drug Testing.

Pakistan-SEATO Cholera Research Laboratory has been approached during the past year by several drug firms to assist in clinical evaluation of drugs. The Technical Committee wishes to register its opposition to such testing unless the action of the agent falls clearly within the central mission of the Laboratory and unless its study is of particular interest to a senior investigator. In all cases it is essential that its use be reviewed by the Clinical Investigation Committee.

4. Use of Consultants.

The Technical Committee approves the policy of spreading the visits of consultants throughout the year, rather than concentrating their visits at the time of the meetings of the Technical Committee. We recommend also that consultants be brought for periods of time long enough to ensure that they will have an impact on the work of the Laboratory.

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