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PAKISTAN-SEATO CHOLERA RESEARCH LABORATORY

DACCA-5, EAST PAKISTAN

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PAKISTAN-SEATO CHOLERA RESEARCH LABORATORY

TECHNICAL COMMITTEE MEETING

17-19 January, 1966

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DIAGNOSTIC BACTERIOLOGY

by Huq, M.I., Kibriya, G., Dey, C., Rahman, R. and Ahmad, W.

The main function of the Bacteriology Section is to support the activities of the Clinical and Epidemiology Sections. For this reason most of our energy is directed towards bacteriological diagnosis of materials collected by them. Practically all our samples are from patients admitted to the hospital, or from the people under surveillance by the Epidemiology Section in the vaccine trial areas, in Rayer Bazar or in the family studies; samples from other sources are very few.

From October 1, 1964 to September 30, 1965 a total of 107,554 samples were received by this section for processing. The following groups of pathogens were isolated:

Vibrio cholera, Inaba	2595
" " Ogawa	239
El Tor Inaba	11
" " Ogawa	25
Non-cholera vibrios	530
Salmonella group A	2
" " B	6
" " C_1	5
" " C_2	4
" " D	18
" " E	3
Salmonella like organisms ?	8
Shigella A	31
" B	184
" C	11
" D	26
Shigella like organisms?	18

Rizvi, S. (Mrs.) Alam, A., and Salek, M.A.,

Vibrio Positivity of Bile Peptone Enrichments.

In the studies reported last year on diagnostic studies, instances were observed in 1108 vibrio positive cases in which virbios were recognized on direct plating but not on enrichment. In view of the fact that the surveillance studies supporting the Matlab Vaccine Trial is based on rectal swabs from the diarrhea cases being placed in tellurite bile peptone enrichment broth and then shipped to Dacca, the question of the effect of delay in transit in the rate of isolation arose. A study was therefore set up in which those enrichment broths found positive after overnight incubation were held at room temperature and streaked daily on gelatine agar (GA) and taurocholate-tellurite-gelatine agar (TTGA) plates for a period upto seven days. The result is given in table I.

TABLE I.

Number of positive cultures on given days of enrichment.

Contaminating organisms.	Day of enrichment						
	1	2	3	4	5	6	7
Pyocyanea Sp.	35	31	28	24	21	18	15
Without Pyocyanea Sp.	333	331	329	327	327	321	317
Total:	368	362	357	351	348	339	332

It is noted that 1.6% (6 out of 368 samples) had become negative after 48 hours and 3% after 72 (11 out of 368 samples). Most Matlab cultures are plated after no more than 24 hours; the longest interval before plating has been 72 hours, indicating a possible maximal loss in enrichment of 3%. As shown last year 2.6% of cultures positive on direct plating were negative after enrichment. When the two losses are combined they may yeild a total maximal error of 6 per cent. Of particular interest is the observation that out of 35 cultures mixed with pyocyanin producing (pseudomonous) bacteria, the vibrios survived in 15 in contact with the pseudomonous, suggesting that there is a diversity in the biological effects of different pyocyanins.

Vibrio growth: Surface versus fluid phase:

Should transfer loop be taken from the surface or in the fluid phase of enrichment broth for recovery of vibrios? The classical injunction to take the loop for transfer from the surface was tested by using 10 ml. syringes as culture tubes whose tips were closed with rubber sleeved stoppers. A loopfull of overnight cultures of vibrios was inoculated using both El Tor (Howrah strain) and a classical Inaba isolated in Dacca (B1). After six and twenty four hours' incubation at 37°C, 1 ml samples were taken from the bottom using the syringe as a pipette. The number of vibrios

(more)

in each ml were adduced by plating serial log dilutions on GA plates. The question whether the loopfull obtained from the surface film might entrap by surface tension more bacteria was tested by comparing the count obtained by passing the loop only through the surface film after six and twenty hours of culture in contrast to the count obtained from a similar procedure performed on the bottom 1 ml of culture material having first been transferred to another tube. The results obtained are shown in the table II.

TABLE II

Vibrio counts in broth cultures at various levels

Size of samples and broth.	Number of sample	Vibrio count at invervals			
		Bl	Hawrah 24	Bl	Hawrah 24
1 ml, T_1N_1 (starting from top)	Top 1 ml	1.9×10^8	2.8×10^8	1.5×10^9	2.5×10^9
	2nd ml	1.8×10^8	2.7×10^8	8.3×10^8	1.5×10^9
	3rd ml	1.8×10^8	2.6×10^8	8.4×10^8	1.4×10^9
	4th ml	1.7×10^8	2.8×10^8	8.2×10^8	1.3×10^9
	5th ml	1.6×10^8	2.8×10^8	8.7×10^8	1.4×10^9
	6th ml	1.8×10^8	2.9×10^8	8.3×10^8	1.5×10^9
	7th ml	1.9×10^8	2.7×10^8	8.2×10^8	1.3×10^9
	8th ml	1.9×10^8	2.7×10^8	8.0×10^8	1.3×10^9
	9th ml	1.7×10^8	2.7×10^8	8.0×10^8	1.2×10^9
	10th ml	1.8×10^8	2.8×10^8	8.1×10^8	1.3×10^9
Loopfull samples from T_1N_1 broth	Top	7.0×10^6	1.3×10^7	1.9×10^7	5.5×10^7
	Bottom	9.3×10^5	9.1×10^5	1.5×10^5	4.7×10^5
Loopfull samples from Bile peptone broth.	Top	1.6×10^6	2.6×10^6	1.2×10^7	1.7×10^8
	Bottom	9.5×10^5	6.4×10^5	4.3×10^6	7.0×10^6

Tanking the average of 3 applications shows that there is an apparent entrapment of vibrios in the loop from the surface film, more evident after 20 hours' culture than after six hours. The effect in terms of the count per ml of depth is only evident at 10-20 hours' growth. The El Tor formed a definite pellicle not seen with the classical strain. It is noted that the El Tor counts throughout were significantly higher than those of the classical strain after six and twenty hours.

Evaluation of Various Media for vibrio growth.

Since the establishment of the entity of Non-vibrio cholera, demonstration of Vibrio cholerae in the stool is an essential requirement before a patient can be diagnosed as a case of asiatic cholera.

Vibrios in most acute cases of cholera are present in such large numbers that their isolation does not cause any problem. However, when the vibrios are few (convalescents, carriers, environmental samples) and are mixed with an abundance of other organisms, isolation is possible only when the material or its enrichment is plated on a selective medium capable of suppressing the growth of the contaminating organisms. A search of old literature will reveal that many such medias have been recommended in the past. Some of these are as follows:

Alkaline laurylsulfate tellurite (ALST) (Felsenfeld and Watanabe, 1958); Alkaline bismuth sulfate (ABS) (Wilson, Reilley, Pandit and Ahuja, 1940); Aronsons agar (Aronson, 1915); Desoxycholate citrate, hynes modification (Felsenfeld 1959-60); Taurochlate - tellurite - gellatine-agar (TTGA) (Monsur 1961); Alkaline Starch -Milk, (Felsenfeld, 1959-60); TCBS Agar (Kabayashi, 1963); cholera medium (Oxoid) (This is essentially ALST Medium).

These media have been used by different people and at different times. No comparative work has been done by one worker using the same materials to test whether one or the other of these was any better than the rest.

As a preliminary step in the direction of comparative study, we decided to study the colonial characters of pure vibrios cultures on the above media and also on four other media routinely employed in our laboratory for the isolation of enteric pathogens. These media are gelatin-agar, SS agar, MacConkey agar and EMB agar.

Colony size on Various Media: Fifteen locally isolated cholera vibrios were grown overnight in T_1N_1 broth. Then they were routinely steaked on the various plates and incubated overnight at 36°C. The results obtained are given in Table III.

TABLE III.

Growth of Vibrio cultures on different media.

Media	Number of strains showing colony characteristics		
	Large colonies	Minute colonies	No growth
GA	15	0	0
Mac Conkey	15	0	0
TTGA	15	0	0
Alkaline Laurul sulfate-tellurite	15	0	0
Cholera	15	0	0
TCBS	15	0	0
Aronsons' Agar	15	0	0
Strach Milk	15	0	0
Bismuth Sulfite agar	3	3	9
EMB	6	4	5
SS	0	1	14
Desoxycholate citrate(Hynes)	0	13	2

SS agar, Desoxycholate citrate agar, Bismuth sulfite agar and EMB agar are not good media for growth of the vibrios isolated in this area. Aronson's medium varied considerably from batch to batch. Colony morphology is masked due to indicators in TCBS unless the vibrio colony is a sucrose fermenter: thus it will miss sucrose negative cholera vibrios as well as Heiberg group V to VIII non cholera vibrios.

Vibrio count on solid media: To assess the sensitivity of the more promising media, pure overnight broth cultures of twenty two local isolates were diluted and suitable aliquots plated on various solid media in order to count the number of colonies appearing on each media. After overnight incubation of plates the colonies were counted. The number of colonies obtained on various media is given in Fig. I.

This figure shows that Desoxycholate-citrate is poor, and oxoid cholera and the TCBS agar media are only slightly inferior to TTGA, lauryl sulfate and MacConkeys.

Practical performance of Media: The actual value of MacConkey's media for routine diagnosis of cholera was available since the routine in the laboratory has been to use MacConkey as a screen for the presence of enteric pathogen on all hospital admissions, so that admission stool samples are concurrently plated on MacConkey as well as GA and TTGA. Comparison of the results of these concurrent isolations (Table IV) reveals that in spite of the good performance with pure cultures, MacConkey serves poorly as the isolation medium for cholera vibrios (whether el tor, true cholera or NCVS) either directly from the rectal swab or after enrichment through a alkaline tellurite bile peptone water.

TABLE IV.

Comparison of MacConkey, GA, TTGA media for the degree of positivity of Known positive rectal swabs and their enrichment.

Material Streaked	Medium	Degree of Positivity				
		0	+	++	+++	++++
Direct (421 samples)	MacConkey	263	68	53	30	7
	GA	10	121	111	107	72
	TTGA	2	78	106	136	99
Enrichment 450(samples)	MacConkey	358	32	35	22	2
	GA	15	76	116	140	103
	TTGA	3	60	92	179	116

As has been noted previously, isolation failures can result from the inability of a culture medium to inhibit the growth of contaminants when the vibrios are in small numbers. To evaluate this, four vibrio. negative rectal swab samples and eight alkaline tellurite bile peptone enrighment cultures were simultaneously plated on GA, TTGA, lauryl sulfate, cholera medium and TCBS medium. Compared to the grouth on GA, all others proved to be inhibitory. However, the TCBS developed yellow colonies resembling vibrios in six of the eight enrichment cultures. The bacteria proved to be cocci and/or proteus. This, coupled with the loss of sucrose negative strains, combine to indicate that it is not a desirable medium for cholera diagnosis despite the clarity with which the typical vibrio colony makes itself evident.

Although TTGA plate (Monsur's medium) has proven to be very satisfactory, preliminary results with the lauryl sulfate tellurite medium (ALST Medium) coupled with its lower cost and lower consumption of scarce ingrediants (lauryl sulfate versus sodium tauro-cholate) make it highly desirable. These two media were put into a comparative study.

Alkaline Lauryl sulphate tellurite medium was prepared as described by Felsenfeld and Watenabe, 1960. The pH of the finished media was found to be 9.5. Five locally isolated vibrios failed to grow when streaked on these plates. However, when the pH of medium was adjusted to 8.5, these strains grew abundantly.

The results of the field trial of these media (Table V) indicate the superiority over gelatine agar of both the other two test media. Between them there was no significant difference in sensitivity or specificity but the vibrios from the enriched samples showed better growth on the TTGA.

TABLE V.

Degree of Positivity of vibrio samples on various media among 1206 samples of rectal swabs examined.

Streaking	Media	Degree of Positivity				
		-	+	++	+++	+++
Direct	GA	1147	31	15	9	4
	TTGA	1144	14	14	25	9
	ALST	1146	15	16	25	4
Enrichment	GA	1135	16	18	23	17
	TTGA	1123	7	18	35	33
	ALST	1122	12	22	28	22

Since the performance of the lauryl sulfate tellurite medium was clearly improved by lowering the pH, a further study was carried out comparing the performance of this medium at a pH of 8.2 with that at pH 8.5 (Fig. 2). Using the number of colonies formed with 15 locally isolated vibrio cholera strains on GA as the basis of comparison, it is obvious that in dealing with pure cultures of vibrios, the recovery of organisms is much better when the pH of the lauryl sulfate tellurite medium is 8.2.

A series of 673 consecutive clinical samples coming from the ward were then streaked directly and after enrichment in alkaline tellurite bile peptone water, on GA, TTGA and lauryl sulfate tellurite at pH 8.2.

The result (Table VI) shows no difference between the performance of the latter two media, both being clearly superior to the gelatin agar.

T A B L E VI

Comparison of Positive isolations on ALST Medium (pH 8.2) with other media

Streaking	Media	Degree of Positivity				
		-	+	++	+++	++++
Director	GA	10	39	22	9	9
	TTGA	0	27	30	21	11
	ALST	0	30	26	23	10
Enrichment	GA	5	21	24	29	10
	TTGA	0	17	23	32	17
	ALST	0	22	35	18	14

To rule out that this lower pH might permit confusion by overgrowth of contaminants, record was kept of the "degree of contamination" on a series of clinical samples as shown in Table VII. It is evident that the lauryl sulfate tellurite medium is actually more suppressant to contaminants than the TTGA on direct plating. While more contaminants appeared after enrichment, the density of contamination is still less on ALST medium than that on TTGA. It is evident that the lauryl sulfate tellurite medium described by Felsenfeld and Watanable equals or possibily exceeds in performance the TTGA medium described by Monsur when the pH is adjusted to 8.2 rather than left as prescribed originally.

I(b)

T A B L E VII

Degree of Positivity of contaminants in vibrio positive and vibrio negative samples.

Sample status	Streaking	Medium	Contaminant Degree of Positivity				
			-	+	++	+++	+++
Vibrio Positive	Direct	TTGA	15	33	30	10	1
		ALST	23	44	18	4	0
	Enrichment	TTGA	12	30	32	14	1
		ALST	9	40	28	12	0
Vibrio Negative	Direct	TTGA	36	202	155	64	19
		ALST	344	107	17	7	1
	Enrichment	TTGA	7	42	117	194	116
		ALST	17	165	137	126	31

The difference in growth of contaminants from direct streakings of vibrio positive and vibrio negative rectal swabs on the ALST medium may be due to either modification of the medium by the growth of the vibrios or due to difference in the contaminants themselves.

These studies have led us to consider TTGA and ALST (pH 8.2) as the preferred selection media for vibrio isolation, and to discard the TCBS medium; the Oxoid cholera medium is essentially the lauryl sulfate tellurite but with a higher pH; Bismuth sulfite medium has served poorly in our hands; Aronson's agar had had too much batch to batch variation; the starch milk medium has been inadequately scrutinized and further studies will be carried out on this.

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Fig. 1. Colony formation by Vibrio Culture on various solid media.

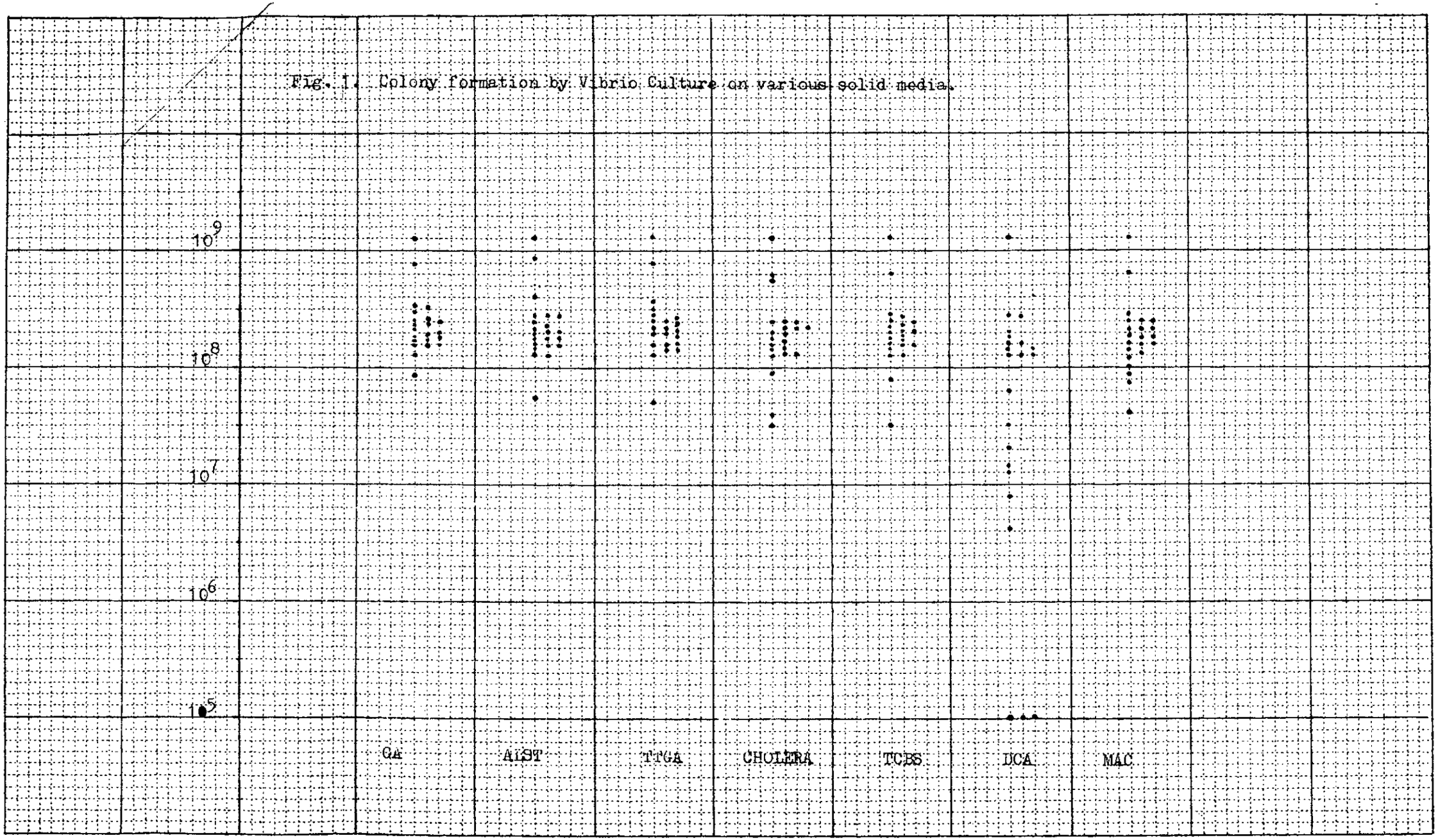
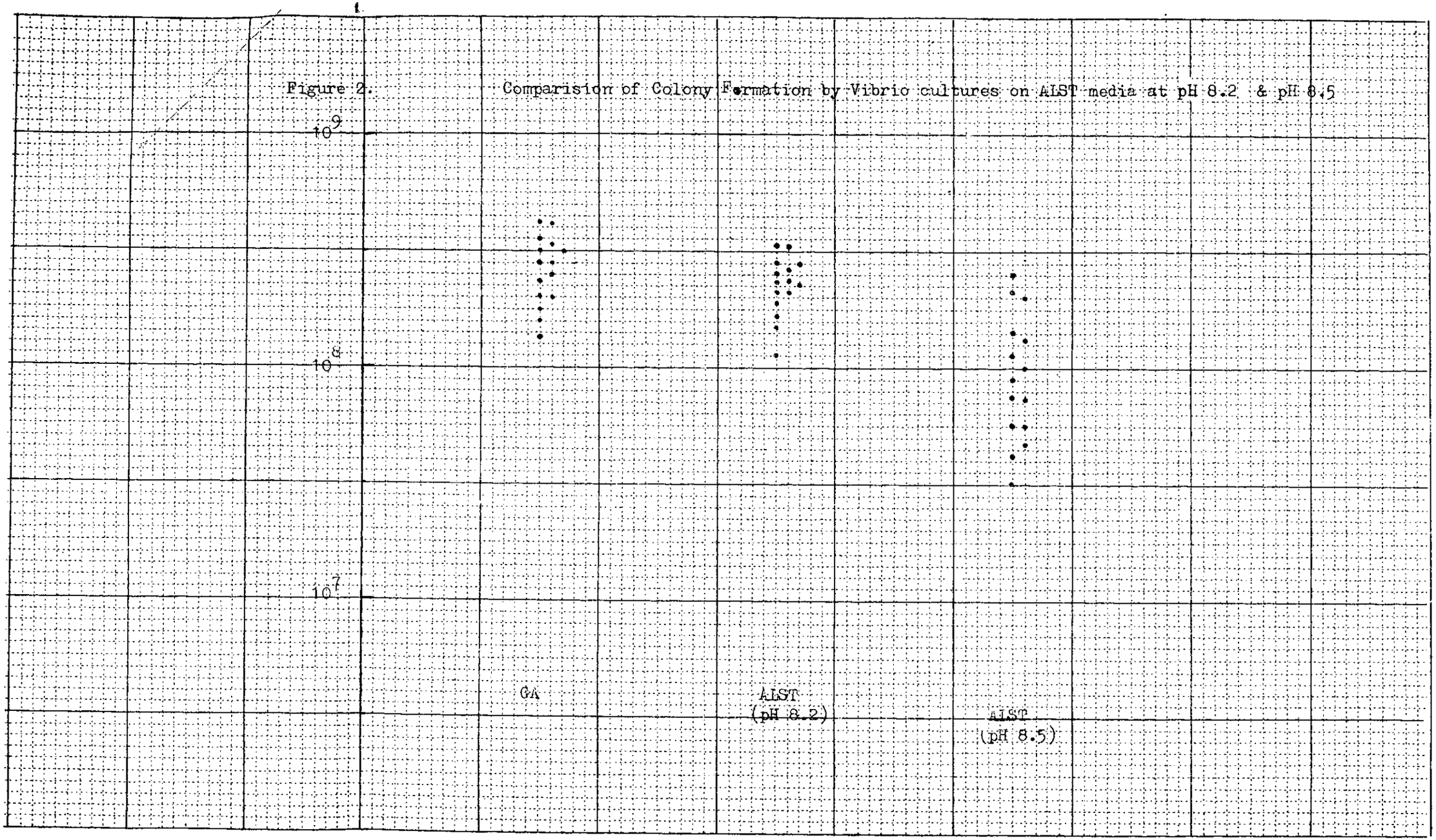


Figure 2. Comparision of Colony Formation by Vibrio cultures on AlST media at pH 8.2 & pH 8.5



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BACTERIOLOGIC STUDIES OF JEJUNAL ASPIRATES

By: Ahmed, A., Alam, J., and Rizvi, S.

A programme of bacteriologic studies of jejunal aspirates from patients admitted to Cholera Research Laboratory has been undertaken from 26th February, 1965.

Necessity for such a study was felt for several reasons:

(1) A group of patients having clinical signs and symptoms of classical cholera (copius rice-water diarrhea, high admission plasma protein, circulatory collapse etc.), did not yield vibrio or any other known pathogens.

(2) No pathogens were isolated from another group of acute gastro-enteritis cases of a milder nature having low or no circulatory collapse, smaller duration and degree of diarrhea, brownish stool instead of rice-water type, etc.

(3) In the faeces of a few cases of acute gastro-enteritis, although vibrio-like motility was present no vibrios were isolated upon culture.

(4) And finally to find out whether malabsorption syndrome is caused by protozoal or helmanthic infection of the upper jejunum.

The first three types of cases mentioned above are imagined to be basterial in origin because most of them show high neutrophilic count with a shift to the left and good response to antibiotics. Therefore, it was felt, particularly in view of the monkey studies reported last year indicating death of vibrios in the rectum, that vibrios and other pathogens may have been isolated if jejunal contents (where infection first occurs and good environment for growth is present), were examined.

Material and methods

The aspirates were collected by Drs. Norbert Hirschhorn and J. Alam with a crosby capsule attached to a thin polythene tube for taking biopsy material. When the tube has reached the desired level of upper jejunum an aspiration is taken by suction with a syringe(when no fluid is obtained on suction a small amount of sterile distilled water is introduced and taken back as a washing).

After noting the approximate quantity, presence of bile by colour and turbidity of the aspirate, microscopic examination (phase contrast and dark-ground illumination) of wet preparation was done and the following characteristics noted:

(i) Motility, vibrio-like or other type;

(ii) Presence of spirochaetes;

(iii) Morphology of the bacterial flora;

(iv) Cells, (Leucocytes, squamous and intestinal columnar epithelial;

(v) Larva of Strongyloides stercoralis and Hookworm;

(vi) Ova of Ascaris lumbricoides, hookworm etc; and

(vii) Fungal hyphae, pseudohyphae, mycelium and spores.

Afterwards serial log dilutions up to 10^{10} were set up in trypticase broth and 0.1 ml from each of these dilutions was plated on blood agar, MacConkey agar, gelatin agar, Monsur plate and S S agar. This was for viable count by the method of surface plating.

Total count of all types of bacteria was made on blood agar and count of the dominating type of bacterium was made on the medium best suited- Monsur plate for vibrio, MacConkey agar for Coliforms, etc.

Anaerobic culture was done in Torbal - B.T.L. Anaerobic jars (Torsion Balance Co.,) in which the paladium catalyst works without electricity to form water from introduced hydrogen and entraped oxygen. Duplicate 0.1 ml undiluted samples were plated by drops on the surface of the agar media for simultaneous aerobic and anaerobic cultivation. Examination of aerobic and anaerobic culture plates helped in separating aerobes, facultative anaerobes and strict anaerobes.

Further verification of the oxygen requirement was done by aerobic cultivation of the isolated bacteria.

Result and Discussion

90 samples of jejunal aspirates obtained from 62 patients were examined. Only 35 were from patients in acute phase of diarrhea (within in 72 hours from onset), 8 samples were from cholera patients, 28 samples from non-vibrio cholera cases, 29 samples from mild diarrhea cases and 17 samples from malabsorption cases. The gross macroscopic and microscopic finding of wet preparation of the aspirates are given in Table I.

TABLE I

CHARACTERISTICS OF JEJUNAL ASPIRATES

Visibly turbid	77	percent
Bile present	58	"
Bacillus in single and irregular arrangement	57	"
Bacillus in chains	17	"
Cocci in clusters	13	"
Cocci in pairs & short chains	38	"
Fungus, Candida Spp. like	4	"
Giardia lamblia	4	"
Hookworm ova	9	"
Ascaris lumbricoides' ova	1	"
Larva of Strongyloides stercoralis	2	"
CELLS (Intestinal epithelial	49	"
CELLS (Squamous	64	"
CELLS (Leucocytes	24	"

It is seen that bacteria, fungi and parasites were generally present in jejunal aspirates, contrary to the findings in normal individuals where these are generally bacteriologically sterile. No spirochetes were seen.

When these aspirates were cultured on various media, growth of many types of bacteria was noted. The result of such experiments is given in Table II.

TABLE II

TYPES OF BACTERIA OBTAINED ON CULTURE FROM JEJUNAL ASPIRATES

1. Vibrios			8
2. Lactose fermenters on MacConkey plate (not differentiated into genera like Escherichia, Citrobacter, Arizona, Klebsiella & Cloaca)			41
3. Non-lactose fermenters on MacConkey plate (Shigella & Salmonella excluded)	: (i) Pseudomonas showing pyocyanin and green fluorescence	:	27
	(ii) Other NLF, mainly consisting of non-fermenting gramnegative bacilli probably belonging to the genera like	:	59
	(a) Achromobactor (Mima polymorpha Herella vaginocola		
	(b) Flavobacterium		
	(c) Lophomonas		
	(d) Alkaligenes faecalis		
	(e) Non-pigmented Pseudomonas		
4. Respiratory tract Commensals:	(i) Pneumococcus (ii) Strep. Viridans (iii) N. catarrhalis (Any one two or three)	:	26

5.	Staphylococcus:	(i)Staph. pyogenes (Coagulase positive) : 2) (ii)Staph. epidermidis :27) : 29
6.	Proteus spp.:	4
7.	Non-haemolytic Streptococcus :	3
8.	Diptheroids :	2
9.	Anaerobic Streptococcus :	1

The above table shows that 8 samples contained vibrios. The number of the vibrios in each of these aspirates was determined by surface plating. Comparison of the actual viable count with the degree of positivity of the rectal swab obtained on the same day has been made in Table III.

TABLE III

COMPARISON OF VIBRIO IN JEJUNAL ASPIRATES WITH RECTAL SWABS TAKEN ON THE SAME DAY AND INOCULATED ON TTGA MEDIA (MONSUR) AND GELATINE AGAR.

Case #	Vibrio Serotype	Jejunal aspirate count/ml.	Rectal swab positivity degrees
1920*	Inaba	9.3×10^8	TTGA +++ Ca +
1949	Ogawa	2.0×10^7	TTGA ++++ Ca +++
1959	Ogawa	2.5×10^4	TTGA ++++ Ca +++
1965	Ogawa	9.3×10^6	TTGA +++ Ca +++
2088	Ogawa	1.4×10^5	TTGA ++++ Ca +++
2092	Ogawa	3.5×10^5	TTGA ++++ Ca +++
2368	Inaba(El tor)	3.0×10^7	TTGA +++ Ca +++

* There were two samples from this case, so one has been omitted.

All the jejunal aspirate samples were found positive for vibrio on culture when same day rectal swabs were heavily positive by direct plating. It was not possible to make simultaneous quantitative determination of the vibrios in all aspirates and corresponding stools. However in one Inaba case (#1920) such simultaneous quantitation was done and the result is given in Table IV.

TABLE IV

COMPARISON OF THE VIBRIO COUNTS IN JEJUNAL ASPIRATES AND LIQUID STOOL

Case #	Date	Jejunal aspirate	Stool
1920	3.3.65	9.3×10^8 /ml	4.3×10^9/ml
	5.3.65	1.5×10^7/ml	$2.6 \times 10^{7/ml}$

In both samples the vibrio counts are slightly higher in the stool as compared to the jejunal aspirates. However, the number of vibrios in the aspirates is quite high. Another opportunity to make such quantitative comparisons was afforded by two autopsy cases. The vibrio and coliform count in different parts of the gut of these cases are given in Table V.

TABLE V

VIBRIO AND COLIFORM COUNTS IN VARIOUS PARTS OF THE GUT IN AUTOPSY CASES.

Part of the gut	Bacterial count per ml. in case numbers.			
	#592		#601	
	Vibrio	Coliform	Vibrio	Coliform
Duodenum	1.9×10^7	2.5×10^7	No growth*	No growth*
Proximal jejunum	1.5×10^8	1.0×10^8	No growth*	No growth*
Small bowel from 200 cm after ligament of Treitz to 275 cm.	1.5×10^9	6.0×10^7	Not done	Not done
Small bowel next 100 cm.	9.0×10^8	3.0×10^8	Not done	Not done
Ileo-caecal valve to mid-transverse colon	Not done	Not done	2.0×10^6	1.5×10^6
Mid-tranverse colon to anus	Not done	Not done	6.7×10^6	4.0×10^7

* Only loopful was streaked and enrichments made.

It is seen that no vibrios were found when loopful was directly streaked or from such enrichment in the duodenum of autopsy case #601. Since the number of these cases is small no definite conclusion can be drawn. It is difficult to explain this because it has been shown in Tables III and IV that vibrios are present in the aspirates.

The result of growth(visible turbidity) in the T_1N_1 tubes inoculated with serial log dilutions of the aspirate fluid is given in Table VI.

TABLE VI

HIGHEST DILUTION IN TRYPTICASE BROTH SHOWING VISIBLE GROWTH ON OVERNIGHT INCUBATION

Number of aspirates showing growth at highest dilutions.											
10^{-10}	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}	Sterile	Total
4	1	3	7	10	16	17	13	9	6	3	89*

* One sample was accidentally lost.

10^{-3} to 10^{-5} dilutions tend to be the highest range where about half of the total samples show growth.

The exact number of bacteria in each aspirate sample was determined by surface plating method. The result obtained is given in the table VII.

TABLE VII

TOTAL VIABLE COUNT IN ASPIRATES OF ALL TYPES OF BACTERIA

Number of aspirates showing viable count in the log region.											
10^{10}	10^{9}	10^{8}	10^{7}	10^{6}	10^{5}	10^{4}	10^{3}	10^{2}	10^{1}	No growth on plating 0.01 ml.	No growth when 0.5ml of aspirate
0	2	1	5	8	25	18	17	6	4	3	1

Two thirds of the aspirates show a viable count in the range from 10^3 to 10^5 and this compares well with the results given in the preceding table showing the growth of serial log dilutions in T_1N_1 broth. Both these indicate majority of the aspirates from diarrheal cases have somewhere between 10^3 to 10^5 bacteria per ml of samples. However, when vibrio infection is present, this number may be much higher (see Table III).

Motility like 'swarming of gnats' of stool is being used in this laboratory for the diagnosis of cholera. Correlation of this characteristic motility with the type of bacteria and actual viable count is given in Table VIII.

TABLE VIII

CORRELATION OF VIBRIO-LIKE MOTILITY OF ASPIRATES WITH THE ORGANISMS ISOLATED UPON CULTURE

Aspirates obtained from cases.	Vibrio-like motility	Vibrio viable count	Pseudomonas isolation
#1920-1	+	9.3×10^8	Negative
#1949	+	1.96×10^7	Negative
#1965	+	9.26×10^6	Negative
#2092	+(few)	3.52×10^5	Negative
#2239	+(few)	Negative	(7×10^7 ml) *
#2368	+	3×10^7	Positive
#2383	+(few)	Negative	(2.52×10^7 ml)
#1959	Negative	2.5×10^4	Negative
#2088	Negative	1.43×10^5	Negative
#1920-2	Negative	1.54×10^7	Negative

Two samples were obtained on different days

* Other NLF_s were found

This table shows that vibrio-like motility may be due to pseudomonas (#2239, #2383) and this motility may be absent when vibrios are either small in number or non-motile (#1959,#2088,#1920(2))

We had frequently isolated as shown in Table II, organisms belonging to the Achromobactor group resembling Mima polymorpha and in maximum number of aspirates they were the predominant organism. To know whether these organisms were coming from hospital environment or were associated with diarrheal diseases, attempt was made to isolate these from the hospital environment. The results are given in Table IX.

TABLE IX

ISOLATION OF ACHROMOBACTOR ORGANISMS FROM

THE HOSPITAL ENVIRONMENT

Materials examined	Method in short	Growth of Achromo-bactors
Patient's glasses for drinking	The wet insideof the glass and its rim swabbed and streaked on MacConkey plate and blood agar; identified by colony morphology. 28 glasses examined.	26
Wash basin for washing patient's utensiles	Swabs from inside basin walls streaked on blood and Mac-Conkey agar. 2 samples examined	2*
Washed utensils	Swabs smeared on wet surface or trypticase broth soaked-swab smeared on	3
Utensils between pre-liminary & final washing	Swabs smeared onwet surface 1 sample examined	1
Tap water taps on kitchen& pantry	10 ml of water enriched in 200 ml of trypticase soy broth- 6 samples examined	5*
Skin over anti-cubital fossa from patients,nurses & doctors	Tryptiease broth soaked-swab rubbed on anti-cubital fossa & streaked on blood and MacConkey agar. 20 samples examined	7

* Pseudomonas also present

It is seen from this table that Mima like organisms are widespread in the environment. Therefore some of these isolated from aspirates, may be contaminants introduced through drinking water or food.

Further work is continuing on this problem.

Summary

The jejunal aspirates of diarrheal patients contain bacteria, fungi and parasites. In the case of cholera patients, the vibrio count in the aspirates is slightly less than in the liquid stool. The number of bacteria in these aspirates is generally of the order of 10^3 -10^5 per ml. Pseudomonas may be responsible for the 'vibrio like' motility of some of these aspirates. The Mima like organisms isolated may be due to contamination of drinking water and food by these organisms and probably has no correlation with diarrhea or cholera. No correlation was found with jejunal organisms and malabsorption.

I(d)

PHAGE RESISTANCE IN VIBRIO CHOLERAE

by : Rizvi, S., Benenson, A.S. and (Mrs) Alam, A.

In recent years, four groups of choleraphages have been used by Mukerjee(1) for separating Vibrio cholerae into types and for identification of El Tor vibrios. Feeley(2) has recommended the use of Mukerjee phage IV for the infraspecific separation of 'O Group I' vibrios into subtypes depending on sensitivity or resistance to phage IV. Therefore, choleraphages have assumed great importance in vibrio cholerae taxonomy. However, frequent laboratory contamination of phage stocks and the reported isolation of phage IV resistant strains of true cholera by Nobechi(3) are the two practical problems that make vibrio classification based on phage work a difficult task.

Hosts specific for the individual standard Mukerjee phages are not available. Mukerjee(4) has described phage resistant mutants of strains 154 and the data presented indicate that the phage resistance in vibrios follows the same principles as apply to other bacteria-phage systems. This project was undertaken to evolve specific host strains in our laboratory, and test the yield and the nature of the phages produced on such hosts; also to prepare phage IV resistant mutants of true cholera and study their properties.

Material and Method:

More than one specific host for each choleraphage is desireable so that if one such host was not satisfactory, another could be used in its place. Therefore, in addition to strain 154, two other strains, Bg 29 (resistant to phage III) and strain 508 (resistant to phage I) were also used. These vibrio cholerae strains and the four choleraphages were kindly supplied by Dr. Mukerjee.

Vibrio strains isolated locally (9 Inaba & 16 Ogawa) were used for the isolation of phage IV resistant mutants. 2 of the Inaba and 6 of the Ogawa were isolated from water and the rest (7 Inaba & 10 Ogawa) were isolated from human sources. 2 of the human Inaba, 4 of the human Ogawa and 3 of the Water Ogawa were chicken cell agglutinating and the remainder chicken cell negative.

The broth used for bacterial growth contained 1 percent trypticase (BBL) and 1 percent sodium chloride in distilled water (T_1N_1). Bacto-agar (Difco) was added to a concentration of 1.5% to prepare basal agar plates and to a concentration of 0.7% to prepare soft agar. All media were autoclaved at 20 lbs. pressure for 20 minutes; the pH was about 7.1 and did not need adjustment.

Two slightly different methods were used for isolation of phage-resistant isolates: (1) 0.5 ml of young 4-5 hrs. bacterial culture in soft agar were mixed with phage enough to cause confluent lysis (after

(more)

overnight incubation) and poured on basal agar plates. If no resistant colonies were visible on these plates in the morning, they were stored at room temperature for 4-5 days. Generally, resistant colonies were not seen after overnight incubation but they appeared after 2-3 days' storage. The supposedly resistant colonies were streaked on T_1N_1 plates for isolation of single coloney cultures completely free from phage. This step of streaking on T_1N_1 helped to differentiate between partially susceptible and completely resistant isolates; if the colony was still susceptible, bacterial growth did not occur at the origin on the plate (the lace of initial application). Phage free single colony isolates from the supposedly resistant streakings were subcultured and tested for their lytic pattern with other phages, as described by Rizvi & Monsur(5). If found to be resistant, they were further treated with other phages and tested in the manner described above.

(2) To 50 ml of young cultures (4-5 hours) in T_1N_1 in a Flask, 0.1 ml of stock phage was added and the mixture incubated overnight. Each day, 0.5 ml of this mixture was mixed with soft agar and poured on T_1N_1 plates.

Whether the resistance of the isolates was due to mutation or lysogenization was tested according to the method described by Rizvi and Monsur (5).

Mutation rate was estimated by treating 5 ml of overnight culture containing about 10^{10} bacteria by 10^{11} viable phage particles contained in 0.3 ml of lysate, incubating the mixture at 37°C for 1 hour, then plating aliquots on T_1N_1 plates and counting the number of resistant colonies appearing in a known quantity of samples.

Result and Discussion:

The evolution of the resistant strains is given in Table I. The nomenclative of Demerec and Fano (6) has been used for describing these mutants.

TABLE I

SCHEME FOR ISOLATION OF PHAGE RESISTANT STRAINS

Bacteria	Phage used	Resistant strain on level	Specific host for phage
Strain 154	I	154/I,II	
" "	II	154/II	
" "	III	154/III	
" "	IV	154/IV	
" " /I,II	III	154/I,II/III	IV
" " /I,II	IV	154/I,II/IV	III
" " /III	IV	154/III/IV	
" " /III/IV	I	154/III/IV/I,II	
" " " "	I	154/IV/I,II	III
" " " "	I	154/III/IV/I	II
" " " "	II	154/III/IV/II	I
" Bg29/III	I	Bg29/III/I,II	IV
" " "	II	Bg29/III/II	
" " "	IV	Bg29/III/IV	
" "/III/II	IV	Bg29/III/II/IV	I
" 508/I	II	508/I/II	
" " "	III	508/I/III	
" " "	IV	508/I/IV	
" " /I/II	III	508/I/II/III	IV
" " /I/II	IV	508/I/II/IV	III
" " /I/III	IV	508/I/III/IV	II

It may be seen from this table that isolates resistant to groups II, III & IV phages are immune to lysis by the corresponding types of phages only but the isolates obtained after action of group I phage were mostly insentive to both group I & II. The effect of subsequent resistance were additive. However, out of about 1000 resistant strains obtained by treatment of 154/III/IV with phage I, one strain was found to be 154/III/IV/I, and twenty were found to be 154/IV/I,II indicating that resistance to phage I does not always give resistance to phage II as well. This also points out the fact that all sequential phage resistance need not be additive as a strain resistant to phage III & IV when treated with phage I could become resistant to phage I & II and lo become susceptible to phage III to which it was originally resistant!

The resistance acquired by the bacterial strains may be due to either lysogeny or mutation. Since the resistant colonies appeared in very low frequencies (1 in 10^8 or lower) it was suspected that they were mutants. To test this, the host specific mutants described in Table I were heat and ultra violet treated for release of the latent phage as described before. When such treated cultures were tested on strain 154, no phages were detected. This suggests that the resistance was due to mutation and not due to lysogenization.

The mutants and the parent strains were found similar in their biochemical properties (growth in TlNl, bile peptone and 0.9% trypsin; sensitivity to polymixin B; Indole production; Nitrite production; V.P. test; and Heiberg group).

As a preliminary step, in order to determine the suitability of the specific hosts for stock preparation, it was decided to compare plaque formation of these hosts with plaque formation on strain 154. Stocks of the four phages were enumerated simultaneously on the specific hosts and strain 154. The counts obtained are given in Table II.

TABLE II

COMPARISON OF PLAQUE FORMING ABILITY OF SPECIFIC HOSTS WITH STRAIN 154 FOR MUKERJEE PHAGES

Bacterial strain	Phage I	Phage II	Phage III	Phage IV
154	2.1×10^{10}	1.9×10^{9}	1.5×10^{9}	4.0×10^{7}
154/I,II/III	-	-	-	4.0×10^{7}
154/I,II/IV	-	-	1.6×10^{9}	-
154/III/IV/I	-	1.8×10^{9}	-	-
154/III/IV/II	2.1×10^{10}	-	-	-
Bg 29/III/I,II	-	-	-	4.2×10^{7}
Bg 29/III/II/IV	2.2×10^{10}	-	-	-
508/I/II/III	-	-	-	4.1×10^{7}
508/I/II/IV	-	-	1.8×10^{9}	-
508/I/III/IV	-	2.0×10^{9}	-	-

Since each stock gave the same number and type of plaques on the specific mutants as on the strain 154, it was concluded that the mutants will support growth of the phages quite normally and these specific hosts can be used as hosts for stock preparation.

There is a possibility that the phages prepared on the mutants may become different after repeated passages when compared to the phages prepared on strain on strain 154. Therefore, each phage was passaged twenty times on its specific host and then the lytic pattern was checked on the standard strains used for typing the phages. The result of the phages passaged on the four mutants of strain 154 is given in table III as example.

TABLE III

LYTIC PATTERN ON TYPING STRAINS ON PHAGES PASSAGED ON SPECIFIC HOSTS.

Phage type	Method of preparation	Sensitivity of typing strains				
		154	508	528	542	Bg 29
I	Stock	+	-	+	-	+
	20 times passaged on 154/III/IV/II	+	-	+	-	+
II	Stock	+	+	-	-	+
	20 times passaged on 154/III/IV/I	+	+	-	-	+
III	Stock	+	+	+	+	-
	20 times passaged on 154/I,II/IV	+	+	+	+	-
IV	Stock	+	+	+	+	+
	20 times passaged on 154/I,II/III	+	+	+	+	+

The table shows no change in the lytic pattern of the original stock phages and of the phages serially passaged twenty times on the specific hosts. However, this does not rule out the possibility of differences in their host range.

Since these strains were evolved for stock preparation, the yields of the phages on the specific hosts were compared with the yield on strain 154. Stocks were prepared by the following three methods:

1. Single plaque method : Normal size plaque from an overnight plate along with the adhering bacteria were put in flasks containing 50 ml T_1N_1 and incubated overnight. Chloroform was added and the preparation titrated for viable counts on strain 154.

2. Confluent lysis method: Bacteria and phage in a ratio to give confluent lysis after overnight incubation were mixed in soft agar and the mixture poured on basal agar plates. After overnight incubation 10 ml of T_1N_1 broth was added on each plate, the upper soft agar was scaped off, chloroform added to it and the agar broken up in a mixer. The thick slurry was centrifuged and the supernant saved for enumerating the viable phage count on strain 154.

3. Aerated broth method: The bacteria were grown for about 5 hours under aeration at 37°C. Phage inoculum was added and the culture maintained under aeration for additional three hours. The flasks were removed, chloroform added and the lysate stored, overnight in the refrigerator. The lysate was then used for counting the number of viable phage on strain 154.

Comparison of the average yield (based on three experiments) obtained with the different methods on the specific hosts and strains 154 is presented in Table IV.

TABLE IV

COMPARISON OF THE YIELD OF PHAGES ON SPECIFIC HOSTS WITH THE YIELDS ON STRAIN 154

Phage type	Host strain	Yield of phage (Titer/ml)		
		Single plaque	Confluent lysis	Aerated broth
I	154	1.0×10^{11}	6.7×10^{10}	5.9×10^{10}*
	154/III/IV/II	9.7×10^{10}	4.3×10^{11}	3.9×10^{11}
II	154	1.2×10^{9}	5.6×10^{10}	7.3×10^{10}*
	154/III/IV/I	3.0×10^{9}	3.0×10^{11}	1.8×10^{10}
III	154	7.5×10^{9}	3.4×10^{10}	8.8×10^{10}
	154/I,II/IV	2.4×10^{9}	8.5×10^{10}	2.0×10^{10}
IV	154	1.7×10^{9}	3.7×10^{10}	1.5×10^{11}*
	154/I,II/III	1.6×10^{9}	5.6×10^{10}	7.0×10^{10}

* These figures are based on experiments performed a year ago.

This table shows that the yield of various phages on the specific mutants is essentially the same as on the strain 154.

Phage IV resistant mutants were obtained from each of the 25 locally isolated strains used. All the mutants were subjected to the tests generally used to differentiate true cholera from el tors as described in a previous paper(7). The result obtained is given in Table V.

TABLE V

COMPARISON OF PROPERTIES OF PHAGE IV RESISTANT MUTANTS WITH THEIR PARENT STRAINS

Test Performed	Number of strains showing positive or negative reactions			
	Parent		Mutant	
	Positive	Negative	Positive	Negative
Serology (Ogawa, Inaba)	25	0	25	0
Heiberg group I	25	0	25	0
Indole production	25	0	25	0
Nitrite production	25	0	25	0
V.P. test	0	25	0	25
Anaerobic glucose growth	25	0	25	0
Growth in 0.9% trypsin	0	25	0	25
Haemolysis (Plate & tube)	0	25	0	25
Chicken cell agglutination	11	14	11	14
Polymixin B sensitivity	25	0	25	0
Gispen test	25	0	25	0
Meyer test	25	0	25	0
Soda serum agglutination	0	25	0	25
Soda sublimate precipitation	25	0	25	0
Phage IV sensitivity	25	0	0	25
Sensitivity to other phages	25	0	25	0

The mutation rate for resistance towards the choleraphages was determined only in a few cases as examples. The data is presented in table VI.

TABLE VI

MUTATION RATE FOR PHAGE RESISTANCE IN SOME STRAINS OF VIBRIO CHOLERAE

Bacterial strain	Type of phage	Mutation Rate
154	Phage I	1 in 10^{8-9}
"	" II	1 in 10^{6-7}
"	" III	1 in 10^{7-8}
"	" IV	1 in 10^{7-8}
Local isolates (10 strains)	" IV	1 in 10^{7-8}

This table shows that resistant strains appear at a rather low frequency indicating that these are probably mutants and not have become lysogenized.

Summary :

The data presented above show that phage resistant mutants of vibrio cholerae (with no change in any other characteristic) can be easily obtained. Such resistant mutants behave normally towards phages to which they are still susceptible. Phages produced on specially evolved specific hosts do not show any change in their lytic pattern or yield when compared with phages produced on strain 154. As the mutants do not show any other difference from the parent strain's, usefulness of phage in taxonomic work and cholera therapy is theoretically questioned. For the identification of el tors, some of the others characteristics must therefore be used and complete reliance on phage reactions in vibrio taxanomy should not be made.

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I(e)

EFFECT OF STORAGE TEMPERATURE ON PHAGE VIABILITY

by Rizvi, S., (Mrs) Alam, A., and Salek, M.A.

Lysates of the four groups of the Mukerjee phages were stored at -45°C +4°C, and 36°C. Also portions were freeze dried and stored at 4°C. At various intervals samples were removed and the number of viable phage enumerated on strain 154. The result is given in the following table:

Phage Groups	Storage time	Initial Count	Final Phage count				
			-45°C	+ 4°C	36°C	Freeze dried 0 weeks	Freeze dried 17 hours
I	17 weeks	2.5×10^{10}	1.1×10^{9}	7.0×10^{6}	0*	2.0×10^{9}	8.9×10^{8}
II	17 weeks	1.6×10^{10}	7.5×10^{8}	1.0×10^{8}	5.0×10^{7}	1.6×10^{9}	7.5×10^{7}
III	17 weeks	6.0×10^{9}	5.0×10^{5}	1.1×10^{6}	0*	3.4×10^{8}	7.5×10^{7}
IV	17 weeks	4.9×10^{9}	5.0×10^{7}	6.0×10^{5}	0*	3.1×10^{8}	1.0×10^{7}

*0.1 ml. samples of the undiluted phages did not yield any plaques; phage I in nine weeks; phage III in 12 weeks and phage IV in seventeen weeks.

It is seen from this table that phage I survives best at -45° or in the freeze-dried condition and dies very rapidly at 36°C. Phage II on the other hand survives well under all conditions. Phage III is inactivated rapidly at 36°C and less so at -45°C; freeze drying and then storing at refrigerator temperature seems to work better. Phage IV also dies out quickly in the incubator and a little slowly at refrigerator temperature. The survival at -45°C or in the freeze-dried state is just about equal.

Thus the best way to store all phage preparations, is in the freeze-dried condition. Storage at -45°C is good except for phage III and at +4°C is good except for phage IV.

I (f)

CHOLERAPHAGE PRODUCTION IN STERILE STOOL FILTRATES

by: Rizvi, S., (Mrs.)Alam, A., Salek, M.A.

Phage therapy of cholera patients can be successful only if the phages multiply normally in the gut and thus initiate cycles of bursts and reinfections. Production of phage in the human gut can not be studied directly for technical difficulties. However, phage production in stool filtrates can be studied and the findings taken as indirect indication of the process as it might be occurring in the human gut. But phage multiplication is dependent upon the growth of the host bacteria, and therefore the effect of the stool filtrate on the host vibrio must be ascertained first.

The present study was undertaken to find out the effect of sterile stool filtrates from cholera and non-cholera patients on the growth of vibrios as well as on the production of choleraphages when the host vibrio is grown in stool filtrates.

Materials and Methods

The fluid stool was first centrifuges at high speed to remove debris. The centrifugate was then filtered through seitz filter of 0.45 μ pore size. It was filtered again through millipore filter of 0.3 μ pore size. Finally the sterility of the filtrate was determined.

Mukerjee vibrio strain 154 and Mukerjee choleraphage I were used.

Result and Discussion

Vibrio growth in stool filtrates:

Loopfuls of overnight vibrio culture in T_1N_1 broth were inocculated in 5 ml. of the sterile stool filtrate. After overnight incubation the number of viable bacteria in each milliliter of the filtrate was determined by surface plating. The result is given in the following table:

..../..

Actual Vibrio Count in Stool Filtrates After Overnight Inocculation

Vibrio Status of patient	Stool filtrate from Case #	Titer of Bacteria per ml.
Positive	530	4.9×10^8
	812	7.6×10^7
	882	6.6×10^8
	1028	1.0×10^9
	1198	3.7×10^7
	1199	4.1×10^9
	1200	1.1×10^3
	1206	4.8×10^8
	1255	8.4×10^6
Negative	823	2.1×10^8
	825-2	1.7×10^7
	1017	1.0×10^9
	1030	1.5×10^9
	1067	3.4×10^9
	1071	1.1×10^8
	1151	2.1×10^8
	1169	7.1×10^6
	1211	1.0×10^8
	1213	0
	1214	1.6×10^9

The above table shows that the host vibrio did not grow in one stool filtrate, and in another the growth was poor. However, 18 of the 20 (90%) filtrates used supported the growth of the host vibrio in a normal manner. This indicated that normal growth of vibrios takes place in stool filtrates of diarrheal patients.

Phage production on hosts grown in stool filtrate:

Phage I production on host strain 154 grown in the presence of stool filtrates was attempted by mixing 1 ml. of overnight culture of the host bacteria in T_1N_1 broth with varying quantities of T_1N_1 broth and/or stool filtrate to give a total volume of 5 ml. The mixture was allowed to grow for four hours at 37°C. Then it was infected with phage and the number of phage enumerated in the routine manner after growth of one hour and three hours. The result of one experiment is given as an example of the general trend since the study is not complete.

Phage Production on Hosts Grown in Stool Filtrates

Bacterial culture	T_1N_1 broth	Stool filtrate	Initial phage titer per ml.	Phage titer per ml. after: 1 hour	3 hours
1 ml.	4 ml.	0 ml.	7.6×10^3	5.5×10^4	1.2×10^8
1 ml.	3 ml.	1 ml	"	2.5×10^5	1.0×10^9
1 ml.	2 ml.	2 ml.	"	1.0×10^6	1.3×10^9
1 ml.	1 ml.	3 ml.	"	2.8×10^5	1.1×10^9
1 ml.	0 ml.	4 ml.	"	3.3×10^5	4.7×10^8

This table shows that stool filtrate does not affect production of phage I.

Summary

Growth of vibrios and production of phage occur normally in bacteria free fluid stools of diarrheal patients sterilized by filtration.

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OTHER STUDIES.

by: Rizvi, S., (Mrs) Akbar, R., Salek, M.A., Kibriya, G., Day, C., amd (Mrs) Alam, A.

Isolation of Vibrios from Stool.

Rectal swabs are used routinely in our studies for the isolation of vibrios. The failure to isolate vibrios from many patients suffering from cholera-like diseases, raised the suspicion that too little stool was being examined and that vibrios may have been found if a bigger inolculum was used.

Therefore a study was started wherein 1.0 g and 0.1 g of stool were enriched in 100 ml of bile peptone-tellurite broth, incubated 8 hours and then streaked on solid media for the identification of the vibrios. The results were compared with the vibrio status of the rectal swab taken on the same day. The data are given below:

Rectal swab vibrio status	Number of samples	Number of Patients	Days after onset	Number of vibrio-positive samples	
				0.1 gm stool	1.0 gm stool
+	9*	7	1-22	6	5
-	95**	29	2-42	12	9
Total:	104	36		18	14

* 1 sample from patient treated with antibiotic.
** 3 samples from patient treated with antibiotic.

The first part of the table shows that when the rectal swabs were positive, out of a total of 8 samples (excluding one antibiotic treated sample), six 0.1 g samples were positive. This indicates that the stool samples are inferior to the rectal swabs for positive vibrio isolations. However, the second part shows that out of 92 samples (excluding three antibiotic treated samples) at the time the rectal swab was negative, 12 samples of 0.1 g stool gave positive isolations indicating that this is a good method for the isolation of vibrios. There is no good explanation to this dilemma. It seems that nature of the contaminating bacteria among other things determines whether a positive isolation is made or not. Obviously this requires further study.

Isolation of vibrios from duedenal aspirate.

We were interested to know whether the vibrios multiply in any large numbers in the upper intestinal tract, since it is here where the infection first occurs, and it is probably here where the nutrients are available in large quantities. A number of samples

were obtained from patients whose rectal swabs were checked for vibrios. The result is given in the following table:

Rectal swab vibrio status	Number of samples	Number of patients	Days after onset	Number of vibrio positive duodenal aspirates
+	2	2	2-4	* 1 (2.9×10^6/ml)
-	17	11	6-41	** 1 (3.0×10^6/ml)

* sample obtained on the 4th day of onset
** sample obtained on the 19th day of onset.

The figure in parenthesis is the actual vibrio count/ml of aspirate.

It is significant to note that out of six samples of duodenal samples taken at a time when rectal swabs were positive, only one duodenal aspirate yielded vibrios. However, in one case the duodenal aspirate was positive while the rectal swab was negative. This indicates that although occasionally vibrios may be found in the duodenal aspirate, they generally do not multiply in great numbers in this region. This aspect is also under further investigation.

Isolation of Vibrio strain lacking in antigen A.

The water studies section isolated a strain (number 2076) that did not agglutinate on the slide with group antiserum (provided by Dr. Goodner) but gave strong agglutination with specific Inaba antiserum. To get a better picture of its antigenic structure, tube agglutination tests were performed. The results are given below:

Antiserum	Titer giving tube agglutination with various bacteria		
	Inaba (B1)	Ogawa (B65)	2076
Goodner's group antiserum (pool of 8 Ogawa and 8 Inaba antisera, unabsorbed, initially diluted for slide agglutination)	1/20	1/10	1
Inaba antiserum, unabsorbed, (prepared at CRL with strain B1)	1/6400	1/3200	1/800
Ogawa antiserum, unabsorbed, (prepared at CRL with strain B65)	1/400	1/6400	<1/100
Specific Inaba antiserum (antiserum against B1 diluted and absorbed with B65)	1/40	<1/10	1/40

Antiserum	Titer giving tube agglutination with various bacteria		
	Inaba (B1)	Ogawa (B65)	2076
Specific Ogawa antiserum (antiserum against B65 diluted and absorbed with B1)	<1/10	1/160	<1/10

< The fractions indicate the lowest dilution of the antiserum used which did not give agglutination.

These findings corroborate the initial observation of the water studies section. Since, according to present knowledge about vibrio antigenic structure the Inaba are considered AC and the Ogawa as AB(C) the conclusion was drawn that this organism has poorly developed or is completely deficient in group antigen A.

When a whole Inaba antiserum (against B1) was, after dilution, was absorbed with 2076, the resultant specific antiserum gave the following reaction:

Antigen	Absorbed antiserum dilutions					
	Neat	1/10	1/20	1/40	1/80	1/160
B1 (Inaba)	++++	++++	++++	++++	+++	++
B65 (Ogawa)	++++	++++	++++	++++	+++	+++
2076	-	-	-	-	-	-

This is consistent with the assumption that 2076 has removed the 'C' antibodies from the Inaba antisera, leaving anti A antibodies which react with both Ogawa as well as Inaba organisms. This also confirms differences in the antigenic structure of 2076 from other Inaba strains, because in such absorptions both 'A' and 'C' antibodies are removed.

Antiserum against 2076 was prepared. The results of a tube agglutination test are presented in the following table:

Antigen	Dilutions of antiserum against 2076					
	1/200	1/400	1/800	1/1600	1/3200	1/6400
2076	++++	++++	++++	++++	+++	+++
B1 (Inaba)	++++	++	++	+		
B65 (Ogawa)	±					

Since the 2076 antiserum agglutinates at a much higher dilution with the 2076 strain than with the Inaba strain B1, it again suggests

a difference in the antigenic structure of these bacteria. This difference may be with regard to lack of antigen A in 2076 and development of another antigen in its place.

The organism was used as an agglutination antigen against a group of routine human sera in parallel with the standard strains, (4650 Ogawa and 466 Inaba). While there tended to be a general agreement at the lower levels in Inaba titers, CRL case number 515 revealed the presence of antibodies against this strain, when there were none against the classical strains. The sequential sera of this patient showed a rise in titer to the standard Inaba strains but no rise in anti-2076 titer occured (Table).

Day of serum collection from onset	Titer		
	Ogawa	Inaba	2076
1	<10	10+	160
2	<10	10+	80
4	<10	20	80
7	<40	40+	80+

The serological finding indicates that while this strain possesses antigen C it also has its own compliment of antigens. This justifies this being considered not a strain of cholers vibrio but a non-agglutinable vibrio as recommended by Feeley; it is noted that Gallut reported 14% of strains containing antigen C, but no antigen A among non-cholera vibrios studied by him.

Identification of Shigella-like organisms.

Last year Dr. Craig reported the isolation of 93 organisms from Meyer Bazar that gave suhar reactions similar to Shigellas but did not agglutinate in any of the antisera when used unheated. Therefore the bacterial cultures were boiled for one hour at 100°C and then slide agglutination tests were performed and the result summarized below:

Shigella type	Number of cultures
Type A	17
Type B	33
Type C	1
AD group	7
Paracolon	3
Non-agglutinating	32
Total	93

Nineteen cultures were sent to Dr. Ewing at CDC for confirmatory identification. The result from his laboratory has been

with the result from our laboratory in the following table:

Shigella type	Ewing report	CRL report
Shigella A	5	5
Shigella B	1	culture died
Shigella B	unidentifiable	7
Shigella C	6	unidentifiable.

The findings agree in the cases of tyoe A only. In the cases of Shigella B or Shigella C our results are the reverse of those of Dr. Ewing. An attempt is being made to explain this discrepancy.

Chicken cell positive true cholera.

Chicken cell agglutination is performed routinely on all our vibrio isolations. In the period reported above, 75 confirmed chicken cell positive vibrio cholera were isolated. Some of the characteristics of these organisms are given below.

These chicken cell agglutinating true cholera strains were never recognised on colonies picked from the primary isolation plate which had been streaked with the rectal swab. They were always observed when colonies were picked from GA plates which we call purity plates on which T_1N_1 broth cultures (prepared for testing the sugar reactions) were streaked for testing the purity of the culture. On these plates, in contrast to El Tor, both chicken cell agglutinating and non-agglutinating colonies of true cholera were generally found as shown in the following table:

Serotype	Number of CCA positive strains	Number of colonies tested per strain	Number of colonies tested Total	Chicken cell agglutination observed: Colony nos. per strain	Chicken cell agglutination observed: Total Nos.	Chicken cell agglutination observed: Colony nos. per strain	Chicken cell agglutination observed: Total Nos.
Ogawa	4	16-25	77	8-25	52	0-9	25
Inaba	71	9-22	1107	1-21	613	0-16	494

To explore this further 45 colonies from the primary SP or GA isolation plate were each individually streaked on GA, TTGA, and also inoculated in T_1N_1 broth. When tested for chicken cell agglutination all the colonies on the two solid media were found to be non-chicken cell agglutinating. However, vibrio colonies from four of the broth samples were found to be chicken cell aglutinating on both solid media. On two further successive plate to plate passages on both

solid media the character of the colonies did not change but broth to broth passages kept on showing positive and negative colonies when streaked on solid media. This suggests that the T_1N_1 broth favours the development of this character.

Attempt was made to ascertain the role of the liquid media and the result obtained is given below:

24 single CCA (-) colonies from direct GA and TTGA plates inoculated in four broths

T_1N_1 (pH7.4)	T_1N_1 (pH 9.0)	Peptone water (pH 7.4)	Bile peptone (pH 7.4)
After 48 hours all tubes streaked on GA; CCA of a sweep from the origin checked	After 48 hours all tubes streaked on GA; CCA of a sweep from the origin checked	After 48 hours all tubes streaked on GA; CCA of a sweep from the origin checked	After 48 hours all tubes streaked on GA; CCA of a sweep from the origin checked.
CCA - 15 CCA + 9	CCA - 14 CCA + 10	CCA - 16 CCA + 8	CCA - 24 CCA + 0

None of the colonies inoculated in the bile peptone broth gave rise to chicken cell agglutinating cultures. However, a good proportion of the same colonies inoculated in the other three broths became chicken cell agglutinating. This again implicates the broth in the development of this character.

The following scheme shows that chicken cell agglutinating colonies give rise only to chicken cell agglutinating bacteria as in El Tore but the chicken cell non-agglutinating colonies keep on throwing chicken cell positive and negative bacteria.

Strain 18626 (b) gave rise to 35 CCA (-) and 10 CCA (+) colonies. One CCA (+) and one CCA (-) colony was subcultured in T_1N_1 broth, and streaked on GA plate for discrete colony formation.

CCA(+) — CCA(-)

36 colonies from the GA plate put in T_1N_1 broth; incubated; streaked on GA; CCA of a sweep from origin checked

24 colonies from the GA plate put in T_1N_1 broth; incubated; streaked on GA; CCA of a sweep from origin checked

cont. . .

CCA(-) 0
CCA(+) 36

47 colonies from one CCA(+) plate put in T_1N_1; incubated; streaked on GA; CCA of a sweep from origin checked

CCA(+) 4
CCA(-) 20

24 colonies from 1 CCA(-) plate put in T_1N_1; incubated; streaked on GA; CCA of a sweep from origin checked

CCA(-) 0
CCA(+) 47

CCA(+) 7
CCA(-) 17

The data presented above may be summarized as follows:- (1) a small number of CCA(-) strains when incubated in T_1N_1 broth give rise to both CCA(-) and CCA(+) bacteria. (2) if solid medium passage is done this separation does not occur. (3) the chicken cell agglutinating strains remain chicken cell agglutinating after repeated passages in the broth.

Since only a small proportion of the total routine samples gave rise to CCA(+) strains vibrio cholerae, one may suspect that these few strains are somewhat inherently different from the majority of the isolations. Thus it may be both the T_1N_1 broth and the genetic characteristic of these bacteria that play a role in the development of chicken cell agglutination as a marker. But it is not proven that the majority of the CCA(-) isolates will not yield CCA(+) cultures.

Therefore, a study was planned to ascertain whether the isolates reported to yield only CCA(-) cultures were actually different in any way from those isolates that had yielded both CCA(+) and (-) cultures. Strain 86654,a known CCA(-) culture was inoculated into a number of broths and incubated. At various intervals, loopfuls were streaked on GA plate. After overnight incubation of the GA plate, 25 individual colonies from each broth streaking were radially subcultured on GA plate in order to get enough bacterial growth for preparing the heavy milky suspension of the bacteria for the CCA test. The agglutination pattern is given in the table on the next page.

Table.

Appearance of chicken cell agglutinating cultures from known chicken cell non-agglutinating isolates.

Broth used	Proportion of CCA(+) and CCA(-) cultures at various days of incubation					
	1 day		3 days		6 days	
	CCA(+)	CCA(-)	CCA(+)	CCA(-)	CCA(+)	CCA(-)
T_1N_1, pH 7.2	0	25	0	25	0	25
T_1N_1, pH 7.2 tellurite	0	25	0	25	0	25
T_1N_1, pH 8.5	0	25	17	8	18	7
T_1N_1, pH 8.5 tellurite	0	25	13	12	13	12
Bile peptone, pH 7.2	0	25	0	25	0	25
Bile peptone, pH 7.2 tellurite	0	25	2	23	3	22
Bile peptone, pH 8.5	0	25	0	25	0	25
Bile peptone, pH 8.5 tellurite	0	25	0	25	0	25
Heart infusion broth	0	25	0	25	0	25
Alkaline peptone water	0	25	0	25	0	25

The table presented above shows that strains that were initially chicken cell negative will give rise to chicken cell positive cultures and thus all Vibrio cholerae may behave in this way. Further work is being continued.

Sucrose negative cholerae vibrios.

On 4 December, 1964, a 2½ years old female child was admitted pulseless, severely dehydrated, with a plasma protein of 10.2. On studies with the family, two siblings were found to be carriers, on the 5th; a third sibling was bacteriologically positive on the 6th., 7th and 8th. On the 7th, another child, a 14 years old girl was admitted to CRL with a pulseless and severely dehydrated condition, with a plasma protein of 14.4. This family shared the same dug well. Three of five members were bacteriologically positive on the 7th and 8th of December. All the isolates from these individuals failed to ferment sucrose in the standard fermentation test.

To determine whether an adaptive enzyme was involved, 18 single colonies isolated from one of the carriers were sub-cultured into 5 ml T_1N_1 broth, 5 ml sucrose broth,, and also streaked on sucrose agar plates. The sucrose broths were examined daily for evidence of utilization of sucrose (change in colour) and the T_1N_1 broth was streaked on sucrose agar plates to detect sucrose positive colonies. In sucrose broth, utilization of the sucrose began on the third day in some tubes; by the fourteenth day, 16 of the 18 cultures had become sucrose positive, while all T_1N_1 tubes continued to be sucrose negative as shown in the table below:

Appearance of sucrose fermenting cultures from non-fermenting cultures.

Days of Incubation	Number of tubes in which sucrose fermenting cultures have appeared after various days in the two broths.	
	Sucrose broth	T_1N_1 broth
1	0	0
2	0	0
3	3	0
4	1	0
5	3	0
6	1	0
7	0	0
8	0	0
9	3	0
10	2	0
11	2	0
12	0	0
13	0	0
14	1	0
Total becoming sucrose positive	16	0

A change of the culture from sucrose negative to sucrose positive was complete; either all colonies were found negative or all positive, when the sucrose broth was streaked on a sucrose agar plate.

All colonies appearing on the 18 sucrose agar plates streaked with the original clones remained sucrose negative for 4 days. Between the 5th and 6th day, 10 to 15 yellow colonies appeared on all of the 18 plates amidst the mass of sucrose negative growth.

Five sucrose positive colonies derived from sucrose negative cultures were passed five times through T_1N_1 broth. They were then put in sucrose broth and also streaked on sucrose agar plates. All

colonies had retained the power of sucrose fermentation.

This episode of water-borne cholera was caused by sucrose non-fermenting vibrios. The clinical disease in the two patients was full blown and would probably have terminated fatally without proper treatment. This outbreak is of particilar importance since commercial media have been introduced and widely used for the isolation of cholera vibrios which are based on the fact that the species normally utilizes sucrose.

Differentiation of Vibrios from Pseudomonas

Identification of vibrios belonging to [1]O group I of Gardner and Venkataraman is not difficult. However, the non-cholera vibrios (NCV's) have given us trouble because many members show differences in the colonial appearance on the routine media employed and some members appear as straight rods under the microscope. Burgey's Manual (p.90) refers to this problem when it says, "The borderline between the straight rods found in Pseudomonas and the curved rods found in vibrio is not sharp: occasionally curved rods may occur in species that normally are composed of straight rods". Shewan and Hodgkiss (1954) studied a total of 200 strains and recommended the use of the vibriostatic compound 2-4 diamino, 6-7 di-isopropyl pteridine to separate vibrio from Pseudomonas. Hugh (1964) after studying 258 strains of Vibrio cholerae, on the other hand, considers anaerobic fermentation of glucose without gas as the most important criteria of the Genus Vibrio. The shortcomings of these two studies are that small number of strains were studied and that no non-cholera vibrios were included among the organisms studied. In addition, the vibriostatic compound is not available commercially.

The present study was undertaken to determine the applicability of the conclusions of Shewan and Hodgkiss and of Hugh to non-cholera vibrios and to correlate these to some other characteristic that can be performed easily in the laboratory. Therefore, the following additional tests were done; morphology, Heiberg group, aerobic glucose fermentation, chicken cell agglutination, polymixin B sensitivity, vibrio-like motility in the dark field, pellicle formation in gluconate broth, growth in trypsin broth and growth on alkaline laurylsulfate tellurite medium. The result is presented in the following table.

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Some Properties of the Members of Genus Vibrio and Pseudomonas

BACTERIA			NUMBER OF STRAINS SHOWING POSITIVE REACTIONS			
Genus	Serotype and Heiberg group	Number of Strains used	Morphology (curved rods)	Sensitivity to vibriostatic compounds	Anaerobic glucose fermentation	Growth on alkaline laurylsulfate medium
Vibrio	Inaba V	4	4	4	4	4
	NCV I	4	4	2	4	4
	NCV II	15	15	14	15	15
	NCV III	1	1	1	1	1
	NCV V	10	0	1	10	1
	NCV VII	8	0	8	8	8
Pseudomonas	VI	7	0	0	0	0

* The result of chicken cell agglutination, aerobic glucose fermentation, polymixin B sensitivity, vibrio-like motility in the dark field, pellicle formation in gluconate broth, and growth in trypsin broth have not been given in the above table because these results were ambiguous.

This table shows that all of the 42 vibrio-like organisms ferment glucose anaerobically but 18 belonging to Heiberg groups V and VII appear as straight rods under the microscope. All the 7 Pseudomonas appeared as straight rods and did now show anaerobic fermentation of glucose. The 7 Pseudomonas and 12 of the NCV's (9 of Heiberg group V, 1 of Heiberg group II and 2 of Heiberg group I) were resistant to the vibriostatic compound. Thus anaerobic glucose fermentation seems to be a better criterion for separating vibrios from Pseudomonas than morphology or reaction towards the vibriostatic compound.

Nine of the Heiberg group V NCV's and all the Pseudomonas did not grow on the alkaline lauryl sulfate tellurite medium (ALST) but the Heiberg group V Inaba and other NCV's showed normal growth on this medium. This means that anaerobic glucose fermentation of NCV's (excepting Heiberg group V) shows a better correlation with growth on ALST medium than with morphology or sensitivity to the vibriostatic compound. Therefore, separation of Pseudomonas from vibrios (excepting Heiberg group V non-cholera vibrios) can be made simply by observing whether or not growth occurs on the ALST medium.

Indicentally, the growth pattern on the alkaline lauryl sulfate medium further points out that Heiberg group V Inaba are biochemically different from Heiberg group V NCV's and that ALST medium, although good for the isolation of true cholera vibrios from field, is not suitable for some NCV's.

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EXPERIENCE WITH DARK FIELD DIAGNOSIS OF CHOLERA

by:

Dr. Rafiqul Islam & Mofizuddin Ahmed.

The Dark field examination is carried out routinely on rectal swabs of all admissions. Direct examination was performed on 424 patients reported as bacteriologically positive for V. cholerae; in 42% the specific infection was correctly diagnosed; in another 37%, vibrios were reported but they were too few to type; in the other 22% vibrios were not recognized.

The rectal swabs of 294 patients were incubated and re-examined after several hours (or overnight). V. cholerae infection was recognized in 60 instances after the direct examination had been reported as negative; in 76% of the samples, the vibrios were recognized. In 2%, vibrios were recognized but were too few to type. 43 of the 177 swabs positive on direct examination were reexamined; 3 positive on direct were reported negative after delayed examination. Swabs of 142 patients were only examined after incubation; vibrios were correctly identified in 87% of these.

The overall performance of the method, including examination immediately and after incubation was excellent 88.7% were correctly identified, 1.2% were recognized as containing vibrios too few to count, and only 10.1% were missed.

In 13 instances, among over 1300 cases, vibrios were reported by the dark field method but bacteriological examination was negative, for a maximum false positive record of 1%. Some of these cases may actually represent bacteriological failures; correlation with the serological results will be helpful.

ANTIBIOTIC THERAPY OF CHOLERA.

II (a)

By Drs. J.Lindenbaum, R.Islam, W.B.Greenough III, R.Akbar, and S.Rizvi.

The effectiveness of tetracycline as an adjunct to intravenous fluid and electrolyte replacement in the treatment of cholera has been established in a series of clinical trials undertaken in Dacca[1] and Calcutta[2] during the past three years. The administration of tetracycline markedly reduces the duration of diarrhea and bacteriologic positivity, thereby diminishing the total amount of intravenous fluid and close medical and nursing supervision required.[1,2] Excellent results are obtained when the antibiotic is given via the oral route, and the addition of intravenous tetracycline to the regimen does not yield any further therapeutic gain.[2]

Several practical questions remain unanswered. Since cholera is overwhelmingly a disease of poor people occuring in countries with limited resources, it is imperative to minimize the cost of the therapeutic regimen without sacrificing its clinical effectiveness. We have therefore undertaken to study a number of different antibiotics and a variety of regimens with particular emphasis on the duration of therapy. These studies are not yet completed, but a report of our preliminary findings on 364 patients studied during the 1964-65 fall-winter epidemic is presented at this time.

Experimental design. In evaluating the therapeutic effectiveness of an agent, it is important that patients in various groups be as similar as possible and that recognized or unrecognized bias not enter into the selection of patients for various groups or into the interpretation of clinical response. Ideally a "double blind" study can be conducted in which randomly selected patients receive either a placebo or active agent and in which neither the patient nor the physician knows the nature of the agent given. Such studies can best be performed on small numbers of patients since they require a great deal of careful supervision. In an epidemic situation, such as that faced by our ward during the cholera season in Dacca, large scale double blind studies have not proved practical. However, if the studies are limited to a few patients, misleading information about the range of therapeutic response in cholera may be obtained. Luckily, objective criteria are available to determine therapeutic response in cholera, i.e., the duration of diarrhea, volume of stool output, and duration of bacteriologic positivity. All of these measurements can be made at periodic intervals in each patient. We

therefore elected to study large numbers of patients using these objective criteria in the absence of a "double blind" situation. In an attempt to further simplify administration of the trial, it was decided to assign all patients admitted on a given day of the week to a given regimen. Thus, for example, all patients admitted on Tuesday were to receive tetracycline 250 mgm six hourly for 48 hours; all admitted on Wednesday, the same regimen for 72 hours; all on Thursday, no antibiotic therapy, etc. In this design the assumption was implicit that the patient population admitted on one day of the week did not differ in any important way (such as age, sex, body weight, duration of symptoms, severity of dehydration, etc.) from those admitted on another. Retrospective analysis of the data has essentially proved the validity of this assumption (vide infra). The first studies were conducted on all patients with proven V.cholerae infection admitted from November, 1964 through February, 1965, and this report will summarize the findings from this period only. From February to September, 1965 only occasional patients were seen with cholera, and antibiotic trials were therefore suspended. The study has been resumed during the Fall 1965 epidemic.

The patients were divided into two groups according to body weight. Those weighing less than 15 kg were given half the dose given to those 15 kg or over. In all patients included in the final analysis the antibiotic was given by the oral route only, and no patient received more than one antimicrobial agent. (Four cases which did not meet these criteria have been omitted). Therapy was started immediately at the time of admission in any suspected cholera case, without waiting for bacteriologic confirmation. (Patients bacteriologically negative for V. cholerae have been omitted from the analysis.) Every patient was placed on a cholera cot and 8 hourly measurement of intake and of stool and urine volume were obtained until end of diarrhea. Other than antibiotic therapy all patients were treated with intravenous fluid and electrolyte replacement as described elsewhere. During the period under analysis, four antibiotics (tetracycline, chloramphenicol, streptomycin, and paromomycin) given at 6 hourly intervals for 48 to 72 hours at varying dosages (see tables III and IV) were evaluated.

Criteria of therapeutic response. In the past, mortality rates have been employed as criteria of therapeutic response in studies of antimicrobial agents given to cholera patients.[3] Since the introduction of the concepts by Phillips and associates of measurement of stool output on the cholera cot and quantitative replacement of fluid and electrolyte loss, the mortality from cholera

treated with intravenous replacement therapy alone should be minimal. Mortality rates of less than 1% in adult cholera patients have been reported by workers in the Phillipines[4], Calcutta[5], Saigon[6], and Dacca[7,8], and we have obtained similar results in children[9]. Therefore mortality rate cannot be used as a criterion of clinical effectiveness of antibiotic therapy. Antibiotic treatment has, however, been shown to affect the duration and volume of stool output, the consequent total intravenous fluid requirement, and the duration of bacteriologic positivity[1,2]. These criteria, as well as the incidence of clinical and bacteriologic relapse, will be applied in the present analysis.

"End of diarrhea" was defined to have occured at the end of the last eight-hour period in which liquid stool was passed. If a patient passed soft or formed stool, thereby reaching "end of diarrhea", and subsequently passed sufficient quantities of liquid stool to require resumption of intravenous fluid therapy, this was considered to be a "clinical relapse". Since diarrhea lasted four days after admission in the average patient who did not receive antibiotics, any case treated with antibiotics in which diarrhea lasted four days (12 eight-hour periods) or more was considered to be a "therapeutic failure".

Patients were termed bacteriologically positive if either direct plate or enrichment medium cultures of rectal swab material grew out V. cholerae. Patients were kept in hospital for daily rectal swab cultures until both direct and enrichment cultures were negative for at least three days. If a patient became bacteriologically negative on both direct and enrichment cultures for at least one day and was subsequently positive, this was considered to be a "bacteriologic relapse".

One tetracycline-treated patient died in pulmonary edema 17 hours after admission and has been excluded from the analysis. There were two other deaths during the period under study, in patients admitted with neurologic complications and coma, who were unable to take oral medications, and who were therefore also excluded.

Comparability of treatment groups. As shown in tables I and II, the various treatment groups were similar as to age, sex, body weight, duration of symptoms prior to admission, as well as degree of dehydration (reflected both in plasma protein elevations and percentage with absent peripheral pulses). There were a few exceptions to this. In the adult group (table I) the mean plasma protein elevation in the group receiving paromomycin was less than in the other groups. Lesser severity of illness would have been

expected, if anything, to favor the antibiotic given to that group; however, the results with paromomycin (table III) were the least satisfactory of the four antibiotics. Among those weighing less than 15 kg (table II) there was a greater preponderance of females in the antibiotic-treated than in the untreated patients. This did not apparently affect the results (vide infra). Finally the degree of dehydration may have been slightly greater in the chloramphenicol-treated children (table III) than in the others.

Results of therapy. The effects of therapy on various clinical and bacteriologic indices are shown in tables III and IV. In patients weighing 15 kg or greater (table III), tetracycline and chloramphenicol proved highly effective. Duration of diarrhea in patients receiving these two antibiotics was roughly half that of the group receiving no antimicrobial therapy, and there were few therapeutic failures and no clinical relapses. Total stool volume and intravenous fluid requirement were markedly reduced. Duration of bacteriologic positivity was also about half that of the untreated group. Paromomycin appeared to have minimal therapeutic effectiveness. Streptomycin was moderately effective clinically and bacteriologically.

In children weighing less than 15 kg, the results were similar, with one exception: the clinical and bacteriological effectiveness of chloramphenicol did not equal that of tetracycline. Only moderate success, similar to that achieved with streptomycin, was obtained in the chloramphenicol-treated children. The reason for the failure to achieve as good results in children as in adults with the latter drug is not certain at this time. While females predominated in the chloramphenicol group (table II), the response to antibiotic was similar in both sexes (table IVa). The dosage of chloramphenicol used appears to have been adequate (in the eight children who had diarrhea for four days or more on this drug, mean dosage was 80.7 mg/kg/24 hours). It may be important, however, that chloramphenicol was administered to children in the form of chloramphenicol palmitate syrup, while patients weighing over 15 kg received unbound chloramphenicol capsules. Chloramphenicol palmitate is itself inert; hydrolysis by intestinal enzymes to the free form of the antibiotic is necessary for an antibacterial effect.[10] It is possible that hydrolysis of the palmitate was incomplete in our patients, either due to rapid transit or poor solubility of the lipid in the voluminous watery intestinal fluid of the cholera patient.

Analysis of specific tetracycline regimens employed reveals certain trends. Increasing the dose of tetracycline did not appear to increase its therapeutic effectiveness (tables V and VI). Duration of therapy appeared to be important. Contrary to the report

of Carpenter and associates[2], therapeutic failures were seen in patients treated with tetracycline for only 48 hours, regardless of dosage. A comparison of 48 versus 72 hour treatment schedules is shown in table Va. While the numbers of patients are too small to draw firm conclusions, there was a higher incidence of therapeutic failure as well as bacteriologic recurrence in those treated for 48 hours even though they received a higher average total dose of the drug. Further information regarding necessary duration of therapy will be obtained during the 1965-66 epidemic. Two patients who did not respond to therapy with tetracycline for 48 hours will be reported briefly:

CRL no. 1686. A.N., a 45 year old Bengali housewife was admitted in a markedly dehydrated state without pulse or blood pressure four hours after the onset of diarrhea and vomiting. Plasma protein was 14.2 gm.%. Immediately after admission she was begun on tetracycline 500 mgm every 6 hours for 48 hours; she did not vomit any of the capsules. Daily total stool output during the first nine days in hospital was 7.1, 17.6, 10.7, 8.0, 15.8, 14.0, 8.0, 3.1, and 0.1 liters. She reached end of diarrhea 7.3 days after admission and became bacteriologically negative on the eleventh day. In this patient there was no apparent therapeutic effect from tetracycline. The growth of vibrios from first and tenth day cultures was inhibited by 0.625 ug/ml (minimal conc.).

CRL no. 1702. G.M., a 30 year old Bengali carpenter, was admitted in profound dehydration, without pulse or blood pressure, 7½ hours after onset of diarrhea and vomiting. The admission plasma protein was 11.0 gm.%. From admission he was treated with 750 mgm tetracycline 6 hourly for 48 hours (total dose 6 gms). During antibiotic therapy, on the second and third hospital day, direct plate cultures of rectal swabs were negative. On the third day, enrichment culture was negative also. Cultures became positive on direct plate after tetracycline was stopped, on the fourth and fifth days, but were negative thereafter. Daily stool volumes for the first six hospital days were 20.0, 19.5, 13.3, 9.6, 6.5, and 0.8 liters. End of diarrhea occured after 5.3 days in hospital. Vibrios from cultures taken on the first and fifth days were equally sensitive to tetracycline (minimal inhibitory concentration 1.25 ug/ml).

While both of these patients passed unusually large quantities of rice-water stool, such cases are not rare in our Dacca experience. Only further experience will tell whether such cases can be controlled with more prolonged therapy. However, the statement of the Calcutta group based on their experience with a smaller series of patients in 1963 and 1964, that "the minimal

oral tetracycline dose which is consistently effective in eradicating vibrios from the stool of the adult cholera patient is greater than 2, but no more than 6, gms" is not universally applicable.

In an attempt to develop a simplified tetracycline treatment schedule for use in epidemic situations, two experimental regimens were tested. In one, four large doses of tetracycline were given over the first 24 hours and no further antibiotic was given (total of 4 gms in adults and 2 gms in children over 24 hours). There were four therapeutic failures in the first seven patients treated in this manner (mean duration of diarrhea 4.7 days, mean duration bacteriologic positivity 6.0 days in the seven patients) and this schedule was not further tested. A second regime, in which the total 24 hour dose of tetracycline was given at one time daily for four days (1 gm daily in adults, 500 mgm in children) was administered to 21 patients. The mean duration of diarrhea and bacteriologic positivity in the group were 2.0 and 3.0 days respectively, but there were two therapeutic failures. It would appear that the time-honoured six-hourly administration of tetracycline over several days will achieve more consistent results than "crash" programs such as these.

Attention should also be called to the relatively high incidence of bacteriologic relapse (4 to 23% even in six-hourly tetracycline regimens given for 72 hours) in all of the antibiotic groups (tables III - VI). This may be of epidemiologic importance if an attempt is being made by means of drug therapy to interrupt and control the spread of cholera in a limited area. However, it is unknown whether more prolonged therapy (e.g., for five or seven days) would reduce or eliminate bacteriologic recurrences.

Bacteriologic resistance. The possibility that bacteriologic and clinical failures or recurrences could be due to the presence or development of antibiotic resistant vibrios was investigated. In 15 antibiotic treated patients who had prolonged bacteriologic positivity usually associated with therapeutic failure and bacteriologic or clinical relapse, vibrios from stool cultures taken on admission and late in the course of the patient's illness were tested for quantitative *in vitro* sensitivity to the antibiotic employed (table VII). In each case the organisms were originally sensitive to the antibiotic received by the patient. Resistance of the organism developed in none; the minimal concentrations of the relevant antibiotic required to inhibit vibrio growth were similar in the admission and late cultures in each of the cases.

Time of onset of therapeutic action. While the average cholera patient treated with tetracycline given 6-hourly reaches end of diarrhea in about two days, it would be of interest to know how soon a beginning therapeutic effect is manifest after antibiotic therapy is initiated. In two studies on small groups of patients reported by Carpenter, et al, the first 24 hour stool volumes in treated patients were less than in untreated controls, though the differences were not statistically significant.[2] In order to obtain more information on this question, an analysis was performed of consecutive 8-hourly stool volumes of cholera patients admitted to the CRL over a period of a year up to and including the time of the antibiotic trials reported herein. All patients with documented V. cholerae infection admitted in a pulseless state weighing over 15 kg in whom there was reasonable certainty of separate collection of stool and urine were included in the analysis. The two groups comprised 28 patients treated with 6 hourly tetracycline for 72 hours and 32 controls who received no chemotherapy. The groups were similar in sex, age, weight, duration of symptoms, ans severity of dehydration (table VIII). Mean 8-hourly stool output in the two groups is expressed in cc/kg in figure 1. Since patients are admitted at various times during a given 8-hour collection of the ward routine, the first collection period is less than eight hours in most cases (mean of 4.3 hours for antibiotic-treated and 4.9 hours for controls). There was no difference in the stool volume between the two groups during the first period. During the second period (i.e., a mean of 5 to 13 hours after admission) the stool output was less in the antibiotic-treated group, though the difference between the means is not statistically significant. During the third period (13 to 21 hours) after admission the difference is statistically significant, and the difference becomes progressively greater with time. These findings suggest that the therapeutic effect of tetracycline in cholera begins to be apparent within a few hours of its administration.

Summary. This analysis of antibiotic trials conducted during the 1964-65 epidemic again demonstrates the effectiveness of antibiotics as an adjunct to the fluid and electrolyte replacement therapy of cholera. Diarrhea ended earlier, stool volume and consequent total fluid therapy was diminished, and the duration of bacteriologic positivity was less in the groups treated with each of four antibiotics under study when compared to controls, though paromomycin gave only limited therapeutic benefit. Streptomycin was moderately effective, though there was a significant incidence of prolonged diarrhea in both adults and children treated with this agent. The best results were obtained in adults with tetracycline and chloramphenicol, and in children, with tetracycline. More uniform success was obtained when

tetracycline was given for 72 hours rather than 24 or 48 hours, regardless of dose. Bacteriologic recurrences occurred in a minority of cases treated for 2 or 3 days with any of the antibiotics tested. Antibiotic resistance did not develop. A reduction in stool volume was apparent within a few hours of the administration of tetracycline.

Plans for the current epidemic. During the current season tetracycline and chloramphenicol will be further evaluated, with emphasis on their relative efficiency in children; a poorly absorbed tetracycline derivative highly active in vitro against vibrios will be tested, in an attempt to determine whether antibiotic absorption is important in its therapeutic effect; sulphonamides, the most inexpensive of chemotherapeutic agents, and long recommended and still widely used in the treatment of cholera, will be assessed; and the effects of combined antimicrobial therapy will be evaluated.

We are grateful for the expert technical assistance of Mr. Chittaranjan Dey and Mr. Raisur Rahman in the antibiotic sensitivity tests and to Mrs. Asia Hussain for help in reviewing patient records.

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TABLE I.

COMPARISON OF TREATMENT GROUPS: PATIENTS WEIGHING 15 KG. OR MORE.

	No antibiotic	Paromomycin	Streptomycin	Chloromycetin	Tetracycline
Number of cases	61	18	11	49	72
Duration of symptoms before admission, hours	19.0 (2-168)	14.6 (1-30)	14.7 (1-37)	16.3 (2-86)	16.2 (1-107)
Age, years	31.1 (7-70)	29.6 (6-62)	25.4 (8-45)	24.5 (8-48)	23.3 (7-65)
Body weight(discharge),kg	41.6 (17.8-63.9)	38.3 (16.0-51.5)	41.4 (18.3-59.5)	37.4 (18.0-54.8)	37.1 (14.7-63.0)
Male: Female Ratio	2:2:1	1:6:1	1:8:1	1:9:1	2:1:1
Number pulseless	25(41%)	8(44%)	6(55%)	26(53%)	31(43%)
Adm. Plasma protein gm%	10.8 (7.1-15.4)	10.3 (7.6-12.8)	11.3 (8.5-13.6)	10.7 (7.8-14.4)	11.0 (7.7-14.2)
Convalesc.plasma protein gm%	7.6 (6.6-10.5)	7.8 (6.6-8.9)	7.9 (7.2-8.8)	7.7 (6.4-9.2)	7.8 (6.8-10.3)
Adm. minus conv. plasma protein, gm%	3.2	2.5	3.4	3.0	3.2

Mean values are given (range in parenthesis)

TABLE II

II(a)

COMPARISON OF TREATMENT GROUPS: PATIENTS WEIGHING LESS THAN 15 KG.

	No antibiotic	Paromomycin	Streptomycin	Chloromycetin	Tetracycline
Number of cases	13	15	22	31	44
Duration of Symptoms before admission, hours.	26.1 (2-128)	21.9 (3-120)	11.5 (2-37)	13.6 (2-63)	19.0 (3-98)
Age, years	4.2 (2-8)	4.3 (2-8)	4.0 (1-8)	4.0 (1-7)	3.7 (2 mo -9)
Body weight (discharge), kg.	11.1 (8.3-14.4)	10.9 (7.5-15.4)	12.1 (6.4-15.9)	11.4 (6.0-16.0)	10.4 (5.1-15.9)
Male: Female Ratio	2.3:1	1:1.2	1:1.4	1:2.9	1.2:1
Number pulseless	6(46%)	5(33%)	9(41%)	11(35%)	19(43)
Adm. Plasma protein gm%	9.4 (7.4-12.4)	9.4 (7.7-12.2)	9.5 (7.2-12.4)	9.9 (7.6-13.2)	9.3 (6.0-12.0)
Convalescent plasma protein gm%	7.1 (5.8-9.0)	7.2 (6.2-8.4)	7.5 (6.7-8.7)	7.2 (6.4-8.4)	7.2 (5.4-8.8)
Adm. Minus Conv. Plasma Protein, gm%	2.3	2.2	2.0	2.7	2.1

Mean values are given (Range in parenthesis)

TABLE III

EFFECTS OF THERAPY : PATIENTS WEIGHING 15 KG or GREATER

	No Antibiotic	Paromomycin	Streptomycin	Chloromycetin	Tetracycline
No. of cases	61	18	11	49	72
Duration of diarrhea					
Mean, days	4.0	3.6	2.9	2.2	2.1
Mean, 8 hr. periods.	11.9	10.7	8.8	6.6	6.3
Range "	(0-23)	(0-23)	(4-15)	(0-14)	(3-22)
Diarrhea 4 d. or more	36(59%)	6 (33%)	3 (27%)	2 (4%)	2 (3%)
Clinical relapses	1	1 (6%)	1 (9%)	0	0
Total Stool Volume, L.					
Mean	21.8	16.0	10.6	6.1	9.9
Range	(0-100.1)	(0-53.5)	(1.2-22.8)	(0-23.7)	(0.2-84.3)
Total intravenous fluids, L.					
Mean	27.7	19.3	15.1	12.8	13.3
Range	(9.2-108.0)	(4.0-61.5)	(3.1-30.9)	(2.5-32.8)	(3.0-105.0)
Days bacterial positive					
Mean	5.8	4.8	3.5	3.1	3.6
Range	(1-13)	(1-9)	(1-8)	(1-9)	(1-10)
Bacteriologic relapses	5(8%)	6(33%)	4(36%)	7(14%)	15(21%)

Dosage schedules: Paromomycin 250 mgm q6h/48 or 72 hr.; 500 mgm q6h/72 hrs.
Streptomycin 1000 mgm q6h/48 or 72 hrs.
Chloromycetin 250-500-750 mgm q6h/48 hr.; 250-500 mgm q6h/72 hrs.
Tetracycline : see Table V.

TABLE IV

EFFECTS OF THERAPY : PATIENTS WEIGHING LESS THAN 15 KG.

	No Antibiotic	Paromomycin	Streptomycin	Chloromycetin	Tetracycline
No. of cases	13	15	22	31	44
Duration of diarrhea:					
Mean, days	4.2	3.3	3.1	3.1	2.0
Mean, 8 hr. periods.	12.5	10.0	9.2	9.3	5.9
Range " " "	(7-18)	(0-23)	(2-23)	(2-23)	(2-11)
Diarrhea 4 days or more	9(69%)	5(33%)	6(27%)	8(26%)	0
Clinical relapses.	1 (8%)	1 (7%)	2 (9%)	1 (3%)	0
Total stool volume,L.					
Mean	8.6	8.1	4.7	6.8	2.8
Range	(1.9-33.1)	(0-24.7)	(0.5-17.8)	(0.5-22.7)	(0.2-10.8)
Total Intravenous fluids,L.					
Mean	9.9	9.4	6.5	7.6	4.0
Range	(3.0-33.8)	(0-29.0)	(1.8-18.9)	(0-29.9)	(0-13.0)
Days bacteriol. positive					
Mean	6.2	4.6	4.9	4.7	2.4
Range	(2-8)	(1-9)	(1-8)	(2-13)	(1-14)
Bacteriologic relapses	2(15%)	5(33%)	10(45%)	10(32%)	10(23%)

Dosage schedules :
Paramomycin 125 mgm q6h/48 or 72 hrs; 250 mgm q6h/72 hr.
Streptomycin 500 mgm q6h/48 or 72 hrs.
Chloromycetin 125-250-500 mgm q6h/48 hrs; 125-250 mgm q6h/72 hrs.
Tetracycline: See Table VI.

TABLE IVa

PATIENTS WEIGHING LESS THAN 15 KG.

		Chloromycetin treated Mean duration, days			Tetracyline treated Mean duration, days			No antibiotic Mean duration, days	
	No.	Diarrhea	Bacter. positive	No.	Diarrhea	Bacter positive.	No.	Diarrhea	Bacter positive
Males	8	3.2	5.4	24	2.0	2.8	9	4.2	6.7
Females	23	3.1	4.5	20	1.9	2.0	4	4.0	5.3

TABLE V

COMPARISON OF VARIOUS TETRACYCLINE DOSAGE SCHEDULES IN PATIENTS WEIGHING 15 KG OR MORE

	500 mgm q6h/48 hr.	750 mgm q6h/48 hr.	250 mgm q6h/72 hr.	500 mgm q6h/72 hr.	Untreated
No. of Cases	25	12	21	13	61
No. pulseless.	9(36%)	7(58%)	7(33%)	8(62%)	25(41%)
Admission plasma protein; g.%					
Mean	11.1	11.1	10.7	11.3	10.8
Range	(9.1-14.2)	(9.5-11.5)	(7.7-13.0)	(9.6-13.6)	(7.1-15.4)
Duration of diarrhea:					
Mean, days	2.2	2.2	1.9	2.2	4.0
Mean, 8 hr. periods.	6.5	6.6	5.7	6.7	11.9
Range, " "	(3-22)	(3-16)	(3-9)	(3-11)	(0-23)
Diarrhea 4 days or more	1(4%)	1(8%)	0	0	36(59%)
Clinical relapses.	0	0	0	0	1(2%)
Total stool volume; L.					
Mean	9.7	10.7	6.3	10.7	21.8
Range	(1.1-84.3)	(0.5-49.5)	(0.2-27.2)	(2.2-23.1)	(0-100.1)
Days bacteriol. positive:					
Mean.	2.8	2.7	2.2	2.6	5.8
Range	(1-10)	(1-6)	(1-6)	(1-7)	(1-13)
Bacteriologic Relapses.	8(33%)	3(25%)	1(4%)	3(23%)	5(8%)

TABLE Va

TETRACYCLINE THERAPY IN PATIENTS WEIGHING 15 KG. OR MORE: COMPARISON OF 48 AND 72 HOURS' THERAPY.

	500-700 q6h/48 hours	250-500 mgm q6h/72 hours
No. of cases	37	35
No. pulseless	16 (43%)	15 (43%)
Mean total dose tetracycline/pt.	4.7 g	4.1 g
Admission plasma protein, g. %		
Mean	11.1	10.9
Range	(9.1-14.2)	(7.7-13.2)
Duration of diarrhea		
Mean, days	2.2	2.0
Mean, 8 hr. periods	6.5	6.1
Range, "	(3-22)	(3-11)
Diarrhea 4 days or more	2 (6%)	0
Clinical relapses	0	0
Total stool volume, L.		
Mean	10.0	7.9
Range	(0.5-84.3)	(0.2-27.2)
Days bacter positive		
Mean	2.8	2.3
Range	(1-10)	(1-7)
Bacteriol. relapses	11(31%)	4(12%)

TABLE VI

COMPARISON OF TETRACYCLINE DOSAGE SCHEDULES IN PATIENTS WEIGHING LESS THAN 15 KG.

	250 mgm q6h/48 hrs.	250 mgm q6h/72 hrs.	125 mgm q6h/72 hrs.	Untreated
No. of Cases.	11	19	14	13
No. pulseless.	4(36%)	8(42%)	7(50%)	6(46%)
Admission pl.protein,g.%				
Mean	9.1	9.3	9.6	9.4
Range.	(6.0-11.3)	(7.0-11.8)	(7.4-12.0)	(7.4-12.4)
Duration of diarrhea:				
Mean, days	2.0	1.9	2.0	4.2
Mean, 8 hrs. periods.	6.1	5.7	5.9	12.5
Range, " "	(2-10)	(2-11)	(3-11)	(7-18)
Diarrhea 4 days or more.	0	0	0	9(69%)
Clinical relapses.	0	0	0	1(8%)
Total stool volume, L.				
Mean	2.3	2.9	3.1	8.6
Range	(0.7-3.9)	(0.5-10.8)	(0.2-9.5)	(1.9-33.1)
Days bacter. positive:				
Mean	3.3	1.8	2.6	6.2
Range.	(1-14)	(1-7)	(1-7)	(2-8)
Bacteriol. relapses.	3(27%)	2(11%)	3(21%)	2(15%)

TABLE VII

ANTIBIOTIC SENSITIVITIES OF VIBRIOS OBTAINED EARLY AND LATE IN THE COURSE OF THE DISEASE

Patient No.	Diarrhea 4 days or more.	Relapse		Days bacter. positive.	Antibiotic received	Minimal Inhibitory Antibiotic Conc. ug/ml	
		Bacter.	Clinical			First day culture	Last positive culture
1686	+	+	0	10	Tetracycline	0.625	0.625
1702	+	+	0	5	"	1.25	1.25
1366	+	+	0	8	Chloromycetin	0.625	0.625
1406	+	0	0	6	"	1.25	1.25
1657	+	+	+	9	"	1.25	1.25
1814	+	+	0	6	"	1.25	1.25
1386	+	0	0	6	Streptomycin	20	10
1388	+	+	0	6	"	20	20
1390	0	0	+	4	"	10	10
1440	+	0	0	6	"	20	20
1442	+	0	0	8	"	20	20
1448	0	+	0	3	"	20	20
1377	+	0	+	7	Paromomycin	10	10
1378	+	+	0	7	"	20	10
1427	+	+	+	9	"	10	10

T A B L E VIII

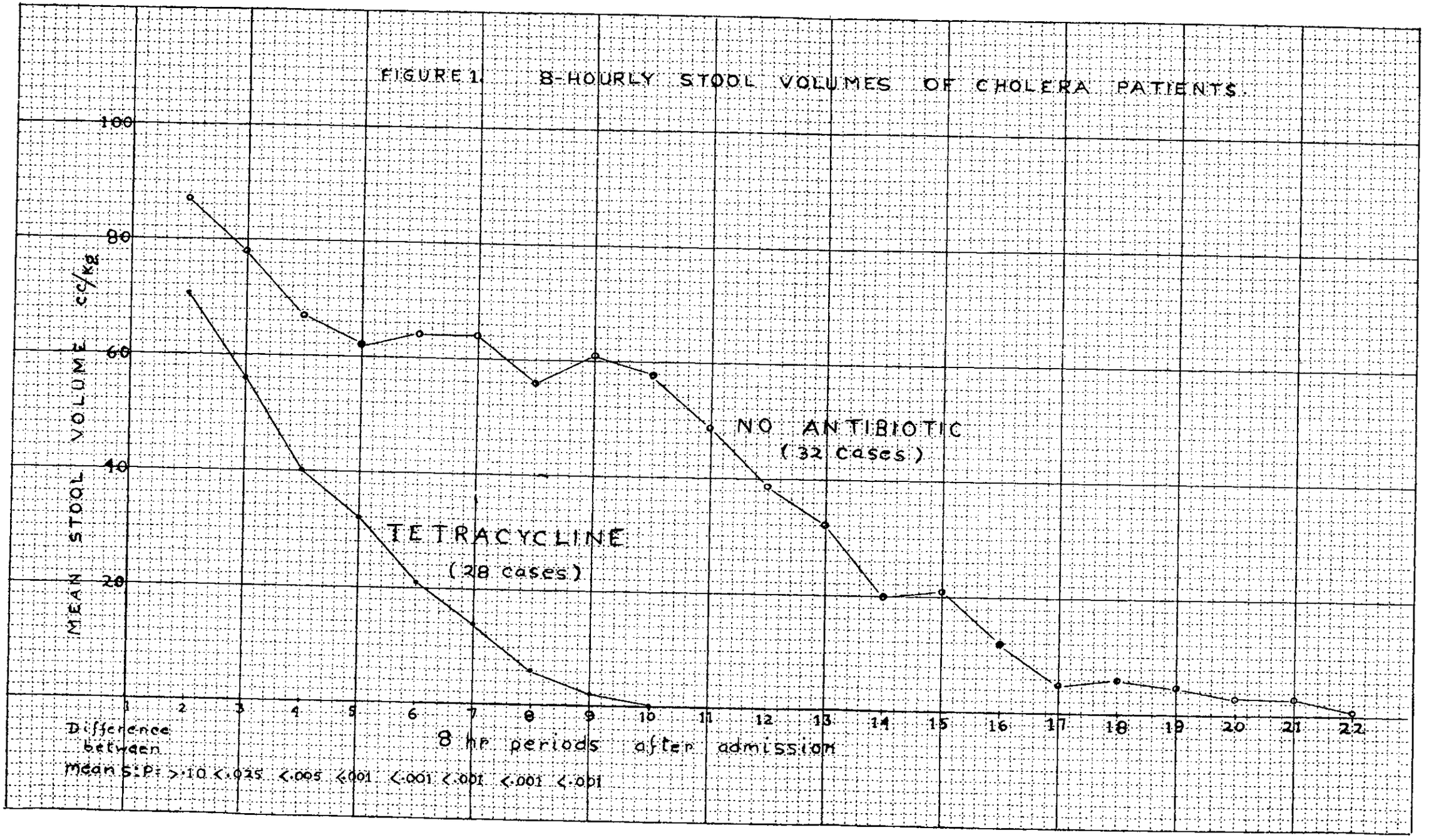
II(a)

COMPARISON OF TETRACYCLINE AND CONTROL GROUPS

	Tetracycline	No Antibiotic
No. of patients.	28	32
Male:Female ratio.	2.5:1	2.6:1
Mean age, years	24.6	28.8
Mean body weight, kg.	38.4	41.4
Mean admission plasma protein, gm.%	11.9	11.7
Mean convalescent plasma protein, gm.%	7.8	7.5
Mean admission Hct.	51.6%	51.1%
Mean admission Blood pressure, mm Hg.	9.3	9.8
Mean number of hours from onset to admission	12.8	17.1

All cases were pulseless on admission.

FIGURE 1. 8-HOURLY STOOL VOLUMES OF CHOLERA PATIENTS.



HYPOGLYCEMIA IN CHILDREN WITH ACUTE DIARRHEA

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W.B. Greenough III, and S.M. Alam

Introduction

Significant hypoglycemia in early childhood is known to occur with a variety of chronic diseases of metabolism, with severe malnutrition,[1] and with chronic diarrhea,[2,3] To our knowledge it has not been described in association with acute diarrhea in children without obvious malnutrition. In the past three years hypoglycemia has been documented in twelve children with acute diarrheal disease admitted to the ward of the Pakistan-SEATO Cholera Research Laboratory in Dacca, East Pakistan. This report is a review of the clinical and laboratory data available in these cases.

Methods and Materials

Between 1 February, 1963 and 7 September, 1965, 573 children with acute diarrheal disease weighing under fifteen kilograms were admitted to the ward. Twelve children in this group presented symptoms suggestive of hypoglycemia, at which time blood sugar determinations were low. Glucose was measured by the glucose oxidase method of Washko and Rice[4] on blood collected in bottles containing heparin and sodium fluoride. Plasma or serum obtained at the time of the hypoglycemia was stored at-60° Fahrenheit. Total ketones and insulin were measured in these samples.(Dr.Ronald Arky, Thorndike Memorial Laboratory, Boston, has generously agreed to do the insulin determinations, but the results are not available at this writing). Total ketones were determined by a modification of Michaels'[5] method. Instead of screw cap bottles with rubber washers, 100 cc volumetric flasks with tight fitting ground glass stoppers very finely wiped with stopcock grease were used. With this method the average recovery of B-hydroxy butyric acid added to blood was 80%(Range 75-84%). An aqueous solution of acetone as the standard yielded very consistent results. The findings are given as ketones in milligrams percent. In addition, serum was tested with Acetest Tablets as a rough index of the level of acetoacetic acid.

Tolerance tests with glucose and glucagon-epinephrine were done on two patients and findings are given in the case histories recorded below.

Results

1. Analysis of hypoglycemia cases

The twelve children with acute hypoglycemia ranged in age from one-and-a-half to six years. Six had bacteriologically documented V.cholerae infection; one had shigellosis; one had typhoid with Salmonella typhi septicemia; three had gastro-enteritis with no recognised bacterial pathogens on stool culture; and one had diarrhea associated with noncholera vibrios. In seven patients (patients 3-5,7-9,12) hypoglycemia occurred within fourteen hours after onset of illness while in four others (patients 1,2,6,10) hypoglycemia occurred within twenty two to thirty five hours after onset(Table 3). One patient (case 11) became hypoglycemic after a week of illness.

Their illnesses were of varying severity (Table 1). Some were in shock and severely dehydrated. Most were acidotic on admission as measured by plasma CO2 content or pH. Patients 1 and 2, however, developed hypoglycemia on the second hospital day, many hours after acidosis and dehydration had been corrected.

The nutritional status of the children was variable (Table 2), as judged by clinical observations, weight percentiles, and convalescent hematocrits and plasma protein concentrations. None of them had edema, hepatomogaly, or other signs of classical kwashiorkor or marasmus.

All were comatose at the time hypoglycemia was documented. A number of other neurological findings, including generalized motor seizures, spasticity, and hemiplegia were present in some (Table 3). All were treated with intravenous glucose. Six showed a rapid clearing of the neurologic signs; two awoke after one-half hour and two hours respectively, and four died. The response to intravenous glucose could not be correlated with the estimated duration of hypoglycemia, or absolute blood sugar levels (Table 4). In some patients who did not respond rapidly to glucose, acidosis (Patients 4,6,7,) or septicemia (Patient 11) may have played a major role in producing coma. Sweating was absent in all of the children at the time of the hypoglycemia.

Hypoglycemia recurred in three patients (Patients 3,5 and 9) within thirty-six hours of the first episode. At the time of recurrence, none of these patients was dehydrated or acidotic. In two of the three there were no symptoms at the time of the recurrent hypoglycemia; the third had recurrent coma (see summary of case 5 below).

There were four deaths; autopsy findings are summarized in Table 5.

2. Laboratory Data (Table 4)

Blood glucose ranged from 1 mg% to 37 mg% at the time of symptoms. All but four were low on admission. Patients 1 and 2 had hypoglycemia nineteen and twenty-eight hours after admission respectively. Patient 12 had hypoglycemia thirty-six hours after admission, just before death. Patient 6 had a blood sugar of 50 mg% on admission and after 400 cc of isotonic bicarbonate solution intravenously the blood sugar fell to 34 mg%.

Total ketones were elevated in each of the eight samples measured, six over 15 mg%. In patients 1 and 2, they were elevated at a time when the CO_2 content was normal. None of the ten sera tested gave a positive reaction with the Acetest tablets, said to be sensitive to as little as 10 mg% of acetoacetic acid, suggesting that most of the ketones were in the form of B-hydroxy butyric acid.

Serum glutamic pyruvic transaminase was measured in patients 8 and 10 and was normal in the first (26 units) and elevated in the second (542 units).

3. Representative Case Summaries

Case 3:

A two-and-one-half year old Bengali boy was admitted with eight hours of diarrhea and vomiting, and lethargy for seven hours. He had had weekly episodes of diarrhea during the previous month. Diet consisted mainly of rice and vegetables, with occasional fish and meat. On admission the blood pressure was 70/50. He was moderately dehydrated and very lethargic. The hematocrit was 45%, and the total plasma protein 7.5 g %. Femoral venous pH was 7.21. The blood sugar was 16 mg%. He received Glucagon (1 mg i.v.,) and aqueous epinephrine (0.01 cc/kg s.c.) as well as intravenous fluid and electrolyte replacement. The blood glucose rose to 58 mg.% (a rise of 41 mg %) after one hour (figure 1), with only slight clinical improvement. Glucose (0.7 g/kg.) was given rapidly by vein, and he became quite alert within minutes. Two hours after the glucose load was given, the blood sugar was 97 mg% and glucagon and epinephrine were again given with a resultant rise of 64 mg% after thirty minutes. Nine-and-one-half hours after the glucose load, the patient was again hypoglycemic (blood sugar 28 mg.%) but was symptom-free. Glucagon and epinephrine were given for the third time and a blood glucose rise of 101 mg.% after thirty minutes was observed (figure 1). He took food orally there-after and was discharged well on the 11th hospital day. Stool cultures grew V.cholerae, Inaba.

Case 5:

A two-and-one half year old Bengali girl was admitted with nine hours of diarrhea and vomiting and four hours of coma. Her diet consisted of cow's milk, pulses, rice and fish. On admission there was no pulse or blood pressure and dehydration was marked. She became apneic very soon after admission, with cyanosis and convulsions. A succession of supraventricular arrhythmias were noted electrocardiographically. Glucose was 37 mg.% but insufficient blood was obtained for other tests. Rapid intravenous infusion of isotonic bicarbonate solution (500 cc), 10% calcium gluconate (10 cc.), and hypertonic glucose (0.9 g./kg.) were given. Within two hours the child was awake and alert. After hydration the arterial blood pH was 7.46. Fourteen hours later she suddenly became comatose and appeared moribund. Blood sugar was 25 mg.% and CO_2 content 23.0 mEq/L. She awoke dramatically immediately after intravenous glucose (0.9 g./kg.). Eight hours later she again became unresponsive, with facial twitchings and irregular respirations. The blood sugar was 18 mg.% and CO_2 content 28 mEq/L. Again she awoke immediately after intravenous glucose. An intravenous drip of 5% glucose was begun as well as oral feedings and she did well thereafter. Two days after a diet of bread, milk and bananas, an overnight fasting blood glucose was 80 mg.%. She was discharged well on the 11th hospital day. Stool cultures were positive for V. cholerae Inaba.

Case 7:

A three year old Bengali girl was admitted with an eight hour history of vomiting and diarrhea and three hours of lethargy followed by coma and convulsions. On examination she appeared comatose, well nourished and moderately dehydrated. Pulse was 160 and temperature 102° F. Scattered rhonchiwere heard over the lungs. The right leg was spastic. The admission hematocrit was 35%, and plasma protein 8.9 g.%. Electrolytes were normal except for a CO_2 content of 13 mEq/L. The blood sugar was 1 mg.%. Glucagon (1 mg. i.v.) and aqueous epinephrine (0.01 cc kg. s.c.) were given, but there was no change in the blood sugar after forty-five minutes (figure 1), and glucose was given intravenously (0.5 g./kg.). A k value(rate of disappearance of glucose) calculated from samples of blood obtained thirty and fifty minutes after the glucose was given was 0.96. Simultaneous capillary blood sugar values were only a few mg. % higher than venous during this period. There was no clinical improvement despite the sustained elevation of blood sugar. The child lapsed into status epilepticus. The cerebrospinal fluid was normal. A constant glucose drip was administered but she died eight hours later with frothing at the mouth and high fever, an antibiotic had also been given.

No pathogens were isolated on stool cultures. At autopsy there was ample subcutaneous fat, the liver and adrenals appeared normal, the lungs were edematous, and there was a right lower lobe pneumonia.

Case 9:

A four year old Bengali boy was admitted with nine hours of diarrhea and vomiting. Diet was said to consist of rice, vegetables, meat and breast milk. On admission he was markedly dehydrated and semicomatose. Temperature was 100° F and pulse 160. The liver was palpable 2 cm. below the costal margin. A right sided hemiplegia was present, with bilateral dorsiflexion of the toes. The hematocrit was 30%, plasma protein 10.6 g.% and CO_2 content 11.3 mEq/L. Serum Na, K and Cl were normal, as was the cerebrospinal fluid obtained on lumbar puncture. The blood sugar was 9 mg.%. He was given a large bolus of glucose intravenously (1.8 g./kg.) and awoke rapidly. The hemiplegia disappeared after several hours. After an initial rapid fall(from 447 mg,% to 282 mg. % over 45 minutes) associated with rapid intravenous infusion of 600 cc of isotonic bicarbonate solution, the glucose level declined very slowly and was still elevated at two-and-three quarter hours (233 mg.%). After thirty-six hours of additional fasting, the glucose level had dropped to 32 mg,%though he remained symptom-free. Oral feedings were begun and his recovery was uneventful. Stool cultures were negative. Three days later, when convalescent, after an overnight fast, the blood glucose was 65 mg.%.

4. Studies of Our Ward Population

To obtain more information on blood sugar levels in our patient population, three groups were studied;

1) Thirty-one convalescent Pakistani children or well children accompanying ill patients on our wards were fasted overnight. The mean age was 4.2 years (range seven months to ten years). The mean fasting blood sugar was 63.3 mg.% (S.D. 11.8) with a range of 36-81 mg.% (figure 2). There was no correlation of glucose level with age. The same study was performed on forty-eight convalescent adults. The mean blood sugar level was 73 mg.% (S.D. 13.5) with a range of 51-109 mg.% (figure 2).

2) Seventeen consecutive children, admitted with an acute diarrheal illness and weighing under fifteen kilograms were fasted for approximately forty-eight hours. Hydration was maintained with glucose-free intravenous electrolyte solutions. The blood sugar was measured on admission, after initial rapid rehydration and subsequently every eight hours,(figure 3); close clinical observations were made. In general, the greatest fall in glucose occurred during the period of rapid rehydration. In nine the blood sugar fell below 40 mg.%, after a mean fast of 30 hours (range 10-48). Two of the children with low blood sugars (34 and 28 mg.%) were mildly lethargic. The remainder were asymptomatic. The nine who developed hypoglycemia did not differ from the eight who did not in age, weight percentile, degree of dehydration, or severity of illness. Fourteen adults with acute diarrheal disease were similarly fasted (figure 2) and only one developed a low blood sugar (35 mg.%), without symptoms.

3) Admission blood sugar was measured in 73 consecutively admitted children with acute diarrheal disease with moderate to severe degrees of dehydration and acidosis (figure 4). They ranged in age from two months to ten years (mean age 4.4 years). The mean admission blood sugar was 104.6 mg.% (range 35-276). Three had blood glucose below 40 mg.% with no symptoms of hypoglycemia.

Discussion:

These findings indicate that significant hypoglycemia is a not infrequent, potentially fatal complication of acute infectious enterides of children in East Pakistan. Cases have been documented in association with cholera, shigellosis, typhoid, noncholera vibrio infection, and acute diarrhea of unknown cause. To our knowledge this is the first time that hypoglycemia has been reported as a complication of these illnesses in children. Minimal hypoglycemia has been reported in biochemical studies of adults with cholera, but no clinical information has been provided to determine its clinical significance,[6,7] In none of our cases was there a history of previous episodes of coma or evidence of any chronic metabolic disease associated with hypoglycemia.

Our studies did not identify the etiology of the hypoglycemia in these cases, but several possible causes bear further investigation. The possible contribution of fasting, malnutrition, ketosis, failure of gluconeogenesis, and anoxia should be considered.

Young children in general are more prone than adults to develop hypoglycemia with prolonged fasting. Kaye[8] reported blood sugar values below 40 mg.% after a twenty-four hour fast in eight of twenty-five normal American infants ranging in age from one week to six months, and in one of eight children from seven months to four years. The hypoglycemia was not associated with

symptoms. When we fasted seventeen acutely ill Pakistani children who were initially normglycemic, eight developed hypoglycemia (two of them mildly symptomatic) after a mean fast of thirty hours. Since our children were acutely ill with diarrheal disease, they are not strictly comparable to Kaye's series of normal Americans. It is possible, however, that prolonged fasting may more readily lead to hypoglycemia in Pakistani children. Though prolonged fasting may have played a role in precipitating severe hypoglycemia in our twelve cases, five of them were without food for only a short period of time, equivalent to an overnight fast.

Hypoglycemia has been reported in association with marked malnutrition[1]. None of our twelve cases had frank marasmus or kwashiorkor, pedal edema or hepatomegaly. Their weights, compared with those of other Pakistani children admitted to this hospital with diarrheal disease and without hypoglycemia, distributed among the low, middle, and high percentile groups. Several of the cases appeared clinically to be well nourished. However, biochemical studies to rule out subclinical protein malnutrition were not performed.

Acute gastrointestinal disorders, , associated with vomiting, diarrhea, and reduced food intake are often associated with elevated blood ketones. Elevation of blood ketones has been shown to depress blood glucose in humans,[9] probably by direct stimulation of insulin release from the pancreas.[10] The minimal blood ketone level effective in lowering blood sugar is stated to be about 3.5 mM/L.[11] In our series, the highest level of ketone bodies was 3.3 mM/L (Patient 9) while the other patients had considerably lower levels. Colle and Ulstrom[12] described several children with recurrent hypoglycemia associated with ketonuria and aggravated by a ketogenic diet; these children had no response to glucagon when hypoglycemic. Hypoglycemia itself may be followed by glucose intolerance (Somogyi effect) and ketosis,[13] so that in our cases ketosis may have been secondary.

Ketosis is, of course, one reflection of glycogen depletion and conversion to a fat-burning metabolism. The initially sluggish response to glucagon by Patient 3 and the absent response to glucagon by Patient 7 suggest that there was liver glycogen depletion. Normally after glycogen depletion, gluconeogenesis from fatty acids, ketones and proteins should supervene. Failure of gluconeogenesis may have occurred in Patient 5, in whom severe hypoglycemia recurred twice, first fourteen and then eight hours after receiving 0.9 g./kg. of glucose intravenously as therapy for the previous episode (this late hypoglycemia makes reactive hyperinsulinism unlikely). Failure of gluconeogenesis may be due to a possible toxic effect on liver enzymes or failure of delivery of necessary precursors to the liver (as might occur with markedly diminished blood flow to the liver), or insufficiency of adrenal hormones or growth hormone,

We have little information regarding hepatic function in our series of cases. None of the children were jaundiced. In one of two sera tested, however, there was a marked elevation in serum glutamine pyruvic transaminase, suggesting hepatic necrosis. There was no clinical evidence of adrenal failure in our patients, other than the absence of sweating at the time of hypoglycemia. But if hypoglycemia develops gradually the typical epinephrine response may be absent.[14] In only one of our four autopsied cases did the adrenals appear abnormal (Patient 12, Table 5). We have no information regarding growth hormone levels.

Anoxia alone is reported to cause a rapid increase of glucose transport into cells in the perfused, isolated rat heart,[15] and may be the cause of acute hypoglycemia in pithed, hypoxic rats.[16] Hypoglycemia has also been reported to be a feature of advanced shock in animals.[17] Several of our children were admitted in circulatory collapse with profound acidosis; others, however, developed hypoglycemia in the absence of these factors, or many hours after shock and acidosis had been corrected. It may well be that the cause of hypoglycemia is not the same in all of our cases, or that the relative contribution of various physiologic derangements varies from patient to patient.

It is uncertain whether the entity of hypoglycemia associated with acute diarrheal disease in children is restricted to East Pakistan, or whether it occurs elsewhere but has been overlooked to date. Since most children in East Pakistan receive herbal or homeopathic medicines before they come to the hospital, the possibility of toxic hypoglycemia must be considered. Most of the parents of these children stated that outside therapy had not been given before admission to the hospital; this point will be investigated more actively in future cases.

It is of interest that during the episodes of acute hypoglycemia, roundworms were passed per rectum in Patient 2 and vomited in Patient 10. It is possible that some of the cases felt by some clinicians[18] to belong to the debatable entity "roundworm convulsions" are in fact unrecognized instances of hypoglycemia associated with acute diarrheal illnesses.

Summary:

Severe hypoglycemia developed in twelve Pakistani children during the course of acute diarrheal illness due to cholera, shigellosis, typhoid, and other acute bowel infections. In three children the hypoglycemia recurred

during the course of the diarrheal illness. The cause of the lowered blood sugar in these patients was not determined. Fasting, malnutrition, ketosis, failure of gluconeogenesis and anoxia may have been contributing factors. Further studies of this phenomenon are required as well as determination of its incidence outside of East Pakistan.

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TABLE I

Bacteriologic Diagnoses and Admission Findings in 12 Hypoglycemic Children

Pt.#	Age	Sex	Diagnosis	Clinical Estimate of dehydration (1-4+)	Acute minus convalscent plasma protein[1] (grams %)	Pulse rate	Respiration	Temperature (Rectal, F)
1	6	M	Cholera	3+	4.7	0	25	103
2	6	F	Cholera	3+	2.2	0	42	98.6
3	2½	M	Cholera	2+	2.0	140	36	99
4	3½	M	Cholera	1+	8.6[2]	0	10	99.5
5	2½	F	Cholera	3+	8.8[2]	0	34 then apnea	98
6	3	M	Cholera	1+	1.1	120	50	97
7	3	F	Acute G.E.	2+	3.5[3]	160	56	102
8	4	M	Shigellosis	1+	0	0	10	96
9	4	M	Acute G.E.	3+	3.8	160	44	100
10	2½	F	Acute G.E. cellulitis clysis site	1+	0	140	50	103
11	1½	M	Typhoid Fever	none	6.1[2]	150	40	103
12	2½	F	Non-cholera vibrio infection	1+	7.0[2]	120	15	97

G.E. gastroenteritis: 1-, and index of dehydration. 2- Admission value only. 3-Rehydration value minus admission valu

TABLE 2 - NUTRITIONAL STATE OF CHILDREN WITH HYPOGLYCEMIA

Pt.#	Clinical	Hepato-megaly. [1]	Age	Weight on Discharge (kg.) [2]	Weight percentile [3]	Convalescent plasma protein (grams %)	Convalescent Hematocrit
1	Cheilosis	None	6	10.6	3rd-10th	6.5	35
2	poor nutrition	None	6	11.2	25th	7.2	34
3	average	None	2½	11.7	90th-97th	5.7	32
4	well nourished	none; 330g.	3½	13	75th-90th	*	*
5	well nourished	none	2½	7.75	10th-25th	*	*
6	average	None	3	9.5	25th	7.2	23
7	well nourished	palpable left lobe; 350 g.	3	10.1	25th-50th	*	*
8	average	none	4	8.75	3rd-10th	7.2	28
9	average	1 f.b.	4	8.15	3rd-10th	6.8	20
10	cheilosis, raw tongue, good pannus	just palpable	2½	11.8 [4]	90th-97th	6.7	26
11	well nourished	none; 290 g.	1½	7.6	25th-50th	*	*
12	well nourished	1½ f.b.; 350 g.	2½	8.2	10th-25th	*	*

1/ In cases 4, 7, 11 and 12, hepatic weight at autopsy is given.

2/ For patients who died an addition of about 10% is made to correct for dehydration and usual weight gain in hospital.

3/ Percentiles are based on 573 consecutive hospital admissions with diarrheal illnesses, using stated age and discharge weight. Weight percentiles for normal Pakistani children are not available at this time.

4/ Admission weight; this child lost weight after admission due to diminished intake.

* = Not available.

TABLE 3 - CLINICAL FEATURES OF THE HYPOGLYCEMIC EPISODES

Pt. #	Hours illness before admission	Hours fasting & hours illness before signs of hypoglycemia		Duration of signs before I.V. glucose given (hours)	Neurologic findings	Response to I.V.glucose	Outcome
		Fasting	Illness				
1	13	33	32	5-8	twitching, grunting, grimacing, teeth-grinding	awoke rapidly	Discharged well.
2	7	35	35	2	coma, stertorous breathing	awoke rapidly	Discharged well
3	8	13	8	8	deeply lethargic	more alert in 15 minutes	hypoglycemia recurred. Discharged well
4	14	11	9	5	coma, fixed dilated pupils, bilateral plantar dorsiflexion, flaccidity	no response	died
5	9	12	4	5	coma, generalized seizures, mouth twitching, apnea	awoke in 2 hrs.	hypoglycemia recurred twice. Disch. well
6	33	25-36	25	8	dilated pupils, un-cooperative and violent	Awoke in 12 hrs after glucose and fluids	Discharged well
7	8	13	4	3-4	generalized seizures, spastic right leg, fixed contracted pupils	No response	died
8	15	20	10	5	generalized seizures, gasping respirations	awoke in $\frac{1}{2}$ hr.	Discharged well
9	9	12	1	8	coma, right hemiplegia, bilateral plantar dorsiflexion	awoke rapidly	hypoglycemia recurred. Discharged well
10	25	24	22	3	generalized seizures	awoke rapidly	discharged well
11	4 days	poor intake during illness	?	6	lethargy, then coma	no response	died
12	24	24-36	14	10	coma, fixed dilated pupils.	no response	died

TABLE IV

LABORATORY DATA OF HYPOGLYCEMIC CHILDREN

Pt. #	Glucose (mg%)	Total Ketones (mg%) (normal 0.3-3mg%)	Acetest Serum = S Urine = U	Co_2 content (mEq/L)
1	16	15.8	S - 0	19.5(13.4 on admission)
2	26 (78 on admission)	15.8	....	23.2(7.9 on admission)
3	16	10.7	S - 0 U - ++	pH 7.21[1]
4	30	16.6	S - 0	6.8
5	37	....	....	....
6	34	15.8	S - 0	6.7
7	1	7.2	S - 0 U - 0	13.0
8	37	....	S - 0	17.8
9	9	34	S - 0	11.3
10	8	....	S - 0	8.8
11	25	....	S - 0	....
12	35	23.8	S - 0	....

1 - Femoral venous blood, CO_2 -, Not done

TABLE V

AUTOPSY FINDINGS

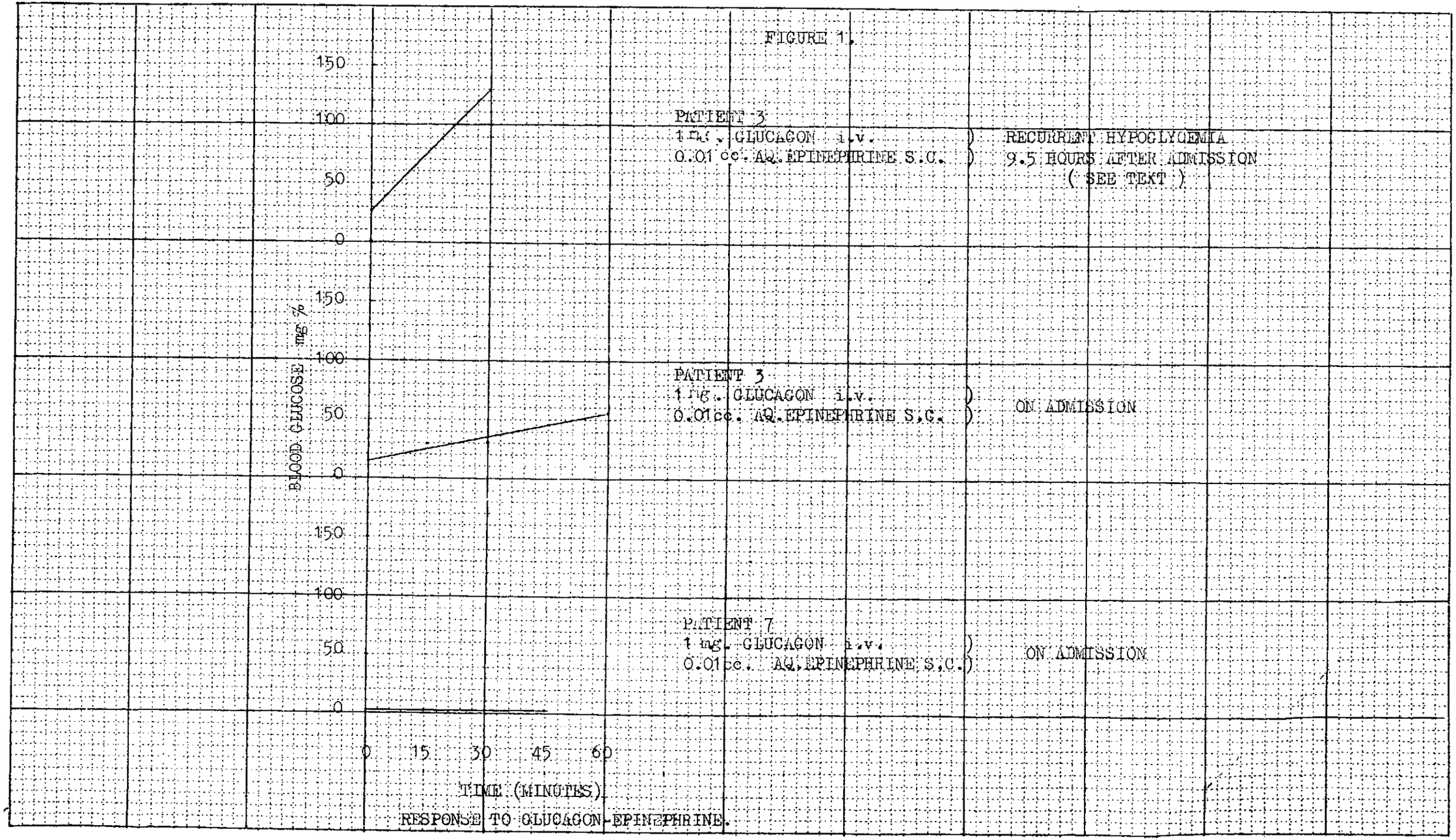
Pt.#	Gross	Microscopic
4	Well developed; thick pannus; pancreas 33 grams, normal on cut surface; liver 330 grams, olive green on cut surface and rather pale and smooth; pleural adhesions. Adrenals normal. Cultures: Liver - 2+ Heiberg Group II non-cholera vibrios and E. coli. Small intestine - V. cholerae, Inaba	Pending
7	Well developed and well nourshied; ample subcutaneous fat; pancreas and liver appeared normal, liver 350 grams; edematous lungs and right lower lobe pneumonia. Adrenals normal.	Pending
11	Well developed, well nourished with relatively thick pannus, and firm well developed muscles; bilateral lower lobe bronchopneumonia; liver 290 grams, pale and fatty on cut section; adrenals normal; enlarged and engorged Peyer's patches in ileum; intestinal mucosa intact. Cultures: Bile, intestines, nodes and lungs - S. typhi; spleen - E. coli	Heart: Anitschkow myocytes, focal edema. Adrenal: Tubular degeneration of cortex. Lungs : Bronchopneumonia Intestinees : Histiocytic infiltrates. Liver : Focal granulomata and necrotic cells in association. Fatty change. Marrow: Granulomata Kidney : Proteinaceous material and subnuclear tubule cell vacuoles.

(more)

Pt.#	Gross	Microscopic
12	Well developed and well nourished; panniculus of 1 cm. Muscles firm and red. Liver weighed 350 grams appeared large, firm and pale; on section, pale and greasy. A yellow gelatinous edema over the lower surface between liver and gall bladder and beneath the diaphragmatic surface.	Heart: Anitschkow myocytes, fibrinoid necrosis at several sites. Fat Stain: Positive. Lungs: Congestion, acute bronchopneumonia. Nodes: Acute lymphadenitis Pancreas: focal necrosis of acinar and islet cells with acute inflammatory cell infiltration. Kidneys: Proteinaceous material in proximal tubules. Fat Stain : Positive. Liver : Vacuolated cells, many necrotic. Fat Stain: Positive. Intestines: Active, acute enteritis. Adrenals: Not remarkable.

The autopsies were performed by Drs. Abram S. Benenson and Robert O. Oseasohn. We are grateful to Drs. Howard B. Goldstein (Captain M.C.) and Helmuth Sprinz (Colonel M.C.), of the Walter Reed Army Institute of Research for the microscopic findings.

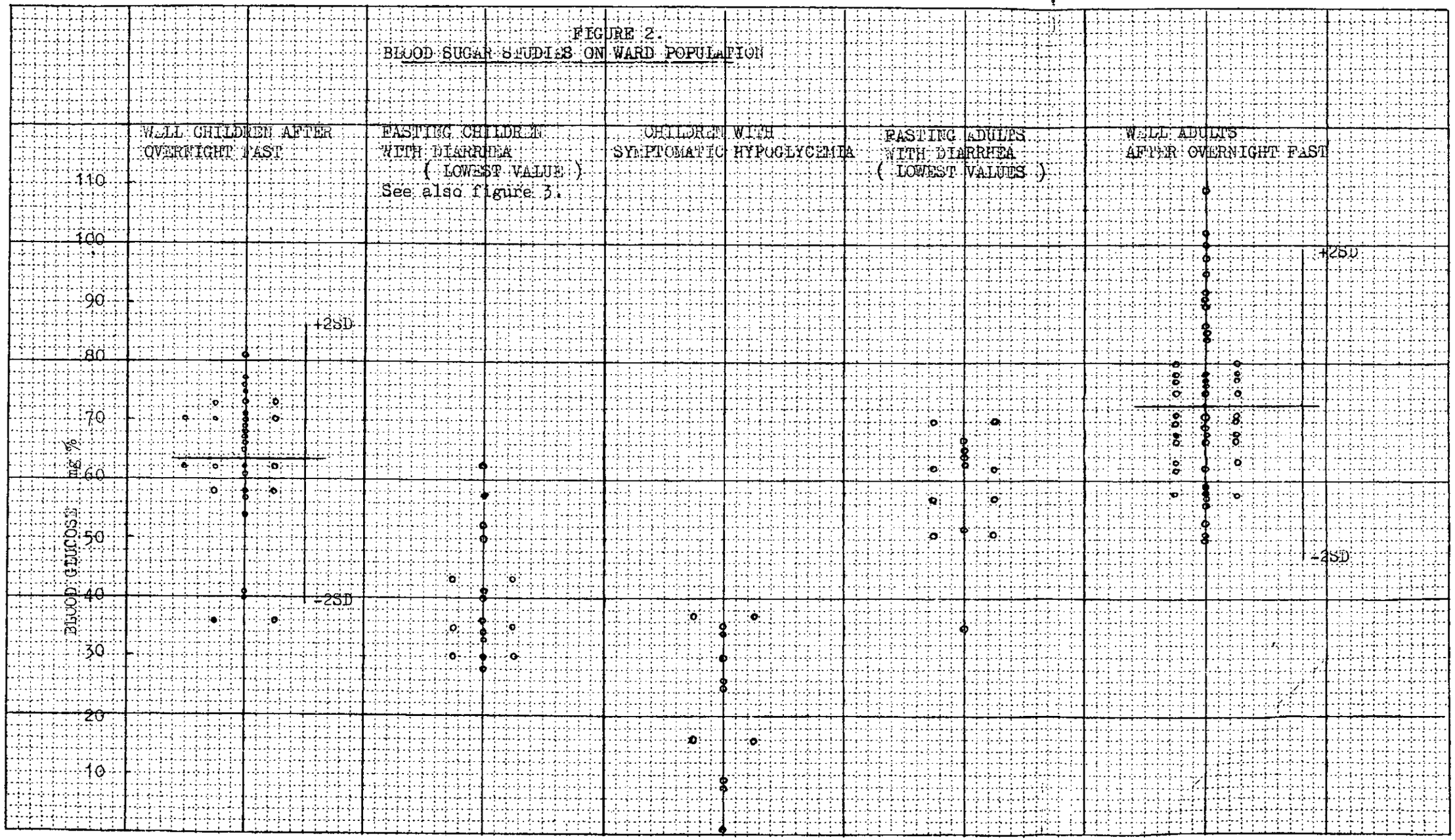
FIGURE 1.



RESPONSE TO GLUCAGON-EPINEPHRINE.

FIGURE 2.

BLOOD SUGAR STUDIES ON WARD POPULATION



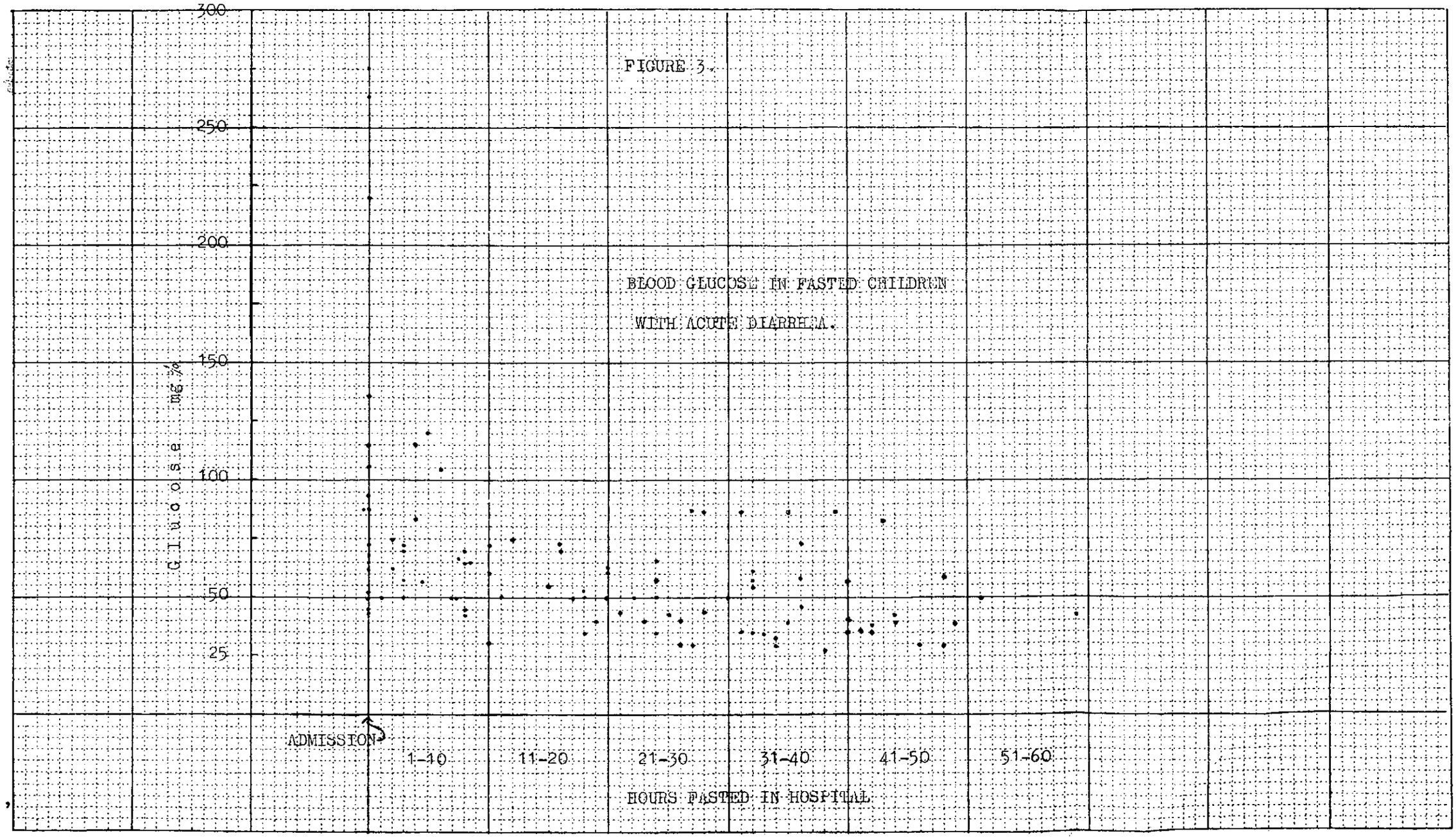
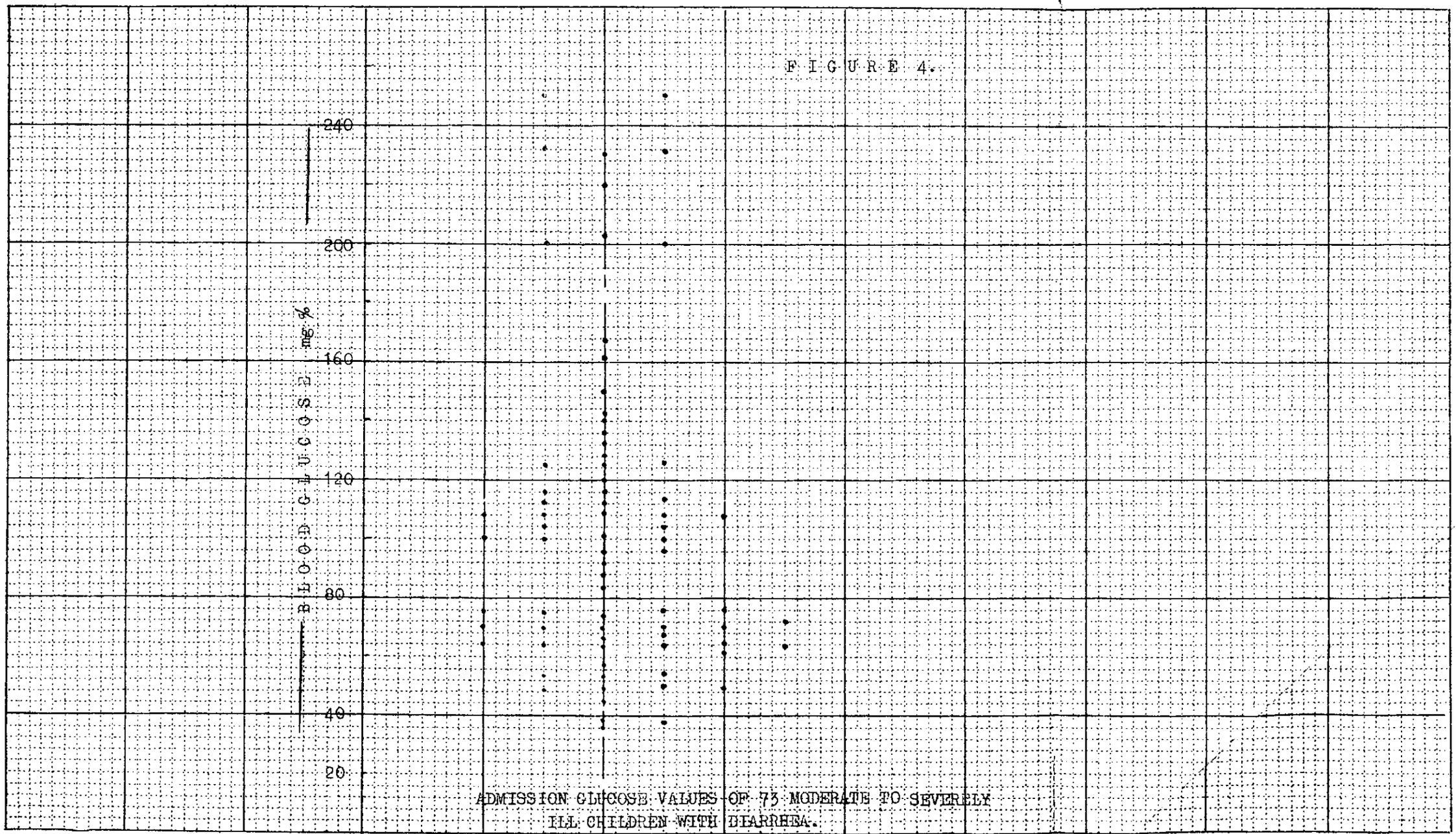


FIGURE 3.
BLOOD GLUCOSE IN FASTED CHILDREN
WITH ACUTE DIARRHOEA.
300
250
200
150
100
50
25
Glucose mg%
ADMISSION
1-10
11-20
21-30
31-40
41-50
51-60
HOURS FASTED IN HOSPITAL

FIGURE 4.



ADMISSION GLUCOSE VALUES OF 73 MODERATE TO SEVERELY ILL CHILDREN WITH DIARRHEA.

The Possible Role of Sustained Pancreatic and Hepatic Hypersecretion in the Fluid Losses of Cholera

by

Dr. William B. Greenough, III.

John Snow in 1855 in his monograph on cholera described the nature of cholera stools in the following way: "The stools and vomited matter of cholera consist of water, containing a small quantity of the salts of the blood, and very little albuminous substance." Although succeeded by other notions, this view has ultimately been shown to be correct. Quantitative studies by Phillips and his colleagues in Bangkok in 1958 firmly established the exact chemical composition of the fluid lost in cholera (Watten et al, 1959). In the experience of our laboratory, if no antibiotic is given, the average cholera patient loses 31 liters of fluid during the course of an illness lasting from 3 to 6 days. The liquid stools are initially colorless with a slight fishy odor, alkaline (pH 7.5), and have a mean electrolyte composition of sodium 139 mEq/l, potassium 24.3 mEq/1, chloride 106 mEq/1, and bicarbonate 48.4 mEq/1 (Lindenbaum et al, 1965). In spite of the detailed knowledge of the composition and volume of cholera stool, its source is still in doubt. In a recent discussion (Gordon, 1965), the various possibilities have been considered.

Previous Theories. Historically, there have been two different views of fluid loss in cholera. The idea championed by Virchow (1879) and subscribed to by the vast majority of workers for over 80 years was that fluid loss in cholera was the result of denudation of the intestinal mucosa with consequent loss of plasma, as in a burn. Although others disputed whether there was actually loss of mucosal integrity (Connheim, 1889) and (Goodpasture, 1923), there was little debate over the idea that fluid loss was due to excessive fluid entering the intestinal lumen. Recently, the denudation hypothesis has been invalidated by three observations:

(1) The protein content of cholera stools was shown to be less than 0.1 gm % (Weaver et al, 1948).

(2) Radioactively labeled macromolecules (I^{131}-PVP) (Gordon, 1959) injected intravenously into actively purging cholera patients did not appear in the stool in greater concentration than in normals, thereby implying mucosal integrity (Gordon, 1960). This has been recently confirmed (Lindenbaum and Gordon, 1965) with a Cr^{51} albumin technique (Waldman, 1961).

(3) The small intestinal mucosa of the cholera patient has consistently been found to be histologically intact in both biopsy and autopsy material (Gangarosa, et al, 1960) and (Freshet al,1964).

With the passing of the denudation theory the mechanism of fluid loss in cholera may be thought of as (1) and overproduction of fluid by all or part of the gut and/or its accessory glands of secretion, (2) an inhibition of normal reabsorptive mechanisms, or (3) a combination of overproduction and malabsoption in the presence of structually intact mucosa. Currently, the most widely accepted hypothesis is that of Phillips and his colleagues (Phillips, 1963 and 1964). They postulate that a toxic product of V. cholerae partially inhibits the enzyme system by which intestinal mucosal cells transport

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sodium from the gut lumen to the blood stream. Since extrapolations from work in dogs suggest that the gut of a 60 kg man, excluding the stomach and duodenum, is capable of transporting sodium ion at the rate of 5.0 mEq/min (Visscher, 1944), Phillips proposes that the volumes lost in cholera may be explained adequately by even a partial blockage of sodium transport. According to this idea there is no necessity of postulating hypersecretion into the gut lumen at any level. Support for this conception has come from observations in cholera patients of an inability to absorb enough sodium ion when it is given by mouth to put them into positive sodium balance (Phillips et al, 1963). In addition, various products of V. cholerae have been shown to inhibit in vitro models of sodium transport (Phillips, 1963).

As appealing as the idea of a specific sodium pump inhibitor produced by V. cholerae causing the massive diarrhea of cholera may be, there are some weaknesses in such a conception that warrant a continuing search for alternate explanations. In accepting the sodium pump inhibition theory, one must accept either that sodium ion enters the gut lumen from the blood by filtration or by an active process driven by a system that is not inhibited by vibrio products. At the present time, although large fluxes have been demonstrated across the gastrointestinal tracts of dogs (Berger et al, 1959; Code et al, 1960), there has not been any direct evidence in an intact animal concerning which direction of flux is actively driven or whether one direction of flux could be inhibited without affecting the other by use of toxic agents. The likelihood that the products of V. cholerae are specific in their interference with the absorption of sodium has been cast into doubt by the demonstration that there is significant malabsorption of a variety of substances tested not only in cholera but also in other diarrheal illnesses (Lindenbaum, 1965). In addition, there has been failure to demonstrate specificity of materials from cholera stool and cultures of V. cholerae in their ability to inhibit the transport of sodium ion in various in vitro systems (Phillips, 1965). Finally, an additional mechanism must be invoked to explain the high concentration of bicarbonate in cholera stool.

Alternate Hypothesis:

Since it is now probable that there is a rather nonspecific interference with a wide variety of absorptive functions in the gut at various levels and that in fact cholera patients often show a milder derangement in absorption than patients with other diseases in which fluid losses are much smaller, it seems less likely that any defect in the reabsorption of sodium ion is likely to be either specific or unique to cholera. Alternatively then, the reason for the remarkable quantities of fluid lost must then reside in the entrance of a large quantity of fluid into the gut at some level. This fluid may enter the gut at multiple levels or it could enter in a localized area. If the fluid entered at a localized site high in the gut, then it should be possible to markedly affect the volume of stool by intubation and quantitative aspiration from that level. A likely site would be above the ligament of Treitz where most of the fluids of digestion enter the gut when it and its accessory exocrine organs are stimulated by the hormones associated with digestion (Borgstrom et al, 1957).

In considering this idea, it was first necessary to ascertain that the common bile duct was indeed patent in cholera. Since the stool in cholera

is colorless, it seemed possible that there might be a functional blockage of biliary flow. However, observations that there were large amounts of amylase (Carraway, 1959), lipase (Seiverd, 1958) and tryptic activity (Seiverd, 1958) in typical "ricewater" stool and that intravenous doses of BSP dye could be detected in the stool within 2-3 hours of injection with the majority of the dose recoverable in the first 8 hours (Cole, et al., 1965) gave assurance that the common bile duct and its pancreatic and biliary radicles were patent. Furthermore, several autopsies in cholera patients exhibited anatomically patent ducts. Thus it appeared entirely possible that a large fluid volume could be entering the gut from the pancreas and liver, as well as the gut itself. Accordingly the following hypothesis was considered:

(1) In cholera large quantities of fluid, rich in bicarbonate, potassium and enzymes, are delivered to the duodenum from the pancreas and liver. In addition, fluid may also be entering from the mucosa of the stomach and duodenum.

(2) This outpouring of fluid is caused by some product or products of V. cholerae which act on the gastric, duodenal, and upper jejunal mucosa inducing a maximal elaboration of the hormones of the mucosal cells. These hormones in turn act on their respective end organs inducing maximal secretory responses. This response is sustained by virtue of the fact that V. cholerae is capable of proliferating to very high density in alkaline bile-rich fluid and thus can remain within the upper intestinal tract for days, thereby exerting a continuing influence.

Alternatively but less likely would be the possibility that V. cholerae itself produces a secretin-like compound which is absorbed and directly stimulates the liver and pancreas.

(3) The quantity of fluid coming into the duodenum exceeds the impaired absorptive capacity of the jejunum, ileum and colon and a copious diarrhea results.

(4) Since the absorptive defects remain for some time after the cessation of diarrhea in cholera (Lindenbaum, 1965), the cessation of diarrhea must result from correction of the state of hypersecretion due to the ultimate removal of vibrios from the duodenum rather than depending on the repair of the absorptive capacity of the gut.

Initial Experiments:

If this hypothesis is correct, one would expect that quantitive aspiration of the duodenal contents during active cholera would produce a marked decrease in stool volume, or even stop diarrhea entirely. Accordingly a Miller-Abbott tube, modified so that there were no openings distal to the obstructing balloon, was positioned in the upper 18 inches of the jejunum of patients who had continuing fluid losses due to cholera. During periods of 8 to 16 hours, the obstructing balloon was inflated and suction applied. All fluid losses were replaced by the intravenous administration of equal amounts of a multiple electrolyte solution (Greenough, 1965). The results in two patients with typical acute cholera are shown in Figs. 1 and 2. A summary of the total amylase output in stool and duodenal contents for one case is seen in Fig.3. The mean electrolyte composition of stool and duodenal aspirate

taken under oil simultaneously is shown in Table I. It is apparent in each of these experiments that there was a prompt and definite reduction in both the stool volume and quantity of amylase in the stools of these patients during the time when fluid was removed from above the first portion of the jejunum. It is also seen that the volumes of fluid obtained in these cases by duodenal aspiration are comparable or greater than those seen after stimulation of the pancreas by secretin (Grossman, 1958).

T A B L E I

		pH	Na	K	Cl	CO_2
Aspirate	Mean	7.3	152	4.6	102	25.3
	Range	6.6-7.8	109-182	1.7-15.4	89-118	20.7-31.0
Stool	Mean	7.9	138	16.3	92	58.2
	Range	6.8-8.2	95-170	4.6-7.7	60-142	38.2-76.0

Table I. A comparison of electrolyte values between aspirated fluid and stool in case #1197.

Discussion:

The usefulness of any hypothesis is limited both to the means by which it may be tested and to the further experimental work it stimulates. The current idea leads to a number of simple experiments by which its validity may be either confirmed or denied. The critical test is of course the demonstration of an elevated level of hormones such as secretin or secretin-like substances in the blood stream of cholera patients. At present, this is possible only by indirect assay in animals. Simpler experiments could establish whether maximal sustained infusions of secretin are capable of producing diarrhea in either normal individuals or convalescent cholera patients. Can the administration of secretin to an actively purging cholera patient be shown to result in any change in either volume or composition of stool or duodenal aspirate? Is Diamox (acetazolamide), a well-documented inhibitor of pancreatic secretion (Dreiling, et al, 1955), capable of decreasing the output of fluid or altering its composition in cholera? Can cholera be seen in the presence of complete obstruction of the common bile duct? Will it be seen in any case of documented pancreating exocrine insufficiency?

The physiologic severity of cholera is reported to be related to the numbers of vibrios in the stool (Huber, 1965). In active cases, the number of vibrios present in the duodenal fluids is equal to that in stool. V. cholerae is peculiarly adapted to survive in the alkaline, bile-salt-rice fluids of the upper intestine under conditions where most other organisms would be inhibited or killed. Thus the organism responsible for producing continuous massive fluid losses has also the almost unique property of being able to enter and thrive in the duodenal fluids of man.

Although no syndrome of excessive primary or secondary production of secretin is as yet described (Grossman, 1958), it is likely that such disorders exist. The primary form would result from an intrinsic disorder of the duodenum, while the secondary form would be due to the presence of some substances within the duodenum which causes it to elaborate secretin. It is possible that the stomach could also participate in or cause such syndromes since pancreatic secretion has been induced by gastrin I (Preshaw, Cooke, Grossman, 1965). Recent reviews of the syndromes of watery diarrhea associated with pancreatic neoplasms have caused those authors to comment that "Surprisingly large columes of fluid are lost daily with water and electrolyte depletion as important sequelae" (Ballard, et al, 1964). "The outcome was fatal in 10 of the recorded cases, and in 9 of them death was due to water and electrolyte depletion" (Deleu, et al, 1964). One patient was observed to lose more than 10 liters of fluid in a 24-hour period due to diarrhea.

Thus it is documented that in a primary pancreatic disorder, volumes of more than 10 liters may be lost in a 24 hour period. The observation by Janowitz that even a single dose of 1 unit of secretin per kg body weight results in a maximal output of 47 ml of duodenal aspirate in a 10-minute period (Janowitz, 1965), indicates that even in physiologic circumstances the pancreas in man can achieve a rate of secretion which, if maintained, would approach 8 liters per day. As yet no studies of the sustained maximal rate of pancreatic secretion in man have been reported.

Summary:

The common bile duct is patent during the stage of cholera when colorless liquid stool is present. The stool output of cholera patients can be drastically reduced or stopped by quantitative aspiration of intestinal contents from above the uppermost jejunum. It is proposed that cholera is a syndrome of hypersecretion of secretin or secretin-like hormones, secondary to stimulation of the upper gut mucosa by large numbers of cholera vibrios. The fluid and electrolyte losses are the result of a combination of an outpouring of fluid into the duodenum, and a nonspecifically defective absorption by the intestine.

Further means of testing this hypothesis have been suggested.

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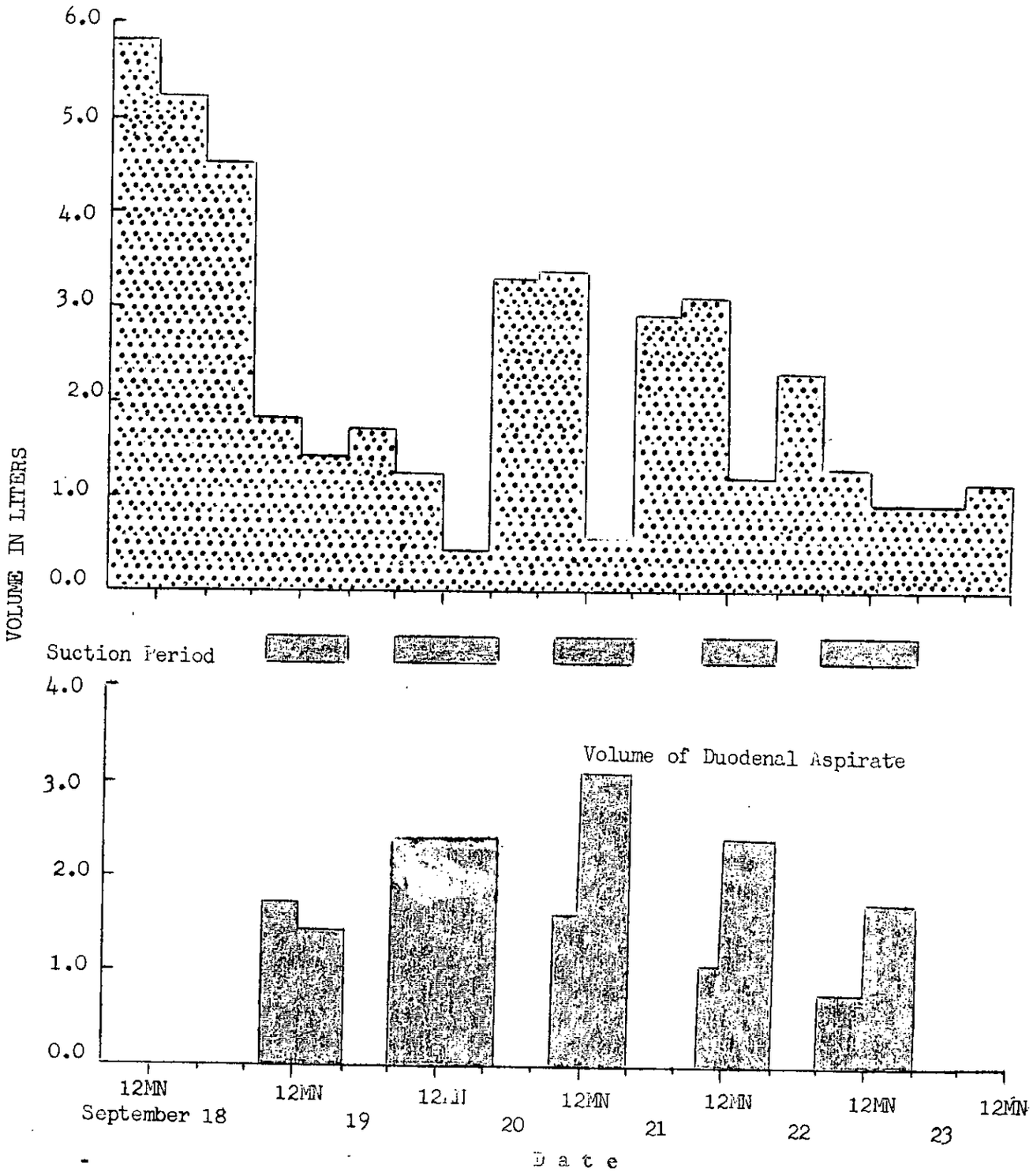
F I G U R E 1

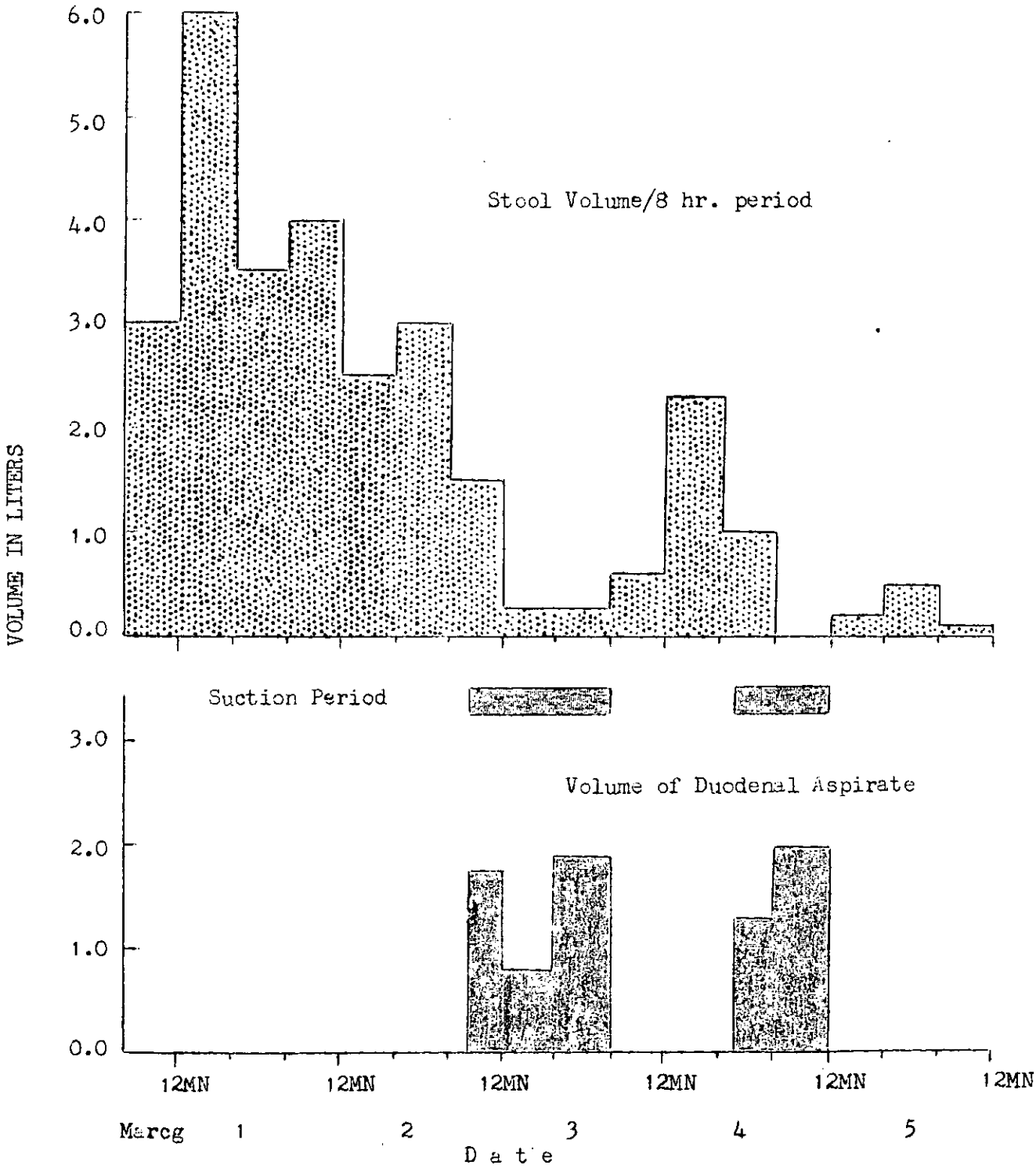
THE RELATIONSHIP OF STOOL VOLUME AND VOLUME OF FLUID ASPIRATED FROM THE DUODENUM AND UPPER JEJUNUM DURING SEQUENTIAL PERIODS ON AND OFF SUCTION IN CHOLERA PATIENT CRL #1197



This study was done on a 50-year old female. The position of the Miller Abbott tube was confirmed by x-ray to be 12-18 inches beyond the ligament of Treitz. In the last two suction periods, some urine was mixed with stool making the volumes recorded as stool for these periods higher than they should be.

FIGURE 2.

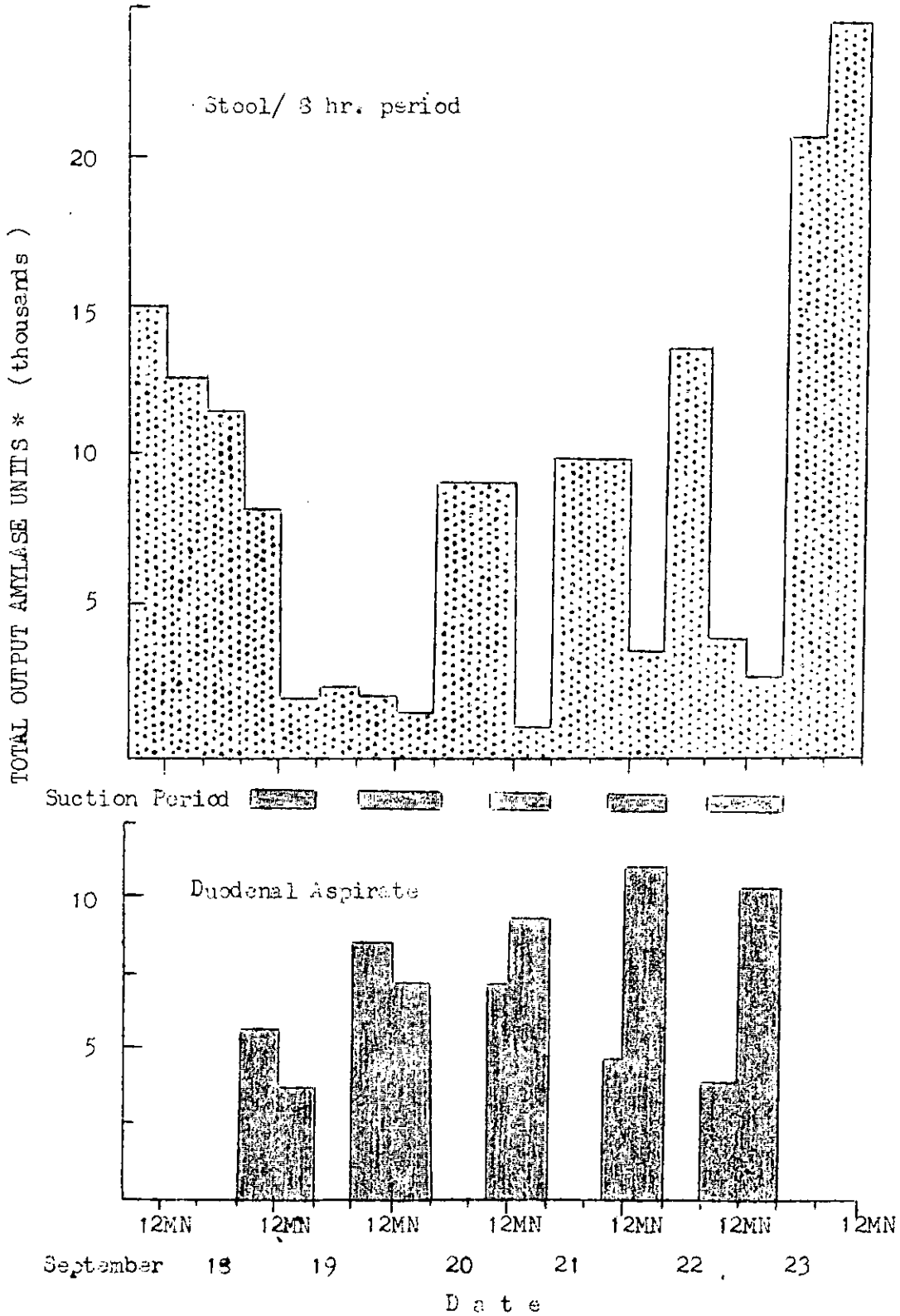
THE RELATIONSHIP OF STOOL VOLUME AND VOLUME OF FLUID ASPIRATED FROM THE DUODENUM AND UPPER JEJUNUM DURING SEQUENTIAL PERIODS ON AND OFF SUCTION IN CHOLERA PATIENT CRL # 1920



This study was done on a 65-year-old male. The position of the tube was confirmed by x-ray to be 12 inches beyond the ligament of Treitz.

FIGURE 3

CHOLERA PATIENT CRL # 1197



Total output of amylase activity in stool and aspirate

* Amylase Units are defined by Carraway (1959).

Demonstration of Patent Bile Ducts at the Time of Colorless "Rice-Water" Stool in Cholera.

by

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(Dr. Cole was at CRL as a fourth year medical student at Columbia University College of Physicians & Surgeons, during the period December 1964 thru March 1965).

The appearance of a characteristically colorless watery stool early in the course of cholera has been a hallmark of this disease. This stool has been described as "rice-watery" because of its color and the presence of floating shreds of mucous. During the evolution of clinical cholera, the color of the stool changes giving way gradually to a deep green or brown. The origianl absence of the deep green or brown color usual in stool has led observers to speculate that there might be a functional obstruction of the biliary tree perhaps at the Sphincter of Oddi (Rogers, 1921; Stoerk, 1916; Chatterjee, 1939). Findings at autopsy had ruled out the idea that any gross pathological obstruction occurs. In fact, early workers had commented on the fact that the gall bladder was usually full of dark green bile that could be easily expressed into the duodenum by gentle normal pressure (Pollitzer, 1956). The current experiment was conceived to answer the question whether or not the bile ducts are functionally patent during the stage of rice-water stool in cholera.

Materials and Methods:

Three patients with bacteriologically proven cholera were studied at a time when normal hydration and circulation had been achieved and they were passing only typical "rice-water" stool. The studies were carried out on the wards of the Pakistan-SEATO Cholera Research Laboratory (CRL) in Dacca, East Pakistan. Sulfobromophthalein (BSP) dye, 5 mg/kg, was injected intravenously. Stool collections were made in eight-hour periods. Volume and color were noted and an aliquot taken from the thoroughly mixed sample for the BSP essay. One milliliter of stool was diluted to 9 ml with distilled water, then made alkaline with 1 ml of 0.1 N NaOH. A blank was prepared in a similar way using 1 ml of stool diluted to 10 ml with distilled water and rendered acidic with 3 drops of 0.1 N HCl. The color was read at 580 millimicrons on a Coleman Junior Spectrophotometer. Results were expressed as total mgm of BSP as read from a standard curve prepared in our laboratory.

Results:

Table I indicates that from 23% to 56% of the injected dye was recovered within the first eight hours after intravenous injection of the dye during a stage when the stool was colorless. In one study, it was noted that the first dye had appeared in the stool within 2 hours of the time of injection.

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TABLE I

Amount of Sulfobromophthalein recovered in the colorless "rice-water" stools of patients with cholera for an 8-hour period after intravenous dose of 5 mgm/kg body weight.

Patient's CRL No.	Age	Sex	BSP injected (mgm)	BSP recovered in stool within 8 hrs of injection (mgm)	Stool volume first 8 hours after injection (liters)
1811	25	M	245	84	3.5
1858	35	F	350	196	4.2
1859	30	M	220	50	1.0

Discussion:

If it is accepted that no major quantity of BSP dye is secreted into the gut lumen by any route other than the biliary passages, one must conclude that the bile ducts are patent and passing large amounts of one material that is known to be secreted by the liver. Hence explanations other than bile duct obstruction must be sought for the lack of bile pigment in the "rice-water" stool of cholera.

The most likely explanation, perhaps, is that the gall bladder does not contract during the early stages of cholera and retains all the bile pigments contained prior to the onset of illness. The small amounts of bile pigment which may be produced by the liver during the vascular collapse-dehydration stage of the disease would be greatly diluted by the large volume of stool produced. A concept of fluid loss because of hypersecretion by liver and pancreas (Greenough, 1965) would insure that any pigments produced would be immediately diluted at their source.

Summary:

Recovery of a large fraction of the injected dose of BSP from colorless "rice-water" stool of actively purging cases of cholera suggests that functional obstruction of the bile ducts is not a tenable explanation for the lack of bile pigments in cholera stool.

Acknowledgement:

It is gratefully acknowledged that the idea for this clinical study was first suggested by Dr. Henry O. Wheeler of the Department of Medicine of the College of Physicians and Surgeons, New York.

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SODIUM-POTASSIUM DEPENDENT ADENOSINE TRIPHOSPHATASE IN THE HUMAN SMALL INTESTINE AND ITS RELATION TO ACUTE DIARRHEAL DISEASE

by: N. Hirschhorn, I. S. Rosenberg
& J.R. Shaha

Introduction

There is now excellent evidence that active cation transport is associated with a membrane adenosine triphosphatase requiring sodium, potassium and magnesium for its activity (Na-K ATPase), as opposed to an adenosine triphosphatase requiring magnesium only (MgATPase). Work by Post, et al[1]., and Dunham and Glynn[2,3] on human erythrocyte membranes revealed identical characteristics of the enzyme and transport systems in their location on cellular membranes, their requirement for sodium and potassium ions together and in their inhibition by oubain. Bonting, et al.[4] demonstrated the presence of the sodium-potassium dependent ATPase (Na-K ATPase) in nearly all tissues of the cat, excepting acellular and low density tissues, with the highest activities in nervous and secretory tissues. They subsequently showed inhibition of aqueous humor formation in the cat[5] and rabbit[6], and cerebrospinal fluid formation in the cat[7] by oubain which paralleled the in vivo inhibition by oubain of the Na-K ATPase of the ciliary body and choroid plexus respectively. In another study Bonting[8] found that oubain inhibited Na-K ATPase in homogenates of toad bladder mucosa and also inhibited active sodium transport by the intact toad bladder preparation only when added to the medium which bathed the serosal surface, in accord with the concept of Frazier and Leaf[9] that the "sodium pump" is present on the serosal surface of the toad bladder mucosal cell.

It has been postulated that an interference with active cation transport of the intestinal mucosal cell results in the copious sodium-rich stool passed by the patient with cholera. Cholera stools and cholera culture filtrates have been shown to inhibit active sodium transport by the frog skin preparation[10]. It would thus be of interest to measure the Na-K ATPase in the jejunal mucosa of patients suffering from cholera and other acute diarrheal illnesses.

This preliminary report describes a method of measuring Na-K ATPase in human small intestinal mucosa and jejunal biopsy material and its application to fifteen patients with acute gastroenteritis.

Materials and Methods

Conditions for the assay of ATPase in human small intestinal mucosa were studied using fresh surgical specimens of grossly normal

terminal ileum (by courtesy of the Harvard Surgical Service of the Boston City Hospital) and jejunal biopsy material obtained from patients on the ward of the Pakistan-SEATO Cholera Research Laboratory, Dacca, East Pakistan.

Ileal mucosa was scraped off the muscularis and washed in isotonic TRIS buffer (pH 7.5 at 25°C) and homogenised in a Potter-Elvejhem homogeniser for ninety seconds with 5 mM EDTA (pH 7.5). The material was spun at 850 xg at 5°C for twenty minutes, the supernate discarded and the sediment resuspended in three volumes of 12mM TRIS-2 mM EDTA (pH 7.6 at 25°C). This material was separated into aliquots and stored at -20°C, and 5°C.

Jejunal biopsy material was processed before assay as described below.

For the assay of ATPase in the ileal mucosa, two solutions were used:

Solution I with Na + 80mEq/L, K+ 16 mEq/L, Mg++ 4 mEq/L;
Solution II with Mg++ 4mEq.L. Both media contained 30 mM/L TRIS and 2.5 mM/L ATP (TRIS salt, Sigma Chemical Company). The final pH of both media was 7.1 at 25°C.

With Mg++and K+ concentrations at 4 and 16 mEq/L respectively, the greatest total activity in ileal specimens were obtained when Na+ = 140 mEq/L. (figure 1). Therefore for the assay of ATPase in the jejunal biopsy material the following solutions were used:

Solution 1	Solution 2
Na+ 140 mEq/L	Mg++ 4mEq/L
K+ 16 mEq/L	
Mg++ 4 mEq/L	

30 mM/L TRIS and 2.5 mM/L ATP (dipotassium salt, from Calbiochem) were present in both media with a final pH of 7.8 at 25°C (7.5 at 37°C).

One tenth cc of homogenate was added to 2.4 cc of each media and the incubation carried out in triplicate at 37°C for thirty minutes (sixty minutes for jejunal biopsy material). The reaction was stopped

with 1.5 cc of 6M perchloric acid. Inorganic phosphate liberated by the reaction was measured by the method of Fiske and Subbarow[11] and protein was measured by the method of Lowry.[12] ATPase activity was expressed as micrograms inorganic phosphate liberated per milligram protein (ugPi/mg prot.). Na-K ATPase activity was expressed by subtracting the activity in Solution II (Mg ATPase) from that of Solution I (total ATPase).

In the ileal mucosa, the peak of Na-K ATPase activity was found to be at pH 7.1 (25° C) and of the MgATPase at 8.0 (25° C). But it should be noted that at 37° C, the temperature of the incubation, the pH of TRIS buffer will decrease by 0.3 units from that at 25°C.[13] TRIS is most efficient as a buffer between pH 8.3 and pH 7.4. We therefore chose pH 7.5 (at 37°C) for work with jejunal biopsy material. This pH will give appreciable activities of both ATPases.

Storage and Deoxycholate:

The proportion of total activity of Na-K ATPase in fresh surgical specimens of ileal mucosa was increased from 9% to 39% by storage at 5°C over 18 days. With further storage in 0.25% sodium deoxycholate for 72 hours at 5°C, the proportion of Na-K ATPase of the total activity rose from 54% to 90% (Table 1). Total ATPase of jejunal biopsy material was considerably inhibited by 0.25% deoxycholate but storage in 0.125% sodium deoxycholate for 24 hours was satisfactory and increased the NA-K ATPase activity from 10% (fresh) to 50% (deoxycholate treated) of the total ATPase activity.

Oubain:

NA-K ATPase was inhibited by Oubain in both the ileal and jejunal homogenates (Table 2).

Patients:

Fifteen adults (thirteen male and two female) admitted to the ward of the Pakistan-SEATO Cholera Research Laboratory, Dacca, East Pakistan, with acute gastroenteritis for which no etiologic agent could be determined (stool cultures negative for known enteric pathogens and no rise in antibody titer to Vibrio cholerae) were studied

at a time when there was little or no cholera present in Dacca. In general there was a tendency to select clinically more severe cases for study. Clinical and laboratory data are given in Table 3.

Biopsies:

The acute phase biopsy was performed on the day after admission (mean of 30.6 hours after onset of illness, range 9-48 hours) when shock and acidosis had been corrected. The convalescent phase biopsy was done on the average of 7.1 days after the last liquid stool (range 4-13). A Crosby-Kugler capsule with radio-opaque tubing was used. The distance of the tubing beyond the Ligament of Treitz at the time of the biopsy was documented radiologically. The subsequent convalescent biopsy in any given patient was obtained at a similar distance from the Ligament of Treitz, within a mean of 5 cm (range 0-12). The mean distance of all the biopsy sites beyond the Ligament of Treitz was 30 cm (range 9-47). The biopsy was placed in ice cold 12mM TRIS-2mM EDTA and examined under the dissecting microscope. It was then thoroughly homogenized at 0° for ninety seconds in an all glass homogenizer in 0.125% solution of sodium deoxycholate (prepared by dissolving in 12 mM TRIS - 2mM EDTA, pH 8.1 at $25^{\circ}C$, in order to bind calcium. The final volume of the homogenate ranged from 0.7 to 1.1 cc depending on the size of the biopsy. The homogenate was stored at $5^{\circ}C$ for 24 hours and assayed as described above.

Each patient received a five gram or twenty five gram dose of d-xylose on the day following each biopsy. Urinary excretion of xylose was measured by the method of Roe and Rice.[14]

Results:

ATPase activities in acute and convalescent biopsies are given in Table 4. There was no significant difference between mean acute and convalescent Mg ATPase activity but there was a statistically significant rise in the mean Na-K ATPase activity (figures 2,3).

The patients may be divided into two groups. Group A (patients 1-9) with an increase in Na-K ATPase of more than two S.E.M. (more than 17 micrograms inorganic phosphate per milligram protein) and Group B (patients 10-15) with a change less than two S.E.M. (figure 4). Four patients in Group A also had significant increases

in Mg ATPase (more than two S.E.M.); of these, two patients (patients 5,6) had increases proportional to the increase in Na-K ATPase and two (patients 2,4) proportionately less than the increase in Na-K ATPase. One patient in Group A (patient 7) and two in Group B (patients 10 and 15) had a significant decrease in Mg ATPase. Patient 15 also had a significant decrease in Na-K ATPase. The mean convalescent Na-K ATPase activity of Group B did not differ significantly from the mean acute Na-K ATPase activity of Group A.

Features of Groups A and B are compared in Table 5. The two groups were similar in most respects, though shock was more common in Group A. The patients in Group B were on the average biopsied earlier in convalescence and most of them had subnormal absorptive function at the time of the second biopsy. For all patients, the convalescent Na-K ATPase value correlated in linear fashion with convalescent xylose absorption (correlation coefficient: 0.87)(figure 5), but no correlation was seen between acute Na-K ATPase activity and acute xylose absorption or between Mg ATPase activity and xylose absorption at either time.

There was no correlation of ATPase activity with morphology as observed under the dissecting microscope.

Discussion:

Mg ATPase may be considered a "marker enzyme" as it may be reduced by various techniques to less than 1% of the total activity,[15] the remainder being Na-K ATPase. This study has shown that in at least some cases of acute gastroenteritis the Na-K ATPase may be selectively affected. Six patients did not recover Na-K ATPase and of these, five had abnormal xylose absorption at that time. The failure to increase Na-K ATPase thus parallels the finding of persistent malabsorption in patients with acute gastroenteritis described in this laboratory by Lindenbaum.[16] It is yet not known if malabsorption precedes (or predisposes to) or is the result of acute gastroenteritis in the patients described in this report. It is interesting to note that in the convalescent state of all fifteen patients the absolute value of Na-K ATPase correlated linearly with xylose absorption. Csaky and Lassen[17] demonstrated that xylose absorption was sodium dependent and inhibited by oubain but only when xylose was in very low concentration. Therefore it is

probable there is no causal relationship between Na-K ATPase and xylose absorption but both are expressions of mucosal integrity. The failure of acute Na-K ATPase activity to correlate with xylose absorption may be due to two causes. First, if these indices are not causally related, acute gastroenteritis may affect them differently. Second, xylose testing was done on the third hospital day, about eighteen hours after biopsy. At this time some patients with acute gastroenteritis who no longer have diarrhea may have already recovered the ability to absorb xylose.[18]

Thus it remains to be determined whether impairment of Na-K ATPase contributes to diarrhea and the malabsorption seen with acute gastroenteritis or is simply another non-specific effect of acute intestinal infection.

We plan to study the ATPase activity in the small intestinal mucosa of cholera patients and additional patients with non-cholera diarrheas. It is also planned to determine the effect of these illnesses on other "marker" enzymes, specifically lactase and sucrase, utilising the same specimen that is analysed for ATPase.

Summary:

A method for studying Na-K and Mg ATPase in jejunal biopsies is presented. It was applied to fifteen patients with acute gastroenteritis and a significant increase of the Na-K ATPase was seen in the convalescent biopsy in nine cases, of whom two also had proportionately significant increases in the Mg ATPase. There is no certain way to distinguish between those with significant rise in ATPase activity from those with none, except in persistant xylose malabsorption. In the convalescent state the level of Na-K ATPase activity correlated with percent xylose absorption. Both measurements may reflect the general functional capacity of the small intestinal mucosa.

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TABLE 1

EFFECT OF STORAGE IN 0.25% DEOXYCHOLATE AT 5°C
FOR 72 HOURS
(HUMAN ILEAL MUCOSA)

ug Pi/mg/prot.	Control	Deoxycholate suspension
Mg ATPase	68.2	15.4
Na-K ATPase	81.8	133.6
Total ATPase	150.00	149.0

TABLE 2

EFFECT OF OUBAIN (1mM/L) ON Na-K ATPase
(Human Small Intestinal Mucosa)

ug Pi/mg prot.	Ileum	Jejunum (mean of 2 biopsies) deoxycholate treated.
Solution I (Total ATPase)	86.6	311
Solution II (Mg ATPase)	54	155
Solution I + Oubain	59.1	157

SODIUM-POTASSIUM DEPENDENT ATPase : PRELIMINARY DATA FROM CHOLERA PATIENTS

TABLE I(a): Results of Acute and Convalescent Biopsies in Five Patients
ATPase, μgPi/mg Prot.

Pt.	Age	Sex	Admission BP	Acute minus Convalescent Plasma Protein grams %	Acute Mg	Conv. Mg	Acute Na-K	Conv. Na-K	Total Stool Output(liters)	Xylose Absorption[a]	
										Acute	Conv.
1	16	M	60/0	1.3	192	230	135	216	3.3	22%	37%
2	30	M	0	4.7	131	144	45	115	8.6	not done	
3[b]	24	F	50/0	2.2	92	92	71	112	3.7	24%	24%
4	22	M	0	4.5	200	188	110	103	33.2	11%	35%
5	26	F	100/80[c]	1.1	185	175	144	116	10	13%	26%

a - Percent absorption of a five-gram dose; all done on day of Biopsy except Pt. 3 done day after Biopsy.

b - Nag Cholera

c - Discharge Blood Pressure 100/70

TABLE II(a) - Results of Multiple Biopsies in Two Patients.

	Patient 6[d]				Patient 7[e]			
Biopsy Number :	1	2	3	4	1	2	3	4
Hours after Onset of Diarrhea	32	57			52	95		
Days after last liquid stool.			2	8			1	10
Stool Volume in 8 hr period in which Biopsy done(lit.)	4.5	5.5			1.5	3.0		
Stool vol.after 2nd Biopsy.		11.4				4.5		
Hrs.Diarrhea " " "		56				40		
ATPase (μgPi/mg Prot.)								
Mg	146	200	105	141	182	141	231	193
Na-K	78	140	130	161	75	84	146	187
Xylose Absorp.(5 grams)	13%	18%	19%	29%	19%	17%	32%	34%

d -BP--; Delta Pl.Prot. 4.8; -Total stool 39.4 lit.
e -BP--; Delta Pl. Prot. 3.1 ; -Total stool 22.8 lit.

TABLE 3

Pt. #	Sex	age	Weight (kg)	Adm. B.P.	Acute biopsy hours after onset	Change in pl. prot. acute-conv. (gram %)	Total liquid stool Vol. in Hosp. (liters)	Liquid Stool Vol. during & after Bx	Days of Diarrhea from onset	Temp on adm. F°	Change in Xylose Absorption acute to conv.	Dissecting Microscope[1] Appearance	Stool guaiac	Stool mucus	Stool microscopic Exam.
1	M	19	38.2	75/60	23	1.4	0.65	0.65	3	104	low to normal	#1 leaves; # 2 leaves	4+	0	RBC+ pus++ macrophage 4+
2	M	22	43.1	0	25	4.9	3.55	0.35	3	101.4	low to normal	#1 fingers, leaves x2	0	1+	RBC occ. pus, macro 1+
3	M	22	35.6	80/60	34	0.4	0.32	0.02	2	101	Normal to normal	leaves x4	0	1+	RBC occ, pus few
4	M	40	39.2	80/55	31	1.5	3.61	2.0	4	98.4	low to low	leaves x2	0	0	RBC occ. pus few
5	M	30	49.9	60/0	41	4.4	0.05	0.05	2	97	low to normal	leaves x2	0	trace	RBC occ.
6	M	50	40.7	80/0	31	2.3	0	0	1	98	normal to normal	leaves x2	-	-	-
7	M	40	43.9	80/0	30	4.1	0.7	0.1	2	101	normal to normal	leaves x2	-	-	-
8	M	20	44.5	70/45	24	2.5	2.5	1.5	3	100.6	low to normal	leaves x2	1+	1+	RBC 1+, pus, macro 1+
9	M	30	40.5	60/40	20	2.7	0	0	1	97	low to low	leaves x2, #1 leaves ridger #2 leaves	2+	0	RBC occ. pus, macro 1+
10	M	26	40.2	100/70	40	3.7	1.3	0.6	4	99	low to normal	#1 fingers leaves, #2 fingers tongues, leaves #3 leaves	1+	1+	RBC few, pus, macro 1+
11	M	50	40.1	80/0	48	3.4	0.055	0.055	2	97	low to low	leaves x2	1+	1+	
12	M	22	44.3	90/60	26	2.4	1.2	0.7	2	101.6	low to low	leaves x2	0	2+	RBC occ.,pus few
13	F	35	34.8	70/0	9	1.7	1.1	1.0	2	101	low to low	leaves x2	3+	2+	RBC 1+,pus 1+, macro few,RBC occ.
14.	F	26	36.4	90/60	29	3.2	1.4	0.8	2	99	low to low	leaves,few fingers x2	0	0	-
15.	M	27	52.4	120/80	48	0	0.250	0.150	5	99.8	low to low	leaves x 2	-	0	RBC 2+,pus 4+ macro 3+

1 Number refers to number of Biopsies.

TABLE 4

JEJUNAL BIOPSY - ATPase activity
(micrograms inorganic phosphate per milligram protein)

Pt.	Mg ATPase			Na-K ATPase		
	Acute	Convales-cent.	Change	Acute	Convales-cent.	Change
1	102	101.3	-0.7	23.3	113.7	+90.4
2	150.7	178.3	+27.6	19	96.4	+77.4
3	130.7	118.8	-11.9	68.3	132.6	+64.3
4.	134.3	172.3	+38	16.7	51	+34.3
5	78.7	101.7	+23	81.3	111.3	+30
6	104.7	126.7	+22	67.6	91.6	+24
7	115.7	91.7	-24	62.6	84.3	+21.7
8	118.3	108.5	-9.8	75	94.5	+19.5
9	106.7	103	-3.7	85	103	+18
10	202.3	133.7	-68.6	60.4	74	+13.6
11	124.3	124.3	0	68.7	71	+ 2.3
12	114.7	103.7	-11	75	72.6	- 2.4
13	137.3	125	-12.3	59	52.3	- 6.7
14	129.3	122	- 7.3	72	59.3	-12.7
15	106.7	91.3	-15.4	89.6	53.7	-35.9
Mean	123.76	120.82	- 3.61	61.57	84.09	+22.52
S.E.M.	± 7.2	±6.7	±6.59	±6.06	± 6.44	±8.79
p	$<0.8>0.7$*		$<0.6>0.5$*	$<0.01>0.005$+		$<0.025>0.01$+

* Two-Tailed Test

\+ One-Tailed Test

TABLE 5

COMPARISONS BETWEEN GROUP A AND GROUP B
(Mean values, Range in parenthesis)

	Group A	Group B
Age (years)	30.3 (19-50)	31 (22-50)
Weight (kg.)	43.4 (35.7-49.9)	41.3 (36.4-52.4)
Biopsy (hours after onset)	28.8 (20-41)	33.3 (9-48)
Convalescent Biopsy days after last liquid stool	8.2 (6-13)	6.1 (4-7)
Systolic B.P. < 90	9/9	2/6
Normal Xylose absorption in either acute or convalescent phase	7/9	1/6
Total stool volume (L)	1.26 (0-3.61)	0.88 (0.055-1.4)
Admission - Convalescent Plasma Protein (g.%) *	2.69 (0.4-4)	2.40 (0-3.7)
Days of diarrhea from onset	2.3 (1-4)	2.8 (2-5)
Fever ($> 100^{\circ}$)F.	5/9	2/6
Stool guaiac +	3/7	4/5
Dissecting Microscope Morphology	8/9 leaves predom. 1/9 fingers and leaves.	5/6 leaves predom. 1/6 fingers and leaves.

* An Index of Dehydration.

FIGURE 1.

HUMAN ILEAL MUCOSA ATPase ACTIVITY WITH VARYING CONCENTRATIONS OF SODIUM ION.

Mg^{++} 4m eq/l

K^{+} 16 mEq/l

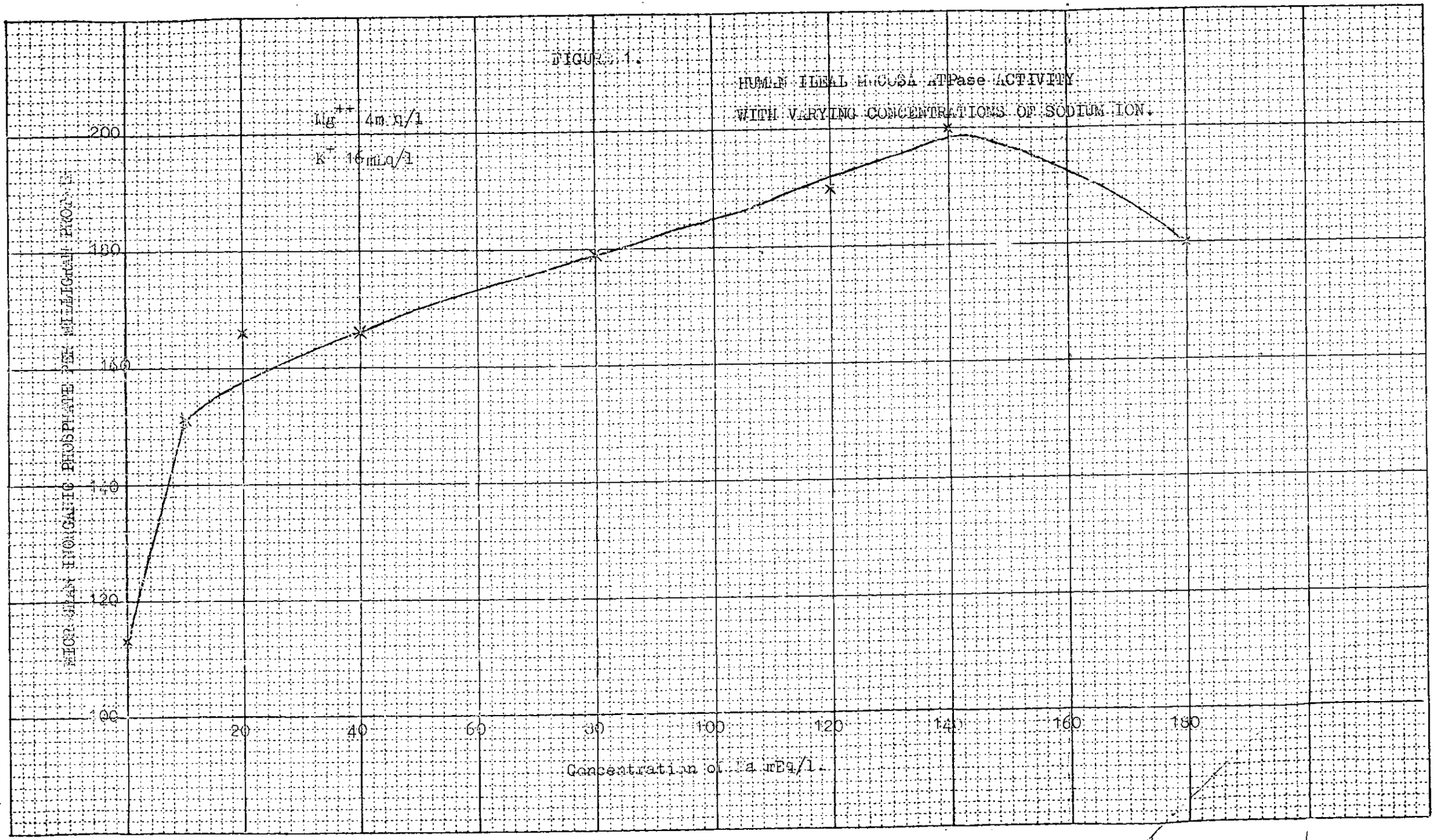


FIGURE 2. ATPase MEAN VALUES ACUTE AND CONVALESCENT

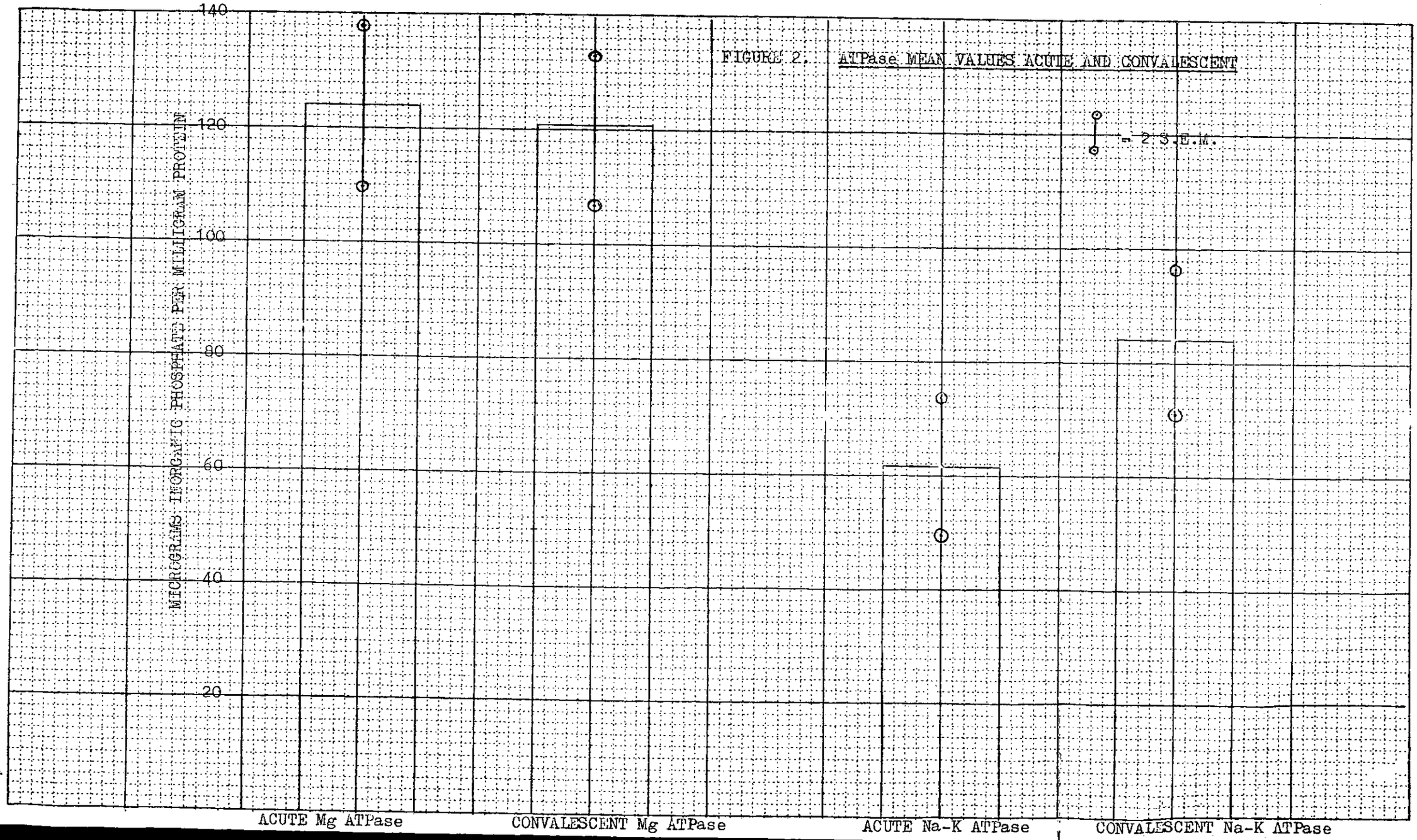


FIGURE 3. ATPase MEAN CHANGE CONVALESCENT - ACUTE

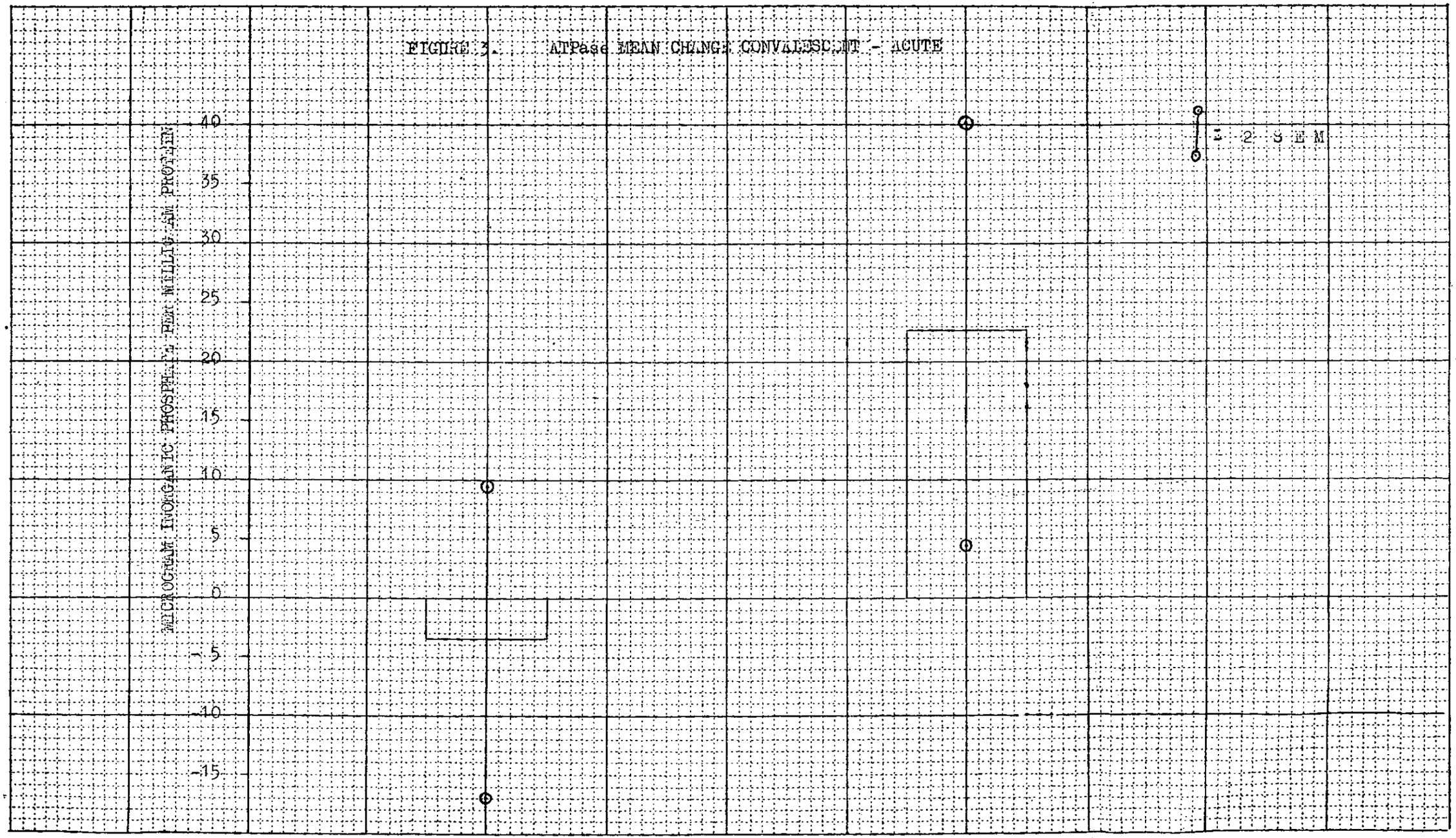


FIGURE 4. Na-K ATPase

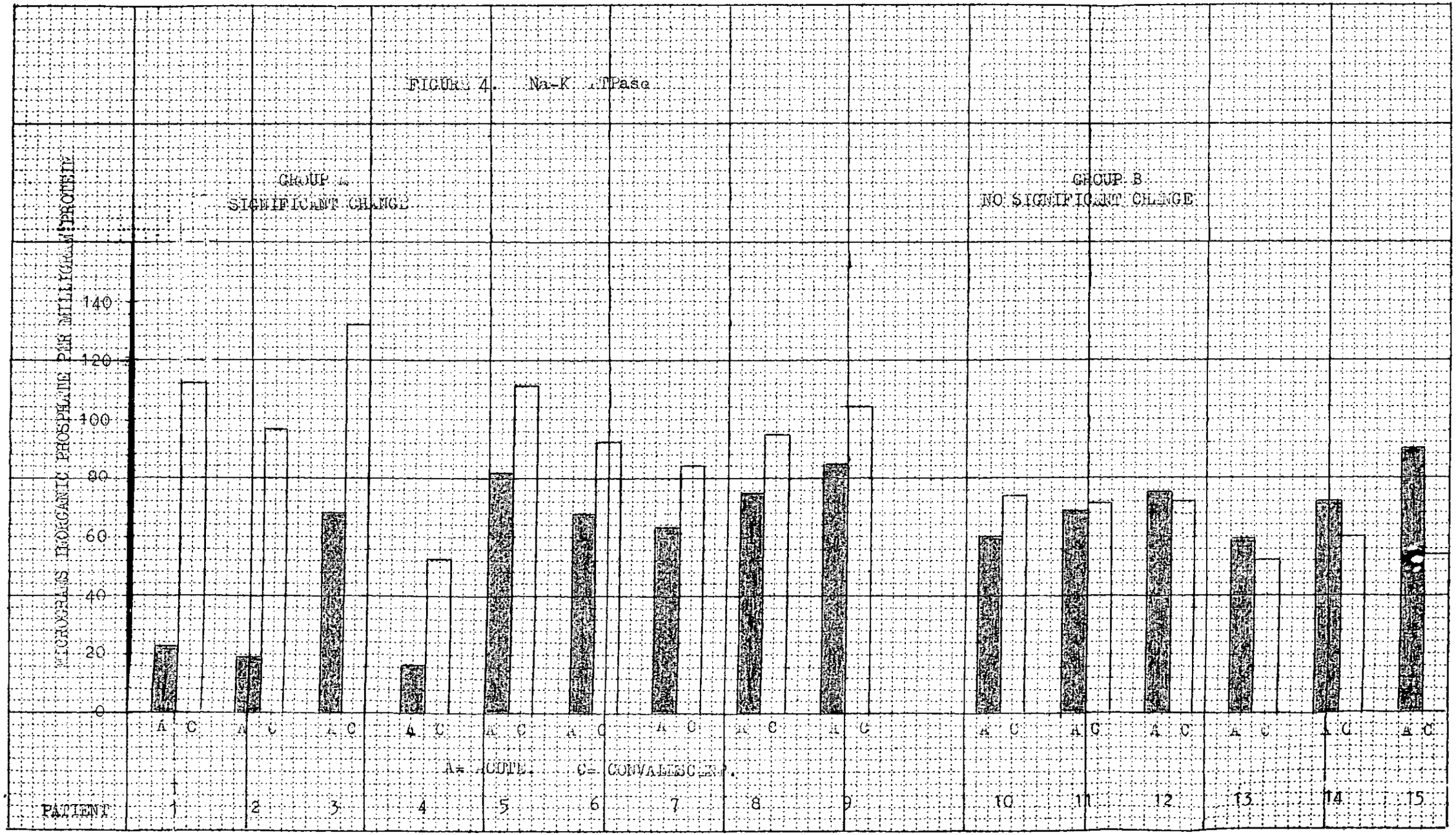
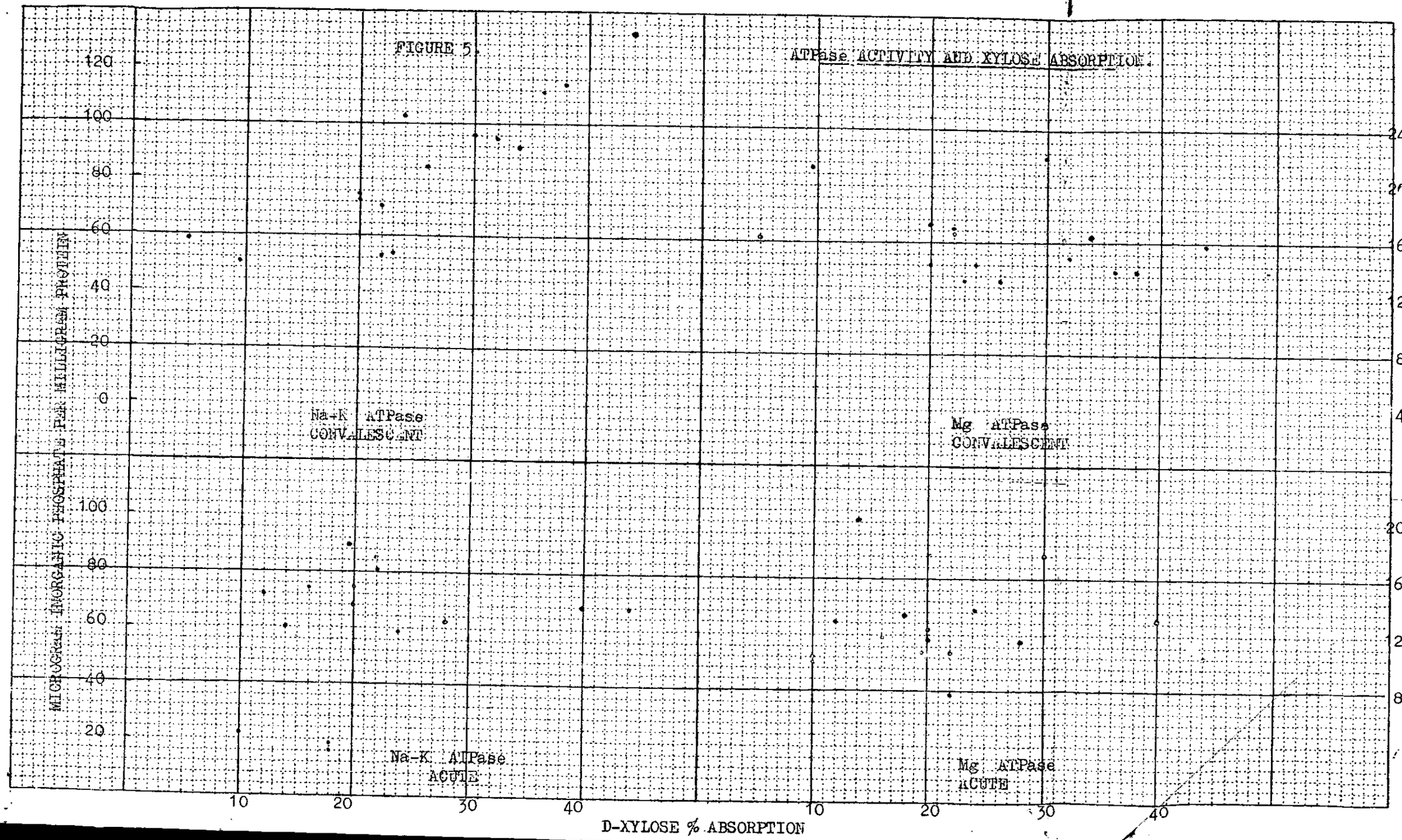


FIGURE 5.

ATPase ACTIVITY AND XYLOSE ABSORPTION



II(f)

SMALL BOWEL FUNCTION AND MORPHOLOGY IN AMERICANS RESIDENT IN PAKISTAN

By J. Lindenbaum, A.K.M.J. Alam, T. Kent and H. Sprinz

Tropical sprue, a syndrome of unknown etiology, characterized by diarrhea, weight loss, malabsorption, and changes in small intestinal morphology, occurs in both native residents and visitors to tropical countries. The syndrome has been frequently described in European and American military personnel stationed in Asia [1, 2] or Puerto Rico. [3,4] During the past year, two cases of severe symptomatic sprue were observed in Peace Corps volunteers in East Pakistan and several cases have been seen among volunteers in Venezuela.[5]

Recent studies from Thailand, India, Pakistan, Taiwan, and Puerto Rico have shown a high incidence of malabsorption and abnormalities of jejunal morphology in asymptomatic native-born individuals.[6-11] The impairment of absorption and abnormality of small bowel appearance in these "normal" persons are often similar in degree to that seen in patients with severe clinical sprue. Since, in accordance with the philosophy of the Peace Corps, volunteers live in a manner as similar as possible to that of the indigenous population, it would be of interest to know whether volunteers develop similar abnormalities of small intestinal function and structure. Therefore, a study of Peace Corps volunteers living in Pakistan was undertaken in order to answer this question.

Methods and Materials.

Studies of intestinal absorption were performed on three groups of adult subjects living in Pakistan during the period 1964-1965.

1) 114 Peace Corps volunteers (PCV's), 47 from West and 67 from East Pakistan; 2) 31 Americans and Europeans employed by various assistance and research organizations, who will be referred to as "protected" Westerners, in view of their tendency to live in air-conditioned homes, eat commissary food, and avoid Pakistani eating places, resulting in minimal contact with the tropical environment; 3) 106 Pakistani employees of the Pakistan-SEATO Cholera Research Laboratory. Subjects included in the study were selected only on the basis of willingness to cooperate in the study. The number of Peace Corps volunteers included comprised 61% of the total PCV

(more)

population resident in Pakistan at the time of the study. The findings in the Pakistani employee group will be reported in greater detail in a separate communication.[9]

The absorption tests were performed in hospital or at the Peace Corps dormitories under the supervision of a metabolic nurse to insure completeness of urinary collections. The five hour urinary excretion of d-xylose after a 25 gram oral dose was measured according to the method of Roe and Rice.[12] Twenty-four hour urinary excretion of Co-58 vitamin B_{12} after an oral dose of 1 microgram of the labelled vitamin given with intrinsic factor was estimated following the method of Schilling.[13] Fecal fat was measured on seventy-two to ninety-six hour collection.[14,15] Jejunal biopsies were obtained with the Crosby-Kugler capsule[16] and examined under the dissecting and light microscopes.

Results

a) Studies of xylose absorption

Forty-five (39.5%) of the 114 volunteers had subnormal xylose malabsorption when first studied after six months' to two years' residence in Pakistan (table I). The incidence was similar in PCV's from the East and West wings of Pakistan. The incidence of xylose malabsorption and the mean xylose excretion were similar to that in the Pakistani employee group but differed significantly from the "protected" Europeans and Americans ($p<.0001$). The xylose tests were repeated one or more times in 39 PCV's one to eight months after the initial study. Fifteen who originally had normal values remained normal; eight subjects with low initial xylose excretion again had low levels. In another nine who were originally low, absorption returned to normal. However, seven initially normal developed xylose malabsorption during a period of several months' observation.

The presence of malabsorption of xylose did not appear to be related to duration of residence in Pakistan. The incidence of malabsorption was similar in subjects studied after six months' residence and in those who had been in Pakistan 18 to 24 months at the time of study (Table II). In addition, two of six volunteers studied within six weeks of arrival in Pakistan failed to absorb xylose normally.

Impairment in xylose absorptive capacity also did not correlate well with the presence or absence of symptoms. Thus the incidence of

weight loss, frequent or infrequent episodes of diarrhea, diarrhea during the two week period immediately preceding the absorption study, and gastrointestinal intolerance to Pakistani food, was similar in those with and without malabsorption (Table III). It should be noted that the incidence of these symptoms was rather high in both groups. While weight loss was no more common in the group with malabsorption, the mean weight loss was greater in this group (12.3 lb) than in the group with normal xylose absorption (8.8 lb.; $p>0.08$). The incidence of intestinal parasites and the specific parasites found were also similar in the two groups. None of the subjects studied was anemic.

b) Vitamin B_{12} absorption (Table IV)

The Schilling test was performed on 23 PCV's. Malabsorption of vitamin B_{12} was present in 12 (52%). In most instances, absorption of cyanocobalamin paralled that of xylose. Thus of 10 PCV's with low xylose values, 8 had low B_{12} absorption; however, 4 of 13 PCV's with normal xylose absorption had low Schilling test values. Seven subjects who initially had low B_{12} absorption were re-studied one to six months later. One had returned to normal and six were still low. Two of the latter group were in the low normal range when studied for the third time six months after the original Schilling test.

c) Fecal Fat (Table IV)

Of 11 PCV's studied, only two had elevated fecal fat excretion (10.6 and 6.2 g/d). Both of these individuals also had malabsorption of xylose and B_{12}; in both the fecal fat excretion later returned to normal.

While impairment of absorption of xylose was usually paralled by malabsorption of vitamin B_{12}, fecal fat excretion was normal in several individuals with poor xylose and/or cyanocobalamin absorption. Similar findings have been noted in our Pakistani employees. Since fat intake was not controlled in these studies, the failure to demonstrate elevated fecal lipids in these individuals may be due to the generally reduced intake of fat in tropical diets.

d) Jejunal biopsies (Table IV)

Biopsies of the upper jejunum were obtained on fourteen PCV's, nine of whom had evidence of malabsorption. Under the dissecting microscope, none of the biopsies showed the predominantly finger-like villous architecture seen in normal American subjects residing in the U.S.A. Two had equal numbers of leaf-like and finger-like villi;

in the remaining 12 the majority of the villi were leaf-like. In 8 of the latter group some ridge-like villi were seen and in 5 there were convoluted forms. Four of the individuals with ridge-like villi had normal xylose and B_{12} absorption studies during the days immediately preceding the biopsy; the four others had impaired absorption.

Histologic slides were available from nine of the PCV's at the time of writing. In general, histologic changes were less striking than those seen under the dissecting microscope. In two, both of whom had normal xylose absorption, there were no histologic abnormalities. The remainder had varying degrees of mild increase in crypt length, round cell infiltrate in the lamina propria and submucosa, and occasional broadening of villi. Only one biopsy (from a PCV with severe clinical sprue) showed diminution in villous height. In none of the subjects, even in two volunteers with marked diarrhea and weight loss, were the changes severe or comparable to those seen in classic celiac disease or nontropical sprue. Also, the histologic abnormalities as well as those seen under the dissecting microscope were in general less severe than those found in the average Pakistani "normal"[9].

Discussion

The Peace Corps represents a radical and often successful social experiment in which Americans live abroad in a manner as similar as possible to the indigenous population, while performing often highly demanding and challenging tasks. It must be recognized, however, that such an attempt at more or less total assimilation of the ways of a tropical country, in which sanitation may be minimal and diseases to which Americans may be immunologically unfamiliar are rife, may involve a certain risk to health. Recognizing this risk, the Peace Corps has instituted a carefully planned health program for its volunteers including immunizations, instructions with regard to preparation of food and water in tropical countries, periodic physical check-ups, etc. Nonetheless a certain minimal, probably irreducible risk remains. For example, the Peace Corps volunteer may well insult his hosts if, on invitation into a local home, he refuses generously offered food on the grounds that it has been prepared without the benefit of modern sanitation and stored without refrigeration.

The findings of the present study indicate that one of the risks to which a Peace Corps volunteer is exposed is the tropical malabsorption syndrome. About 40% of PCV's stationed in Pakistan during the period of this report developed malabsorption during their stay there. Since xylose is predominantly absorbed in the duodenum and upper jejunum,[17] and B_{12} in the ileum,[18,19] and malabsorption of both substances was common, a lesion involving the entire small bowel is implicated.

In recent years, reports from a number of tropical countries have indicated a high incidence of bowel damage in supposedly normal members of the indigenous population. Sprinz and colleagues reported malabsorption of xylose in 44 of 66 asymptomatic Thai army recruits without known intestinal disease.[6] Small bowel biopsies from this and similar groups of control subjects showed varying degrees of histologic abnormality suggestive of chronic enteritis. Baker et al reported a high incidence of dissecting microscope and histologic abnormalities in jejunal biopsies taken from control South Indian subjects without symptoms of gastrointestinal disease.[7] Similar histologic abnormalities were reported by Frosh and associates on 3 of 10 biopsies of Taiwanese patients hospitalized for nondiarrheal diseases.[10] Angel and colleagues found xylose malabsorption in 25% or more of Puerto Rican villagers and army recruits.[11] Russell et al reported histologic abnormalities indicating a chronic enteritis in all of 88 duodenal and jejunal biopsies taken from 47 asymptomatic West Pakistani subjects, the majority of whom had low or borderline xylose absorption.[8] Similar findings have been obtained in a study of East Pakistanis employed in this laboratory.[9]

It is against this background of a high incidence of subclinical small intestinal disease among native-born inhabitants of tropical countries, that the development of similar though generally less marked changes in Peace Corps volunteers living under conditions closely approximating those of the indigenous Pakistani population must be viewed. On the other hand, the incidence of malabsorption was negligible in the control group of "protected" Americans and Europeans, living in comfortable airconditioned homes, eating predominantly imported food prepared under more sanitary conditions, and rarely or never eating in Pakistani homes or roadside "restaurants".

It is of interest that none of the jejunal biopsies obtained from PCV's appeared normal under the dissecting microscope, even in those with normal absorptive function, and normal morphology under the light microscope. In all 14 biopsies, as viewed under the dissecting microscope, leaf shaped villi were present in equal or (more often) greater numbers than the finger-like forms seen in normal individuals residing in the U.S.A. Our experience with native-born Pakistani exactly parrallels this, since in none of more than 200 biopsies examined to date from 86 Pakistani subjects have finger-like villi predominated. Others have reported the occurence of predominantly leaf-shaped villi in patients with idiopathic steatorrhea after successful treatment with a gluten-free diet,[20] and in jejunal biopsies obtained distal to the gastrojejunal anastomosis in patients who have undergone gastrectomy.[21] Several workers have suggested that leaf-

shaped villi represent the earliest stage of mucosal abnormality, or represent a transitional stage between normal and abnormal.[20,21] If this concept is correct, and if further studies in other PCV's bear out our initial findings, the incidence of at least minimal bowel damage in Volunteers living in Pakistan may be 100%.

Malabsorption was commonly present in PCV's within six months or arrival in Pakistan and its incidence was no greater after eighteen to twenty-four months residence in the country. The onset of a tropical malabsorption syndrome within a few months (or even weeks) after arrival has frequently been reported in military personnel stationed in tropical countries.[2-4,22]

The etiology of these changes in bowel function and structure among indigenous subjects and PCV's in Pakistan is obscure. Evidence is accumulating that infection may play a role in the tropical sprue syndrome though no causative organism has been isolated. The occurrence of sprue in epidemic form in various parts of the subcontinent has been repeatedly reported,[2,22-24] and responses to antibiotic therapy have been observed in sprue cases from Hong Kong,[25] Malaya,[25] Puerto Rico,[26] and Pakistan.[27] Transient malabsorption, more commonly without associated symptoms, is a common sequelum to acute small bowel infection and may persist for weeks or months.[28] The incidence of episodes of acute gastroenteritis as well as bacillary dysentery and other parasitic infestations is high in both the Peace Corps volunteers and native population.[27] It is possible that repeated bouts of infection may have resulted in delayed recovery of bowel structure and function. Possibly these changes may favour bacterial overgrowth in the small bowel. Of the two Volunteers in Pakistan with severe sprue mentioned earlier, one responded dramatically to tetracycline therapy and the other showed an equivocal improvement. Further studies of the effects of antibiotics in PCV's with symptomatic and asymptomatic small bowel disease are planned.

The prognostic significance of the changes in bowel function and structure observed in these PCV's is uncertain. It should be emphasized that the dissecting microscope and histologic changes were minimal to moderate in severity and in no individual was severe flattening of the mucosa with loss of villi noted. The clinical picture also was generally not a severe one. More than a quarter of the Volunteers with malabsorption had not lost weight and had only infrequent episodes of diarrhea. At the other end of the clinical spectrum, the two PCV's with severe weight loss and marked diarrhea did not have glossitis or anemia, characteristic features of advanced cases of tropical sprue. On the other hand, the degree of malabsorption as measured by xylose or Schilling tests was often marked.

Clinical follow-up as well as further studies of these individuals after their return to the United States are obviously needed to determine the long term significance of these changes.

SUMMARY

Peace Corps volunteers resident in Pakistan commonly develop a malabsorption syndrome, which is usually mild to moderate in clinical severity. Malabsorption of xylose and B_{12} was frequently found. All of 14 jejunal biopsies taken from PCV's, including 5 with normal absorption, were abnormal when viewed under the dissecting microscope. Malabsorption was also common among Pakistani subjects but rare in Americans and Europeans whose contact with the local environment was minimal. The etiologic and prognostic significance of these abnormalities of small bowel function and structure in Peace Corps volunteers is uncertain.

T A B L E 1

XYLOSE ABSORPTION IN SUBJECTS RESIDING IN PAKISTAN.

	No.of subjects	Mean xylose excretion, gm. *	S.D.	Range	No.of subjects 5.0 gm.
Peace Corps Volunteers	114	5.32	1.81	1.0 - 9.4	45 (39.5%)
"Protected" Americans and Europeans	3	7.1	1.19	4.6 - 9.1	1 (3.3%)
Pakistani Employees	106	5.38	1.57	0.5 - 9.3	42 (39.6%)

* Normal range 5.0 - 10.0 gm.

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T A B L E II

XYLOSE ABSORPTION AND LENGTH OF RESIDENCE OF PCV'S IN PAKISTAN

	Length of residence in Pakistan			
	0-6 mos.	7-12 mos.	13-18 mos.	19-24 mos.
No. of subjects	17	17	35	74
No. with xylose 5.0 gm	8	7	9	31
% with xylose 5.0 gm.	47%	41%	27%	42%

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TABLE III

RELATION OF XYLOSE ABSORPTION TO SYMPTOMS IN PEACE CORPS VOLUNTEERS IN PAKISTAN.

	PCV's with xylose <5.0 gm.	PCV's with xylose 5.0 gm. or >
Lost weight while in Pakistan	72%	78%
Mean weight loss	12.3 lb.	88 lb.
Episodes of diarrhea while in Pakistan:		
less than once/month	29%	23%
1 - 2 times/month	25%	43%
more than twice/month	46%	34%
within two weeks before xylose test	53%	51%
Gastrointestinal symptoms after eating Pakistani food.	37%	40%
Parasites present in stool* (on any of 6-12 concentrated specimens)	41%	43%

* The parasites most commonly found on stool examination in both groups were E. histolytica and Ascaris lumbricoides.

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TABLE IV

PEACE CORPS VOLUNTEERS - ABSORPTION STUDIES AND BIOPSIES

Subject	Months in Pakistan	Symptoms	Weight Loss.lb.	Xylose g.	Schilling Test	Fecal fat G/D	Jejunal biopsy: Dissecting Microscope	Jejunal biopsy: Histology
K.H.	16	Severe diarrhea, weakness	35	2.0	0.6%	10.2	L>F	INF
M.B.	11	Severe diarrhea, abdom. pain, flatulence	40	2.4	3.1%		L>>F	CL;DVH;INF
S.E.	6	Diarrhea	22	5.9	9.2%	5.0	L=F	CL;INF
K.C.	6	Minimal Diarrhea	0	5.1	11.5%	1.3	L>F,R	normal
H.T.	6	Diarrhea	0	3.8	4.5%	2.2	L>F,C,R	CL;INF
E.H.	7	Diarrhea, flatulence	2	4.3	0.8%	6.2	L>F	INF
J.F.	6	Diarrhea	0	6.7	5.5%	5.4	L>F	CL;INF
L.E.	17	Diarrhea	43	5.8	2.9%	2.6	L>>F.R	INF
D.S.	23	Diarrhea	5	6.0	5.1%		L>R,C,rare F	pending
J.R.	23	Diarrhea	5	5.3	15.4%	2.0	L>>R,C,rare F	"
W.M.	23	Minimal diarrhea	5	6.7	14.7%	3.6	L>R,C,F	"
D.N.	6	Diarrhea	0	2.6	4.7%		L>>F	CL;INF
W.C.	19	Diarrhea	30	4.3	7.6%		F=L,R,C	pending
F.W.	19	Diarrhea	20	7.9	8.8%		L>>F,R	"
M.D.	6	None	gained	1.4	5.9%	2.9		
F.R.	19	Diarrhea	40	2.6	0.7%			
D.B.	19	Diarrhea	25	3.4	8.8%			
N.A.	14	Diarrhea	10	4.1	11.5%			
D.P.	19	Diarrhea	5	8.6	9.7%			
T.B.	19	Diarrhea	18	5.8	2.3%			
J.A.	19	Diarrhea		6.7	13.7%			
W.S.	19	Diarrhea	7	5.2	8.8%			
G.L.	19	Diarrhea	40	6.1	11.4%			
				normal 5-10 g	normal 8-26%	normal <6.0g/d	L=leaves C=convolutions F=fingers R=ridges	DVH=decreased villous height CL=lengthening of crypts INF=increased mucosal or submucosal infiltrate

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SMALL BOWEL FUNCTION AND MORPHOLOGY IN PAKISTANIS

By J. Lindenbaum and A. K. M. J. Alam.

Investigators in Thailand, South India, Puerto Rico, and West Pakistan have noted a remarkably high incidence of malabsorption and histologic evidence of chronic enteritis in surveys of asymptomatic, apparently normal members of the native-born population.[1-4] These studies have usually been performed as control observations in connection with studies of patients with diarrheal illnesses. Reduced absorptive capacity for xylose has been demonstrated but the absorption of other substances has not been widely studied, and there has been little in the way of systematic inquiry into the problem. In the current investigation, it was elected (1) to determine the incidence of malabsorption in a group of asymptomatic East Pakistanis (2) to test the absorption of substances other than xylose to gain a fuller picture of small bowel function (3) to attempt to correlate the presence of malabsorption with factors such as economic status, body weight, parasitism, and anemia, and (4) to observe the natural history of subjects with evidence of subclinical bowel disease and the effects of therapy with tetracycline and folic acid on small intestinal function. A report of preliminary findings is presented at this time.

Methods and materials.

Adult Pakistani employees of the Pakistan-SEATO Cholera Research Laboratory were asked to volunteer for the study. Of 113 employees approached, 106 agreed to take part in the study. They represent a cross section of our employee group, with a wide range in occupation (table 1) and economic status (fig. 1). Studies of xylose and vitamin B12 absorption, fecal fat excretion, and jejunal mucosal biopsies were performed according to methods described in the preceding report. Folic acid absorption was tested according to the method of Chanarin, et al, using Streptococcus faecalis as the organism for microbiological assay of serum samples, after an oral dose of 40 ug/kg of folic acid.[5]

Results

1. Xylose absorption The mean xylose excretion of the 106 employees after a 25 gram oral dose was 5.38 grams (S.D. 1.81). The values follow a normal distribution curve (fig. 2). Forty-two (39.6%) of the employees excreted loss than 5.0 grams, the lower limit of normal for the test.

2. Vitamin B12 absorption The Schilling test was performed on 30 employees, 14 of whom had low xylose values (table 2). Absorption of vitamin B12 was subormal in six (20%), four of whom had xylose malabsorption. The distribution of the 30 Schilling test values was irregular, with a suggestion of two peaks, one in the low and one in the normal range (fig.3).

3. Fecal fat Average daily fecal fat excretion (based on three to four day collections) was normal in 14 subjects and borderline (5.8 g) in one (table 2). The borderline value was in an employee with low xylose and

Schilling values. Of the 14 with normal fecal fat excretion, four had xylose malabsorption and another absorbed both xylose and B12 poorly.

4. Folic acid absorption In eleven individuals (of whom six had low xylose values and another had impaired absorption of both xylose and B12) peak serum folic acid levels after the oral test dose were well within normal limits in every instance (table 2).

5. Jejunal biopsies. Small intestinal biopsies from the first or second loop of jejunum were obtained from twelve subjects, four of whom absorbed xylose poorly. Under the dissecting microscope, leaf-like villi predominated in all of the mucosal specimens. The presence of varying numbers of finger, ridged, and convoluted forms did not appear to correlate with abnormalities in absorptive function. Histologic sections were not available at the time of writing.

6. Correlation of malabsorption with other factors There was no correlation apparent between inability to absorb xylose normally and occupation (table 1), salary (fig.1; $r_s = 0.06$), age, weight, blood hemoglobin level, eosinophil counts, number of daily bowel movements, presence of constipation, or brief episodes of diarrhea during the year preceding the time of study (table 3). There were a few subjects with mild chronic recurrent diarrhea among the employees who absorbed normally and among those who did not (table 3).

The presence of parasites on stool examination was a more common finding in the subjects with impaired xylose absorption than in those with normal absorption (table 4). The differences were statistically significant for both ascaris and hookworm infestation.

7. Variability of xylose absorptive capacity in individual subjects. In 23 subjects with initally low xylose absorption, the test was repeated one to four times at intervals during a period of 18 months (table 5). In 14 of the 23 subjects, malabsorption of xylose was demonstrated at each repetition of the test; in five, xylose absorption was impared on three of four occasions; and in the remaining five xylose absorption became normal five to twelve months after the initial test and remained normal on subsequent testing. The data can be summarized in another way: in 50 repeat xylose tests on 23 individuals with initially low values, 36 (or 72%) were again low. None of the changes could be related to changes in body weight or symptomatology.

In twelve subjects with initially normal xylose values, the test was repeated 9 to 18 months later (table 6). In nine xylose was again absorbed normally; three had developed malabsorption, though they were asymptomatic.

8. Effects of therapy with tetracycline or folic acid Six asymptomatic subjects with consistently impaired xylose absorption over a baseline period of 12 to 14 months were given a three week course of oral tetracycline (1 gram/day). Repeat xylose tests immediately after the course of therapy showed no improvement in five, and an increase from 4.1 to 5.7 g. in the sixth. Appetite increased while on the antibiotic in four of the six subjects and weight gains of 1.1 to 1.8 kg were noted in three; weight remained stable in the other three.

Four of those who showed no improvement in absorption after the three week course of tetracycline were subsequently given folic acid 15 mgm daily by mouth for 21 days. In three of the four there was no improvement in absorption over baseline values after folic acid therapy; in one xylose excretion rose from 4.7 to 6.4 g. In none of the four was there a change in symptoms or weight during the course of folic acid.

Discussion

Jejunal biopsies obtained from "control" subjects free of gastrointestinal symptoms have revealed abnormalities of small intestinal morphology in the majority of native-born inhabitants of tropical countries. In South India, for example, jejunal villous architecture was abnormal under the dissecting microscope in all of 25 control subjects studied by Baker and associates, and abnormalities were present in histological sections in most.[2] These investigators felt that jejunal biopsy could not differentiate patients with severe tropical sprue from apparently normal individuals. Similar abnormalities were found in the majority of a group of Ugandan subjects without known gastrointestinal disease.[6] Histologic changes consistent with a chronic enteritis were present in almost all of 27 asymptomatic Thai individuals studied by Sprinz and colleagues,[1] in all of 47 asymptomatic West Pakistanis biopsied by Russell and associates,[4] and in three of ten Taiwanese without known gastrointestinal disease reported by Fresh, et al.[7]

Xylose absorption was subnormal in 20 to 68% of asymptomatic subjects studied in South India,[2] Thailand,[1] West Pakistan,[4] and Puerto Rico.[3] Little information concerning the absorption of substances other than xylose has been provided in these reports, though Baker and colleagues found fecal fat excretion to be normal in balance studies in 17 of 18 control subjects in South India.[2]

It is apparent, therefore, that both morphologic and functional evidence of subclinical small intestinal disease is readily demonstrable in most if not all residents of tropical countries. In the present study a group of asymptomatic East Pakistanis was studied in an attempt to characterize these changes more fully.

In terms of absorptive function xylose was the substance most commonly absorbed poorly by our subjects. Vitamin B12 absorption was less frequently impaired and folic acid and fat absorption were usually normal. The last finding is consistent with the fat balance studies reported from South India.[2] The high incidence of xylose malabsorption points to a lesion predominantly involving the upper small intestine.[8] It is of interest that folic acid was absorbed normally in individuals with poor absorption of xylose, since both substances are believed to be absorbed predominantly in the upper small bowel,[8-10, 23] and in patients with clinical bowel disease, impairment of xylose absorption is usually accompanied by malabsorption of folic acid.[11] The exact site of the absorption of folic acid in man is unknown; clinical evidence suggests that the vitamin may be partly absorbed in the lower jejunum and perhaps the ileum.[10,11] The sparing of fat absorption in our subjects may be another indication that the whole length of the small bowel is not involved,[12] though in 20% of the individuals studies, B12 was absorbed poorly even in the presence

of added intrinsic factor, suggesting ileal disease.[13,14] We are unable to resolve these discrepancies at this time, though it should be noted that the physiologic circumstances associated with each of the tests employed are different. Thus, for example, with the 25 gram xylose test a massive dose of a pentose unessential for human nutrition and absent from most foodstuffs is administered; in the Schilling test a quantity of vitamin B12 roughly equal to the daily physiologic requirement is given; and folic acid is given in a dose well beyond the physiologic requirement and in a form different from that predominantly occurring in foodstuffs. In any case it is not surprising that most substances tested were absorbed well, in view of thelack of clinical symptomsaincluding weight loss in our subjects. The xylose test may merely be more sensitive than the others in detecting minimal bowel disease.

The etiology of these changes in bowel function and morphology is at present obscure. They appear to occur at all economic and social levels of the Pakistani population, as malabsorption was as common in physicians as in bottle washers or guards (table 1). While longstanding protein malnutrition has been suggested as a possible cause of these abnormalities,[15] their presence in employees at all economic levels (including some individuals from upper class families) would appear to make even subclinical malnutrition an unlikely cause. The lack of correlation between malabsorption and body weight further argues against this hypothesis.

Present evidence suggests that these intestinal lesions are acquired rather than genetic. This is supported by their widespread distribution (including Asia, Puerto Rico, and Africa), their absence in South Indian and Ugandan still-born fetuses, [2,15] and the rapid development of malabsorption by American Peace Corps volunteers after a few months' residence in Pakistan.

The significance of the higher incidence of parasitism in Pakistani employees with malabsorption (table 4) is uncertain. It is doubtful whether infestation with hookworms or roundworms, the most common parasites found in these subjects, is directly responsible for malabsorption.[16] Possibly the presence of bowel dysfunction predisposes to the establishment of a parasitic infestation. On the other hand, the high incidence of parasites may indicate exposure to a more heavily contaminated environment, suggesting the possible role of bacterial infection or microbial toxins in producing bowel damage. Staphylococcal enterotoxin, for example, has been shown to cause reversible histologic damage to the small bowel of animals.[17] The absence of response to therapy with tetracycline in our subjects argues against a primary bacterial infection. The possible contribution of irritating foods, such as spices or betel nut, has been suggested by Sprinz and associates,[1] and requires further investigation.

It has been postulated the small bowel abnormalities present in asymptomatic persons in tropical countries represent a subclinical form of tropical sprue.[18] The tropical sprue syndrome often responds to antibiotic therapy.[19-21] In five of a series of six Pakistani patients with symptomatic steatorrhea, improvement in symptoms as well as return of xylose and fat absorption to normal were noted within two to three weeks of beginning treatment with tetracycline.[22] In contrast, xylose absorption failed to improve after tetracycline therapy in five or six asymptomatic employees with malabsorption in the current study.

Considerable variability in xylose absorption in asymptomatic subjects was noted when the test was repeated at intervals of several months. Absorption of the pentose changed from subnormal to normal or vice versa in about a quarter of the individuals under study, without apparent associated changes in well-being, weight, or other symptomatology. Careful attention was paid to rule out inadequacy of urine collection as a possible artefactual cause of these variations. The tendency for such spontaneous fluctuations will require repeated baseline studies over a prolonged period as well as cautious interpretation of results in evaluation of any attempts to improve absorptive function with therapeutic measures. In future studies, the effect of changes in protein content of the diet, or, if feasible, elimination of spices or other irritating foods might be evaluated.

Summary

Preliminary results of a study of small intestinal function and morphology in a group of 106 East Pakistanis are presented. Malabsorption of xylose was found in 40%, and of vitamin B12, in 20%. Subjects with xylose malabsorption absorbed folic acid and fat normally. Villous architecture was predominantly leaf-like in jejunal biopsies viewed under the dissecting microscope. Some asymptomatic individuals showed marked variation in their ability to absorb xylose when studies were repeated at intervals of several months. Therapy with tetracycline or folic acid did not appear to improve absorptive function.

This study would not have been possible without the careful nursing support of Mrs. Madhabi Ghose and the accurate technical assistance of Mr. Mujibur Rahman and Mr. Bazlur Rahman.

TABLE I

Occupation	Number Studied	Number with xylose less than 5.0 g.
Technician	29	11
Nurse	18	8
Administrative work	10	2
Sweeper	10	1
Bottle washer	7	2
Physician	6	4
Guard	6	4
Field worker	6	3
Miscellaneous	14	7
Total	106	42

TABLE 2

PAKISTANI EMPLOYEES - ABSORPTION STUDIES AND BIOPSIES

Subject	Xylose, g.	Schilling test	Fecal fat g/d	Peak folic acid level. mug/cc	Jejunal biopsy diss. micros.
M.G.	4.2	13.9%	1.6	>100	
M.I.K.	3.9	20.0%	2.3	>100	L>>R,F
A.H.C.	2.1	4.8%	1.7	>100	
M.A.	7.0	20.0%	4.3	>100	L
K.	5.6	11.4%	1.7	80	L>F
M.A.	4.3	11.8%	1.1	100	
I.C.	5.2	14.9%	5.0	>100	
A.R.S.	6.2	12.3%	1.4		L>>F
M.R.	7.7	11.9%	3.3		L>>F
S.I.	6.5	14.3%	2.4		L
S.K.	4.7	5.2%	5.8		L>> F,C
A.R.	6.8	4.3%	3.9		
H.	5.2	16.1%	1.8		L> R,C,F
M.M.	7.9	8.6%	2.8		R and L>> F
N.R.	3.7	12.5%		80	
N.D.	6.8	11.8%		72	
O.A	3.5	9.1%		100	
K.M.A.	1.2	7.8%		>100	
A.M.	6.1	12.0%			
G.G.	3.6	13.2%			
S.S.	4.7	16.1%			
S.	4.0	3.8%			
A.K.H.	7.5	21.3%			
C.	5.5	15.3%			
A.M.	5.7	15.9%			
A.H.	2.8	21.5%			
D.O.R.	3.1	3.0%			L>> R,F
M.I.	9.3	3.7%			
M.S.	2.7	9.8%			L> R,C,F
K.A.	6.5	9.7%			
J.A.	5.4				L> F
J.R.S.	4.5		3.0		
normal range	5.0-10.0	8-26%	less than 6.0	more than 40	F>> L

F= fingers
L= leaves
C= convolutions
R= ridges

TABLE 3

COMPARISON OF LABORATORY AND CLINICAL DATA ON 100 EMPLOYEES

	Xylose less than 5.0 g	Xylose 5.0 g or more
Number of subjects	41	59
Male: Female ratio	4.1:1	7.4:1
Mean age, years	30.1	30.3
Mean Hgb., g. %	13.2	13.3
Mean weight, kg	50.7	52.0
Mean Eosinophils, % of total WBC	6.9	6.5
Mean number of bowel movements/d	1.3	1.3
Episode of diarrhea/ past year	36.6%	30.0%
Chronic recurrent diarrhea	9.8%*	4.0%*
Constipation	34.1%	28.0%

* difference not statistically significant; $p > 0.2$.

TABLE 4

STOOL EXAMINATIONS FOR OVA AND PARASITES

	Xylose less than 5.0 g	Xylose 5.0 g or more	"p" value
Mean no. stool examinations/subject	1.6	1.6	
Ova or parasites present	80.5%	57.6%	<0.01
Ascaris	56.1%	27.1%	<0.01
Hookworm	46.3%	27.1%	<0.05
Trichuris	24.3%	15.3%	>0.2
E. histolytica	4.8%	8.5%	>0.2
Giardia	4.8%	3.4%	>0.9
More than one species present	41.5%	25.4%	>0.05

TABLE 5

SERIAL STUDIES OF XYLOSE ABSORPTION IN SUBJECTS WITH INITIALLY LOW VALUES

Subject	Xylose, grams: Initial test	Months after initial test: 0-3	3-6	6-9	9-12	12-15	15-18
B.	2.4			4.6			
N.K.	4.6			4.3			
M.G.	3.5			4.5		4.2, 4.1	3.6
A.H.C.	4.0					2.1, 3.9	2.6
R.	4.4					4.9	
G.G.	3.6		4.6			4.1	
S.S.	2.7				4.7	2.9, 4.5	
S.	4.5		3.7		4.0	4.8	
O.A	3.3		3.9			3.5	
M.H.	2.7		4.1			3.7	
K.M.A.	4.4					1.2	
M.A.	4.8					4.3	
A.M.	2.5		3.1				
N.R.	3.7	4.0	4.9				
J.G.	3.7		1.6			5.3	4.9
A.R.	3.7		4.2		6.7	3.5	
G.	3.9		4.8		6.6	4.3	
A.R.D.	4.0	2.7		5.0	3.8		
I.K.	2.9			5.7			5.7
A.	3.5			5.6			8.5
B.	3.1		7.4			6.2	
G.	4.6		4.8			5.7	5.8
F.B.	4.4			5.9	5.6		

TABLE 6

REPEAT XYLOSE TESTS IN SUBJECTS INITIALLY NORMAL

Subject	Xylose, grams: Initial test	Mo. after initial test: 9-12	12-15	15-18
J.A.	6.1	6.4		
M.A.	5.7	5.8		
S.	8.8	6.2		
S.S.	6.0	7.5		
B.R.	5.5	6.3		
R.	8.2	7.7		
M.M.	5.6	7.9		
K.	5.8		6.6	
M.P.	7.1			7.3
L.T.	8.3		4.5	
R.T.	6.2	1.8		
S.P.	5.2			3.4

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FIGURE 1

DISTRIBUTION OF XYLOSE VALUES IN GRL EMPLOYEES

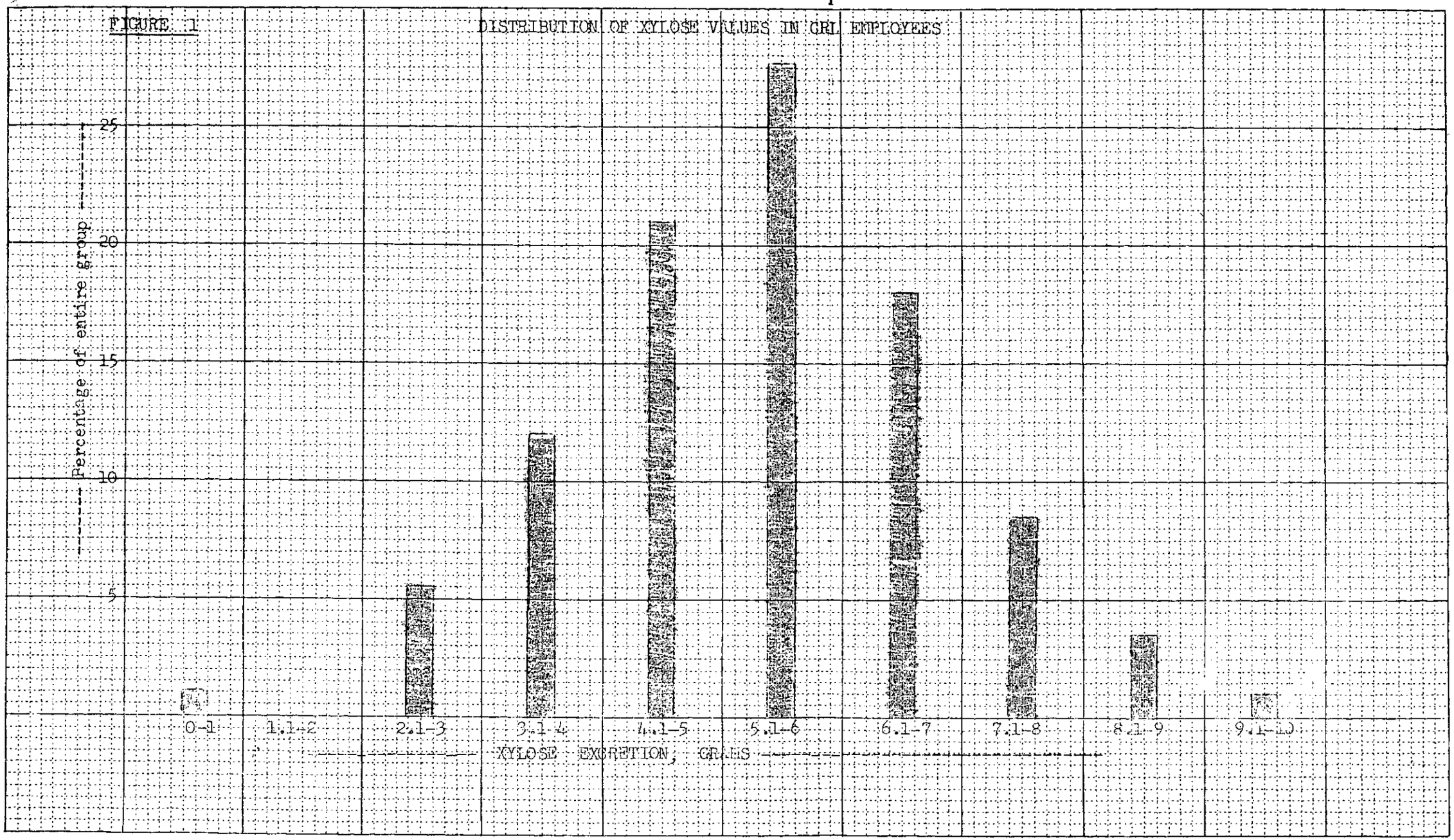
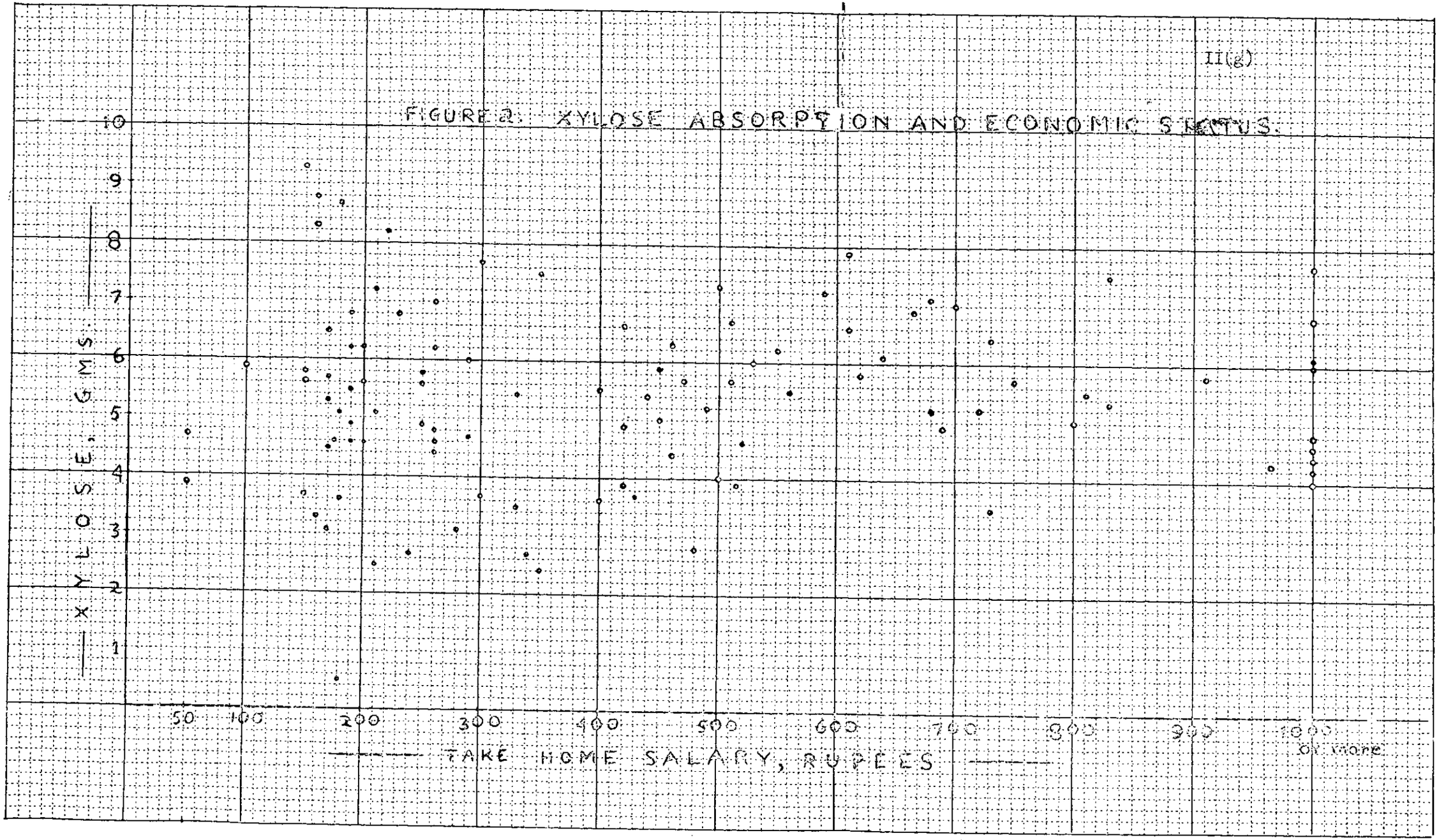


FIGURE 2. XYLOSE ABSORPTION AND ECONOMIC STATUS.



STUDIES OF GASTROINTESTINAL PROTEIN LOSS IN CHOLERA AND OTHER ACUTE DIARRHEAL ILLNESSES.

By J. Lindenbaum, A. Firoze, and R. S. Gordon, Jr.

In recent years it has been recognized that significant amounts of albumin and other proteins may be lost in man via the gastrointestinal tract in both normal and pathologic states.[1,2] Considerable interest has been stimulated in the association of hypoproteinemia with excessive gastrointestinal protein loss, or the "protein-losing enteropathies." Increased fecal loss of protein has been described in a number of conditions associated with mesenteric lymphatic obstruction,[2] as well as the sprue syndromes,[3,4] and inflammatory diseases of the bowel, such as regional enteritis[5] and ulcerative colitis.[5] Two cases have been reported in which an acute intestinal infection was associated with transient hypoproteinemia, edema, and massive gastrointestinal protein loss.[1,2] In addition, during the past two years at CRL, we have encountered several patients with severe, ultimately fatal amebic colitis associated with hypoproteinemia and edema.

An ideal radioactive marker for the study of all aspects of albumin metabolism has not yet been developed, but at present, for the purposes of evaluating gastrointestinal protein loss, Cr-51 labelled albumin according to the method introduced by Waldmann[6,7] yields reliable results, and its half-life of 27.7 days makes it suitable for use in a field station such as Dacca. Cr-51, whether bound to protein or in inorganic form, is poorly absorbed from the gastrointestinal tract, so that after Cr-labelled protein that has been administered intravenously has passed into the gastrointestinal lumen, the radioactive marker will appear in the feces.[6]

It was decided to study gastrointestinal protein loss in a number of acute intestinal infections using this technique in order (1) to determine whether increased gastrointestinal protein loss is a common feature of such infections, or can be used as a way of physiologically differentiating acute diarrheal states (such as cholera, nonvibrio cholera,[8] and other noncholera diarrheas) and (2) to establish whether protein loss through the inflamed colon was responsible for the hypoproteinemia associated with amebic colitis in Dacca.

At the time of writing the study is not yet completed, but prelininary results will be presented. Unfortunately we have not yet been able to successfully study a patient with the fulminating amebic colitis syndrome; the single patient admitted to the ward at a time when radioactive material was available expired within a few hours of initiating the study.

Methods and materials

Cr-51 albumin prepared by Mr. William Briner at the radiopharmaceutical section of the NIH Clinical Center was sent to Dacca by air express. Patients received intravenous doses varying from 30 to 120 microcuries. Studies were restricted to alert, cooperative adult males in order to avoid admixture of urine and stool specimens. All stool and urine were separately collected

over 8 hour periods for 96 hours after the injection. The 8 hour specimens were placed in plastic bottles each containing 800 ml liquid, protected by a heavy lead shield, and counted over a thallium activated sodium iodide scintillation counter.

Patients studied up to the time of writing included three with bacteriologically documented severe classical cholera; two with nonvibrio cholera; one with the full-blown cholera syndrome associated with noncholera vibrio infection (with a convalescent antibody titer rise against the homologous noncholera vibrio organism but not against V. Cholerae); one with mild amebic dysentery; and eight with acute diarrheal illnesses not associated with recognized pathogens on stool culture ("acute gastroenteritis"). These patients received the intravenous dose within 24 to 48 hours of admission in the presence of continuing diarrhea. Seven patients (three with cholera, three with acute gastroenteritis, and one with nonvibrio cholera) were studied in early convalescence during the first week after end of diarrhea. In four patients (two cholera, two gastroenteritis) who were first studied while actively purging in the acute phase, the test was repeated 4 to 23 days after end of diarrhea.

Results

The results of studies conducted on 15 patients who were actively purging are shown in table I. A four-day excretion of 0.63% or less has been taken as normal, as established by Waldmann.[6] (However, it is intended to determine the range of normal for Pakistanis, pending the arrival of more radioactive material from Bethesda). In two patients (one with noncholera vibrio infection and one with gastroenteritis) the percentage excretion was within the normal range. In a third, the value was borderline (0.83%). In the remaining twelve patients, the values were slightly but definitely above normal (1.4 to 3.4%). Severity of dehydration on admission or volume of stool output did not appear to be related to the presence or degree of gastrointestinal protein loss. In none of these patients was gross exudate seen in the stool, though in most a few white cells were present on microscopic examination of the stool; this was true in patients with normal as well as abnormal values.

Paper electrophoresis of serum proteins was performed by Mr. Samin Ahmed on serum obtained in the first week after end of diarrhea from 12 of the 15 patients. Serum albumin levels varied from 3.9 to 5.5 grams per cent.

In the seven cases who were studied during the first week after end of diarrhea, the percent protein loss in the stool ranged from 0.67 to 1.4%.

The peak excretion of radioactivity occurred within 24 hours of administration of the marker in the majority of the patients studied during the acute phase, and at 24 to 48 hours in most of the patients studied during convalescence. There were a few in both groups whose peak excretion was at 48 to 78 hours.

The results of paired studies done during acute diarrhea and in the convalescent period in four patients are shown in table II. In each case, the convalescent values are less than during the acute phase, and are within or near the normal range.

Discussion

These preliminary findings suggest that in most patients with acute intestinal infections there is a slight increase in protein loss via the gut as measured by the Cr-51 albumin technique. In none of the patients studied was the loss as great as that usually recorded in typical cases of "protein-losing enteropathy," and noen of the subjects studied developed hypoproteinemia or edema during the acute or convalescent period. The findings in the first three cholera patients studied would appear to be consistent with those obtained by Gordon in Bangkok with I-131 labelled polyvinylpyrrolidone (where six cholera patients given the intravenous macromolecule excreted less than 1% of the dose over the first 24 hours after the injection).[9] Previous evidence [9,10] as well as that obtained in this study indicates that massive protein loss is not a feature of cholera, which is consistent with the maintenance of mucosal integrity as demonstrated in small bowel biopsies from acute cholera patients in Bangkok,[11] Manila,[12] and Dacca.[13]

Whether the slight increase in protein loss demonstrated in the majority of our patients with cholera and non-cholera diarrheas corresponds to a real increase in gastrointestinal permeability to protein appears questionable. It should be noted that the peak appearance of marker in the stool tended to be within 24 hours of its administration in patients studied during acute diarrhea, while in those studied during the convalescent phase peak radioactivity appeared in the stool at a later time during the 96 hour collection. These observations are consistent with the decreased transit time present during acute intestinal infections. In view of the reduction in transit time, even if the amount of protein passing into the gastrointestinal lumen were normal, a greater amount might appear in the feces during the 96 hour collection period than in normal subjects. If this interpretation is correct, then the administration of a purgative such as magnesium sulphate during a Cr-51 albumin study in a normal individual should result in similar increases in fecal radioactivity.

The possibility cannot be excluded, however, that a slight increase in gastrointestinal permeability to protein exists in some patients with acute intestinal infection, and that exagerration of this tendency results in the occasional cases of hypoproteinemia associated with acute gastroenteritis such as those that have been reported in the literature.[1,2,14]

Summary

Preliminary results of studies of gastrointestinal protein loss using Cr-51 albumin in patients with cholera and other acute diarrheal states are presented. Most cases showed slight increases in the percentage excretion of the intravenously administered dose during acute diarrhea with a tendency to return to normal during the convalescent period. Whether these findings are attributable to decreased transit time alone or whether there is a real increase in the amount of protein passed into the lumen of the bowel during acute infections is uncertain.

* * *

The authors are grateful to Mr. M. A. Quddus and Mr. Bazlur Rahman for reliable technical assistance.

TABLE 1

CR-51 ALBUMIN LOSS IN PATIENTS WITH ACUTE DIARRHEAL DISEASE

CRL No.	Diagnosis	Adm.pl. protein, g.%	Total stool vol. during study. cc	% Cr-51 in 96 hr. stool
2730	V. cholerae	12.6	26,800	3.1
2744	V. cholerae	12.0	2,800*	2.1
2707	V. cholerae	11.8	5,900	1.4
2165	Nonvibrio cholera	12.8	7,500	3.4
2183	Nonvibrio cholera	11.8	5,500	0.83
2200	Cholera syndrome due to nonchol. vibrios	14.6	6,000	0.48
2262	amebic dysentery	8.0	1,630	1.9
2163	gastroenteritis	8.8	1,500	2.6
2168	gastroenteritis	11.2	2,500	1.7
2175	gastroenteritis	8.7	500	3.3
2176	gastroenteritis	9.8	2,900	1.5
2204	gastroenteritis	10.4	6,940	2.4
2209	gastroenteritis	8.7	1,400	0.55
2231	gastroenteritis	9.2	1,360	2.1
1887	gastroenteritis	7.8	300	2.4**

* tetracycline treated. Diarrhea ended 24 hours after Cr-51 albumin administered.

** 48 hours collection only.

TABLE II

SERIAL CR-51 ALBUMIN STUDIES: ACUTE AND CONVALESCENT

CRL No.	Diagnosis	% Cr-51 in stool during acute diarrhea	Days after end of diar. at time of second study.	% Cr-51 in stool during second study
2730	V. cholerae	3.1	18	1.2*
2744	V. cholerae	2.1	20	1.1*
2163	gastroenteritis	2.6	6	0.69
2231	gastroenteritis	2.1	4	1.2

* Corrected value after subtracting background counts based on daily average for five days collection preceding second injection.

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A PRELIMINARY REPORT ON HEMODYNAMIC OBSERVATIONS IN PATIENTS WITH CHOLERA

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Supported in part by HE-02001-10 and HE-05741 from the National Heart Institute, U.S. Public Health Service and by a Public Health Service research career Program award (5-K6-HE 16,603-03) from the National Heart Institute, and by Research Agreement 196802 between the National Institutes of Health, Bethesda, Maryland and the Pakistan-SEATO Cholera Research Laboratory, Dacca, East Pakistan.

Recent studies in patients with chronic obstructive lung disease have shown that an increase in the hydrogen ion concentration of the blood augments the pressor response in the pulmonary artery to hypoxia (1,2). The effect of severe acidosis on the heart and circulation in man, free of cardiopulmonary disease, is not known. Certain observations in patients with cholera have suggested that an increased hydrogen ion concentration is contributing to the hemodynamic abnormalities induced by dehydration in these patients (3). The purpose of this investigation was to describe, in part, the hemodynamic state of patients with cholera and to define, if possible, the role of acidosis, utilizing the technique of cardiac catheterization for the measurement of cardiac output, systemic and lesser circulation pressures.

Material and Methods:

Twenty-three patients suspected of cholera were studied: bacteriologic identification of Vibrio cholerae, Inaba, was subsequently made in 18 and of cholerae, Ogawa, in 2. The remaining 3 patients resembled the others in all respects, but the organism could not be demonstrated by stool culture or by dark field examination. In only 2 individuals were other diseases suspected; one was found to have an abnormal glucose tolerance curve and the other a neurologic picture suggestive of multiple sclerosis. Seventeen subjects were male and 6 female. The average age was 33 years with a range of 16 to 63. These patients were small, the average height was 61 inches and the average weight at time of study was 98 pounds.

* Recipient of investigatorship of the Health Research Council of the City of New York under contract I-176.

(more)

The patients chosen for study demonstrated severe dehydration, severe acidosis or both. Preliminary examination of serum proteins and arterial blood pH aided in the selection. For the most part patients with a pH greater than 7.25 were eliminated from this investigation. The severity of the illness from which these patients suffered can be estimated by a consideration of their blood pressure and pulses on admission. In 12, blood pressure was unobtainable, in 7, the systolic pressure was 80 mm Hg or less and the diastolic could not be elicited; in 3, the pressure was 90/60; and in the remaining one, the pressure was normal. In 10 patients only the femoral pulse could be felt, but was too weak to be counted, in the remaining 13 the pulse was weak, frequently thready, the rate varying from 76 to 146. Only 2 patients had received some intravenous fluids prior to admission; to 3 others some unidentified tablets or powders had been administered.

Since cholera produces dehydration and acidosis concomitantly, it was necessary to vary the protocol so that the influence of each could be identified. The fluid balance of each patient was estimated by observation of intake and output from the time of admission to any given time during the procedure.

Four patients were studied immediately upon admission in the dehydrated, acidotic state prior to any therapy. After the initial measurements were made, 2 were hydrated with solutions of isotonic saline and 2 with solutions of isotonic alkali administered intravenously. Three additional patients were treated with intravenous solutions containing alkali until an optimal state of hydration and acid-base balance was felt to have been achieved. Over the next 8 to 9 hours they were allowed to dehydrate through purging, while blood pH was maintained by the intravenous administration of alkali. Initial measurements were then made and were repeated after further dehydration and, in 2, subsequent rehydration, the blood pH being maintained at essentially the same level throughout the study. Fourteen patients were studied after the administration of isotonic saline had corrected in whole or in part their dehydrated state. Following initial measurements, one patient received further infusions of saline before measurements were repeated; 3 received further infusions of saline, followed by infusions of alkali with measurements being made in each infusion period; and in 7 observations were repeated during alkalinization.

The alkalinizing solutions used were variable in their content and consisted of the following:

1) 12 grams of $NaHCO_3$ in 1000 ml water.
2) 10 grams of $NaHCO_3$, 2 grams KClin 1000 ml water.
3) 5 grams of NaCl, 4 grams $NaHCO_3$, 1 gram KCl in 1000 ml water.
4) 6 grams NaCl,4 grams $NaHCO_3$ in 1000 ml water.

All patients were studied fasting and without sedation. Nine patients had received no food for at least 12 hours, while the remainder gave a history suggesting abstinence for over 2 to 12 hours. The rectal temperature at time of study varied from 96° F, to 100.4° F, only 2 patients had over 100°, F. Although the patients were weighed prior to study, the weight at time of discharge was used in estimation of body surface area.

The patients were brought by stretcher to the fluroscopic room and transferred to a cholera cot which replaced the top of a horizontal fluoroscope. This arrangement permitted effortless collection of the often copious stool without contamination of the catheterization field or disruption of the various procedures. A 12 lead electrocardiogram was then taken. Cardiac catheterization was carried out in the usual fashion through an antecubital vein. The size of the patients' veins precluded using a Cournand catheter greater than #7. A brachial or infrequently a femoral artery was cannulated with a Cournand needle. While there was no difficulty in entering these vessels, the length of the needle prevented complete threading in many subjects.

Measurements were made of pulmonary artery, pulmonary "wedge", right ventricular, right atrial, and systemic arterial blood pressure by means of Statham gage pressure transducers and were recorded by an oscillographic technique (Model IR-4 monitor with accompanying 4 channel camera, Electronics for Medicine, White Plains, New York). Cardiac output was measured by the direct Fick principle or by the dye dilution method using T-1824, or both, using techniques previously described from the Cardiopulmonary Laboratory at Bellevue (4,5). Plasma concentration of T-1824 was measured with a Unicam-500 Spectrophotometer. Expired air was collected in neoprene balloons from which 30 ml aliquots were immediately drawn for analysis. The remaining volume was then measured in a gasometer (CD-1 meter, Parkinson Cowan, Industrial Products, London, England). This ventilometer had been previously calibrated against a Tissot spirometer. The patients' lack of familiarity with any type of scientific equipment made utilization of gas collecting equipment, notably mouth piece and noseclip, difficult. Furthermore, female patients had nose jewelery which prevented the use of a noseclip. For these reasons, in a few instances systemic blood flow was measured solely by the dye dilution technique. The initial estimate of plasma and total blood volume was made from the plasma concentration of T-1824 10 minutes after injection and the hematocrit. Subsequent calculations of these volumes were based on the change in plasma protein concentration, as compared with the concentation of the time of the control determination, and the hematocrit according to the considerations of Noble and Gregerson (6) and Phillips (7). Arterial and mixed venous blood pH were determined on a model 33B Vibron Electrometer pH Meter (Electronic Instruments LTD., Richmond-Surrey, England). Plasma proteins were measured by means of a Bausch and Lomb Serum Protein Meter.

After the initial measurements were secured, the patients were allowed to purge, or were hydrated, or alkalinized as described previously. The electrocardiogram, and hence the heart rate, were constantly monitored. Blood pressures in the systemic and pulmonary circulations were recorded every 10 to 15 minutes. Plasma proteins and pH of the arterial blood were measured frequently to provide a guide as to the state of the patient. Measurements of cardiac output and total blood volume were repeated at the end of each period and before a new state was initiated.

One patient became sufficiently restless after the initial deterination to preclude completion of the study. In one other, the calculations of cardiac output by the Fick and dye methods, after the preliminary measurements had been made, were so divergent as to warrant exclusion of these data from further consideration.

Results

The results will be presented in two sections. The findings in the initial period will be described irrespective of the state of hydration or level of blood hydrogen ion concentration, followed by presentation of data secured in subsequent periods when dehydration or hydration had been furthered or acidosis ameliorated.

Initial Period

The arterial blood pH varied from 6.98 to 7.35 pH units in the 20 patients who had not received alkali prior to study. In the remaining 3, it varied from 7.36 to 7.49 units. In both the initial and subsequent periods, the pH of mixed venous blood was on the average 0.02 pH units lower than that in the arterial blood, the range was 0 to 0.05. The variation between mixed venous and arterial blood pH was no greater at high levels of hydrogen ion concentration than at low levels.

The total blood volume varied widely from low to above normal, 1576 to 3563 ml/m^2 BSA. The values of Samet and others have been utilized to estimate the normal range (7). There was a significant correlation between the fluid balance (net intake minus net output since admission) and the total blood volume, $r = 0.565$, p 0.005. The lowest blood volumes were encountered in the untreated subjects and those allowed to dehydrate .

Cardiac output showed a wide range, 1.61 to 6.33 L/min./m^2 BSA, the lowest values being found on those who were untreated or allowed to dehydrate. The cardiac output appeared to vary as did the total blood volume, the correlation between these two variables was high, $r = 0.761$, p 0.001. Female patients demonstrated a higher blood flow per unit of blood volume than did males. The significance of this difference is yet to be tested. It was also found that the higher the hydrogen ion concentration of the blood, the higher the cardiac output, $r = 0.511$, p 0.02. No significant relationship could be demonstrated between the stroke volume and blood pH. Preliminary calculations suggest that a simultaneous consideration of total blood volume and hydrogen ion concentration will give a better prediction of cardiac output than either of the two former variables above, $r = 0.830$, p 0.001. This remains to be proven.

The pulmonary artery systolic, diastolic, and mean pressures varied from very low to moderatley elevated, 10/4,6 to 42/18,26, again the lowest being obtained in those individuals who were untreated or allowed to dehydrate. The "wedge" pressure similiarly varied from 0 to 18 mm Hg. An unusual finding was that the pulmonary artery mean pressure varied in the initial period as did the cardiac output; $r = 0.744$, p 0.001. The stroke volume, however, did not show a correlation with the mean pressure. It was also of note that the higher the blood hydrogen ion concentration, the higher the mean pressure in the pulmonary artery, $r = 0.561$, p 0.01. A consideration of pulmonary artery mean pressure as a function of both blood flow and hydrogen ion concentration yields a multiple correlation coefficient of 0.767, p 0.001. The total blood volume had no direct influence on this pressure, as a function of both blood flow and hydrogen ion concentration yields a multiple correlation coefficient of 0.767, p 0.001. The total blood volume had no direct influence on this pressure, $r = 0.460$, p 0.05, although the central blood volume may prove to be related to it.

The range of heart rate was wide, 60 to 155 beats per minute. Only the total blood volume was found to have any correlation with the heart rate, the lower the blood volume, the higher the rate, $r = -0.683$, p 0.001. Apparently neither the hydrogen ion concentration nor the cardiac output were influencing this variable.

Pulmonary vascular resistance ($R = \frac{\overline{PA}_m - \overline{PA}_w}{CO} \times 1332$ dynes cm.$^{-5}$)

did not vary with the hydrogen ion concentration but showed some relationship to the total blood volume, $r = 0.570$, p 0.01.

Changed State

Those patients who were allowed to dehydrate while alkali was being infused showed a slight fall in blood pH. The hydrogen ion concentration of those receiving isotonic saline remained virtually constant or rose slightly. On the other hand, those who received alkalinizing solution had an average rise on pH of 0.23, with a range of 0.10 to 0.43 pH units.

Changes in total blood volume were apparently more influenced by changes in fluid balance, $r = 0.685$, p 0.001, than by the amount of fluid infused, $r = 0.477$, p 0.01. As the net fluid balance rose or fell, so did the total blood volume.

The cardiac output rose strikingly in the patients who had previously received no therapy prior to study, in the patients who were allowed to dehydrate and then were rehydrated without change in blood pH, and in all who were further hydrated with isotonic saline. Those patients who were hydrated with saline and then with alkalinizing solutions, or those who were hydrated primarily with the latter showed an initial increment in blood flow, to be followed by a decline as the blood pH rose toward normal. Hence, it would appear that, although a close relationship exists between cardiac output and total blood volume ($r = 0.656$, p 0.001) and blood hydrogen ion concentration ($r = 0.628$, p 0.001), a better expression of the influence of these 2 variables on blood flow may be given by an equation which considers all 3 parameters simultaneously ($r = 0.795$, p 0.001). In this manner, it is possible that not only will be actual level of blood flow be estimated, but also the magnitude and direction of change.

Pulmonary artery mean pressure showed a close correlation with both blood flow and hydrogen ion concentration, much as in the initial period, $r = 0.820$, p 0.001, and $r = 0.447$, p 0.01 respectively. Further analysis may reveal the relative contribution of a change in each of these parameters in the regulation of pressure in the changing state.

The pulmonary artery " wedge" pressure also rose as hydration was carried out; in some patients this reached abnormal levels. With the correction of the acidosis these elevated pressures fell.

In the initial period only the total blood volume was shown to have some relationship to the heart rate. In subsequent periods, increases or decreases in rate could not be correlated with any other hemodynamic factor analyzed to date. However, some trends seem apparent. In the 12 studies in which the cardiac output rose, heart rate fell in 8 and rose in 4. In the 18 observations in which blood flow decreased, rate rose in 9 and fell in 9. In those patients, in whom

hydrogen ion concentration either rose slightly or did not change, heart rate rose 5 out of 6 times. In 22 studies in which blood pH fell, 15 showed a slowing of rate and 7 an increase.

Discussion

This early report on the studies in patients with cholera in the Pakistan-SEATO Cholera Research Laboratory in Dacca during the months of January, February, and March of 1965 will give some indication of the type of hemodynamic data secured. Since a large bulk of material has not yet been analyzed, final conclusions are not warranted. Furthermore, it is obvious that many of the data have not yet been commented upon even briefly, e.g., systemic blood pressure, systemic and pulmonary vascular resistances, central venous pressure, central blood volume, and the electrocardiogram.

It is, however, not too early to thank the various members, past and present of the staff of the Pakistan-SEATO CRL: Doctors Robert Gordon, Abram S. Benenson, John Lindenbaum, and Mrs. Doris Parkinson and of the Cardiopulmonary Laboratory of the Columbia Medical Division of Bellevue Hospital: Doctors M. Irene Ferrer, Harry W. Fritts, Jr., and Miss Madeline A. Bohman whose combined interest, knowledge, preserverance, and industry made it possible to undertake this study.

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STUDIES ON CHOLERA INFECTION IN THE RHESUS MONKEY

by Abram S. Benenson, S. Ahmed.

Studies on the monkey have continued with the hope that the riddle of disease initiation could be solved; particular stress was paid toward the part that might be played by the toxin reported last year br Dr. Craig. This was tested by introducing bacterial-free filtrate into the intestinal tract, as well as by growing bacterial challenges in 5% peptone in Kolle flasks, the condition in which maximal toxin production occurs.

Both Ogawa and Inaba strains were used for the production of toxin. It proved to be feasible to pass a polyethylene tube through the nose of the monkey and hold him in the monkey chair for prolonged challenge and observation. When alkaline bile-stained fluid was recovered, suggesting that the tube had passed the pylorus, an initial volume of 200 ml. of filtrate was introduced and then a drip at a rate of 6 to 13 drops per minute was maintained, until the total dose had been given. Four monkeys received 200 to 540 ml. of filtrates (with toxin content to produce 5 to 8 mm. indurated and blueing lesions in the rabbit skin), but there was no evident effect on the monkey beyond the passage of up to 85 ml. of green coloured liquid stool (monkeys were on rabbit pellets) during the period that the toxin was being administered. To retard gut motility, tincture of opium was given to two monkeys in a volume of 1.5 ml. and two others in 7 ml. 43 minutes to 3 hours before toxin introduction was started. Again there was rectal passage of liquid green fecal material but in a volume less than half of that introduced orally. Thus we failed to establish the cholera syndrome in the monkey by the administration of filtrates to eight monkeys, four with and four without pre-treatment of tincture of opium. In three of these four monkeys, the pH ofthe aspirates before toxin was given was between 7 and 9 (test paper method) and in one was frmkly bile. The pH of the aspirates from the fourth monkey was 6.

Previous studies had suggested that monkeys more frequently succumbed to infection when the challenge consisted of several repeated introductions of vibrios. Accordingly two monkeys were challenged every hour for six doses with 25 ml. of a 5% peptone water culture of a locally isolated Ogawa strain, totalling 9.6×10^{10} vibrios, with no adverse effect. Two other monkeys were challenged with 50 ml. daily for six days to a total dose of 1.8 x

10^{11} Inaba organisms, again with no adverse effect. Four monkeys were challenged with 3-50 ml. doses at an 8 hour interval, totalling 2.3×10^{11} organisms, with no symptoms supervening. On the basis that the bacterial strain might be important, V cholerae (Ogawa Ike), the strain which had produced the disease in the second Walter Reed patient, was procured and this too failed to produce any effect in 16 monkeys other than the usual asymptomatic passage of vibrios for up to 10 days after challenge, even after doses as large as 3.5×10^{12} organisms were given in 50 ml. doses every 8 hours two to four times.

Two monkeys (154 & 155), receiving 3.5×10^{12} organisms (Ogawa Ike) in two doses, appeared ill (inactive and refused food) the day after challenge but were normal on the third day. They were bacteriologically positive for only 2 and 4 days; on the 7th day one monkey again appeared sick and was sacrificed – no vibrios were recovered at autopsy. The organisms were no more effective when grown in T_1N_1, which had been used last year, instead of 5% peptone water.

Based on earlier negative results on the effect of diet, our monkeys had been placed on a full diet including rice, dahl, fish and supplementary vitamins, or on rabbit pellets imported from the U.S.A., supplemented with vitamin C. However, as a last try, a group of monkeys was placed on a rice only diet, some supplemented with ascorbic acid and others not. In the following three months, these monkeys lost 27 to 40% of their body weight. Four of these monkeys were put in a protocol for four doses of 50 ml. Ogawa Ike of 8 hours. After the first two doses totalling 1.1×10^{11} organisms, one monkey was dead and another moribund. Both of these monkeys had been receiving ascorbic acid in addition to rice. The other two monkeys survived all four doses, receiving a total of 2.1×10^{11} organisms but both did poorly and died 21 days later. One, whose rectal swabs had been negative for 9 days, was found on autopsy to be vibrio positive from the stomach through the colon, but negative in the rectum; no vibrios were recovered from the other. One other starved monkey was exposed to 9.3×10^{10} vibrios; this monkey died on the 15th. day and on autopsy after 13 days of consecutive negative rectal swabs, vibrios were recovered only from the duodenum and in small number. To check whether a change in organism or culture conditions was responsible, three monkeys on the current adequate diet of rice, dahl, fish and vitamin C (rabbit pellets had been shunted off to Ethiopia) were exposed to 1.9×10^{11} vibrios given in four divided doses 8 hourly, resulting only in stool positively for 6 to 7 days. These monkeys were sacrificed on the 15th and 27th day: no vibrios were recovered from the two who were autopsied bacteriologically. Over the course of these studies, bacteriological autopsies were performed on five monkeys sacrificed on days 3,4,6,6, and 6 for quantitative bacteriology (only one positive); one 155 was sacrificed on day 7 (see above); seven sacrificed for space and ten who died, two of pneumococcal pneumonia and the other with a bloody stool, but free of shigella, salmonella or parasites. Seven of the

23 were bacteriologically positive,(Table 1), the duodenum being vibrio positive in every instance. The liver was positive only once; this occurred in a monkey who died incidental to the challenge; the stomach and duodenum were positive, the bile negative and the remainder of the intestinal tract negative. Infected bile was not found in any of these monkeys. Two of three animals whose intestinal tracts were positive after relatively long periods of negative rectal swabs, were from the starved group.

Electrophoretic studies of sera taken just prior to challenge disclosed no difference between the rice fed monkeys and their well fed brothers in albumen, alpha 1, alpha 2, beta and gamma globulins, total protein or A/G ratios.

Agglutination and vibriocidal antibodies have been studied on 14 monkeys. Vibriocidal antibodies were present at the time of challenge in 8 of these monkeys. No relationship could be established between the presence or absence of antibodies and the duration of positive rectal swabs. After challenge, the two monkeys who developed cholera and died were found to have low level (1:10 and 1:20) vibriocidal antibody and no agglutinating antibody at the time of challenge. Comparision of the serum electrophoresis analyses of these two groups revealed a clear cut difference in beta-globulins; the mean value of the antibody positive group was 0.88 grams in contrast to 0.65 grams for the vibriocidal negative group. In the former, all seven values but one fell between 0.84 and 1.14 with the aberrant value of 0.60; the four in the other group fell between 0.59 and 0.69 grams.

Repeated liver biopsies 24 - 120 hours after challenge were performed on 8 monkeys; none were positive.

Two monkeys are of particular interest:

Monkey #138 was given 540 ml. filtrate prepared with an Inaba organism, on 26th March. This filtrate was contaminated with 5 organisms per ml., a total dose of approximately 2.5×10^3 Inaba vibrios; on the following day rectal swab was positive for Inaba. On 28th March, 50 ml. of 5% peptone broth containing 3.9×10^{10} Ogawa vibrios was administered. Rectal swabs were positive for both organisms daily thereafter. On sacrifice on 1st April, 10^5 organisms were isolated from the stomach and small intestine in counts ranging from 5×10^2 to 4×10^5 per ml; both Inaba and Ogawa strains were present but Inaba predominated. The count in the caecum was 3.5×10^4 ml; all types being Inaba. Cultures below this were negative for vibrios, even on enrichment.

Monkey #156 was challenged four times over a 2 day period with 50ml. of 5% peptone broth contaminated with 1.5×10^{12} vibrios Ogawa Ike. This strain had been isolated on the second Washington case (Ike). So far as is known the first Washington case had only been exposed to an Inaba strain but an Ogawa isolate strain was obtained as the third positive culture and "Ike" became infected working with this strain..The monkey challenged produced no evidence of disease

but the rectal swabs were positive for Ogawa until the fifth day, then from the 7th to the 12th day, all isolates were Inaba. Neither agglutinating or vibriocidal antibodies were present at a 1 to 10 level at the time of challenge; by the 12th day agglutinating antibodies were present to a titer 1 to 10 and to a titer of 160 in the vibriocidal test against both Inaba and Ogawa strains.

Further studies will test whether the rice diet and starvation is the key to the problem, using a new supply of monkeys in numbers adequate to answer the questions and perhaps evaluate effect and specific supplementation.

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TABLE I

BACTERIOLOGICAL TABLE AND FINDINGS ON MONKEY AUTOPSIES

Monkey No.	Days after challenge	Days R/S +	Days R/S -	Died or Sacrif.	Liver	Bile	Stomach	Duod	Jejunum	Ileum	Caecum	Colon	Rectum
133	0(2d)	0	0	D	+	-	++++	+++	-	-	-	-	-
144*	1	0	0	D	-	-	+	+	+	+	++	+++	+
143*	2	1	0	D	-	-	2.5×10^4 +	++	++	+	++	++	2.9×10^8 +++
138	4	4	0	S	-	-	4×10^4 +	5×10^2 +	4×10^5 +	5×10^4 +	3.5×10^4 +	+	+
147*	15	2	13	D	-	-	-	±	-	-	-	-	-
150*	21	12	9	D	-	-	±	±	±	+	±	±	-
153	(45) 34	4 4	32	D	- 1/7	- 0	+ 6	+ 7	+ 5	- 4	- 4	- 4	- 3
139	3	0	3	S	-	-	-	-	-	-	-	-	-
140	6	2	4	S	-	-	-	-	-	-	-	-	-
141	6	1	5	S	-	-	-	-	-	-	-	-	-
142	6	2	4	S	-	-	-	-	-	-	-	-	-
132	7	1	6	S	-	-	-	-	-	-	-	-	-
155	7	2	5	S	-	-	-	-	-	-	-	-	-
154	9	4	5	S	-	-	-	-	-	-	-	-	-
159	12	6	6	S	-	-	-	-	-	-	-	-	-
149*	21	8	13	D	-	-	-	-	-	-	-	-	-
160	27 (38)	7 3	15	S	-	-	-	-	-	-	-	-	-
137	28	1	27	D	-	-	-	-	-	-	-	-	-
157	34	9	18	D	-	-	-	-	-	-	-	-	-
156	43	10	31	D	-	-	-	-	-	-	-	-	-
131	53	2	50	S	-	-	-	-	-	-	-	-	-
134	59	9	50	S	-	-	-	-	-	-	-	-	-
135	101	8	93	S	-	-	-	-	-	-	-	-	-

SEROLOGICAL TECHNIQUES

By : Abram S. Benenson, Anisa Saad & technical assistance of Manik Paul & Mrs. S. Pashi.

a. Vibrio Agglutination Test :

Studies were continued to compare results obtained in testing routine sera in microtiter system with those in the standard tube test. In only 8 of 526 (1.5%) did the value differ by more than two fold (Table I). In over 70% of determinations, identical values were obtained. The titer rise in 149 positive pairs of sera was identical in both systems (Table II), the rise in titer differed by 1 tube only in all others except one pair with an 8 tube rise in the tube test but a 6 tube rise in the microtiter system. This degree of agreement, combined with the simplicity, rapidity and large number of sera that can be processed with this system, justified the elimination of the tube titer method.

The use of finger tip blood was evaluated by taking finger tip samples from patients on the same day that venous blood was obtained. The results (Table III) show the same degree of agreement noted above the serum tube titers tend to be one tube higher than that obtained with finger tip blood; a finding consonant with the volume of the original finger tip sample occupied by red cells.

Accordingly the finger tip technique has been standardized for use in the field or in small children in whom venipuncture is difficult or undesirable. 50 lambda Drummond "microcap" capillary tubes are used to add .050 ml of blood to 0.450 ml of saline; on arrival in the laboratory this is centrifuged and the 1:110 blood dilution is stored in the deep freeze if not immediately examined.

The only problems encountered with the microtiter technique have been the necessity for very thorough clearsing of the plates to remove all traces of detergent for disinfectant an difficulty in reading when plates begin to get scratched. The solution to both of these problems seemed to lie in the use of disposable plates. However.....

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the agreement in readings between the disposable plate and the fixed plate was relatively poor (Table IV). It was difficult to read reactions in the disposable plates grossly, but they could be read satisfactorily in the stereoscopic microscope, but this slows up the technique appreciably. Rises in titer seemed to be comparable with those in the fixed plates. Therefore, disposable plates have not proven to be satisfactory and they are only used to accomodate an excessive number of clinical pairs.

Reproducibility of the results has proven to be good. On each test run, a control serum is included. The mean agglutinating titer obtained in 72 tests done on this serum with Ogawa antigen was 8.8 tubes, with a standared deviation of 0.07. In 65 tests with the Inaba antigen, the mean value was 8.0, with a standard deviation of 0.08.

TABLE I

COMPARISON OF MICROTITER AGGLUTINATION WITH TUBE AGGLUTINATION TITERS ON ROUTINE CLINICAL SERUM SAMPLES

Tubes Positive	0	1	2	3	4	5	6	7	8	9
9	-	-	-	-	-	-	-	-	-	1
8	-	-	-	-	-	-	1	-	1	-
7	-	-	-	-	-	-	2	5	-	-
6	-	-	-	-	-	5	10	1	-	-
5	-	-	-	1	9	38	3	-	-	-
4	-	-	2	18	36	10	-	-	-	-
3	-	-	12	39	8	-	-	-	-	-
2	1	5	47	8	-	-	-	-	-	-
1	11	41	15	-	-	-	-	-	-	-
0	157	36	3	-	-	-	-	-	-	-

Microtiter Cups Positive
(1 = 1:40 dilution)

TABLE II

COMPARISON OF MAGNITUDE OF TITER RISE IN MICROTITER AGGLUTINATION AND TUBE AGGLUTINATION TESTS

Number of Tubes of Titer Rise	0	1	2	3	4	5	6	7	8	9
9	-	-	-	-	-	-	-	-	-	1
8	-	-	-	-	-	-	1	-	1	-
7	-	-	-	-	-	-	2	5	-	-
6	-	-	-	-	-	2	7	1	-	-
5	-	-	-	-	4	21	2	-	-	-
4	-	-	-	6	24	2	-	-	-	-
3	-	-	7	22	6	-	-	-	-	-
2	-	3	25	3	-	-	-	-	-	-
1	-	1	3	-	-	-	-	-	-	-
0	-	-	-	-	-	-	-	-	-	-

Rise in titer in Microtiter cups

TABLE III

AGGLUTINATION TITERS OF FINGER TIP BLOOD IN MICROTITER SYSTEM AND SERUM IN TEST TUBES

Tubes Positive with Serum	0	1	2	3	4	5	6	7	8	9
9	-	-	-	-	-	-	-	-	1	-
8	-	-	-	-	-	-	-	-	1	-
7	-	-	-	-	-	-	4	-	-	-
6	-	-	-	-	-	5	1	-	-	-
5	-	-	-	1	12	6	-	-	-	-
4	-	-	-	18	6	-	-	-	-	-
3	-	1	11	12	-	-	-	-	-	-
2	-	5	16	-	-	-	-	-	-	-
1	6	9	1	-	-	-	-	-	-	-
0	136	12	-	-	-	-	-	-	-	-

Microtiter Cups Positive with saline dilutions of fingertip blood
(1 = 1:40 dilution)

TABLE IV

VIBRIO AGGLUTINATION TITERS OF SERA SIMULTANEOUSLY TESTED IN DISPOSABLE AND NON-DISPOSABLE MICROTITER PLATES

Cups Positive in non-disposable Microtiter Plates	0	1	2	3	4	5	6	7	8	9	10	11	12
12	-	-	-	-	-	-	-	-	-	-	1	1	2
11	-	-	-	-	-	-	-	-	-	-	-	2	-
10	-	-	-	-	-	-	-	-	-	-	3	1	3
9	-	-	-	-	-	-	-	-	1	9	2	1	-
8	-	-	-	-	-	-	-	1	3	3	1	-	-
7	-	-	-	-	-	-	-	-	3	1	-	-	-
6	-	-	-	2	-	-	2	-	-	-	-	-	-
5	-	-	-	-	1	3	4	-	-	-	-	-	-
4	-	-	-	2	12	2	-	-	-	-	-	-	-
3	-	-	-	17	9	-	-	-	-	-	-	-	-
2	-	-	6	7	1	-	-	-	-	-	-	-	-
1	1	1	7	1	-	-	-	-	-	-	-	-	-
0	86	14	6	-	-	-	-	-	-	-	-	-	-

Cups Positive in disposable Microtiter Plates

C. Toxin Neutralisation Test

(with Miss Anisa Saad, Dr.Ansaruddin Ahmed, Abdullah Al-Mahmood & Ahmad Rashid)

The toxin neutralisation test is done essentially as described by Craig in the Technical Committee report for last year. Threefold dilutions of serum are incubated at 37°C for 30 mm with an equal volume of toxin at twice the dilution which produces an 8 mm area of induration on the skin of a guinea pig. 0.1 ml. is injected into the clipped skin on the gridded back of an albino rabbit, or the depilated skin of a guinea pig. The injection sites are randomized but each serum is tested in the front and the rear. After 18 hours, the area of induration at each site is recorded in mm, and 0.5% pontamine blue is injected at a dose of level of 1.2 ml per kg. (maximal guinea pig dose of 0.72 ml). After 1-1½ hours, the intensity of blueing at the site of injection is recorded as + to ++++, and the area of bluing recorded in millimeters. On each rabbit, control injection of toxin are made and a positive neutralization is manifested by an average area of bluing (and induration) less than half size of the control sites. Approximately 90 sites can be used on an adult rabbit, and about 25 on a guinea pig.

389 of sera taken on admission from 88 patients exhibited no toxin neutralizing power at a 1:10 dilution; 26% were effective only at the 1:10 level. In convalescence from proven cholera, only one of 111 patients failed to neutralize toxin at the 1:10 level and 4 had a titer of 1:10.

Toxin was prepared from both Ogawa and Inaba strains; similar results were obtained regardless of the serotype infecting the patient. Animals cannot be reused; blueness persists for months, and antibodies are developed. If such an animal is reused, bluing tends to be vague, and in an occasional animal bluing does not appear.

The procedure can be carried out on finger tip blood samples, but the number of sera that can be handled is limited.

SEROLOGICAL TECHNIQUES

b. Vibriocidal Antibody Determination:

An attempt to establish the vibriocidal technique described by Finkelstein, which is based on the reduction in number of colonies growing on agar plates, failed because of difficulty in getting consistent bacterial counts in the working dilution of bacteria, probably due to failure to dry the agar plates consistently.

Accordingly it was felt that a tube technique would be necessary. The method used by Feeley, a modification of the original bactericidal antibody method of Muschel and Treffers, was set up and found to function satisfactorily. However, the number of sera that could be run per day was disappointingly small. Therefore the attempt was made to adapt this procedure to the microtiter system. Several doubling dilutions of the sera were made in the plates at 0.025 ml volume. Bacterial antigen (saline suspension of a 4 hour growth on heart infusion slants adjusted to density of one opacity unit) is mixed with an equal volume of 1:5 dilution of guinea pig complement and 0.025 ml added to each cup with the micropipette. After 1 hour incubation at 37°C, 0.15 ml Bacto heart infusion broth is added to each cup and the plates are re-incubated for another 2 - 4 hours (the time is determined by the interval required for control tubes of 0.5 ml saline and 0.5 ml antigen-complement mixture plus 3.0 ml heart infusion broth to reach an optical transmittance of 60-70%). A standard serum is included in each test, and the serum is tested for anti-complementary by adding 0.025 ml antigen -complement mixture to an equal volume of 1:5 serum, incubating for 1 hour and then adding 0.025 ml sensitized sheep cells. If the cells are not hemolyzed after 30' incubation, serial dilutions are tested to titrate the degree of anticomplementary activity. At this time a clear distinction in turbidity is evident in those cups in which the vibrios grew and those in which they were killed. Essentially similar quantitative readings were obtained after overnight refrigeration, but the reading is facilitated by the presence of buttons of sedimented organisms as well as turbidity, clarifying unclear end points. No complication was introduced by the use of non sterile plates; the growth of contaminants, on the rare occasions when they did appear, looks different after overnight than does the growth of vibrios.

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Initially serum dilutions were started at 1:80 level; with it was found that not infrequently there was no evidence of vibriocidal activity in 1:10 dilutions of serum. This was confirmed by experience; in 811 tests of the routine admission sera of 406 patients, 29.2% were found to be negative throughout the dilution series, and only 1% were vibriocidal at the 1:1280 dilution, i.e., across the entire row of 8 cups. On the other hand, among 305 tests from 153 convalescent cholera patients, 43% were vibriocidal across the entire row. For diagnostic purposes, the routine test revealed a significant rise in all but a very few cases which required retesting at higher dilutions.

The test system can handle large numbers of sera, and gives comparable results on finger tip blood. The reproduceability is high; in 76 routine tests, the mean anti Ogawa titer of the reference goat serum was 10.6 tubes, with a standard deviation of 0.11, and the mean anti Inaba titer of 10.4, with a standard deviation of 0.11.

Convalescent sera

Age group	<10	10	20	40	80	160	320	640	1280 or more	No. tested
0-5	6	2	-	8	6	17	11	9	41	64
6-14	3	-	-	-	-	7	14	12	65	58
15+	1	1	-	1	5	12	13	18	48	83

The effect of vaccination is most clearly seen in the 0-5 age group in the admission sera. There is no difference in distribution between the bacteriologically positive and negative groups-

The effect of age is clearly evident in the increasing mediam titer. The titers of the convalescent sera are higher than seen the agglutinating antibody; they tend to be lower in the younger age group.

Toxin Neutralization : The toxin neutralizing antibody appears at about the same time as the others, one case had developed a 9 fold rise by the fifth day. The percentage distribution of titers, based on relatively few patients is :

	Titer								
	10	10	30	90	270	810	2430	2430 or more	No tested
					Admission Sera				
0-5	20	23	26	11	17	-	3	-	35
6-14	26	15	40	11	7	-	-	-	27
15+	45	27	20	4	3	-	-	-	95
					Convalescent Sera				
0-5	4	9	13	9	26	26	13	-	23
6-14	-	-	6	33	39	17	6	-	18
15+	-	4	25	25	23	12	9	4	57

SEROLOGICAL STUDIES

B. Antibody levels in normal Dacca population & response to infection:

Abram S. Benenson, Miss Anisa Saad, Manik Paul
and Mrs. S. Pashi

Serum pairs were examined by two or more of the serological methods in use at CRL on 228 patients infected with V. cholerae, 138 negative by bacteriology and dark field, and 38 with a single positive bacteriological report, either on dark field or culture. A positive test was considered a rise in titer of 2 dilution; i.e. a four fold rise in the agglutination and vibriocidal techniques but a nine fold rise in the toxin neutralization test. In the latter, ± is a three fold rise. Results obtained, including a line for three fold rises in the toxin neut test (excluding changes from less than 1:10 to 1:10) were

	Vibrio Positive		Vibrio Negative	
	No. tested	% Pos.	No. tested	% Pos.
Agglutination:	228	91%	138	2.9
Vibriocidal	228	96%	138	3.6
Toxin neut- +	96	78%	35	2.8
±	96	91%	35	5.7
Any positive	228	96.9	138	3.6

The vibriocidal test is evidently the most sensitive, little improvement in test sensitivity results from adding the other procedures. Only one vibrio positive case was negative on vibriocidal test and positive on agglutination. Four cases failed to develop an vibriocidal antibody rise after the fifth day; all were in the 0-4 age group. Two in this group and two in the 15 or over group showed only a one tube rise. Rises in the vibrio negative group may represent cases missed bacteriologically or received vaccinations - they do not necessarily imply false positivity.

Analysis of the antibody status of patients on admission, and of the response to infection shows considerable effect of age. Only Inaba titers will be considered since this was the strain infecting the vast majority of cases.

(more)

Agglutinating antibody : Significant antibody rises occured by the fourth day in one case, and the fifth day in four. The percentage distribution of anti Inaba titer levels is :

Titer

Age group	< 10	10	20	40	80	160	320	640	1280 or more	Total tested
					Admission sera					
0-5	79	15	4	2	-	-	-	-	-	126
6-14	53	30	9	5	3	-	-	-	-	100
15+	33	34	19	8	5	1	0.5	-	-	254
					Convalescent sera (Vibrio Positive Cases)					
0-5	11	6	13	17	27	11	8	2	6	64
6-14	2	3	3	13	18	11	18	18	13	61
15+	4	2	6	15	11	20	18	11	14	107

The age difference is very evident in the admission sera; among 0-5 convalescent cases, 47% have titers less than 80, while only 21 and 27% sera in the 6-14 and 15+ groups fall in this range.

Prior vaccination does not have any clear effect on the antibody levels, either before or after vibrio infection.

Vibriocidal antibody: In one instance a significant antibody rise occured by the third day; one by the fourth day, and four by the fifth day. Percentage distribution is

Titer

Age group	<10	10	20	40	80	160	320	640	1280 or more	Total tested
					Admission sera					
0-5 No vaccine	76	11	6	4	1	2	-	-	-	102
Vaccine	27	27	23	5	5	5	-	5	5	22
6-14 No vaccine	34	16	23	10	10	3	2	-	2	61
Vaccine	15	15	27	12	21	3	6	-	3	34
15+ No vaccine	11	17	16	23	21	7	3	1	-	107
Vaccine	7	8	14	22	20	13	8	2	3	122

The point of greatest interest is the distribution of admission titers among the younger age group, with high titers not associated with other antibodies. This is in contrast to findings in the older age groups, where the higher titers are associated with high vibriocidal antibody.

Despite the high spectrum of titers seen on admission, this antibody, like the others, begins to fall by the 14th day after onset of disease.

Summary :

Serological methods provides a sensitive and specific means to recognize exposure to vibrio antigens. The vibriocidal technique is most sensitive; the toxin neutralization test may prove to be the most specific for recognition of infection with the living organism but proper analysis is necessary to determine whether a three fold rise includes a significant number of false positive reactions.

SEROLOGICAL STUDIES

Anisa Saad, Sushoma Pashi, Abram S. Benenson, M.D.

Relation of Agglutinating Antibody to Cessation of Symptoms:

Fingertip blood was taken daily (except for weekends) from patients on the CRL Ward who were positive on dark field examination (confirmed by culture) for cholera vibrios, and negative for agglutinating antibodies on admission; 88 were followed over a 7 day period, and 83 over a 10 day period. All bloods collected from a single patient were run in one protocol in the microtiter agglutination test. 58 of these patients were treated with antibiotics (13 on tetracycline; 25 on chloromphenicol; 12 on humatin and 8 on streptomycin).

Agglutinating antibodies were present by the fifth day in almost half, as indicated in Figure I. Ages ranged between 8 months and 62 years. There was no definite evidence that either antibiotic or age had an influence on the time of appearance of antibodies. Two patients failed to develop antibodies by the 10th and 12th day, they were an 8 month old and a 3 year old child treated with tetracycline (with a total dose of 0.20 and 0.19 g/kg total drug). However, a $2\frac{1}{2}$ year old child who received 0.22 g/kg developed antibodies on the 6th day.

The day of antibody appearance in those who did not receive antibiotic was related with the last day the stool was positive for vibrios (Table 1) with the day diarrhea stopped (Table 2), andwith the day the stool volume showed an abrupt drop, a phenomen not infrequently seen in cholera (Table 3). A better correlation is found between the time of appearance of the agglutinating antibody and the drop in stool volume ($r_{24} = .605$), than with the last day of the stool was positive for vibrios ($r_{25} = .492$), or the cessation of diarrhea ($r_{24} = .333$). This would suggest that the circulating antibody may be of more importance in counteracting some pathogenetic factor than it is in eliminating the bacterial source of that factor

However, it must be noted that when the comparison is done between the appearance of antibodies and the duration of diarrhea in eight hour periods, a very highly significant correlation is obtained ($r_{24} = .721$) (Table 4).

Day of Antibody Rise	3	4	5	6	7	8
7				3		
6		1	3	3		1
5		2	5	3		
4	1	2	1	1		

Days of Drop in Stool Volume

TABLE I

Relation of time of appearance of agglutinating antibody and drop in Stool Volume.

r_{24} = .605 p <.005

Day of Antibody Rise	4	5	6	7	8
7			1	2	
6		2	5	1	1
5		3	5	1	1
4	2	2	1	-	-

Day of last vibrio Isolation

TABLE II

Relation of time of appearance of agglutinating antibody and day of Last vibrio isolation from stool

r_{24} = .492 p <0.01

Day of Antibody Rise	3	4	5	6	7	8	9
7					2		1
6		1	2	3	-	1	1
5		-	5	2	2	-	1
4	1	2	1	1	-	-	-

Day Diarrhea stopped

TABLE III

Relation of time of appearance of agglutinating antibody and day diarrhea stopped

r_{24} = .333 p > 0.05

	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
7											2	1					
6	1					1		2	1		1	1					1
5	-					2	1	2	-		3	1					1
4	-	1	2	1	-	1	1	-	-	-	-	-	-	-	-	-	-

8 hour period of diarrhea

TABLE IV

Relation of time of appearance of agglutinating antibody and the duration of diarrhea in eight hour periods

r_{24} = .721 p <.0005

Figure- 1.

TIME OF APPEARANCE OF AGGLUTINATING ANTIBODY IN VIBRIO POSITIVE CHOLERA CASES WITH AND WITHOUT ANTIBIOTIC TREATMENT.

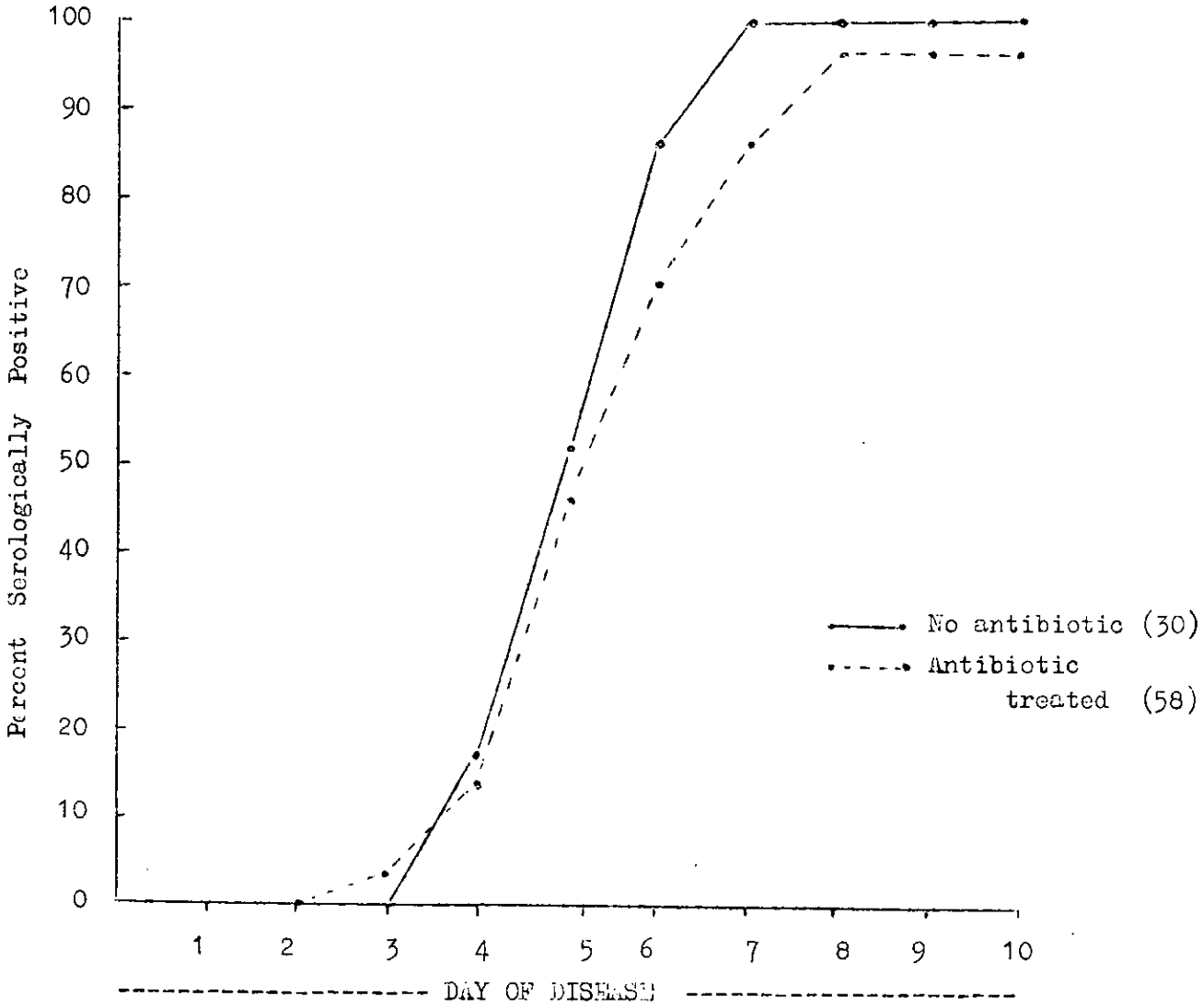

TABLE I

DISTRIBUTION OF 765 CONSECUTIVELY ADMITTED CASES OF DOCUMENTED CHOLERA

Age group	Number of cases	Male:Female Ratio
0 -	14	2.5:1
1 -	30	1:1
2 -	35	1.1:1
3 -	42	1:1
4 -	45	1:1
5 -	43	1:1.7
6 -	34	1.1:1
7 -	34	1:1.3
8 -	32	1.9:1
9 -	14	1:2.5
10 -	77	1.6:1
15 -	53	1.5:1
20 -	262	1.3:1
50 - 80	50	1.9:1

Age	No. of cases/year
0-	33.2
5-	31.2
10-	15.6
15-	10.6
20-	8.8
50-80	1.7

TABLE II

ADMISSION FINDINGS

	Age Group						
	0-	2-	5-	10-	15-	20-	50-70
Number of patients	44	122	157	77	53	262	50
Hours from onset to admission, mean	20.6	21.2	15.9	11.7	18.7	16.5	24.1
Percent pulseless	22.7	44.2	51.6	45.4	49.0	52.2	56.0
Admission plasma protein, g. % mean	8.4	9.3	10.1	10.6	10.8	11.1	10.5
Convalescent plasma protein, g. % mean	6.8	6.9	7.1	7.3	7.6	7.5	7.5
Adm. minus conv.protein, g. %, mean	1.6	2.4	3.0	3.3	3.2	3.6	3.0
Percent comatose on adm.	4.7	13.1	14.0	5.2	3.8	10.7	10.0
Respiratory rate on adm., mean	29.9	29.4	27.9	25.6	25.1	26.3	26.6
Adm. CO_2, mEq/L, mean	13.0	13.4	13.8	14.5	15.5	14.7	13.9
Adm. K. mEq/L, mean	4.2	4.1	4.3	4.5	4.3	4.6	4.6

TABLE III

INCIDENCE OF FEVER AND HYPOTHERMIA

	Age Group 0-	2-	5-	10-	15-	20-	50-70
Percent with admission temp. 97° or less	30.2	40.1	42.0	53.2	36.5	41.3	50.0
Percent with admission temp. 100° or more	20.9	13.9	12.1	11.6	9.4	11.8	8.0
Percent with temp. 100° or more after admission	23.2	31.1	26.1	24.6	17.0	35.9	36.0

TABLE IV

COMPLICATIONS AND REQUIREMENT FOR INTRAVENOUS FLUIDS

	Age group 0-	2-	5-	10-	15-	20-	50-70
Percent with prolonged drowsiness	11.9	10.7	10.2	1.3	1.9	2.7	2.0
Percent with oliguria 24 hours or more	2.3	3.3	6.3	10.0	15.3	14.2	16.0
Percent requiring intravenous fluids	70.4	91.8	98.0	96.1	96.1	98.0	98.0
Percent mortality*	0	0.8	0	0	0	0.8	2.0

* Mortality for entire series: 4 deaths in 765 cases, or 0.52%.

To be discussed: tetany, hypoglycemia, pneumonia.

TABLE V

DURATION OF ILLNESS AND RELATED FACTORS

	Duration Diarrhea, days		Stool volume, cc/kg		Intraven. fluids, cc/kg		Days bacteriol. positive	
	Contr.	Antib.	Contr.	Antib.	Contr.	Antib.	Contr.	Antib.
0-	2.9	2.4	407	400	537	477	4.2	3.3
5-	3.3	2.2	513	322	577	450	5.3	2.7
10-	3.5	1.8	446	178	513	263	5.1	2.3
15-	3.0	2.4	355	201	397	288	5.0	3.1
20-	4.0	2.6	477	258	578	355	5.2	3.0
50-80	4.4	2.6	516	236	680	318	7.7	3.0

Contr. = no antibiotic received.

Antib. = received tetracycline, chloromycetin, streptomycin or paromomycin.

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SEVERE ACIDOSIS AS A CAUSE OF HEART FAILURE IN CHOLERA

by: W.B. Greenough, III, M.D., K.M. Ally, M.B.B.S.,
Robert S. Gordon, Jr., M.D., N. Hirschhorn, M.D.,
& John Lindenbaum, M.D.

Introduction:

The clinical results of severe acidosis are ordinarily difficult to separate from the effects of the underlying disease which has produced the acidosis. Therefore the significance of severe acidosis as such, and the urgency with which it must be corrected is seldom appareciated. During recent years there has been an increasing interest in defining the effects and biological significance of acidosis. In experimental animals there is no information available regarding severe acidosis sustained for more than a few hours. Experiments have generally been of short duration, or the acidosis has not been very severe. In man, acidosis of the degree and duration seen in association with many clinical states has not bee, and cannot properly be achieved in experimental situations using normal volunteers. There is a need, then, to seek both experimental and clinical situations which will enable us to define better the consequences of acidosis as such.

The cholera patient provides an unusual opportunity to observe the specific effects of low pH in the blood and body fluids. The metabolic acidosis of cholera originates as a result of the loss of large amounts of bicarbonate ion in the stool (1). This acidosis may be effectively corrected by the rapid infusion of sodium bicarbonate (2). All cholera patients with clinically significant cholera become acidotic. The degree of acidosis depends on a variety of factors, the most important of which initially is the amount of bicarbonate lost in the stool. If a patient is then treated with infusions of isotonic saline alone, his diarrhea may continue and his total loss of bicarbonate increase. If poor circulation has been sufficiently prolonged, oliguria or anuria can ensue. This state permits accumulation of metabolic acids and aggravates the existing acidosis due to loss of base. Finally, prolonged circulatory insufficiency will result in an acidosis manily due to the accumulation of lactic acid (3).

In the severest state of acidosis frank congestive heart failure ensues, refractory to digitalis therapy and responding only to large quantities of intravenous dodium bicarbonate. It is this syndrome in particular that we wish to describe in five patients with cholera. The five case studies following are presented chronologically and show how our understanding of the syndrome of acidosis and its treatment evolved.

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Case Reports:

Case I - CRL # 26.

A.M. a 22 year old laborer, was apparently well until he was attacked by cholera. He was admitted to Mitford Hospital, unconscious, after 12 hours of illness and trasferred to CRL three days later. At the time of transfer he was disoriented, restless and moderately dehydrated, with a blood pressure of 108/50. He was severely hyperpneic, but not in obvious heart failure. The plasma protein concentration was 9.0 gram %. V. cholerae, type Inaba, was found in stool culture. During three days at CRL, he received 14.5 liters of intravenous fluid, containing a total of 290 mEq base. Approximately the same number of mEq of sodium bicarbonate were given by mouth. His hydration was adequate as judged clinically and by serum protein concentration. However, he developed a cough, increasing pulmonary rales, and died in pulmonary edema which failed to respond to digitalization, phlebotomy, diuretics and tourniquets.

Acidosis had been inadequately treated, as shown by a fall of plasma CO_2 content from 9.6 mEq/L on admission to 5.7 mEq/L at death, and a corresponding rise in chlorides from 115 mEq/L to 132 mEq/L. No autopsy was done.

Case II - CRL # 95.

D.M., a 40 year old farmer, was admitted directly to the CRL ward three days after the onset of cholera. His clinical picture was typical of the algid stage of the disease. He was conscious, but restless and disoriented. His pulse was feeble, his heart sounds almost inaudible, and there were moist rales and rhonchi in the lungs. Hyperpnea was marked. Initial infusion of only one liter of saline was followed by an alarming increase in pulmonary rales, although dehydration and hypotension had not been corrected. A slow infusion of isotonic sodium bicarbonate was started, but four hours later, after 350 cc, his blood pressure was unobtainable and it was decided to administer epinephrine intravenously. As epinephrine is unstable in alkali, the infusion of isotonic sodium bicarbonate was stopped and normal saline containing the drug started. His further course was marked by continuing weakness and disorientation, complete anuria, but no significant diarrhea. The rate of infusion was regulated in an attempt to maintain blood pressure without precipitating pulmonary edema. The total intravenous fluid intake was only three liters. The plasma protein concentration, which had been 10.2 on admission, was 7.0 gram% on the fourth day, while CO_2 content dropped slightly from 12.8 mEq/L to 11.7 mEq/L. Plasma chloride first rose from 88 mEq/L to 112 mEq/L but then returned to the former level. The patient finally died in shock on the fourth hospital day despite digitalization, hydrocortisone, antibiotics, and norepinephrine.

Autopsy findings of particular interest in the present context included heavy lungs that showed an abnormal amount of fluid on the cut surface, and a soft, flabby heart with punctate epicardial hemorrhages. Microscopic sections revealed diffuse myocarditis with focal necrosis and hypostatic pneumonia. The kidneys showed focal tubular degeneration, dilatation of tubules, and interstitial infiltrates of inflammatory cells, consistent with ischemic nephrosis. Stool culture was positive for V. cholerae Inaba.

Case III - CRL #102

A.K. was a ten year old boy who had been in good health until the onset of cholera. He entered Victoria Hospital in collapse eleven hours later where he received 5% glucose in saline, and was transferred to CRL late in the second day of illness. On arrival he was obtunded and weak, with no palpable pulse at the wrist. Blood pressure could not be measured with certainty, but it was felt to be 50/0. He appeared only mildly dehydrated but was markedly hyperpneic. Heart sounds were of poor quality and nearly inaudible; there were bilateral crepitant rales, but no sign of increased peripheral venous pressure. An initial dose of 200 ml intravenous fluid produced an increase in rales, but no improvement in B.P. He was than given epinephrine, and enough further fluid to offset stool and insensible losses. His total intake by vein was 2.6 liters, which contained 220 mEq sodium bicarbonate and 170 mEq chloride. During this time he passed 2.7 liters of stool and no urine. His course was gradually downhill, with persistent hypotension and pulmonary rales, and he died 24 hours after admission. His plasma protein level was 9.9 gram % on admission and was unchanged at death. CO_2 content had risen from 3.0 mEq/L to 11 mEq/L while chlorides had fallen slightly from 87 to 83 mEq/L.

An autopsy showed hyperemia of the ileum, and vascular engorgement of the kidneys. Heart and lungs were not grossly abnormal. Microscopic sections however, demonstrated focal myocarditis similar to that seen in the preceding case. Stool culture was positive for V. cholerae Inaba.

Case IV - CRL #433

M.C., a 28 year old fisherman was brought to the Matlab Hospital in a moribund state fifteen hours after the onset of cholera. He had previously been in good health. Initial therapy consisted of five liters of saline and three liters of Darrow's solution intravenously. With tetracycline his diarrhea soon ceased, and he did not require intravenous fluids after the second day, when he received two liters saline and one liter Darrow's He was judged to be well hydrated, but remained anuric until the third day. He took food and fluids by mouth, but did not do well, with continuing hyperpnea, oliguria and weakness. Puffiness of hands and face and hiccough developed and seven days after the onset of his illness, he was transferred to the CRL ward in Dacca. On arrival he was obtuneded. His

face was edematous, his neck veins distended and his liver large and tender. Heart sounds were of poor quality with an accentuated P2, and there were rales, rhonchi and wheezes in both lungs. Laboratory data were consistent with mild uremia, severe acidosis and excessive hydration. Of note were creatinine 8 mg%, CO_2 contents 8.4 mEq/L and K 6.8 mEq/L. The chest x-ray showed cardiomegaly, pulmonary vascular congestion and patchy infiltrates consistent with pulmonary edema for which he was given 1 mg of digoxin parenterally. Intravenous isotonic sodium bicarbonate was given despiate the obvious overhydration and signs of congestive failure. Two liters were given during the afternoon of his arrival, and three liters the following day. The results were dramatic, as the signs of heart failure and pulmonary congestion cleared. His urine volume rose to 2.3 and then 4.7 liters per day, and his blood chemistries and chest x-ray reverted to normal. Digoxin was discontinued after the initial dose. At discharge he showed no signs of heart disease. Stool culture was positive for V. cholera Inaba.

Case V - CRL # 1587.

A.H., a 46 year old businessman, contracted a cholera like illness on a trip to Jessore. He was brought to the Matlab treatment center six days after onset, with anuria and semicoma, but without diarrhea. Records of treatment received during his illness were not available. He was felt to be moderately dehydrated, but his pulse rate was 60, respiratory rate 20, and BP 90/50. Four liters of saline were given by vein within the first 24 hours. He still failed to pass urine, his mental state worsened, his pulse and blood pressure became undetectable, and hyperpnea became apparent. In this virtually moribund state he was transferred by jet boat to Dacca. On arrival at the CRL ward, his condition was critical. He appeared well hydrated, but was unresponsive. Femoral pulse was slow and irregular; the pulse was not palpable peripherally. His breathing was irregular and deep, with expiratory grunting and wheezing. Chest examination revealed bilateral rales and rhonchi, and an enlarged heart with sounds of poor quality, mostly obscured by rales. A bedside chest x-ray showed pulmonary edema and vascular congestion. ECG showed idioventricular rhythm and A-V dissociation. Arterial blood pH was 7.00, plasma CO_2 content 5.9 mEq/L, Na 150 mEq/L, K 5.9 mEq/L and plasma protein 6.9 gram%. An infusion of isotonic sodium bicarbonate was begun in one vein and a slow drip of 10% glucose in another. During the first two hours with two liters of sodium bicarbonate, his color improved, pulses became palpable and the heart rate increased. The respiratory rate increased to 30/minute but there were no signs of increasing pulmonary edema. Arterial pH was now 7.32, CO_2 content 14 mEq/L, and K 5 mEq/L. The infusion was continued at a slower rate and by the fourth hour his pulse rate was 70, and his ECG showed normal conduction. By the twelfth hour after three liters isotonic sodium bicarbonate he was alert but weak, had a systolic B.P. of 90 and was free of signs of pulmonary edema. Diuresis began on the tenth day of illness, the third day after coming to CRL. His course thereafter was one of improvement, although a troublesome pulmonary infection with B. proteus and probably D. pneumoniae developed and delayed his recovery.

In all, he received 800 mEq/L of bicarbonate by vein in seven liters of fluid. With no diarrhea and the temporary oliguria his weight, which was 33.1 kg in convalescence was forced to a high of 42.3 Nevertheless serious pulmonary edema was relieved, his urine output increased and subnormal hematocrit and plasma protein levels rose, suggesting a decreasing plasma volume. By the ninth day all blood chemistries, ECG and Chest X-ray were normal, and he was discharged at the end of three weeks.

Discussion:

The beneficial effects of solutions containing sodium bicarbonate in cholera patients with acidosis and circulatory collapse were first suggested in 1832 by O'Shaughnessy (4). Latta demonstrated their efficacy in practice (5) and other practitioners adopted this new mode of therapy. A particularly dramatic account of these first attempts at intervenous fluid therapy is given by Weatherhill (6) who infused in less than 24 hours 480 ounces (15 liters) of fluid of the following composition: " Muriat. Sodae 2 drams," (about 8 grams sodium chloride) and "Carb. Sodae 2 scruples," (2.6 grams of sodium bicarbonate) in two quarts of water. This was hypotonic containing about 75 mEq/L NaCl and 16 mEq/L of $NaHCO_3$. Nevertheless the responses were dramatic as circulation was restored, breathing returned to normal and the petient survived. Dr. Weatherhill remarked, " I shall hereafter consider myself as not doing my duty, if ever again I suffer a patient to die of the cholera, without giving it a fair and full trial." In 1908-1910 in a series of observations on cholera patients in the Philippines, A.W. Sellards dramatized the magnitude of the base deficit in cholera by demonstrating that more than 1,000 mEq/L of sodium bicarbonate did not completely correct it (7). He then showed that the mortality of cholera could be significantly reduced by using sodium bicarbonate in the treatment of cholera patients (8). Shortly thereafter two papers on the severe acidosis of pediatric diarrhea and its correction with sodium bicarbonate, were published (9) (10).

In diseases of primary importance in adult medicine, such as diabetic acidosis or uremia, treatment of the underlying basic defects often ameliorates all the sequellae at once including acidosis and therefore the effect of acidosis itself cannot always be appreciated. In the past decade, however, several observations have refocussed medical interest on the deleterious effects of low blood pH. It was noted that patients were often severely acidotic after open heart surgery and that regulation of acid-base balance resulted in improved survival (12) (13). Studies with dogs indicated that accumulation of lactic acid secondary to inadequate perfusion was mainly responsible for the acidosis (3). Treatment of this acidosis was extended to other states of circulatory arrest and shock (14) (15) (29). Syndromes of lactic acidosis in a variety of other situations were described (16) (17). Finally there has been increased interest in animal experimentation in acidosis, its pathophysiology and correction (20) (21) (22) (30). The importance of direct treatment of the acidosis in diabetic coma is still under discussion (11).

Latta's Weatherhill's and Sellar's observations in cholera patients were apparently forgotten until 1958 when Phillips and his colleagues quantitatively measured the losses of water and electrolytes in cholera stools and instituted a rational treatment regimen based on replacement of these losses (18). In many areas where cholera is endemic the urgency for the replacement of bicarbonate is not widely recognized, and fear of pulmonary edema following use of saline alone has led many physicians to use inadequate fluid replacement in the treatment of cholera. As a result many patients die in shock and dehydration. It is true that if saline alone is used in initial rehydration some cholera patients may develop pulmonary rales and a diastolic gallop rhythm which we have ourselves observed. But when acidosis is corrected even considerable overhydration is tolerated without signs of heart failure in an otherwise normal patient.

Studies carried out by Drs. Harvey, Enson, Lewis and Ally this past year at CRL have indicated some of the mechanisms in man by which acidosis may exert its deleterious effects. By means of right heart catheterization Dr. Harvey's group measured blood pressures in the right heart and pulmonary arteries. Cardiac output and blood gasconcentrations were measured at the same time. The results indicate that for any specified cardiac output, an increase in hydrogen ion concentration is correlated with an increase in pulmonary artery pressures. In patients rehydrated with saline, abnormally elevated pulmonary artery pressures were common at arterial blood pH values below 7.25. On the basis of data analyzed so far it is not known whether pulmonary hypertension is due to spasm of the vascular bed or due to left heart failure. But studies in dogs suggest the most likely effect of increased hydrogen ion concentration in the blood is on the myocardium itself: acidosis reduces the muscular force of contraction (2) (2). However one study using a different animal and different measurement techniques failed to show this effect (22). Acute experiments have indicated that cardiac arrest occurs at blood pH of 6.2 - 6.4. Identical effects are produced by carbonic acid, lactic or hydrochloric acid (23), (24). However, animal experiments which have been reported do not reproduce the situation often found in cholera patients since they deal with either a very severe acidosis that lasts only a few hours or a much milder acidosis of longer duration.

It is apparent that acidosis may play a central role in many common medical problems. One of the more perplexing diseases in which acidosis has been implicated as an important feature is the respiratory distress syndrome in infants associated with a pulmonary hyaline membrane. Recently the use of sodium bicarbonate in therapy has been shown to reduce the mortality in infants with this illness (25). It is possible that immediate correction of acidosis could be life saving in other disease as well, among them the post operative period in open heart surgery (27), diabetes (11), and pulmonary insufficiency (28).

Summary:

We have reported five cases of severe acidosis occurring as a consequence of cholera. Each patient had heart failure with hypotension, pulmonary vascular congestion and pulmonary edema, and less often with signs of right-sided heart failure. All were disoriented. Three of these patients died having received inadequate therapy for acidosis. The two survivors received adequate amounts of sodium bicarbonate intravenously.

We strongly recommend further observations in cholera, which makes available a naturally occurring clinical state where pure metabolic acidosis can be observed in the absence of other complicating diseases. There is cause to suggest that acidosis may play a central role in many disease states, and that its correction may improve the patient's chances for survival.

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III(a)

Rayer Bazar Diarrhea Morbidity Study

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The diarrhea morbidity study in the village of Rayar Bazar was continued until May 31, 1965 employing the methods described in the Annual Progress Report of the Pakistan-SEATO Cholera Research Laboratory, October, 1964. Thus the total duration of the study was from February 1, 1965, or 16 months, and the bacteriologic methods employed were constant for a twelve-month period, from June 1, 1964 to May 31, 1965. (Annual Progress Report, 1964, IV(e)).

The data which were collected are still being studied in an attempt to answer a number of the questions concerning the natural history of diarrheal disease in this area of East Pakistan. The present interim report deals with the analyses completed as of August, 1965.

General Environmental Data

Records of daily rainfall, relative humidity, and maximum and minimum temperatures for Dacca City were collected from the local weather station and converted into monthly means for the 16 months of the study. These data are presented in Table I and Figure 1 in order to provide a background against which to view seasonal changes in morbidity and environmental contamination with potential enteric pathogens.

I. The Role of Non-cholera Vibrios in Diarrhea Morbidity.

Water samples were collected each week from April 1, 1964 to May 31, 1965, from all tube wells in the village, and from all dug wells used by study families. All samples from dug wells were collected within 24 hours of the routine weekly rectal swabbing of the associated families, and in most cases they were collected at the same visit. All 50 ml. samples were incubated at 37°C with one-half volume (25 ml.) of triple-strength alkaline bile peptone medium for six hours, then streaked out on gelatin agar and taurocholate-tellurite-gelatin agar and incubated overnight. All suspected vibrios were tested for agglutinability with anti-group, anti-Ogawa and anti-Inaba sera by the slide test, and were transferred to sugar media for Heiberg grouping. Vibrios of any Heiberg group which filled to agglutinate with anti-O Group I serum were considered non-cholera vibrios. The majority fell into Heiberg group II, but members of all six groups were isolated during the study period. All tube well samples and monthly dug well samples were also cultured for heat-resistant coliforms by incubation in lactose media at 45.5°C. Lactose-fermenting and gas-producing organisms were considered to be Escherichia coli.

(more)

At the time samples were collected, the ground-water level at each well was estimated by measuring the distance in feet of the well-water surface below ground level. The pH of all water samples was estimated at the time of collection by the use of from thymol blue indicator and a Lovibond comparator.

A total of 4446 water samples were collected from 137 dug wells during the 14 month study period. Of these, 1790 samples yielded non-cholera vibrios on culture and 4 samples were positive for V.cholerae. The monthly distribution of isolations in relation to depth of well water and pH is shown in Table II and in Figure 2. Well-water levels are also plotted in Figure 1, in order to relate temperature and rainfall to well depth. It is clear that dug wells are less frequently contaminated with non-cholera vibrios during the cooler and drier months of the year, which is also the time well-water surfaces are greater than 25 feet below the ground surface. It would appear that vibrio contamination is closely correlated with rainfall since there is an increase in contamination after each rainfall (Figure 3). Percentage positive rose in March and April, 1965, when temperature was rising even though rainfall was scanty and wells continued to remain very low. The pH of well water showed slight but definite shift from a low of 6.25 in August, when ground water was highest, to 6.50 in December to March, when water was lowest.

It is not possible from these data to determine which environmental factor(s) is primarily responsible for changes in frequency of non-cholera vibrios, but it is clear that these organisms are not available for human consumption with equal frequency throughout the year. From the few isolates of V. cholerae which were made, it appears that their presence arises from different determinants from those influencing non-cholera vibrio rates. Isolates were made in October, December and January, during both high and low water periods.

In order to determine the relationship between contamination of well water with Escherichia coli and non-cholera vibrios, bacteriologic results from all samples tested for both groups were analyzed. The findings are presented in Table III. Although the majority of wells were positive for E. coli and only half contained non-cholera vibrios, it is clear that in the absence of E. coli, vibrios were very rarely found. The association is consistent with the notion that fecal contamination of wells is the chief source of the vibrios. On the other hand, in tube wells where only one seventh of the samples contained E. coli, and only one out of twenty contained vibrios, there appears to be no association between the two organisms, suggesting that vibrios do not enter tube wells by fecal contamination (Table IV).

The association between non-cholera vibrios in water and the incidence of diarrheal disease was investigated by comparing diarrheal disease incidence in households using contaminated and uncontaminated water. Periods were selected during which three consecutive weekly samples from a single dug well yielded non-cholera vibrios. For each such period a well was selected which was negative for non-cholera vibrios during the same three-week period. The diarrheal disease incidence per 1000 person-weeks was determined for the second and third weeks of both groups of three-week periods. The results are given in Table V. which shows that diarrheal disease was actually less frequent among users of contaminated wells. Total non-diarrheal disease was also slightly less frequent among contaminated well users. Rectal swab isolations of non-cholera vibrios were few, but were more commonly found among persons using contaminated wells.

The rates by age in each group are given in Table VI. Diarrhea was never more frequent among the contaminated well users, but the age distribution is unusual in that highest incidence was found among children 5-14, whereas the users of uncontaminated wells showed the usual pattern of decreasing attack rates with increase in age.

These data suggest that non-cholera vibrios are ingested by dug well users with great frequency throughout the year, and that more than half the wells are contaminated with these organisms during eight months of the year. Yet, there is no evidence that diarrheal disease is associated with well contamination. On the other hand, these data provide no evidence for or against the occasional pathogenicity of non-cholera vibrios in certain individuals. Indeed, data presented below suggest that the presence of these organisms in the gut <u>is</u> associated with a higher incidence of diarrhea than that found in non-infected individuals. Taken together, these findings indicate that in an environment where these organisms are abundantly available, the chief determinant of both infection and disease is the individual host's response or the pathogenicity of the vibrio strain rather than the intensity of exposure.

II - An Estimate of the Relative Pathogenicity of Selected Enteric Organism in the Study Population.

<u>Methods :</u>

The method used was a modification of that which had been employed by Watt et al. (19) to estimate the relative pathogenicity of certain <u>Shigella</u> and <u>Salmonella</u> species among residents of south-western United States.

During weekly visits to households of study families a history of diarrheal and non-diarrheal disease with onset since the last visit was obtained from each member. Additional morbidity data were obtained when study family members presented themselves at the daily clinic maintained in the village by this laboratory, and were confirmed by subsequent house visits. Rectal swabs were collected from all willing members at each weekly visit, and from all clinic patients with gastrointestinal symptoms. In nearly all instances, swabbing was repeated within 3 to 4 days for confirmation.

Swabs were streaked directly on SS (Shigella-Salmonella) agar plates in the field and then placed in alkaline bile peptone broth tubes. Plates and broths were exposed to ambient temperatures varying with the season (See Figure 1) for one to three hours between collection and incubation at 37°C. Non-lactose fermenting colonies on SS plates were picked and subcultured to TSI (Triple-sugar-iron) slants and trypticase broth for subsequent re-streaking and inoculation of sugars, urea, indol and motility media. Suspected Salmonella or Shigella were tested by slide agglutination against group sera. Shigella-like organisms which failed to agglutinate with Group A, B, C or D antisera were boiled for two hours after which many showed satisfactory group-specified agglutinogens. Since testing with boiled antigens is not yet complete, all strains which failed to agglutinate with group antisera on primary isolation are classified tentatively in this report as "indeterminate Shigella".

Alkaline peptone water was streaked on gelatin-agar and taurocholate-tellurite gelatin agar after 6 hours of incubation, and examined for vibrios.

The isolation of Shigella, Salmonella or Vibrio from a family member served as the index event. All cases of diarrheal disease with onsets during the week preceding, the week(s) of isolation, and the week following the last positive culture of a given organism in that family, were selected arbitrarily as the diarrheal disease associated with that particular isolation. Rates were calculated in cases per 1000 person-weeks of observation.

For each positive isolation period, a control family was selected using a table of random numbers. If during the same period of weeks no isolations of Shigella, Salmonella or Vibrio were made from any members of that household, it was selected as a control. If infection was present during the period, the family was passed over and the next family selected from the random number table was used as a control.

Results :

The overall rates in all family members for the 16 month period of observation are shown in Table VII. Diarrheal disease was 4 to 6 times as frequent among members of families in which one or more individuals harbored Shigella than among control families. Rates were similar for all four Shigella groups. Rates in families harboring indeterminate Shigellas, non-cholera vibrios and Salmonella were all roughly three times that of controls. These findings suggeststhat the indeterminate group of Shigella, as presently classified, are a mixed group including some true Shigella and some non-pathogenic enteric organisms.

The higher incidence of diarrheal disease in households infected with non-cholera vibrios is interesting in the light of the findings reported in Part I of this report. These data suggest that if infection is established with non-cholera vibrios, disease is more frequent than among non-infected individuals; in other words, infection with these organisms is not always inapparent. This is consistant with the findings of McIntyre et al (Am. J. Trop. Med.) and Lindenbaum, et al (Lancet, 1964), which demonstrate clearly that acute diarrheal disease may be associated with the presence of non-cholera vibrios in high concentration in the stool, and accompanied by a rise in titer of homologous agglutinins during convalescence.

On the other hand, it appears that the development of diarrheal disease, as such, is not associated with the frequency of contamination of dug wells. It is possible, of course, that the chief source of enteric pathogens, including non-cholera vibrios in the environment is not from dug-well water. Water consumption data collected from all families indicate that the vast majority of individuals in this community claim to drink only tube well and dug well water, but tank and canal water is generally used for bathing (which includes rinsing the mouth and gargling), laudering and occasionally cooking (which includes use of raw water for preparation of Panta Bhat). Dishes are frequently washed in tank water, and it is reasonable to assume that such dishes when used wet or air-dried may contaminate otherwise clean food or drink with tank organisms. Although tank, canal and pond water was cultured weekly throughout the study, it was felt that histories of usage of these water sources by individual households were too unreliable to include in the present analysis. Food itself may be a source of pathogens, but no food was cultured during this study.

Diarrheal disease rates for Shigella and Salmonella-infected households are given by age in Table VIII. These data indicate that disease associated with both these groups of organisms is much more frequent in children, and that the age distribution of disease is similar whether associated with known "pathogens" or not. The declining

incidence with increasing age in all three categories is consistant with an endemic pattern in which all the important agents associated with diarrhea are continually present in the community and early infection resulting in enhanced resistance is the rule.

The influence of family size upon the incidence in infected households was investigated by calculating the overall rates for Shigella-infected household and in control households, in families with less than eight members, and in families with eight or more members. In Table IX it can be seen that rates were actually somewhat less in the larger families, both for diarrhea associated with Shigella, and for non-specific diarrhea. Mean family size is slightly greater in the Shigella group, but diarrhea rates in the two bacteriologic groups do not approach one another regardless of the size of families. Thus there is no evidence that the size of the household group above and below eight members has only influence upon the number of people who will sicken once Shigella is introduced into the household. Data have not yet been studied to determine whether family size influenced the likelihood of introduction of Shigella into the household.

An estimate of the total impact of Shigella infection upon the studied community was sought by determining the frequency with which Shigella were isolated from individuals with and without diarrheal disease. The results are presented in Table X. Organisms were isolated from only 0.54% of samples obtained from asymptomatic individuals*, an inapparent infection rate much lower than that demonstrated by Hardy, Watt and DeCapito (1942) who found as many as 3.8% of asymptomatic carriers in an endemic area. Furthermore, in both study areas, Shigella flexneri was the most frequently isolated organisms, both in health and disease. In Thailand, Noyes recently reported the isolation of Shigella from as high as 12% of symptomfree individuals (personal communication). The technique employed in the present study for the isolation of Shigella from rectal swabs would seem to be atleast as sensitive as that used by other workers. Thus the results suggest either that the actual swabbing was less satisfactory in the present study, or that the frequency and/or intensity of inapparent infection is lower in the population under study in East Pakistan than in areas previously investigated or that marked seasonal variations occur.

In contrast to the findings in inapparent infection, Shigella was isolated with great frequency (20.1%) from cases of acute diarrheal disease, indicating that it is a major cause of diarrhea in this area.

* 163 isolations of Shigella were made on repeated sampling of 2078 persons, an infection rate of 7.8% at sometime during the studies.

Viewed from the standpoint of the clinical spectrum evoked by Shigella infection in this community, it appears that the majority of infections are not associated with diarrhea. Thus, of 278 infections recognized, 163, or 58% were in asymptomatic persons, while 115 were suffering from diarrhea at the time or within 7 days of the swab collection. Data were collected concerning the severity of diarrhea but these have not yet been analyzed. In general, however, the frequency of disability, as manifested by inability to work or confinement to bed, was very low; the vast majority of cases were able to continue their usual activities.

Data concerning the seasonal distribution of diarrheal disease by age, sex and associated agents, and the aggregation of disease in household and areas within the village are currently being analyzed and will be presented in a later report. Also, the state of nutrition of all children was estimated in terms of body weight, and a study of the association of diarrheal disease with nutritional status will be made.

III. Cholera

During the study period, 33 cases of bacteriologically confirmed cholera appeared in the village. With one exception, all cases appeared in families which had not been under surveillance before the onset of the index case. These cases will be discussed in a separate report since they did not occur in study families as here defined, and, hence, no data concerning their experience prior to the onset of cholera is available.

No instances of inapparent infection with V. cholerae were detected in persons under prior surveillance, except for the single case in a 10 year old boy reported in the Annual Progress Report last year (1964). Thus one of the aims of this study: namely, to obtain data concerning the incidence and distribution of inapparent infection with cholera vibrios in a group of families under previous surveillance, was not fulfilled. The failure to isolate more than one cholera vibrio from more than 30,000 rectal swabs is evidence of the infrequency with which inapparent infection is detectable in these families, except from members of households in which frank clinical cholera is occuring(Annual Progress Report, this laboratory, 1964, Section IV(d)). In consideration of these negative findings it is important to recall that a sharp cholera outbreak had occured in this village during the two weeks preceding the onset of the study on February 1, 1964, with an estimated overall attack rate of 15.6 per 1000; that a single case and a single inapparent infection appeared in April, 1964, and that 33 cases appeared in the village in previously unobserved families between October, 1964, and January, 1965. It is clear that cholera vibrios were present in the community during these times, and is possible that they existed in intervening periods. Our failure to detect them in human or environmental

materials, except after the appearance of frank clinical cholera suggests either that they were absent, were present in location in human hosts or in the environment which were beyond the reach of our sampling methods, or that our present techniques do not possess sufficient sensitivity to reveal their presence.

It is suggested that future studies on the spectrum of infection evoked by V. cholerae, which must include the frequency and nature of inapparent infection, include a search for cholera vibrios in all parts of the gastrointestinal tract, especially the small intestine, rather than being confined to rectal swabbing. There is reason to believe that the rectal environment is inimical to cholera vibrios and that they are present there in numbers suitable for detection only during the onrush of acute diarrhea when the normal flora of the large bowel are flushed out. It is a distinct possibility that the permanent natural reservoir of V. cholerae is the upper intestinal tract of man, but of only a few individuals who provide the proper substrate, as yet unknown, for their survival and multiplication; and that their reintroduction into the environment at a given moment is dependent upon the occurence of inter-current diarrheal disease of a variety of causes, which allows the vibrios to pass the large bowel "barrier" and contaminate the environment, much as the louse, appearing at the right moment, provides the means for releasing the rickettsia from a case of Brill's disease, thus introducing typhus to other members of the community.

TABLE I

Weather Data, Dacca, East Pakistan 1964-65

Month (28 or 35 day periods)	Weeks of Study	Means of Maximum Daily Temp C°	Means of Minimum Daily Temp C°	Means of Average Daily Rel. Humidity(%)	Mean Daily Rainfall in inches
1964					
JAN	1-3	22.6	10.8	59.4	0.00
FEB	4-7	28.8	16.2	52.8	0.028
MAR	8-11	33.9	21.2	47.3	0.018
APR	12-15	32.8	23.2	67.8	0.000
MAY	16-20	32.1	24.4	71.3	0.255
JUNE	21-24	31.4	25.7	82.4	0.242
JULY	25-29	30.3	26.1	84.1	0.162
AUG	30-33	30.8	26.3	82.1	0.125
SEPT	34-37	30.9	26.1	84.8	0.162
OCT	38-42	30.5	24.5	85.4	0.177
NOV	43-46	28.5	19.3	74.3	0.011
DEC	47-50	25.5	13.9	69.2	0.000
1965					
JAN	51-55	25.2	11.7	63.7	0.000
FEB	56-59	27.4	14.3	62.6	0.032
MAR	60-64	31.9	17.9	44.5	0.000
APR	65-68	34.5	24.0	64.4	0.007
MAY	69-72	32.9	25.3	69.4	0.160

TABLE II

Frequency of Isolation of *V. cholerae* and Non-cholera Vibrios from Dug Wells, pH of Dug Well Water, and Depth of Water Surface below Ground Level, by month*, Rayar Bazar, Dacca, April 1964-May 1965.

Month (28-35 day period)	Weeks of Study	Number of Samples Examined	Number Positive for NCV	Percent of samples Positive for NCV	Number of Samples Positive for *V. cholerae*	Mean Dug Well Water level below Surface	Mean pH of all Samples
1964							
APR	12-15	152	58	38.2	-	24.9	6.43
MAY	16-20	247	149	60.3	-	18.5	6.34
JUN	21-24	251	130	51.8	-	18.9	6.33
JUL	25-29	348	174	50.0	-	10.9	6.27
AUG	30-33	293	184	62.8	-	7.3	6.25
SEPT	34-37	308	181	58.8	-	9.9	6.30
OCT	38-42	413	249	60.3	2	10.5	6.31
NOV	43-46	329	133	40.4	-	17.1	6.37
DEC	47-50	321	77	24.0	1	23.0	6.50
1965							
JAN	51-55	382	58	15.2	1	26.4	6.47
FEB	56-59	335	43	12.8	-	29.0	6.50
MAR	60-64	425	129	30.4	-	28.4	6.48
APR	65-68	340	122	35.9	-	29.7	6.44
MAY	69-72	302	103	34.1	-	26.4	6.39
TOTAL :		4446	1790	40.3	4		

* Based on Weekly Sampling.

TABLE III

Relationship between Non-cholera Vibrios and *Escherichia coli** in Dug Well Water Samples, Rayar Bazar, Dacca, East Pakistan, 1964-1965

		Non-cholera Vibrios		
		Present	Absent	Total
Escherichia coli	Present	220	190	410
	Absent	2	30	32
	Total	222	220	442

* Heat resistant (45.5°C) coliforms.

TABLE IV

Relationship between Non-cholera Vibrios and Escherichia coli* in Tube Well Water Samples, Rayar Bazar, Dacca, East Pakistan, April,1964 -May, 1965

		Non-cholera Vibrios		
		Present	Absent	Total
Escherichia coli	Present	2	59	61
	Absent	18	343	361
	Total	20	402	422

* Heat Resistant (45.5°C) Coliforms

TABLE V

Disease Rates*, Infections with Non-cholera Vibrios, and family size of Households according to presence or absence of NCV in Dug Well Water

NCV in Dug Well Water	No. of 3-week periods examined	No. of Families	No. of persons	Average family size	Diarrheal Disease cases per 1000 persons-weeks	Non-diarrheal Disease cases per 1000 persons-weeks	Rectal Swab Isolations of NCV during observation periods
Present	103	152	1072	7.1	12.6	8.4	5
Absent	103	139	1006	7.2	17.9	10.4	2
Total	206	291	2078	7.1	15.2	9.4	7

* Based on diarrheal disease with onset during second and third weeks of periods during which three consecutive weekly samples of dug well water were either positive or negative for non-cholera vibrios.

TABLE VI

Diarrheal Disease Rates per 1000 Person-Weeks*, by age in households according to presence or absence of Non-cholera Vibrios in water of dug wells, Rayar Bazar, Dacca, 1964-1965

Age Group	Non-cholera Vibrios present in Dug Well Water			Non-cholera Vibrios Absent in Dug Well Water		
	Person - Weeks	Cases	Rate per 1000 persons- weeks	Person weeks	Cases	Rate per 1000 persons- weeks
0-4 years	434	6	13.8	368	9	24.5
5-14 years	806	16	19.9	718	14	19.5
15 and over	904	5	5.5	926	13	14.0
Total	2144	27	12.6	2012	36	17.9

* Based on diarrheal disease with onset during second and third weeks of periods during which three consecutive weekly samples of dug well water were either positive or negative for non-cholera vibrios.

TABLE VII

Diarrheal Disease Rates* in Families of infected individuals, per 1000 Person-weeks, All ages, by Infecting Organism, Rayar Bazar. Dacca, February 1964 - May 1965

Organism Isolated during Observation period	Person weeks	Cases	Rates
Shigella A	209	12	57.4
Shigella B	3165	124	39.2
Shigella C	120	5	41.7
Shigella D	637	29	45.5
Total Confirmed Shigella	4131	170	41.2
Shigella Indeterminate	1562	44	28.2
Non-cholera Vibrios	827	21	25.4
Salmonella	703	20	28.5
None (control)	5186	47	9.1

*Based on diarrheal disease with onset during period from week preceding to week following isolation of organism from one or more family members.

TABLE VIII

Diarrheal Disease Rates* in Families of infected individual per 1000 Person-weeks, by Age, in relation to Infection with Certain Enteric Organisms, Rayar Bazar, Dacca, February 1964-May, 1965

Age Group	Organism Isolated during Observation Period								
	Shigella all groups			Salmonella all groups			Control Periods		
	Person-weeks	Cases	Rates	Person-weeks	Cases	Rates	Person-weeks	Cases	Rates
0-4	851	60	70.5	103	7	70.0	934	17	18.2
5-14	1570	71	45.2	303	9	29.7	1905	19	10.0
15 and over	1720	39	22.7	297	4	13.5	2347	11	4.7
Total	4131	170	41.2	703	20	28.5	5186	47	9.1

* Based on diarrheal disease with onset during periods from week preceding to week following isolation of organism from one or more family members.

TABLE IX

Relationship of Family Size to Diarrheal Disease Rate in Families with one or more members Infected with Shigella, Rayar Bazar, Dacca, February 1964 - May 1965

Infecting Organism	Family size Members	Total Family Members	Number of Families	Mean Family Size	Person-Weeks Observation	Cases of Diarrhea	Rate per 1000 Person-weeks
Shigella all Groups	< 8	428	78	5.5	1413	77	54.5
	8 and over	751	76	9.9	2718	93	34.2
	Total	1179	154	7.6	4131	170	41.2
No Organism isolated	< 8	481	97	5.0	1672	15	9.0
	8 and over	481	55	8.7	1634	13	8.0
	Total	962	152	6.3	3306	28	8.5

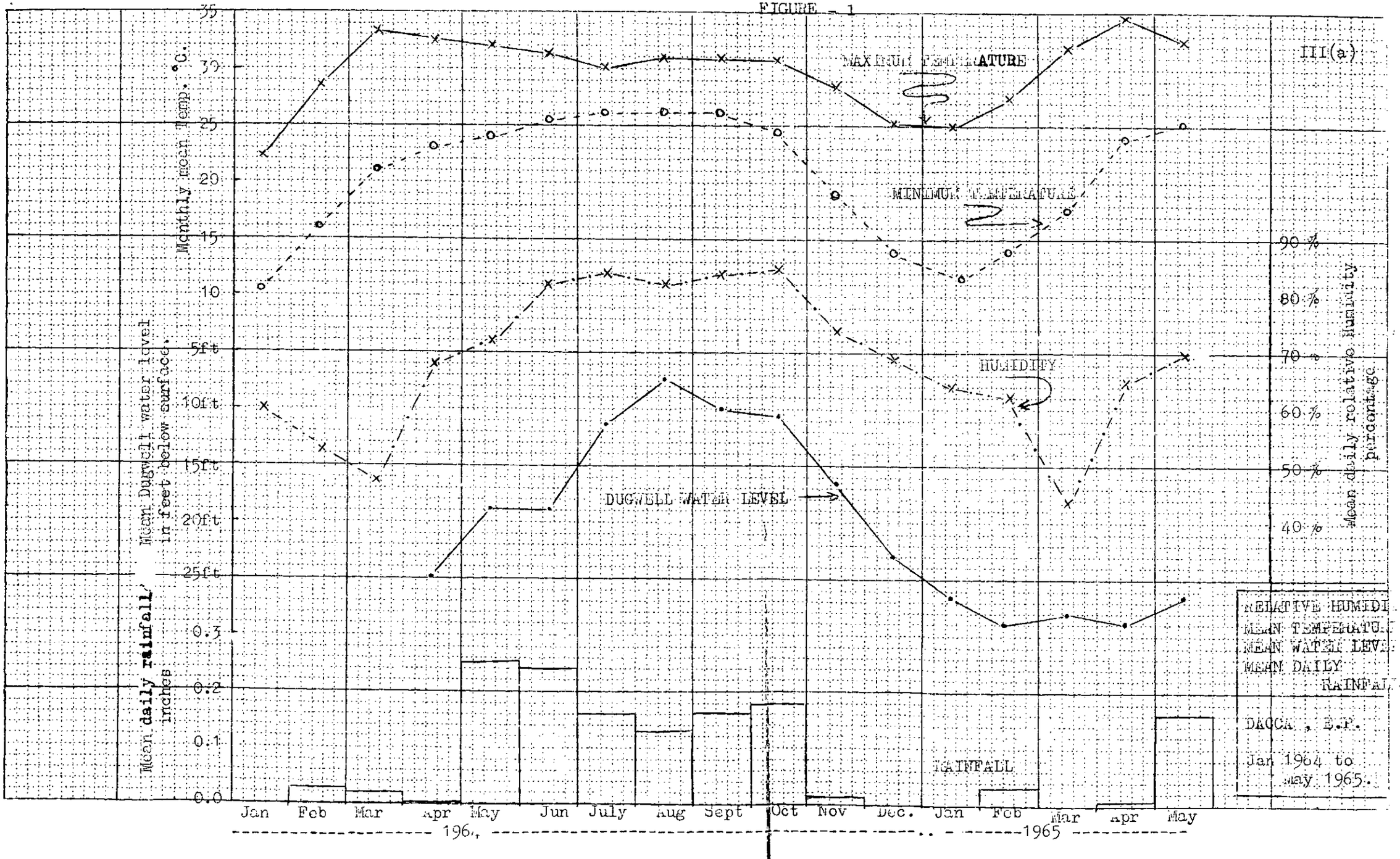
TABLE X

Association of Diarrheal Illness and Isolation of Shigella, All study Families, Rayar Bazar, Dacca, East Pakistan, February, 1964-May, 1965

Symptoms within one week of Swabbing	Number of Visits	Rectal Swabs Collected	Percent Swabbed	Shigella Isolated	% Shigella of total Swabbed
Diarrhea Absent	52,187	29,775	56.8	163	0.54
Diarrhea Present	607	571	94.1	115	20.14
TOTAL:	52,794	30,346	57.5	278	0.92

FIGURE - 1

III(a)



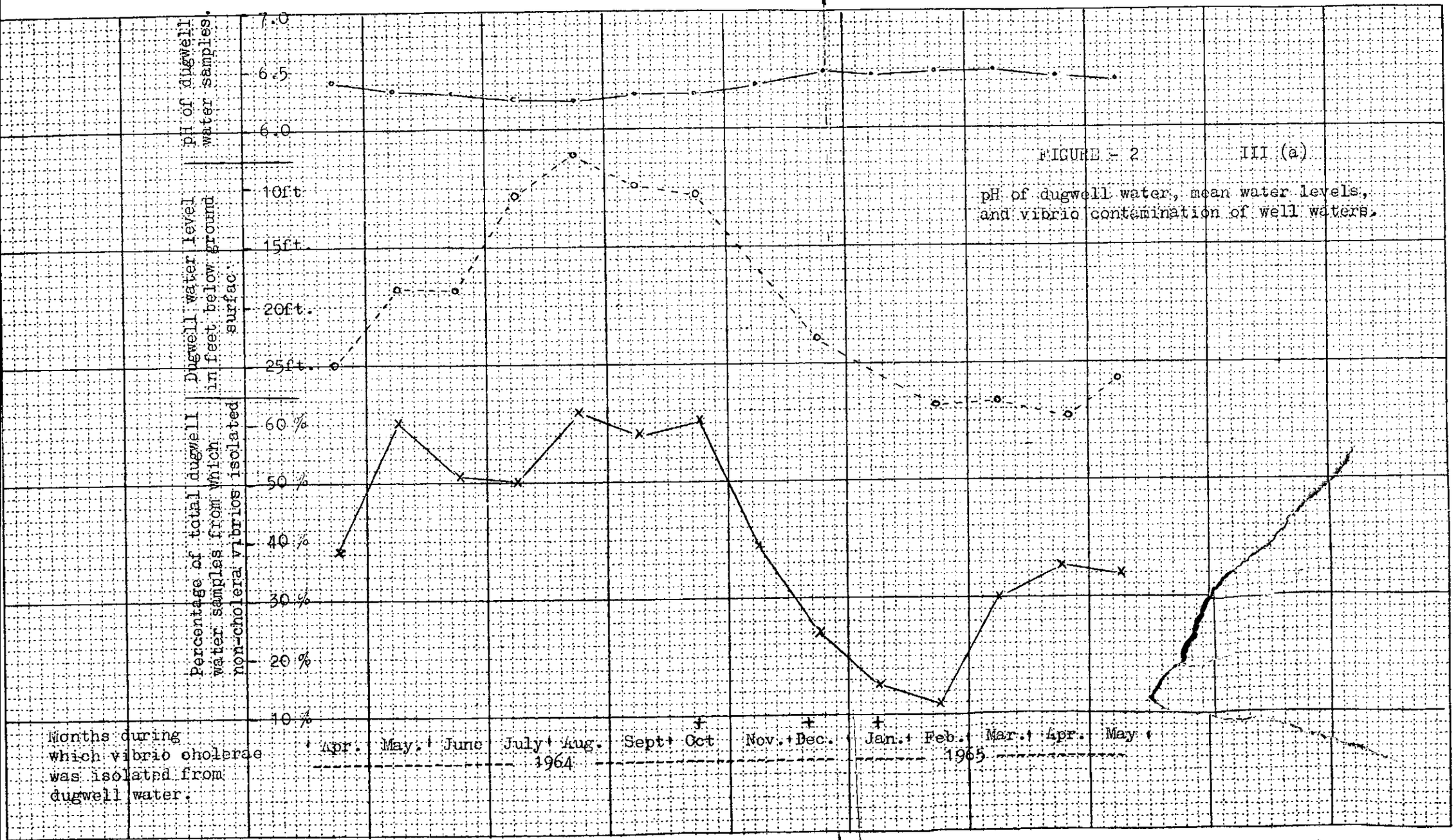


FIGURE - 2 III (a)

pH of dugwell water, mean water levels, and vibrio contamination of well waters.

FIGURE - 3


PERCENTAGE OF TOTAL DÉCHELL WATER SAMPLES FROM WHICH NON-CHOLERA VIBRIOS WERE ISOLATED
80%
70%
60 %
50 %
40 %
30 %
20 %
10 %
TOTAL RAINFALL PER WEEK IN INCHES.
3
2
1
47
49
51
53
55
57
59
61
63
65
67
69
71
73
75
77
79
81
W E E K S .

CLINICAL AND BACTERIOLOGICAL FINDINGS AMONG FAMILIES OF CHOLERA PATIENTS

by
Dr. Robert O. Oseasohn and Dr. Shamsa Ahmad.

Introduction.

A rational plan for the control of cholera in endemic areas such as East Pakistan requires further information concerning patterns of occurrence and determinants of infection. Crude incidence data presently available are based on urban hospital admissions and death reports provided by village watchmen. Hospitalized cases represent, however, an undefined fragment of total disease. Village statistics are based on instances of fatal diarrheal illness which may or may not be associated with V. cholerae.

Initial study of a group of families of cholera patients admitted to the Pakistan-SEATO Cholera Research Laboratory (CRL) hospital in Dacca during 1962-3 revealed the frequent occurrence of diarrheal disease associated with V. cholerae among household contacts. Children particularly were affected. These observations were based on the occurrence of illness among family members and the extent of inapparent infection was not measured.

Another group of families were selected for study, and clinical and bacteriologic data were collected from the entire population at risk. The present report describes the distribution of subsequent apparent and inapparent cholera infection in these families.

Methods:

Families of cholera patients hospitalized at CRL and living in Dacca were selected for study within two days of onset of illness in bacteriologically confirmed index cases. Families, defined as units consisting of two or more persons who lived and ate together, were observed daily for 10 days by teams comprised of one physician and one health visitor. Family members were queried daily about the occurrence of diarrhea or any symptom which interfered with usual duties. When either diarrhea or disability was encountered, a detailed history of illness was obtained. Rectal swabs were collected daily from each family member, were placed in bile-peptone transport medium and incubated overnight at 37°C. Subcultures were made on plain gelatin-agar and on taurocholate-tellurite gelatin agar (Monsur) and incubated overnight at 37°C. Suspicious colonies were tested for agglutination by specific antisera. Cholera cases were defined as persons with acute diarrheal disease from whom V. cholerae were recovered from one or more fecal samples; all other isolations of V. cholerae were regarded as instances of inapparent infection.

(more)

Results.

Ninety-two patients and their families were observed between 6 November 1963, and 4 February 1965. Subsequent cholera infection occurred in 49 of the 92 families. Cholera cases occurred in 32 of the 49 families, whereas in the remaining 17 only inapparent infection was observed. The rate of subsequent infection in families was not related to family size. The rate (Table 1) was higher in households with 3 or more persons under 15 years of age than in those with less than 3 young individuals. However, the difference was not significant at the 5% level. The relationship between age and sex of index cases and infection rate is shown in Table 2. Rates were higher in all age groups when index cases were females than when index cases were males.

The prevalence of inapparent infection was more frequent during the first 5 days of observation than after the fifth day and always exceeded clinical disease except on the first day of observation. A few persons with diarrhea were observed each day from whom no vibrios were recovered. On the first day of observation, 17 individuals with diarrhea associated with V. cholerae sought hospitalization while only one was observed in the home. On each day thereafter, the number of cholera cases found in the community exceeded the number hospitalized.

Cumulative clinical and bacteriologic findings by age groups are summarized in Table 3. During the 10-day study interval, V. cholerae were found in samples collected from 115 of 545 persons at risk. Frequency of infection was similar among males and females. Sixty-one of the 115 were free of symptoms. The remaining 54 experienced diarrhea, and 31 of these sought hospitalization. The proportion of infected individuals with and without diarrhea was similar among all age groups and did not differ by sex. Infection was most frequent among children less than 5 years of age. This group included 30 infants under 1 year of age of whom 9 were infected. Four of the 9 experienced mild diarrhea but none was hospitalized, Table 3a presents the vibrio infection of the contact group.

Onset of symptoms among the 54 individuals with diarrhea associated with V. cholerae is shown in Table 4. The majority had onsets within 5 days of the index case. As a rule, vibrios were isolated within a day of the appearance of symptoms. However, in 5 persons, onset of diarrhea did not appear until 3 to 5 days after the first positive culture.

Admission to hospital was sought more frequently by persons whose symptoms appeared less than 2 days following onset of illness in index than among those who became ill 2 or more days after index cases. Twenty-three of the 54 remained at home and did not receive antibacterial drugs or fluid replacement. One died on the first day of the family episode and 16 of the remaining 22 reported cessation of diarrhea by the close of the study interval.

Appearance of first positive cultures by 2-day intervals and age groups is shown in Table 5. Most positives appeared by the fifth day, but 16 of the 115 appeared on the sixth day or later. Similar trends were observed in all age groups. Table 6 summarized the duration of inapparent infection by age groups. Thirty-one of the 61 were negative after 1-2 days and all except 4 by the seventh day. Inapparent infection tended to persistlonger in children than in adults.

Discussion:

These data provide an estimate of risk of infection among families of hospitalized cholera patients in a large urban community in an endemic area. One-fifth of household contacts were affected and nearly half of those developed diarrheal disease. The rate of infection was highest among children less than 5 years of age. Contrary to previous observations in Dacca based on the occurrence of overt disease, rates did not increase significantly with an increase in the number of family members under 15 years of age.

Higher rates among contacts of female index cases than male index cases may be related to cloistered living patterns (the purdah system) observed in a predominantly Muslim society. Female children remain close to older female relatives within the home. Women and children collect water and prepare food and, consequently may be better "spreaders" than men and boys.

Daily prevalence data showed that the majority of those with severe diarrheal disease were hospitalized soon after onset of illness in index cases. Thereafter, the majority of infections among families were asymptomatic.

Athough evidence of infection usually appeared within 5 days of onset in index cases, the initial isolation of vibrios was delayed to the sixth day or later in 14% of those at risk. Present international quarantine regulations require close scrutiny of contacts for 5 days. These findings may not be directly applicable, however, since persons at risk in the present study continued to live in their usual environment. The occurrence of subsequent infection in families may differ when contacts leave that setting. The onset of diarrheal disease one or more days following the detection of inapparent infection has been reported previously.

Further information is needed concerning mechanisms responsible for the variety of host responses to cholera infection and the manner in which it is spread. Family studies are in progress using serologic techniques, bacteriologic examination of water and food supplies and experimental control of environmental factors.

TABLE I

SUBSEQUENT INFECTION AND AGE COMPOSITION OF FAMILIES

Number of family members under 15 years	Vibrio present	Infection absent	Total	Rate(%)
0-2	16	92	108	14.8
3+	99	338	437	22.7
Total:	115	430	545	21.1

TABLE II

SUBSEQUENT INFECTION AND AGE AND SEX OF INDEX CASES

Age of Index case-Male	No infected/No. at risk	% infected	Age of Index case female	No.infected/No at risk	% infected
<5	3/33	9.1	< 5	37/107	34.6
5-14	13/101	12.9	5-14	28/115	24.3
15+	13/112	11.6	15+	21/77	27.3
Total:	29/246	11.8	Total:	86/299	28.8

T A B L E III

CUMULATIVE CLINICAL AND BACTERIOLOGICAL FINDINGS BY AGE

Finding		Age groups (years)							
Diarrhea	V. cholerae	5		5-14		15+		Total	
		No.	%	No.	%	No.	%	No.	%
0	0	56	51.4	106	67.9	245	87.5	407	74.7
0	+	21	19.3	25	16.0	15	5.4	61	11.2
+	0	8	7.3	7	4.5	8	2.9	23	4.2
+	+	24 (11)*	22.0	18 (13)*	11.5	12 (17)*	4.3	54 (31)*	9.9
Total		109	100.0	156	99.9	280	100.1	545	100.0

* No. hospitalized in each group

T A B L E IIIa

	Age Groups			Total
	0-4	5-14	15 or over	
Total Number	109	156	280	545
Asymptomatic	19.3	16.0	5.4	11.2
Diarrhea	22.0	11.5	4.3	9.9
Total infected	41.3	27.5	9.7	21.1
Hospitalized	10.0	8.3	2.5	5.7

Percent with Vibrio infection and Symptom Complex in Family Contacts of Vibrio Positive Cholera Cases.

T A B L E IV

ONSET OF INITIAL SYMPTOMS IN SUBSEQUENT CASES BY AGE.

Age Group	Days of onset after index case					
	Same-1	2-3	4-5	6-7	8+	Total
< 5	8	6	4	4	2	24
5 -14	11	5	2	0	0	18
15+	4	4	2	1	1	12
Total:	23	15	8	5	3	54

TABLE V

FIRST POSITIVE CULTURE AND AGE GROUPS

Age group (years)	Day of First Positive Culture Following Onset of Index Case					
	Same-1	2-3	4-5	6-7	8+	Total
< 5	16	16	6	4	3	45
5-14	25	10	5	1	2	43
15+	10	8	3	3	3	27
Total	51	34	14	8	8	115

TABLE VI

DURATION OF INAPPARENT INFECTION BY AGE GROUPS

Age group (years)	Number of days				Total
	1-2	3-4	5-6	7+	
< 5	8	3	7	3	21
5-14	10	7	7	1	25
15+	13	2	0	0	15
Total	31	12	14	444	61

PATTERNS OF MEDICAL CARE BASED ON A FOLLOW-UP OF DEATHS IN DACCA CITY

by: Drs. Shamsa Ahmad & Robert Oseasohn.

Purpose of study:

A study was carried out in a village of West Punjab in 1956, by Gordon, Singh and others, in which they concluded that males got priority over women and children in availing of medical facilities. We, too wanted to know whether these observations held good in East Pakistan. Secondly, we wished to ascertain whether age, sex and causes of illness had any relation to the facilities used, as the vaccine trials at Matlab showed cholera to be a disease of children. Furthermore, in order to introduce control measures, any social bias should be borne in mind.

Method used:

Statistical reports revealed that 78.7% deaths in East Pakistan were among Muslims, while in Dacca City 91% were Muslims. An estimated 99% of Muslims dying in Dacca were buried in either of the two public graveyards at Azimpur and Zurain. Therefore, we selected 3 cases daily from the graveyard registers using a random number table. The deaths in the city had a seasonable variation being highest in the months of November and December, so 100 cases were chosen every three months, so as to cover the year. After selection of the cases, the homes of the deceased were visited and a standard proforma was completed by interviewing the family members of the deceased. The questionnaire covered data regarding housing, literacy in the family, age, sex, cause of death and medical facilities used by the deceased during the fatal illness.

Results:

Table I illustrates the percentage of the families available for study, by age group of the deceased. Overall 72% of the families were contacted, and there does not seem to be any significant difference according to the age groups. The failure to interview the remaining 28% was due primarily to difficulty in locating the homes of the deceased because of wrong or insufficient address. In the majority of cases, it was because a friend or relative registered the death at the graveyard rather than an immediate member of the bereaved family.

In comparing the percentages of diarrheal and non-diarrheal deaths (Table II), we see that only 27% of all deaths had diarrheal at the time of death. There is a variation in the proportion of diarrhea in the different age groups. In children under 5, thirty-two percent had diarrhea, while in adults over 15, only twenty percent had diarrhea in their fatal illness.

(more)

Among the 80 diarrheal deaths, the medical facilities used differ according to age, as is seen in Table III. In children, 86% saw the doctor and only 20% went to hospital, whereas in adults, 96% visited the doctor and 46% went to hospital. This shows that children are not hospitalized as readily as adults. Secondly, it is clear that people do not restrict themselves to a single source of medical facilities, but use a combination of sources, at the time of their fatal illness.

In the case of non-diarrheal deaths, the observations on the use of medical facilities yield similar results, in as much as the children visit the hospital less frequently than adults. (Table IV). There is also a higher percentage of children visiting the homeopaths and spiritualists, when compared with the adults. The sex of the ill person causes no significant difference in the medical care received by the deceased (Table V), and therefore differs from the observations of Gordon, Singh and others made earlier in 1956. As this study was carried out in the urban population, a pilot study was undertaken in the Matlab Vaccine Trial area. As there are fewer doctor and hospital facilities available, the village folk there depend much more on homeopaths, spiritualists and kaviraj for medical care, in contrast to people of Dacca.

Discussion:

Several points should be considered in looking at medical care received by persons at the time of their fatal illness. Firstly, though the majority of hospitals give free treatment, the poor cannot avail of this facility always, as the admissions to the hospital are many and it takes weeks before a vacant bed is available for chronic cases, especially among adults.

As regards children, they need their mother as their attendant during their stay in hospital, but as this involves domestic problems, the poor hesitate to bring their children to hospital.

This study has been on dead people, so is it worthwhile doing one on living persons? It may be argued that the present standard of treatment of diarrhea is so good, that the number of deaths has deceased among the hospitalised cases. This study based on data obtained from the graveyard would focalise on those who did not receive hospital treatment and therefore bias the overall medical care pattern.

TABLE I

PERCENTAGES OF FAMILIES AVAILABLE FOR STUDY

age of deceased	Total selected cases	Cases available for study	Percentage
< 5	194	136	81
5-14	24	17	71
+ 15	182	135	74
Total	400	288	72

TABLE II

PERCENTAGES OF DIARRHEAL AND NON-DIARRHEAL DEATHS

	Diarrheal Present		Diarrheal Absent	
Age	No. of cases	% age	No. of cases	% age
< 5	50	32	86	63
5-14	2	12	15	88
+15	28	21	107	79
Total	80	28	208	72

TABLE III

SOURCES OF MEDICAL CARE AMONG DIARRHEAL DEATHS

Age of deceased	Total deaths	Doctor	%	Hospital	%	Homoe-path	%	Spiri-tualist	%	Others	%	None	%
< 5	50	43	86	10	20	32	64	26	52	8	16	2	4
5-14	2	2	100	0	0	0	0	0	0	0	0	0	0
+15	28	27	96	13	46	12	43	11	39	3	10	0	0
Total	80	72	90	23	29	44	55	37	46	11	14	2	3

TABLE IV

SOURCES OF MEDICAL CARE AMONG NON-DIARRHEAL DEATHS

Age of deceased	Total deaths	Doctor	%	Hospital	%	Homoepath	%	Spiritualist	%	Others	%	None	%
<5	86	62	72	34	40	37	43	45	52	9	10	8	9
5-14	15	13	87	7	47	4	27	4	27	3	20	2	13
+15	107	85	79	61	57	26	24	30	28	13	12	10	9
Total	208	160	77	102	49	67	30	79	38	25	12	20	10

TABLE V

MEDICAL SOURCES USED BY THE DECEASED BASED ON SEX AND AGE

	Diarrheal deaths			Non-diarrheal deaths		
Source	Sex	< 5	+15	Sex	< 5	+15
Doctor	M	25	14	M	33	51
	F	18	13	F	29	34
Hospital	M	6	6	M	16	39
	F	4	7	F	18	22
Homoepath	M	19	6	M	22	13
	F	13	6	F	15	13
Spiritualist	M	12	6	M	23	16
	F	14	5	F	22	14
Others*	M	5	2	M	2	8
	F	3	1	F	7	5
None	M	2	0	M	4	3
	F	0	0	F	4	7

* This term includes all herbal medicines, Kabiraj and and wearing of tahsinans.

EPIDEMIOLOGICAL ANALYSIS OF THE CHOLERA AND ACUTE DIARRHEA ADMISSIONS TO THE PAKISTAN-SEATO CHOLERA RESEARCH LABORATORY, DACCA, EAST PAKISTAN.

July 1964 - June 1965.

Wiley H. Mosley, S.Z. Ahmad, Yousuf A. Saeed, Moslemuddin Khan, and Samsa Ahmed.

Introduction:

Essential to the development of hypotheses concerning the factors involved in the transmission of cholera is a background of the basic epidemiological patterns of the disease. Specifically, one needs to know the distribution of cases in time, place, and persons, as well as a detailed look at other factors associated with the cases. In the past much of this type of information on cholera has been based on analysis of either diarrhea deaths, or unconfirmed cases. While this type of data is often satisfactory in epidemic situations where the proportion of non-cholera diarrheas may be small, it may be quite misleading in endemic areas. Two recent studies from this laboratory on cholera-like syndromes, one group associated with non-agglutinable vibrios (1), the other group with no known pathogen present (2), illustrate the need for bacteriological confirmation in classifying a case as cholera in this area.

The CRL hospital now provides a unique opportunity for detailed study of bacteriologically confirmed cholera cases occurring in Dacca Municipality and adjacent areas. Since April 1964, arrangements have been made with the Director of Health Services of the Government of East Pakistan for the CRL to be designated the official treatment center for all cholera cases occurring in the Dacca area. Since September 1964, all suspect cholera cases have been treated at CRL. To implement this, an ambulance is maintained at the Sir Salimullah Medical College Hospital (formerly Mitford), where patients are accustomed to seek treatment. Also, suspect cholera cases presenting at the Dacca Medical College Hospital are transferred to the CRL. Further, when localized outbreaks occur in any accessible area, an ambulance is sent to that area frequently, accompanied by a physician, to collect cases. In addition, epidemiological teams frequently locate additional cases and refer them for hospitalization.

It is recognized that analysis of hospital data has its limitations, particularly in a community where a large portion of the population do not seek hospitalization; however, because of the dramatic, well recognized symptoms of cholera, treatment is sought much more frequently than for many other illnesses.(3) In addition, the CRL is establishing a reputation for curing cholera which is becoming widely known. This has a considerable influence on the patients' willingness to come to the hospital. Deaths occurring before seeking hospitalization, as well as mild cases will not be included in a study based on hospital admissions. In spite

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of the limitations imposed by the factor of hospitalization, the active effort being made to seek out cases, coupled with the bacteriological confirmation of every case, make the CRL hospital experience the best data to date on the occurrance of cholera in Dacca Municipality and adjacent areas.

Background:

East Pakistan is divided into 17 Districts. Dacca District is centrally located, with an area of 2882 square miles, and an average population density of 1768 per square mile. Administratively, Dacca District is divided into 37 Thanas, and the Thanas further divided into Unions, each with a population of about 10,000 to 20,000 persons. Dacca City, located on the Buriganga River includes 4 Thanas and is divided in 33 Union Committees. Twenty five Union Committee comprise the old city, with 8 Union Committee being annexed in 1961. (These Union Committees are approximately equivalent to the Census Tracts in American Cities). Dacca City had a population of 556, 712 in the 1961 census and is the oldest and largest city in East Pakistan. Its growth has been particularly rapid in the past 15 years due to industrilalization and migration of population from other areas of East Pakistan, and India. Based on 1951 and 1961 cenus reports the city population had increased 64% in that decade. Because much of this increase has been due to people coming in to work in various industries and leaving their families in the villages, the sex ratio of the population is 1.50 males per female, primarily due to an excess of adult males.

The climatic conditions in the Dacca area can best be decribed as subtropical, however, the seasonal variation in rainfull is marked as illustrated in Table 1. The monsoon begins in late May and extends through September; while the remainder of the year is quite dry. The cool season extends from December through February.

Methods:

All hospital charts of patients admitted to the CRL from July 1, 1964 through June 30, 1965 were reviewed, and the cases of bacteriologically confirmed cholera, and of all other acute non-specific diarrheas were selected for study. Chronic diarrhea cases as well as confirmed bacillary and amoebic dysentary and non-diarrheal diseases were excluded. For all cases selected, the age, sex, religion, occupation, date and hour of onset, vaccine history, outcome and bacteriological results were coded for machine processing. In addition family aggregations were identified by notes on the hospital records as well as by checking the name of the household head, and address. For the cholera cases only, the geographic location of the case at the time of onset was also coded. When available, information from various field investigations made during the course of the year was also utilized.

Results.

During the 12 months from July, 1964 through June 1965 there were 537 admissions with bacteriologically confirmed cholera, 498 Inaba serotype, and 39 Ogawa serotype. Over this same period of time there were 587 admissions with acute diarrhea from whom no bacterial pathogen was isolated. Non cholera vibrios (NAG) were isolated as the predominent organism from 29 of the 587 acute diarrhea patients. Figure 1 gives the epidemic curves by month of onset for the cholera and acute diarrhea cases for the year of study as well as the preceding year. Cholera was virtually absent during the monsoon season then cases increased as the flood waters receded, peaked sharply during the cold dry season, and fell to low levels during the hot dry season. Acute diarrheas occured throughout the year, with a sesonal pattern quite distinct from cholera; cases occured with maximal frequency during the hot, dry season.

The distributions of the cholera and diarrhea cases by age and sex are given in Tables 2 and 3. The preponderance of males among the adult cholera patients is primarily a reflection of the sex distribution in urban Dacca. Among the acute diarrhea patients, there was an excess of males in all age groups, which in children probably reflects some preferential treatment of males by their families if they do not suspect a severe disease such as cholera (3). Most interesting is the fact that 50% of the cholera cases were under the age of 15, as compared to only 24% of the acute diarrhea admissions.

Figures 2 and 3, showing the frequency distributions of the cholera and diarrhea cases by age, further illustrates the differences in age pattern, particularly in the first 10 years of life. Acute diarrheas showed the pattern found generally in children, with most cases being under the age of one, and a marked fall in frequency with age. (The small peak at age 5 is most likely due to digit preference, as birthdays are not generally recorded by the population). Cholera had a distinctly different age pattern, with few cases under the age of one year, and an increase in cases till the age of 4 years, then a rapid fall with increasing age.

Based on the 1961 census data, it is possible to estimate the cholera attack rates by age and sex for Dacca city. Three hundred four of the 537 cholera cases occurred within the city, giving an attack rate of 5.46 per 10,000 population. Table 4 gives the attack rates by age and sex. Though there were more made than female cases, the overall attack rate was slightly higher among females. The highest rates were in the 1-4 and 5-9 year age groups of both sexes.

Geographic Distribution of Cholera Cases:

The epidemic curve of cholera admissions illustrated in Figure 1 represents a composite picture of a number of smaller community and neighborhood outbreaks as well as many apparently unrelated cases. Figure 4 breaks the epidemic curve by areas contributing the majority of cases; Dacca city, the adjacent Thanas of Keraniganj and Tejgaon, and Gazaria Thana which had a village epidemic about 30 miles from Dacca. The distinct epidemics occurring early in Tejgaon Thana and late in Gazaria Thana are apparent. Also, the more progressive rise and fall in the epidemic curve in Keraniganj, a rural area, contrasts with the "peaks and valleys" of the epidemic in Dacca city.

Table 5 shows the cases by week of onset in each Union Committee in Dacca city. The wide distribution of cases throughout the city during the course of the epidemic is apparent. At the same time, localized clusters of cases occurring within 1 to 4 week intervals in the various Union Committees can also be seen. Table 6 presents the distribution of cholera by week of onset in the Unions of Keraniganj and Tejgaon Thanas. While there is more of a selection factor of hospitalization influencing the data in Table 6 because of the distance of some communities from the hospital, the pattern of small epidemics in various localities is evident. Table 7 summarizes the attack rates in the Union Committees within Dacca city. The rates confirm in general the rather uniform distribution of cholera over the city. Those Union Committees with unusually high rates represent areas from which clusters of cases came to the hospital.

A more detailed examination of these localized outbreaks is given in Table 8. Within Dacca city there were 11 neighborhood or small community epidemics, a group of 8 cases from the Central Jail, and a series of cases among adult male brickfield workers. This brickfield epidemic provided 13 of the 39 Ogawa isolations made in Dacca during the year. Outside Dacca city, 56 of the 85 cases in Keraniganj came from 7 villages, and 3 villages were involved in outbreaks in Tejgaon, Gazaria, and Fatualla Thanas. In addition, there were 2 outbreaks involving only adult males, one among jute mill workers, and another among brick field workers. As Table 8 indicates, some of the small epidemics were explosive, with all the cases occurring within a few days. In other areas, particularly the rural villages, the spread was more leisurely, taking several weeks. Also of interest is the high frequency of families with multiple cases, particularly in the explosive epidemics. Among the 32 families involved in 8 outbreaks which terminated in 10 days of less, 15 (47%) had multiple cases. This is in contrast to the overall hospital experience where only 14% of the families had multiple cases (vide infra).

Figures 5 and 6 illustrate 2 of the explosive outbreaks occurring in Dacca city. Figure 5 shows by day of onset the 12 cases occurring in 3 families in Rayer Bazar during October 1964. The simultaneous occurrence of 6 cases on the same day, and 11 cases within a 48 hour period pointed to a common source. Field investigation revealed these three families and a guest shared a common meal on the evening of October 18. The earliest case, a 20 year old female who assisted in the preparation of the food developed mild diarrhea within 10 hours after the meal, but did not seek hospitalization for 2½ days. The subsequent cases developed within 28 to 83 hours after the meal. The last case on 25 October was a 5 month old breast fed baby who did not eat any of the food. Investigation did not positively identify any suspect vehicle, though the well was found contaminated with V. cholerae 3 days after the meal.

Figure 6 demonstrates an epidemic occurring in 4 families living on Dhalkanagar Lane in Dacca city. In this outbreak there was a progessive occurrance of cases in the first family until November 25, when there was the simultaneous occurrence of cholera in 10 persons in 4 families. These 4 families all shared a common dug well which was confirmed bacteriologically positive for V. cholerae that same day.

The Rayer Bazar Brickfield epidemic represented the only Ogawa cholera cases in that locality, and thus provided a conveniently marked organism to trace the probable introduction of the infection and the pattern of spread. The index case was a boatman who had come to Rayer Bazar from Faridpur, and developed cholera 2 days following his arrival. He had diarrhea for about 24 hours while on his boat, which was located at the junction of 2 canals in the center of a large lowland area containing about 50 brickfields employing about 50 to 150 workers each. Contamination of the canal with V. cholerae, Ogawa serotype, was bacteriologically confirmed at the site of the boat, the day after the index case occurred. These canals served as the only water source for the brickfield workers. Over the next 20 days, 12 cases of Ogawa cholera developed among the brickfield workers in widely separated brickfields along the canals, one almost a mile away from the location of the boat.

Altogether, 240 (45%) of the 537 cholera cases hospitalized at the CRL came from these localized outbreaks listed on Table 8. The possible sources of infection for the remaining 297 cases remains less well defined. The hospital records reveal, however, that 60 of these cases (20%) had a history of travel outside their locality within the preceding 7 days as compared to only 19 (8%) of the 240 cases in localized epidemics. However, only 22 (7%) of the 297 cases gave a history of contact with persons with "cholera" in the few days preceding their illness.

Multiple Case Families.

There were 58 families with multiple hospitalized cases, contributing a total of 87 secondary cases. If one excludes the epidemics occurring among the jute Mill workers, the brickfield workers, and in the jail, there are remaining 353 families with only a single hospitalized case. Thus, based on hospital experience, 14.1% of the families had multiple hospitalized cases. The number of hospitalized cases per household is summarized in Table 9. Forty two (72%) of the 58 families with multiple cases had only a single secondary case. The hospital records were inadequate to determine the number of family members at risk in the 411 families with cases; however, by assumming an average of 5.5 persons per household (based on 1961 census data), and subtracting 411 Index cases there would be approximately 1850 family members exposed to a case of cholera. With 87 "secondary" cases, the secondary attack rate would thus be 4.7%.

The pattern of spread within families based on the intervals between the index and secondary cases is summarized in Figure 7. Examination of the individual families in more detail reveals that in 21 of the families, all the cases occurred simultaneously, or within 24 hours of the index case, in 11 families the majority of the secondary cases occurred on the second day after the index case, while in the remaining 26 families the majority of the secondary cases occurred 3 or more days after the index case, with none developing on the same day as the index case. In the 37 families in which there were cases 2 or more days after the index case, the pattern of onset suggested a peak occurrance of secondary cases at 2,4 and 6 days after the onset of the index case. Figure 5 and 6, presented earlier to illustrate outbreaks involving several families demonstrate well the two patterns of spread seen within families - progressive and explosive.

Hour of Onset.

It has been a frequent clinical observation that cholera characteristically has an onset in the early hours of the morning. Figure 8, illustrating the time of onset of 534 cholera cases where this information was available, confirms this clinical impression, revealing the most frequent time of onset of diarrhea in the cholera patient to be between the hours of 3 A.M. to 6 A.M. Figure 8 also demonstrates, however, that this pattern is not unique for cholera, as this is also the most common time of onset for all other acute diarrhea admissions !

Acute Diarrhea Associated with Non-cholera Vibrios.

In 29 (4.9%) of the 587 acute diarrheas, non-cholera vibrios (NAG, or non agglutining vibrios) were isolated as the predominant organism on direct plating of the stool specimen (without prior enrichment). In 24 of these cases, these non-cholera vibrios were also demonstrated as the predominant organism on direct examination of the stool specimen by dark-field microscopy. Though the number of these cases was small, the distribution by month of onset, given in Table 10, suggests a seasonal trend paralleling the acute diarrheas of undetermined etiology, with a paucity of cases during the peak of the cholera season. The age distribution of these cases, given in Table 11, reveals the majority of these cases were adult males. This pattern is quite distinct from cholera, however it corresponds to the pattern seen in acute diarrheas of undetermined etiology, with the major exception of a complete lack of cases under the age of one year.

Discussion.

In this analysis of the CRL Hospital experience with bacteriologically confirmed cholera and with acute diarrhea of undetermined etiology in an endemic cholera area, several distinctive epidemiological patterns emerge. Cholera had a well defined seasonal pattern, with peak occurrance in the cold dry season, a marked fall in cases as the hot dry season entered and essentially no cases during the humid monsoon season. Acute diarrheas, which are present throughout the year, showed peak incidence in the hot dry season as the cholera cases were disappearing. Identical seasonal patterns for these two entities have also been observed in the CRL Field Hospital in Matlab Bazar, which serves an entirely rural population, suggesting that this pattern is not unique for urban areas. Additionally, the virtual absence of cholera through the hot dry season, and the monsoon season has been supported in the Matlab Field Trial area by failure to isolate the organism from approximately 4000 rectal swabs collected monthly from a population of 39,000 in 59 rural villages under continued surveillance.

The age distribution of cholera revealed very few cases under the age of 1 year, with cases increasing in frequency to the age of 4, then falling to low levels in adolescence. Acute diarrheas, by contrast, had the highest incidence in children under the age of 1 year, with a progressive fall in cases with age. This distribution of cholera in children with a paucity of cases in infants and adolescents, and a peak incidence at age 4 years has also been demonstrated in a recent analysis of 4296 bacteriologically confirmed cholera admissions to San Lazaro hospital in Manila (4), a nonendemic area. This resistance to clinical cholera in young children and adolescents has also been observed by Yen in Taiwan in 1962, when

he noted that the "carrier: case ratio" was 4.2 and 2.7 to 1 in the 0-4 and 10-14 age groups respectively, compared to values of 0.33 to 0.75 to 1 in adults over age 35(6). This pattern must relate to some host factors favouring resistance to manifest clinical illness in infancy and adolescence since inapparent infection has been demonstrated in these age groups (5).

The high proportion of cases in children as compared to adults, with correspondingly higher childhood cholera case rates is characteristic of endemic cholera, quite in contrast to the pattern reported from non-endemic areas. For example, while 50% of the cholera admissions to the CRL (and 69% of the cholera admissions to the field hospital in Matlab Bazar) were children under the age of 15, only 10% of the confirmed cholera admissions to the San Lazaro Hospital in Manila in 1962 (7), and 20% of the confirmed cholera cases in Taiwan in 1962 (6) were under the age of 15 years, with correspondingly higher attack rates in adults. This age pattern is consistent with acquired immunity as a result of living in an endemic area. Support is given to this hypothesis by the results of the serological survey recently conducted in Matlab, demonstrating an increasing proportion of the population with vibriocidal and agglutinating antibodies with increasing age(8).

In studying the geographic distribution of the cholera cases admitted to the CRL, 45% of the cases were found to come from 26 localized epidemics. In 2 of these localities common sources of infection were demonstrated by field investigation. The pattern of spread in several other localities also suggested a common source epidemic because of the explosive occurrance of cases, with multiple cases within the families. It should be noted, however, that in other localities listed in Table 8, the epidemics had a more leisurely course suggesting contact spread. In spite of this pattern, and the close geographic association of the cases, a history of direct case-to-case contact was rarely present, suggesting that inapparent infections may have played a role. The role of inapparent infections is further supported by the fact that the majority of the cholera admissions (55%) did not come from areas with localized outbreaks, but appeared "sporadically".

Based on hospital experience, 14% of the families with cases had multiple hospitalized cases, with the large majority of these families having only 2 cases. This frequency of multiple cases within a family is seven times higher than had been observed in a similar analysis of hospitalized cholera cases in the Philippines (7), and contrasts impressively with the recent report from Hong Kong noting the rarity of multiple cases from the same family(9). One possible explanation for these differences is the heavy environmental contamination observed in Dacca around the cases possibly leading to larger doses of the infecting organism. For example, 69% of 58 dug wells belonging to cases and their families that were examined during 1964-65 were found contaminated with V. cholerae

with a higher frequency of contaminated wells found among families with multiple cases (10). Two other hypotheses are that there may be a higher case: infection ratio because the organism is more virulent in East Pakistan, or because of a more susceptible population.

It should be noted that in spite of the fact that 14% of the families had multiple cases when one considers the number of family members exposed, the "secondary attack rate" for hospitalized cases among family contacts has been estimated to be only 4.7%. This should be contrasted with the results of the Family Studies conducted in 1964-65 which revealed that the total infection rate among family contacts of cholera cases determined by multiple rectal swabs was 21% (5). Thus, based on the pattern of illness and infection in families, the case: infection ratio can be estimated to be at least 1:4. This serves to emphasize the high frequency of individuals with mildly symptomatic or inapparent infections surrounding the recognized case that may play a role in the spread of infection.

An estimate of the incubation period of cholera is afforded by the outbreak in Rayer Bazar related to a common meal. The 12 persons developing cholera had onsets of diarrhea between 10 to 83 hours following the meal. The first case, developed only mild diarrhea within 10 hours after the meal. The next earliest case was 28 hours after the meal with 6 cases occurring 49 to 56 hours after the meal. A median incubation period of about 48 hours is consistent with the pattern of spread seen within 37 of the 58 families with multiple cases, where the distribution of onsets of the secondary cases had modes at 2,4 and 6 days after the index case.

The incubation period is also possibly influenced by the pattern of nocturnal or early morning onset of diarrhea seen in cholera. For example, among the 12 persons eating the meal in Rayer Bazar, on the evening of October 18, the first case had onset on 7 AM October 19; the second case 1 AM October 20, followed by a case at noon that same day. The next 6 cases had onsets between 10 PM October 20 and 4 AM October 21. The last 3 cases didn't develope diarrhea until the following morning 24 hours later, between 3 AM and 8 AM October 22. Possible host factors influencing this characteristic early morning pattern of onset, including diurnal changes in intestinal motility, and in the pH and chemical contents of the bowel have not yet been studied.

Special mention has been made of acute diarrhea associated with the bacteriologic finding of non-cholera vibrios as the predominate organism in the stool, because of previous observations from the CRL suggesting an etiologic relationship between this organism and diarrhea (1) as well as the continuing speculation regarding the relationship between these organisms and V. cholera.

As this study has revealed hospitalized acute diarrheas associated with these organisms are rare, accounting for only 4.9% of all acute diarrhea admissions. Epidemiologically, the seasonal and age pattern suggest no relationship with V. cholera. In fact, it is difficult to define these cases as a separate entity based on the epidemiological characteristics since the seasonal and age pattern seem to be primarily a function of the fact that these non-cholera vibrios are found in about 5% of the persons with acute diarrhea, and thus parallel the acute diarrhea patterns. With the ubiquitous finding of non-cholera vibrios in the rivers, tanks and dug wells, it is difficult to attach pathogenic significance to the presence of these organisms in 4.9% of persons with acute diarrhea; however, as with E. coli, there may be only certain strains that are pathogenic, and more careful definition of the organisms associated with diarrhea cases by means of serotyping or phage typing may elucidate the problem.

The importance of bacteriological confirmation in establishing the diagnosis of cholera in an endemic area, has been emphasized by an earlier report from the CRL on "Non-vibrio Cholera" an acute diarrheal illness indistinguishable on admission from classical Asiatic cholera (2). Epidemiologically, the importance of distinguishing between bacteriologically confirmed cholera and other diarrhea can be seen in the seasonal and age patterns discussed above. Without bacteriological confirmation, the CRL hospital experience would suggest that "cholera"occurs throughout the year with peak incidence in two seasons, and that the disease was much more common in adults. Indeed, previous observations, based on unconfirmed cases have suggested two cholera seasons in the same year with one peak in cold dry season, and a second peak in the hot dry season (11). This has led to many hypothesis regarding the role of the tanks and impure water as the reservoir of cholera, with the termination of the epidemic with the onset of the purifying rains. The present observations demonstrate that in the Dacca area cholera disappears as the hot dry season reached its peak, and thus one is forced to reevaluate the role of such factors as stagnant water in explaining the epidemic and endemic pattern of cholera. Likewise, though "non-vibrio cholera" is characteristically seen in adults, bacteriologically confirmed cholera is primarily a disease of children in endemic areas. This suggests that acquired immunity may play a role, a concept that had often been questioned because of the many adult "cholera" cases reported from endemic areas.

Summary.

The data from this study indicate that cholera has a distinct seasonal pattern, so that in Dacca city (and in the rural area of Matlab Bazar) endemic cholera is actually characterized by recurrent annual epidemics, rather than by a low level of cholera cases throughout the year. In addition, endemic cholera is primarily a disease of young children, suggesting that acquired immunity develops with age among those persons living in an endemic area.

Based on therelative frequency of multiple hospitalized cases in the families versus mild or inapparent infections found by family studies, it has been estimated that the case to infection ratio is at least 1 to 4. These persons with mild symptoms, or inapparent infections are apparently the reservoir of cholera. Water plays an important role as a vehilce in the dissemination of infection, as illustrated by the localized outbreaks, and supported by the results of bacteriological examination of water sources around cases. However, as studies in this laboratory have demonstrated, water cannot serve as a reservoir of infection, since examination of contaminated wells and tanks have repeatedly revealed negative cultures within a few days after the cholera cases or infected individuals have been removed from the area (12). The frequently reported finding of V. cholerae in tanks and rivers in East Pakistan in the absence of clinical cases only serves to illustrate the importance of individuals with mild cholera or inapparent infections repeatedly "inoculating" these areas.

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TABLE I

AVERAGE DAILY MAXIMUM AND MINIMUM TEMPERATURE, TOTAL RAINFALL, AND NUMBER OF DAYS OF RAIN, BY MONTH, DACCA CITY JULY, 1964-JUNE, 1965

Month	Average Daily Temperature		Total Rainfall (inches)	Days of rain
	Maximum (°F)	Minimum (°F)		
July	86.5	78.6	12.1	15
August	87.4	79.2	3.6	12
September	87.8	78.6	6.9	8
October	86.7	76.3	3.8	11
November	83.5	66.7	0.3	1
December	77.7	55.8	-	0
January	77.5	53.4	-	0
February	81.3	57.2	0.9	1
March	89.4	64.2	0.1	1
April	91.6	74.1	0.2	1
May	89.8	77.7	4.6	5
June	88.2	77.9	8.4	13

TABLE 2

DISTRIBUTION OF CHOLERA CASES BY AGE AND SEX
CRL HOSPITAL
JULY 1964 - JUNE 1965

Age Group	Sex		Total	% of total cases	Cumulative %
	Male	Female			
Under 1	6	3	9	1.7	1.7
1	8	9	17	3.2	4.9
2	19	15	34	6.3	11.2
3	18	16	34	6.3	17.5
4	19	19	38	7.1	24.6
5	12	18	30	5.6	30.2
6	13	9	22	4.1	34.3
7	9	8	17	3.2	37.5
8	11	7	18	3.4	40.9
9	4	3	7	1.3	42.2
10-14	30	14	44	8.2	50.4
15-19	30	9	39	7.2	57.6
20-29	70	43	113	21.0	78.6
30-49	48	26	74	13.8	92.4
Over 50	31	10	41	7.6	100.00
Total :	328	209	537	100.00	

TABLE 3

DISTRIBUTION OF ACUTE DIARRHEA CASES BY AGE & SEX
CRL HOSPITAL
JULY 1964 - JUNE 1965

Age group	Sex		Total	% of total cases	Cumulative %
	Male	Female			
Under 1	22	13	35	6.0	6.0
1	11	6	17	2.9	8.9
2	9	3	12	2.0	10.9
3	7	3	10	1.7	12.6
4	3	2	5	0.9	13.5
5	10	2	12	2.0	15.5
6	2	2	4	0.7	16.2
7	2	4	6	1.0	17.2
8	5	2	7	1.2	18.4
9	4	2	6	1.0	19.4
10-14	21	8	29	4.9	24.3
15-19	28	14	42	7.2	31.05
20-29	94	54	148	25.3	56.8
30-49	127	64	191	32.5	89.3
50 +	41	22	63	10.7	100.00
Total :	386	201	587	100.00	

TABLE 4

CHOLERA CASE RATES BY AGE AND SEX
DACCA CITY
JULY 1964 - JUNE 1965

Age Group	Population[a]			Cases			Rates per 10,000 pop.		
	Male	Female	Total	Male	Female	Total	Male	Female	Total
Under 1	7221	7106	14327	3	2	5	4.15	2.81	3.49
1-4	34870	34739	69609	25	34	59	7.17	9.79	8.48
5-9	42410	42026	84436	28	28	56	6.60	6.66	6.63
10-14	34484	25959	60443	19	11	30	5.50	4.24	4.96
15-19	31174	19597	50771	13	6	19	4.17	3.06	3.74
20-29	82170	39947	122117	39	23	62	4.75	5.76	5.08
30+	101578	53431	155009	51	22	73	5.02	4.12	4.71
Total:	333907	222805	556712	178	126	304	5.33	5.66	5.46

[a] 1961 Census.

TABLE 5

Dacca Municipality - Cholera Cases by Union Committee and by Week of Onset
July 1964 - June 1965

Union Committee	July-October	November							December					January					February				March					April - June			
	27-41	42	43	44	45	46	47	48	49	50	51	52	53	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17-26
1 Faridabad			1		1		2	19	5	2	1										1	1					1			
2 Farashganj								1			2							1												
3 Rokanpur	1					1			1	2	2																			
4 Patuatuli		1		2					1	3	1		1		3	1	1	2												
5 Islampur										3	1			1					2	1		1								
6 Nawabpur									2	2	3							1												
7 Sarafatganj			1	2		1	4	1	2	1				2																
8 Wari				6	4					2	3		1																	
9 Narinda			1					2	6	1	2							1												
10 Gopibag								2		3																				
11 Dilkhosa									1			1																		
12 Siddique Bazar	2								3	1		3					1													
13 Purana Moghal T.							1																							
14 Samsabad																														
15 Nazira																														
16 Ramna	1					1	4																				1	1		
17 Maulavi Bazar														3	5	1														
18 Chota Katra				1	1						3		2														1			
19 Rahmatganj										1																				
20 Bakshi Bazar							1	1		1						1														
21 Chawk Bazar													1																	
22 Azimpura					1			1	1																					
23 Lalbagh					1		3	1	1																					
24 Nawabganj																														
25 Hazaribagh				1			1	1			1		1	2			1													
26 Dhanmandi					1			1		3	2					1											1		1	
27 Khilgaon								1			1	1	1	3					.1											
28 Mohammedpur	1		13	8	5	2			1	2		1	3		1							1	8	2	5					
29 Jatrabari							3	2	1	2		1	1														1			
30 Rajarbag										1												1								
31 Purana Paltan			1					1		1	1	1			1			1							1				1	
32 Naya Paltan					3					1																				
33 Tejgaon		1	2	5	1					1		2	2						.1											
Unknown						1			2				1																	
TOTALS :	5	2	20	24	18	6	19	34	27	33	23	10	14	11	11	4	3	6	4	1	1	4	8	2	6	0	5	1	1	1

TABLE 6

Tejgaon Thana and Keraniganj Thana
Cases by Union and by Week of Onset, July 1964 - June, 1965

Union	June-Aug. 27-35	September 36	37	38	39	October 40	41	42	43	44	November 45	46	47	48	December 49	50	51	52	53	January 1	2	3	4	February 5	6	7	8	9	March-June 10	11-26
Tejgaon Thana																														
Beraid																														1
Cantonment												2																		
Dakshin Khan																														
Demra																	1	1	2	5	3	6	2	1			2			1
Harirampur																														
Joar Shahara																														
Mirpur	2			8	3									1	1		1	1	1	1	3									
Mutuail														1						1						1	2			
Satorkul																														
Uttarkhan																														
Shympur																													1	1
TOTAL	2			8	3							2		2	1		2	2	3	7	6	6	2	1	1	2	2	1	3
Keraniganj Thana																														
Basta																					1									
Hazratpur																														
Kalatia																		1		2	1									
Kalindi																	1	2	1							1				
Konda															1															
Rohitpur																			1								1			
Sakta																														
Subhodya												1	2	7	6	6	3	2				1	1							
Taranagor																														
Tegharia																				1		1	1							
Zinzira																	3	4	6	9	6	4	1	4	3	4			1	2
Unknown																			1	1										
TOTAL												1	2	8	6	10	10	11	13		8	6	3	4	3	5	1		1	2

TABLE 7

CHOLERA CASE RATE BY UNION COMMITTEE, DACCA CITY, JULY 1964 - JUNE 1965.

Union Committee	Population[a]	Case	Rate per 10,000	Union Committee	Population[a]	Case	Rate per 10,000
1 Faridabad	15,466	34	22.0	18 Chota Katra	8,969	8	8.9
2 Farashganj	17,798	4	2.3	19 Rahmatganj	9,635	1	1.0
3 Rokanpur	13,919	7	5.3	20 Bakshi Bazar	18,511	4	2.2
4 Patuatuli	15,488	16	10.3	21 Chawk Bazar	10,987	1	0.9
5 Islampur	21,254	10	4.7	22 Azimpur	16,749	3	1.8
6 Nawabpur	12,846	8	6.2	23 Lalbagh	16,031	6	3.7
7 Sarfatganj	21,541	14	6.5	24 Nawabganj	8,064	0	-
8 Wari	15,944	16	10.0	25 Hazaribagh	12,597	8	6.4
9 Narinda	10,388	13	12.5	26 Dhanmandi	21,236	10	4.7
10 Gopibag	14,178	5	3.5	27 Khilgaon	30,021	8	2.7
11 Dilkhosa	19,500	2	1.0	28 Mohammadpur	31,155	53	17.0
12 Siddique Bazar	21,250	10	4.7	29 Jatrabari	20,155	11	5.5
13 Purana Moghultuli	10,769	1	0.9	30 Rajarbag	28,424	2	0.7
14 Samsabad	9,391	0	-	31 Purana Paltan	17,424	9	5.2
15 Nazira	11,474	0	-	32 Naya Paltan	7,811	4	5.1
16 Ramna	16,676	9	5.4	33 Tejgaon	33,177	15	0.5
17 Moulavi Bazar	13,161	8	6.1	Unknown		4	
				Total :	551,969	304	5.6

a 1961 Census Data

TABLE 8

SUMMARY OF LOCALIZED CHOLERA OUTBREAK, DACCA CITY AND NEIGHBOURING AREAS, JULY 1964 - JUNE 1965.

A. DACCA CITY

Union Comm.No.	Locality	No.of cases	Duration of outbreak (days).	REMARKS
1	Dhalkanagar Lane	17	7	Four families with multiple cases sharing common well positive for V. cholera.
1	Nabin Ch. Goshami Road	6	5	Four cases in 1 family. River positive for 7 days.
6	Nawabpur Road	6	12	Neighborhood epidemic; 6 different families. Dug well posivive in 1 family. Water jar of another family positive.
7	A.K. Das Road	7	23	Three cases in 1 family. Three wells shared by 5 of the families positive for V. cholera.
8	Johali Mandir Road	6	8	Two cases in each of 3 families. One Dug well positive for V. cholera.
9	Dayaganj	8	16	Two cases in 1 family.
10	Shawmibagh	5	15	Two cases in each of 2 families. Water negative.
12	Kazi Alauddin Road	3	5	Three separate families. Two Dug Wells positive for V.cholera
17	Central Jail	8	8	All adult male prisoners.
28	Rayer Bazar	13	6	Three families with multiple cases following a common meal. Dug well positive.
28	Rayer Bazar	16	65	Progressive spread through predominently Hindu, pottery community.
28	Rayer Bazar Brick Field.	13	21	All adult male brick field workers. (Only Ogawa epidemic). Canal positive.
33	Rajar Bazar	8	9	One family with 2 cases; 1 family with 3 cases. Dug well positive for V. cholera.

T A B L E 8

SUMMARY OF LOCALIZED CHOLERA OUTBREAKS, DACCA CITY AND NEIGHBOURING AREAS.

July 1964 - June 1965

a. OUTSIDE DACCA CITY

Locality	No. of cases	Duration of outbreak(days)	R E M A R K S
Adamjee Nagar Jute Mill	9	47	Jute Mill workers and their families.
Demra - Latif Bawani Jute Mill	10	16	All adult male jute mill workers.
Mirpur - Refugee colony	8	10	4 cases in 1 family, 2 cases in 1 family.
Mirpur - Refugee Colony	4	24	Second group of cases in same area after 10 weeks.
Mirpur - Brick Field	5	15	All adult male brick field workers. Lowland tank positive for V. cholera.
Baushia - Gazaria Thana	32	44	Rural village 30 miles from Dacca that ambulance visited daily.
Villages in Keraniganj Thana.			
Rasulpur	3	18	
Kaliganj	11	34	1 Family with 5 cases; 1 family with 2 cases.
Par Gandaria	7	10	
Subhodya	4	42	
Mandail	9	41	1 Family with 2 cases
Zinzira	16	89	1 Family with 2 cases
Choto Gaon	6	14	1 Family with 2 cases.

T A B L E 9

NUMBER OF HOSPITALIZED CHOLERA CASES PER FAMILY CRL HOSPITAL ADMISSION.

July 1964 - June 1965.

Number of Hospitalized cholera cases.	Number of Families.
1	353
2	42
3	9
4	3
5	2
6	2
Total:	411

***

T A B L E 10

DISTRIBUTION OF ACUTE DIARRHEA CASES ASSOCIATED WITH NON-CHOLERA VIBRIOS BY MONTH OF ONSET.

July 1964 - July 1965.

Month of Onset	Number of Cases
July	3
August	0
September	0
October	3
November	3
December	0
January	1
February	1
March	1
April	8
May	4
June	4

T A B L E 11

DISTRIBUTION OF ACUTE DIARRHEA CASES ASSOCIATED WITH NON-CHOLERA VIBRIOS BY AGE AND SEX.

Age Group	Number of Cases		
	Male	Female	Total
Under 1	–	–	–
1-4	–	1	1
5-9	1	1	2
10-19	1	–	1
20-29	4	1	5
30-49	11	3	14
50+	4	1	5
Total:	21	7	28

FIGURE 1

CHOLERA AND ACUTE DIARRHEA ADMISSIONS
TO THE CRL HOSPITAL BY MONTH OF ONSET
JUNE 1963 - JULY 1965

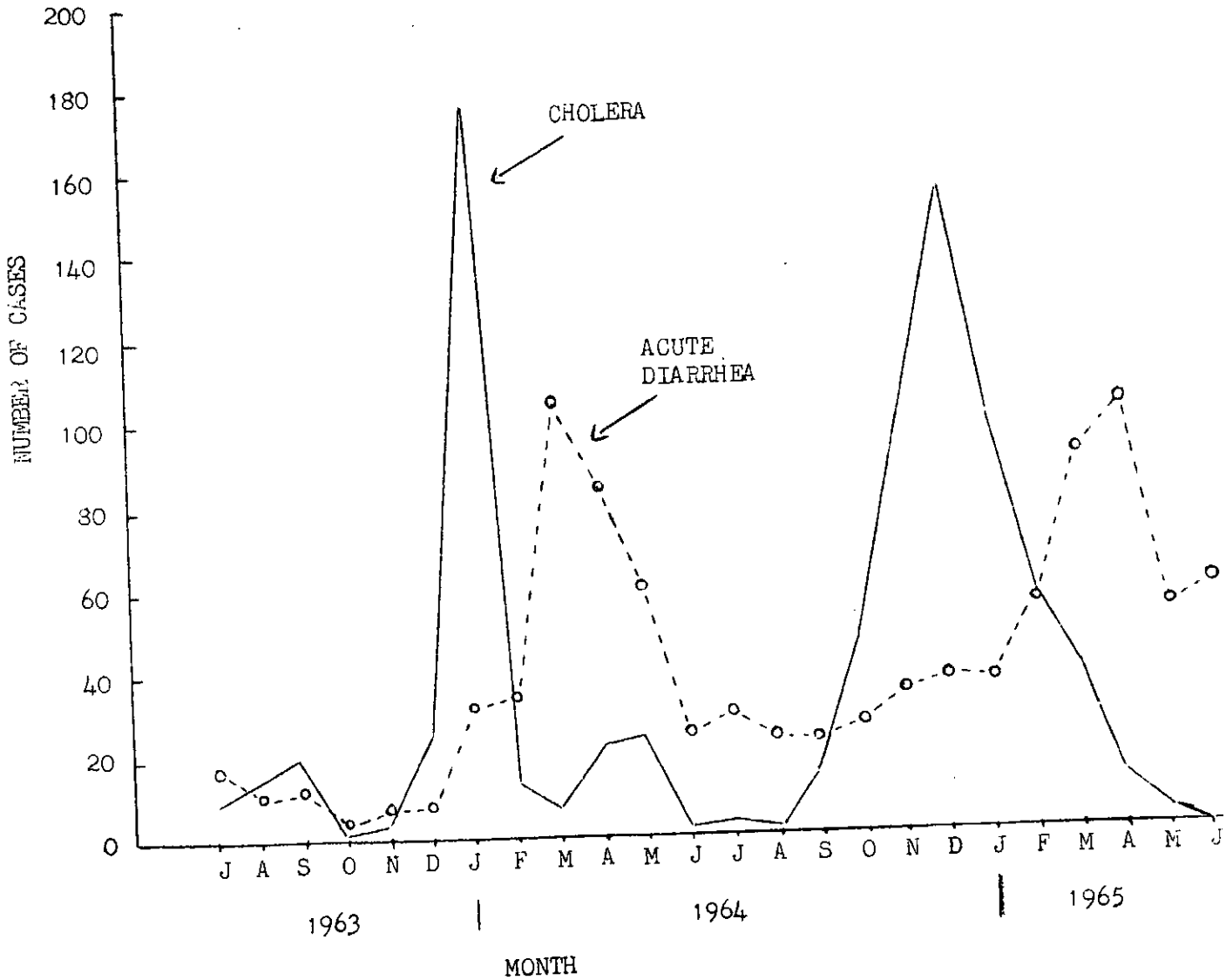


FIGURE 2

FREQUENCY DISTRIBUTION OF
537 CHOLERA CASES BY AGE.

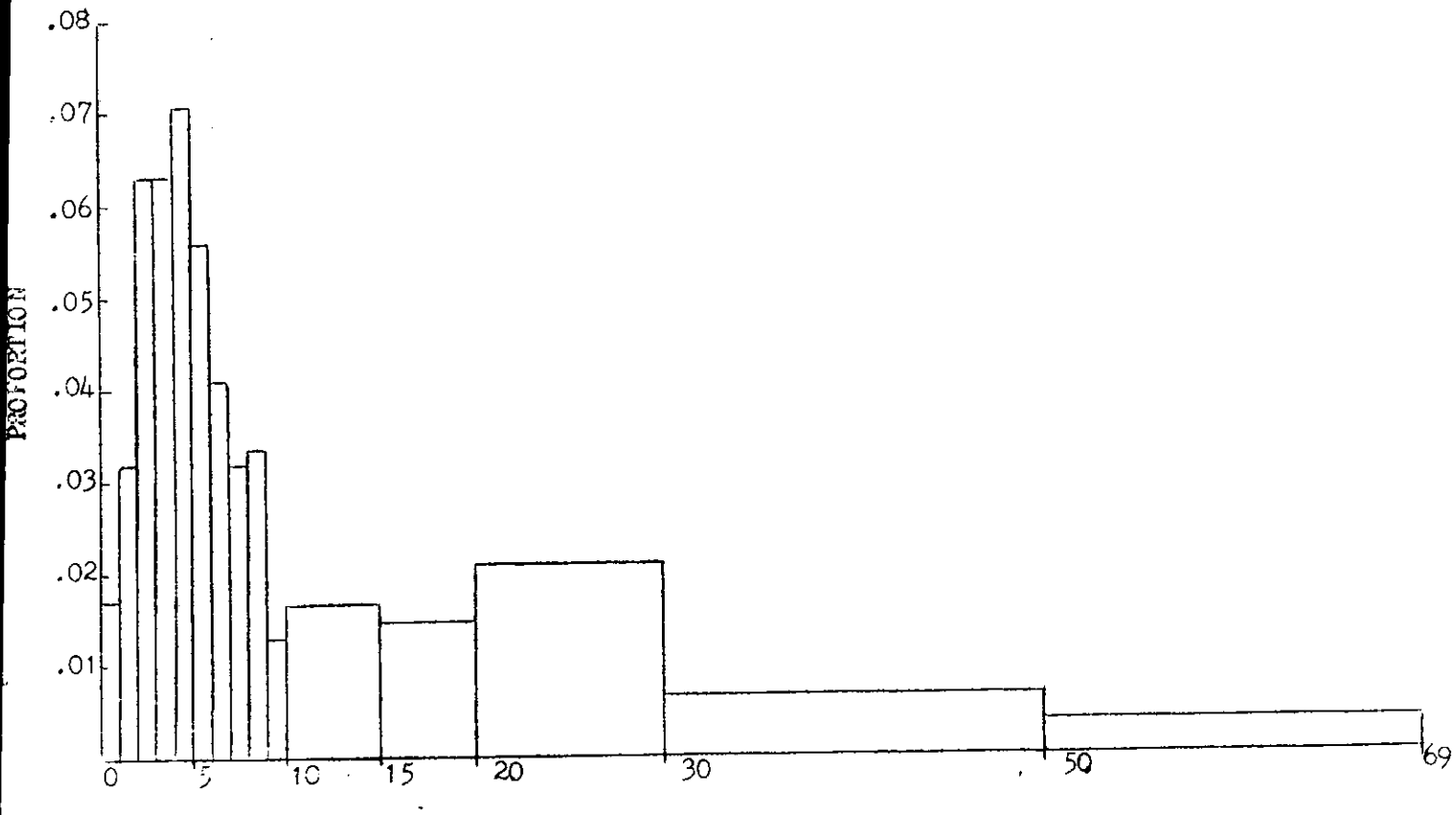


FIGURE 3

FREQUENCY DISTRIBUTION OF 587
ACUTE DIARRHEA CASES BY AGE

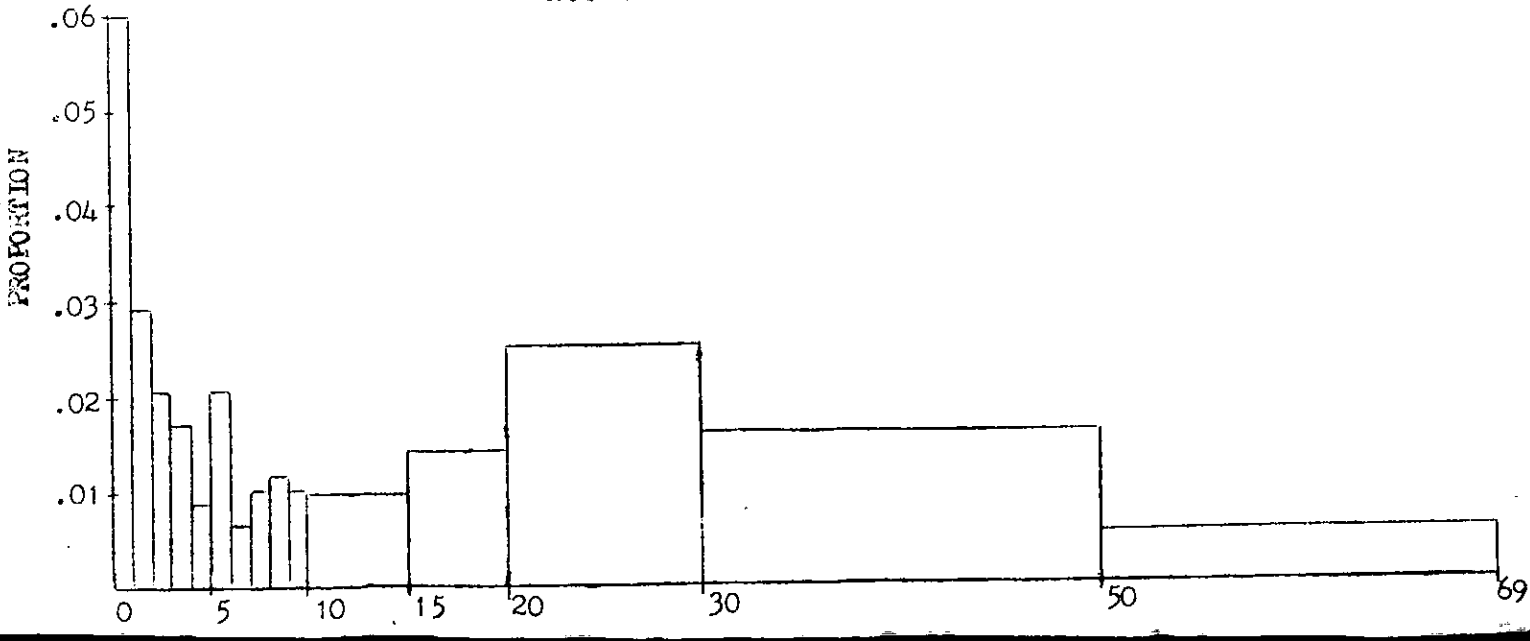


FIGURE 4

CHOLERA CASES BY WEEK OF ONSET DACCA MUNICIPALITY AND SELECTED THANAS

JULY 1964 - JUNE 1965

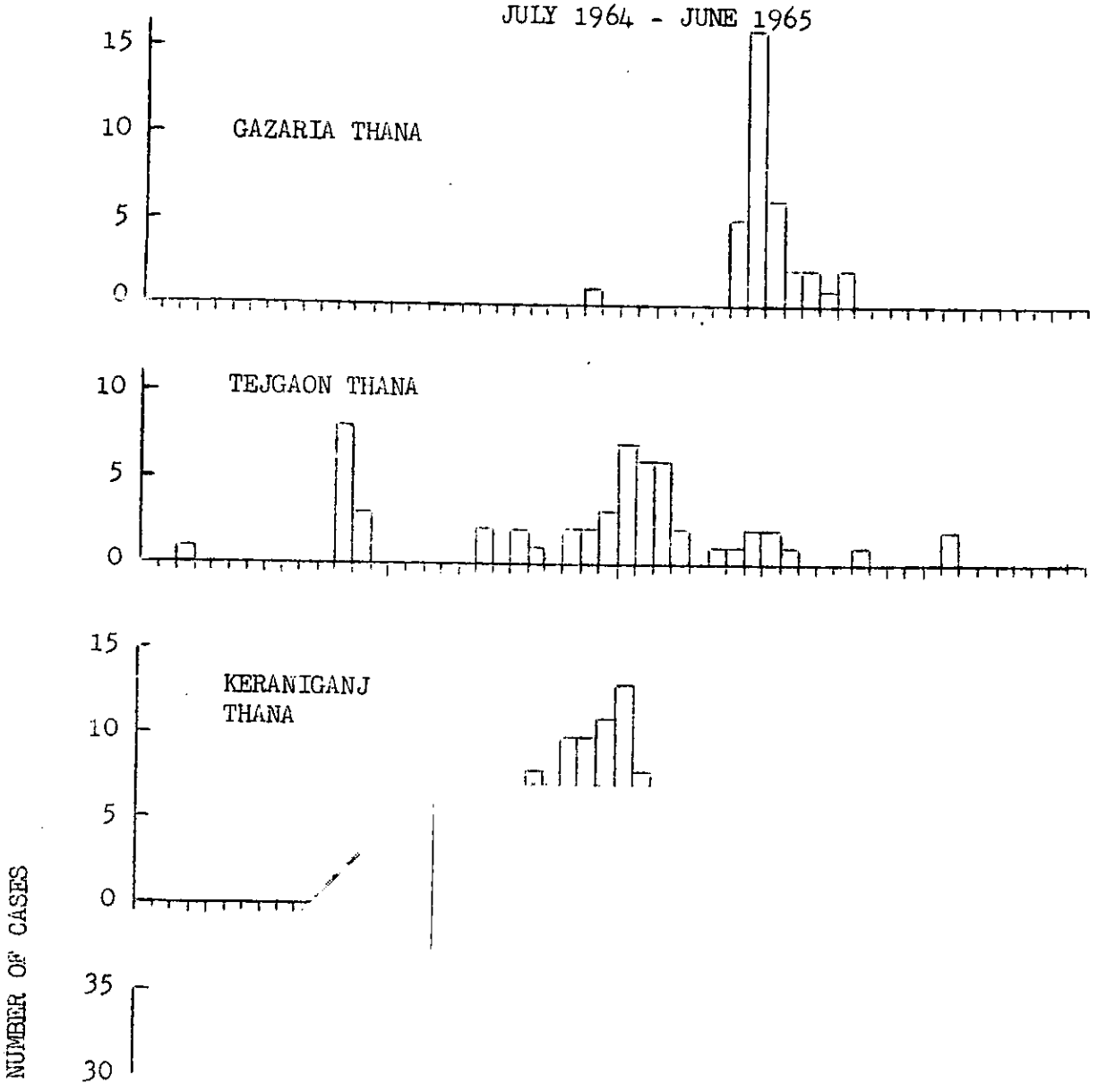


FIGURE 5

RAYER BAZAR OUTBREAK
CASES BY DAY OF ONSET

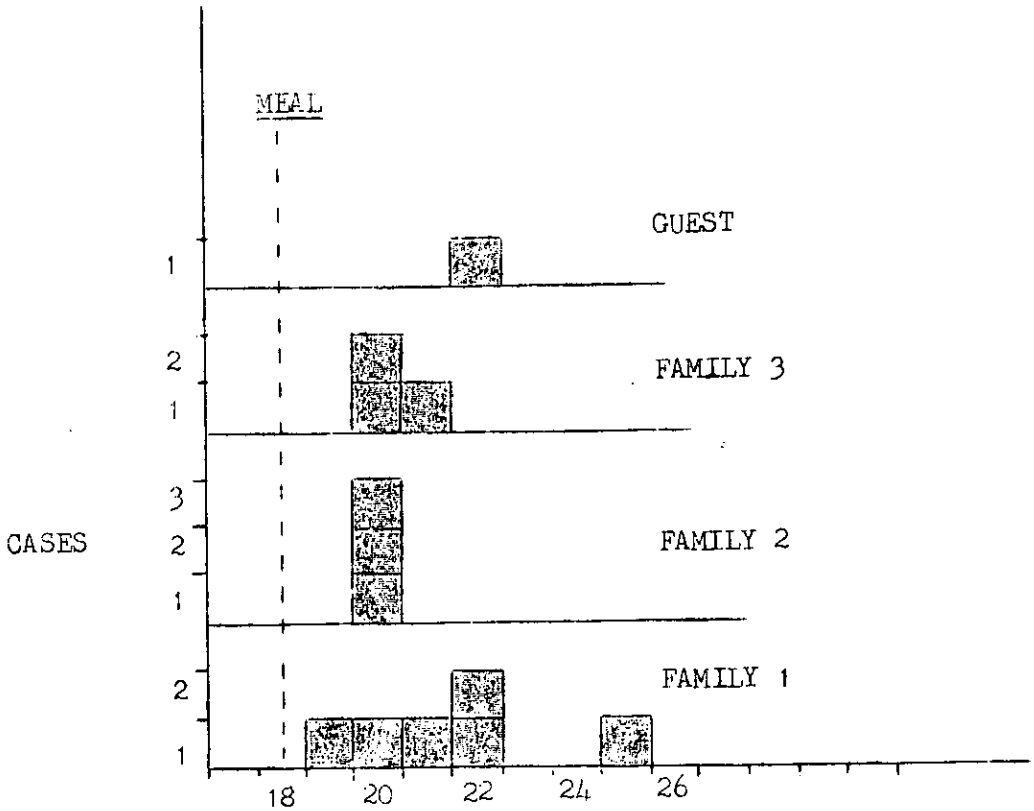
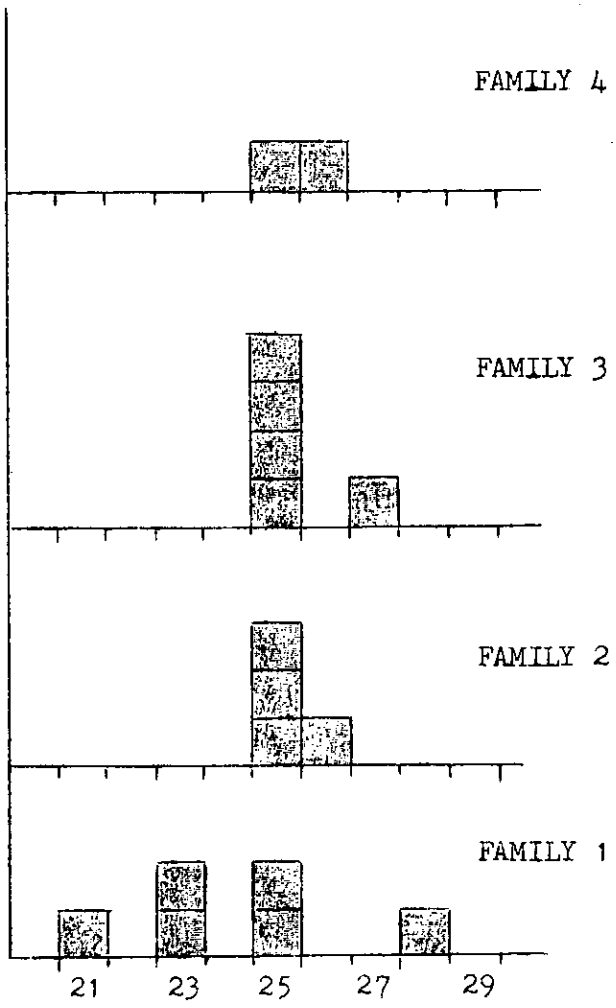


FIGURE 6

DHALKANAGAR LANE OUTBREAK
CASES BY DAY OF ONSET.



NOVEMBER 1964

FIGURE 7

INTERVAL BETWEEN ONSETS OF INDEX AND SECONDARY CASES IN 58 FAMILIES WITH MULTIPLE CHOLERA CASES.

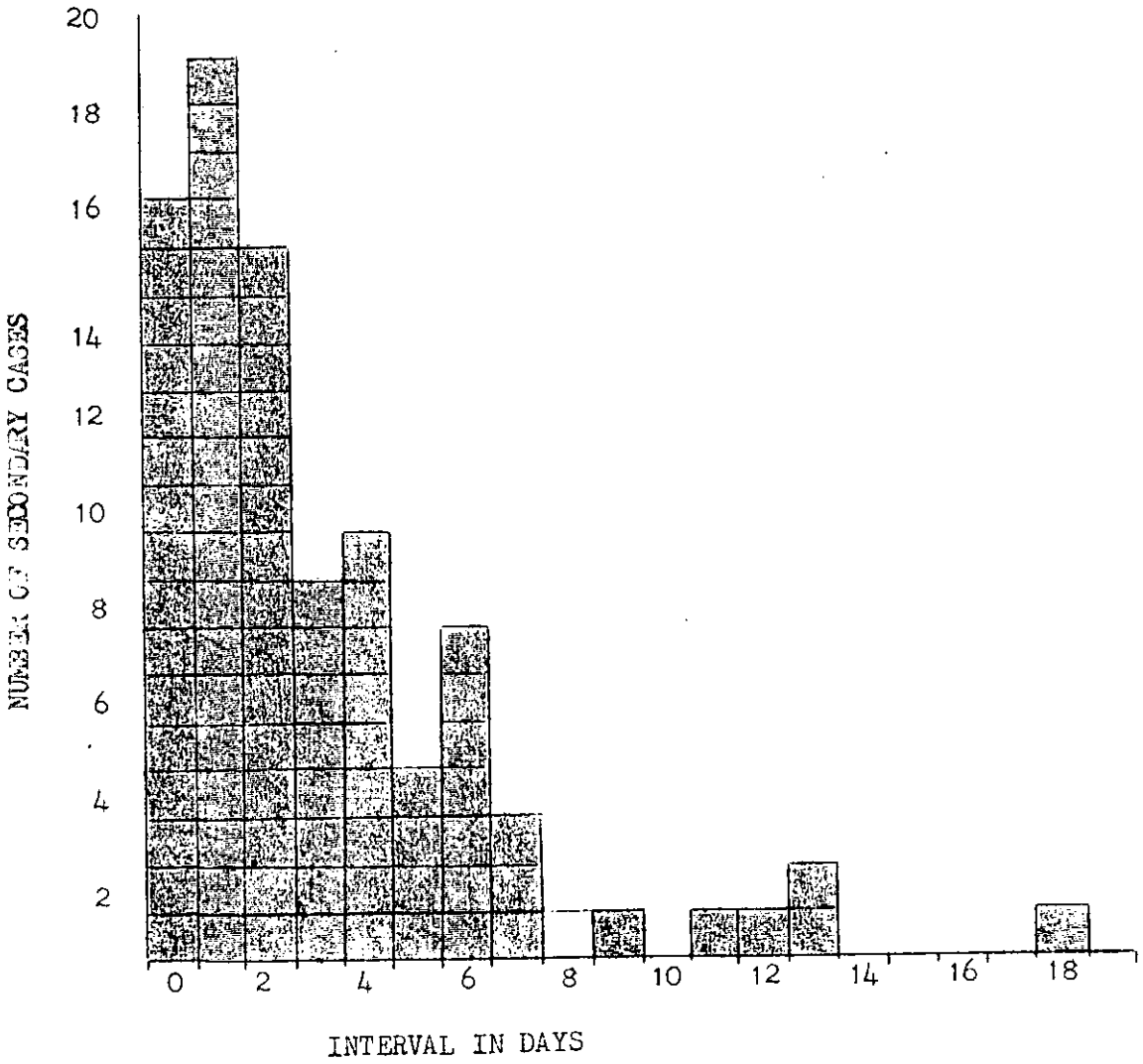
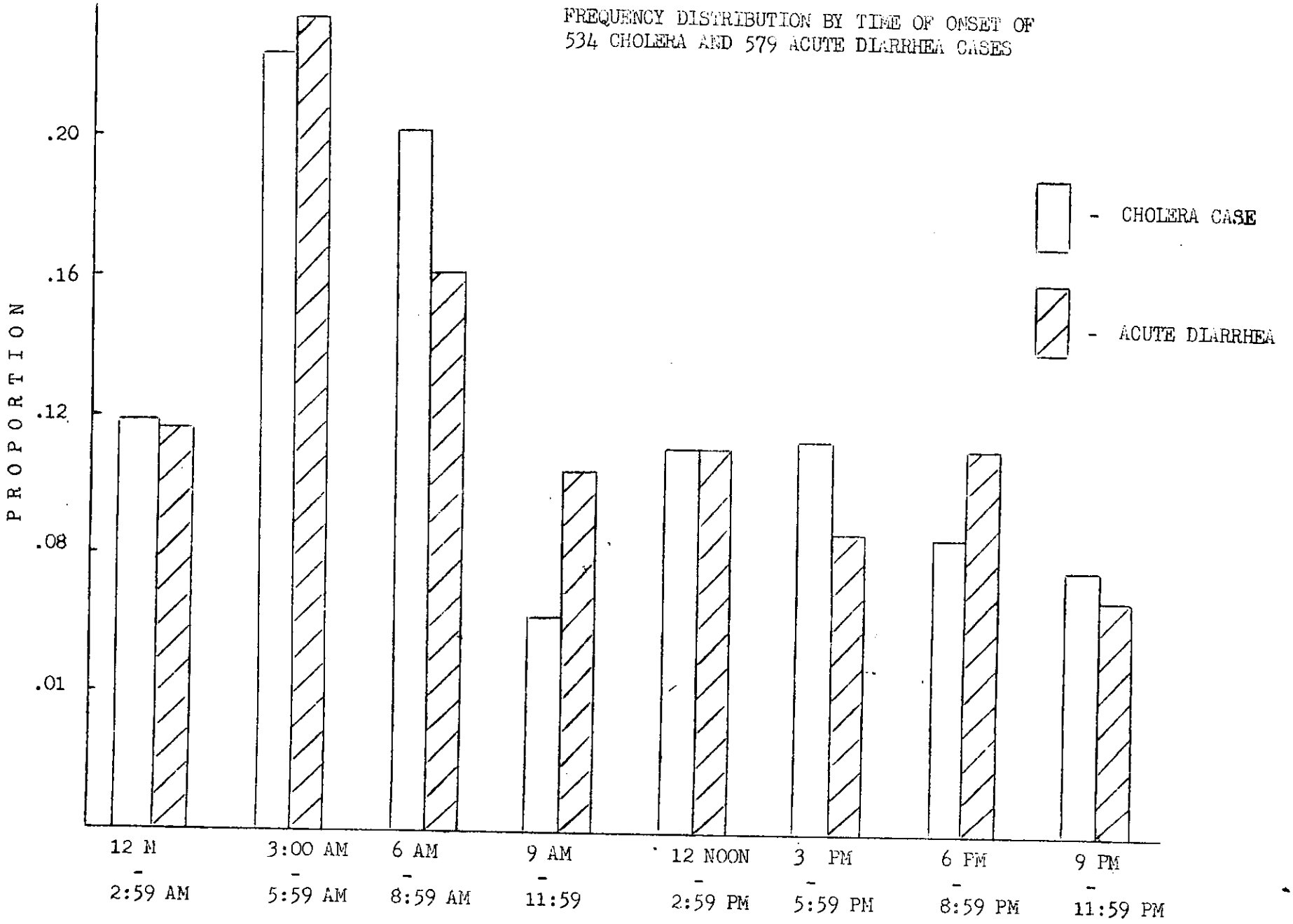


FIGURE 8

FREQUENCY DISTRIBUTION BY TIME OF ONSET OF
534 CHOLERA AND 579 ACUTE DIARRHEA CASES



PRELIMINARY ANALYSIS OF A SEROLOGICAL SURVEY FOR CHOLERA ANTIBODIES IN THE MATLAB BAZAR VACCINE TRIAL POPULATION.

Wiley H. Mosley, Abram S. Benenson, Ranjit K. Barui, with Technical Assistance of Miss Anisa Saad, Manik Paul and Mrs. Sushama Pashi.

Implicit in the establishment of the cholera vaccine field trials by the Pakistan-SEATO Cholera Research Laboratory has been the selection of a defined population in rural East Pakistan, the allocation of the cholera vaccine and the control vaccines to the population in a random fashion, and then the continued surveillance to detect as far as possible every cholera case subsequently developing in the population. Thus, in Matlab Bazar, in September-November, 1963 a highly antigenic cholera vaccine (based on laboratory tests), and a typhoid vaccine control were given randomly to 14,064 persons in 23 villages. Again, in September-December, 1964 the same cholera vaccine, a tetanus toxoid control, and a purified Ogawa antigen preparation were given randomly to an additional 25,264 persons living in 35 other villages. The surveillance of these 39,328 persons since injection has confirmed the efficacy of the cholera vaccine in reducing the cholera case rate, even after 2 years, as compared to the controls, while the purified Ogawa antigen has shown only a minimal effect. Matlab Bazar can therefore be considered as having 5 defined populations which may be considered different as regards cholera based on the vaccines received, the intervals since injection, and the cholera case rates.

A serological survey was conducted in these Matlab Bazar vaccine trial populations during August-September, 1965, in order to examine several questions relating to the role of circulating antibodies and cholera. The control populations would provide information on the levels of cholera antibodies in various age groups in persons residing in an endemic area. By comparing the control and immunized populations, it would be apossible to determine whether these groups could be distinguished by the levels of circulating antibody present. More importantly, because of the highly reliable data on the incidence of cholera in these populations that is available, it will be possible to determine whether the differences in case rates that are related to age, and to the various vaccines are associated with corresponding differences in the level of circulating antibodies in these populations.

Methods:

Selection of the sample - The sample was drawn from the entire population of 39,328 originally vaccinated with no effort to exclude deaths or migrations which may have subsequently occurred. Instead, an allowance for 40% non-participation was made for such losses as well as for refusals. This obviated the problem of finding unreported deaths or migrations, and having to make substitutions in the course of the

(more)

survey. (It also permitted the survey to serve as a follow up sample census of the population.) Using the individual census cards, the population was stratified into the 5 vaccine groups, into the age groups[a] 0-4, 5-14, 15-29, and 30+, and into males and females, as indicated in Table 1. From each of these 40 strata, 50 persons were selected with the use of a random number table. First one 10th of the group was selected based on the last digit of the census number, and then 50 persons were selected based on the next to the last digit of their census number. This method of sampling insured proportionate representation from each village, and avoided familial aggregation of the sample. The 2000 persons selected were listed by census number, name, age, and sex, in order that they could be correctly identified in the field.

Collection of the specimens - All specimens were collected by one physician assisted by a nurse between July 26, and October 6, 1965. Finger-prick blood was collected in a 50 lambda (0.05 ml) capillary tube,* and immediately diluted 1:10 in 0.45 ml of saline in a screw capped vial. The specimen was labeled with the individual's census number, dated and placed in a portable field ice box. At the time of collection of the specimen, the history of any other cholera vaccine was rechecked.

Serological Methods - The specimens were submitted to the laboratory identified only by the census number, so that the age, sex and vaccine group of the individual was unknown to the technicians. Upon receipt by the laboratory, the cells were separated and the diluted sera frozen until titrations were performed. Titrations for vibriocidal and agglutinating antibodies were done by the microtiter techniques previously described (1, 2.) The lowest dilution of sera tested for antibodies was 1:20. Vibriocidal titers were determined on all sera collected. Agglutinating titers were determined on only the first 978 sera submitted.

Statistical methods - Geometric mean titers were calculated by assigning a value of 0 to titers of less than 1:20, 1 to titers of 1:20, 2 to titers of 1:40, 3 for 1:80, 4 for 1:160, etc. The levels of significance(p) for the differences between means was calculated using Students t-test.

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a Except where indicated otherwise, the age groups referred to throughout this preliminary analysis will be the age groups at the time of injection, 1 and 2 years prior to the survey.

* Microcaps, Drummond Scientific Co., Broomall, Pa., U.S.A.

Results:

Sera were collected from 1522 (76.1%) of the 2000 persons in the original sample. The number of sera collected from the 50 persons selected in each of the 40 age, sex, and vaccine groups is listed in Table 2. Only 2 individuals refused to give a specimen when contacted, 24 were dead, and the remainder were out of the locality at the time of the survey. The highest percentage of collections were made in the 0-4 age group, while the fewest collections were in the 15-29 age group, primarily due to young men at work away from Matlab.

Figure 1 combines the results of the vibriocidal titers of the 609 persons who were sampled from the 2 control populations to illustrate the distribution of antibodies found in various age groups in an endemic area. The age groups represent the age at the time of the survey; males and females are combined, as sex was not a significant factor influencing antibody level. The distribution curves of vibriocidal titers in each age group tend to be bimodal with the Ogawa and Inaba titers closely paralleling one another. In children ages 1-2 years, 82 to 86% had no detectable vibriocidal antibody (less than 1:20); with increasing age, vibriocidal antibody was detectable in a large proportion of the population so that by the age of 30 and over, 87 to 90% of the population had detectable titers, with a mode at 1:80. A similar trend of higher titers with increasing age is present for agglutinating antibody, however, since the agglutinating titers are about 3 to 4 fold lower than the vibriocidal titers, this resulted in 100% of the children having no detectable antibody (less than 1:20), and only 25% of adults having detectable agglutinating antibody.

In order to evaluate the effect of previous cholera injections on the antibody levels, the control populations were divided into two groups based on whether or not there was a history of any previous cholera injections. There was no significant difference in the distributions of the antibody titers among those who gave a history of immunizations as compared to those who denied ever receiving a cholera injection.

Figures 2 and 3 compared the distributions of vibriocidal antibody titers in the populations that received the cholera vaccine in 1963 and in 1964 with their corresponding controls. It is apparent that in each age group, the distribution of titers is higher in the immunized populations than in the controls, even in the population that received cholera vaccine in 1963, 2 years prior to the survey. It should be noted, however, that even among those receiving cholera vaccine the distribution of titers is much lower in children than in adults. Table 3 and 4 giving the distributions of the agglutinating antibody titers in the cholera vaccinated and control populations, show the same relationships discussed above for the vibriocidal titers, though at lower levels.

Figure 4 compares the distributions of vibriocidal titers in the population receiving the purified Ogawa antigen in 1964 with the corresponding controls. The distributions of titers are only slightly higher among those who received the Ogawa antigen, being most evident in the youngest and oldest age groups. Most interestingly, the distributions are higher for both Ogawa and Inaba vibriocidal antibody. Table 4 giving the distribution of agglutinating antibody for this population shows essentially no difference from the controls.

The geometric mean vibriocidal titers are given in Table 5 and 6 and are also illustrated in Figure 5, 6, and 7. Again, these data illustrate the increasing antibody level in all 5 populations with increasing age. Comparing the cholera vaccinated populations with their corresponding controls, reveals significantly higher titers among the immunized persons in all age groups, even in the population immunized 2 years prior to the survey. Of additional interest are the higher Inaba titers among the cholera immunized populations, both in comparison to the control population, as well as in comparison to the Ogawa titers in the same persons.

The geometric mean vibriocidal titers of the population receiving the purified Ogawa antigen were not significantly different from the control population, except in the oldest age group, where both Ogawa and Inaba titers were higher. The significantly higher Ogawa as compared to Inaba vibriocidal antibody titers among the persons receiving the purified Ogawa antigen in the 5-14 and 15-29 age groups appears to be primarily a reflection of a similar pattern in the control populations.

The geometric mean agglutinating titers are given in Table 7. Significance levels have not been calculated because the majority of persons had titers less than 1:20 but the pattern of higher levels with age, and of higher levels in the populations receiving cholera vaccine as compared to the controls can be seen.

Relationship of Vibriocidal Antibody Levels to Cholera Case Rates:

By continued surveillance of the 5 defined populations through the 1965-66 cholera season, having this baseline of serologic information, it may be possible to establish the relationship between the antibody levels of the population, and the cholera case rates. A retrospective examination of the 1964-65 cholera experience in these populations in the light of the present serological survey is of interest, however, as it suggests a relationship of vibriocidal antibody to protection against cholera. An assumption in this type of retrospective analysis, is that the population antibody levels determined in this survey (July-September, 1965) represent the levels at the time of the cholera epidemic the preceding year (November 1964 - March 1965). This assumption can be considered to hold reasonably well for the control groups as well as for the population given cholera vaccine in 1963, as a marked change in antibody level of the population between 12 and 24 months after injection is unlikely,

however, for the populations given cholera vaccine in 1964, immediately prior to the epidemic, the presently determined antibody levels one year after injection may not approximate as closely the levels one to 3 months after injection.

Table 8 gives the 1964-65 cholera case rates in each of the 5 populations by age, and serotype of infecting organism. Because there were very few Ogawa cases, the discussion will be restricted to the Inaba cholera experience. As Table 8 illustrates there was a reduction in the cholera case rates with age, irrespective of the vaccine group of the population. This suggests an inverse relationship with the proportion of the population with detectable vibriocidal antibody (titers of 1:20 or greater) which increased with age in all groups irrespective of the vaccine received. One further relationship can be seen, particularly in the populations injected in 1963. While the 0-4 age group that received cholera vaccine had a 45% reduction in case rate as compared to the 0-4 as controls, that case rate was 16 times higher than the unimmunized adults age 30+. Similarly, as regards vibriocidal antibody titers, while 52% of the 0-4 age group that received cholera vaccine in 1963 had detectable vibriocidal antibody as compared to only 36% of the 0-4 age controls, vibriocidal antibody was found in 95% of the unimmunized adults age 30+.

Figure 8 illustrates the relationship between the Inaba cholera case rates and the proportion of the population with Inaba vibriocidal titers of 1:20 or greater in the populations injected in 1963. It can be seen that for the control population, the reduction in attack rate (with age) is directly related to the percentage of the age group with vibriocidal antibody. Correspondingly, in the population given cholera vaccine, the reduction in attack rate in each age group as compared to the controls, is similarly directly proportional to the rise in vibriocidal antibody titer elicited by the vaccine in that age group.

Figure 9 illustrates the relationship between the Inaba vibriocidal titers, and the Inaba cholera case rates for the populations injected in 1964. The same general relationship between the reduction in the attack rates with age and the increase in the proportion with vibriocidal antibody can be seen, particularly in the control population. In the population receiving the cholera vaccine, there is a marked discrepancy in the 0-4 age group, in that case rate is much lower than would have been expected, based on the antibody level. One explanation is that the titers were higher in this age group at the time of the cholera epidemic, shortly after injection, but that since the injection represented the initial exposure to the antigen, the titers have fallen rapidly. If this were true, one will expect that the cholera case rate in this age group will be much higher in the 1965-66 epidemic, because the antibody titers are now lower. Evidence that this is a reasonable prediction is the fact that in the population given cholera vaccine in 1963, the cholera case rate in the 0-4 age group did indeed rise markedly in the second epidemic year, so that the vaccine effectiveness in this age group fell from 80%

the first year to 45% the second year, while the vaccine remained effective in adults.

In the population that received the purified Ogawa antigen in 1964. Figure 10 illustrates that the same general relationship between the vibriocidal antibody titer and case rate exists. Again the same discrepancy in the 0-4 age group discussed above can be seen. Of particular interest is the fact that in the 5-14 age group, where the case rate was almost identical to the control population, the vibriocidal titer was also almost identical to the control population. In the 15-29 year age group the complete lack of cases is unexpected, based on the antibody titer in the group, however, since the control population in this age group had only 4 cases, this difference is not significant.

Discussion:

The role of immunity, and therefore of vaccines, in protecting a population against cholera has been a subject of considerable debate through the years. The observation that endemic cholera is a disease of children has suggested that acquired immunity was a factor, however, lack of bacteriological confirmation of diarrhea deaths, has often led to the report of high "cholera" case rates in adults in endemic areas Likewise, though vaccines have been reported as effective in the past by some observers, others have reported no effect, and all of these reports have been criticized because of defects in the study design required in modern investigations.

The Pakistan-SEATO Cholera Research Laboratory has now conducted 2 cholera vaccine field trials in Matlab Bazar in rural East Pakistan, which have established the efficacy of immunization in the prevention of cholera. Thus it is clear that immunity plays an important role in risk of a population developing cholera. The serological survey was conducted on the vaccine trial populations to determine whether the age pattern of cholera was related to the level of immunity in the population, as measured by circulating antibodies, and further whether the effect of cholera vaccine on the case rate corresponded to similar effect on the population antibody level. The data presented has demonstrated in the control population the direct relationship between the higher vibriocidal antibody level and the lower case rate with increasing age. While this could be attributed to two independent variables both being correlated with increasing age, the fact that in the immunized population of the same age group, the fall in the case rate maintains the same relationship to the rise in the antibody level, suggests that these two factors are indeed truely related. Prospective observations on these populations through the 1965-66 epidemic year will provide the data to define more clearly this relationship.

There are several important implications of these observations. First, they suggest that circulating vibriocidal antibody is a protective antibody in cholera. It should be noted, that in the case of the population receiving cholera vaccine, this is vibriocidal antibody detected approximately 1 and 2 years after injection and thus it probably represents "7S" immunoglobulins, which are more persistent thatn "19S", however, this has not been studied. If this hypothesis is true, then standardization of vaccines could be based on the production of persistant vibriocidal antibodies by the product. This would be a more reliable procedure than the presently recommended mouse protection test.

Evidence suggesting that the mouse protection test may actually be a poor test for a standardizing vaccines is the failure to find significantly higher vibriocidal titers in all but the oldest age group in the population that received the purified Ogawa antigen, which has a high mouse protection activity. If this preparation produced primarily a "19S" antibody response, it is conceivable that it could give a high mouse protection titer, which involves an intraperitoneal challange of vibrios, and yet not produce significant protection to an intestinal challenge, as Feeley has suggested in the suckling rabbit model (3). In addition, the antibody response would be transient, and there would be no significant persistence of the antibody 1 year after injection.

Further questions regarding the relationship of immunological studies in animal models to the immunological response in man are raised by the relative Ogawa and Inaba potentencies of the vaccines used in the Matlab Field trials. The whole cell cholera vaccine has been shown in the mouse protection test to be more protective against an Inaba challange relative to an Ogawa challange (4), and this has apparently been reflected in the serological survey by the significantly higher Inaba relative to Ogawa vibriocidal titers in the populations receiving this product. On the other hand, there is the parodoxical observation that though the purified Ogawa antigen has high Ogawa specificity in the mouse protection test, and in the serological response produced in the rabbit(5), in the field trial in Matlab there was some protection against Inaba cholera, particularly in the youngest and oldest age groups. Likewise, in the serological survey conducted 1 year after the administration of this product, the slight increase in vibriocidal antibody, seen primarily in the youngest and oldest age groups, is similar against <u>both</u> Ogawa and Inaba antigens!

The serological survey also raises a practical point regarding effective cholera immunization of a population. The relationship between the vibriocidal antibody titer and case rate suggests that in order to reduce case rate to less than 1 per 10,000, 95% or more of the population must have a vibriocidal titer of 1:20 or greater (as determined by the present microtiter technique). In the two vaccine trials that have been conducted in Matlab Bazar, only a single injection of the vaccine was given, and furthermore, children ages 2 to 12 received only ½ the adult dose, and children under 2 only 1/4 the adult dose. It is apparent, that while these doses were effective relative to the unimmunized population

in the same age group, the children receiving the cholera vaccine were actually poorly protected relative to unimmunized adults. With the role of immunization in the prevention of the cholera case established, and with the present study suggesting the relationship of the level of circulating antibodies to immunity, the next problem in cholera immunization may be practical, rather than theoretical; how to administer a cholera vaccine of known potency to a population, particularly to children who have never had experience with the antigen, in order to get uniformly high, and presumably protective vibriocidal antibodies over a long duration. The Ludua vaccine study (7) has been designed to provide information on this question as a foundation for future vaccine trials.

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7. Mosley, W.H., 1965 Technical Committee Report.

TABLE 1

Distribution of Matlab Vaccine Trial Population by Age, Sex, and Vaccine status.

Age	Sex	Cholera Vaccine 1963	Typhoid Vaccine 1963	Cholera Vaccine 1964	Tetanus Toxoid 1964	Ogawa antigen 1964
0-4	M	652	620	712	791	834
	F	596	667	796	797	737
5-14	M	1294	1345	1484	1487	1465
	F	1085	1075	1396	1411	1366
15-29	M	497	558	560	547	612
	F	715	684	967	879	950
30+	M	980	1056	1144	1199	1147
	F	1134	1101	1291	1342	1344
Total:		6953	7106	8356	8453	8455

****

TABLE 2

Number of serological specimens collected from each age and vaccine group. (original sample was 50 in each group).

Age	Sex	Cholera Vaccine 1963	Typhoid Vaccine 1963	Cholera Vaccine 1964	Tetanus Toxoid 1964	Ogawa antigen 1964
0-4	M	38	38	45	47	44
	F	45	43	45	43	38
5-14	M	36	36	39	43	40
	F	37	37	41	47	38
15-29	M	22	23	23	25	30
	F	45	38	42	37	40
30+	M	27	32	36	34	34
	F	43	42	43	44	42
Total:		293	289	314	320	306

***

TABLE 3

Percent distribution of Ogawa and Inaba agglutinating antibody titers by age group in the population that received.

Cholera Vaccine September-November, 1963.

Age Group	Number of Specimen.	Antigen	Percent with titers (reciprocal of: <20	20	40	80	160	320
0-4	47	Ogawa	93.6	6.4				
		Inaba	93.6	6.4				
5-14	41	Ogawa	90.2	9.8				
		Inaba	87.8	12.2				
15-29	31	Ogawa	61.2	35.4	3.2			
		Inaba	71.0	22.5	6.5			
30+	32	Ogawa	62.5	37.5				
		Inaba	65.6	28.1	6.3			

Typhoid Vaccine September - November, 1963

Age Group	Number of Specimen	Antigen	Percent with titers (reciprocal) of: <20	20	40	80	160	320
0-4	40	Ogawa	100					
		Inaba	95.0	5.0				
5-14	28	Ogawa	100					
		Inaba	100					
15-29	27	Ogawa	74.1	25.9				
		Inaba	81.5	18.5				
30+	34	Ogawa	85.3	14.7				
		Inaba	85.3	11.2		2.9		

***

TABLE 4

Percent distribution of Ogawa and Inaba agglutinating antibody titer by age group in the population that received:

Cholera Vaccine September-December, 1964

Age Group	Number of Specimen	Antigen	Percent with titers (reciprocol) of: <20	20	40	80	160	320
0-4	67	Ogawa	100					
		Inaba	98.5	1.5				
5-14	56	Ogawa	73.2	23.2	3.6			
		Inaba	76.8	17.9	5.4			
15-29	48	Ogawa	47.9	33.3	16.7	2.1		
		Inaba	53.1	33.3	12.5	2.1		
30+	61	Ogawa	34.4	32.8	26.2	4.9	1.6	
		Inaba	31.1	36.0	24.6	6.6	1.6	

Tetanus toxoid. September-December 1964

Age Group	Number of Specimens	Antigen	Percent with titers (reciprocal) of: <20	20	40	80	160	320
0-4	65	Ogawa	98.4	1.6				
		Inaba	100.0					
5-19	66	Ogawa	93.9	4.5	1.5			
		Inaba	95.5	3.0	1.5			
15-29	45	Ogawa	73.3	22.2	2.2	2.2		
		Inaba	82.2	15.6	2.2			
30+	56	Ogawa	60.7	26.8	10.7		1.8	
		Inaba	66.1	19.6	8.9	5.4		

Ogawa purified Antigen. September-December, 1964.

Age Group	Number of Specimen	Antigen	Percent with titers (reciprocal) of: <20	20	40	80	160	320
0-4	56	Ogawa	94.6	5.4				
		Inaba	94.6	3.6	1.8			
5-14	59	Ogawa	88.1	11.9				
		Inaba	98.3	1.7				
15-29	43	Ogawa	65.1	27.9	7.0			
		Inaba	79.1	18.6	2.3			
30+	57	Ogawa	59.6	35.1	3.5	1.8		
		Inaba	66.7	21.0	10.5	1.8		

****

TABLE 5.

Geometric Mean Vibriocidal Titers by Age in the population that received cholera vaccine and typhoid vaccine - Sept. - Nov.1963, and levels of significance for differences between vaccine groups, and between Ogawa and Inaba titers within vaccine groups.

Age Group	Antigen	Typhoid Vaccine G.M.T.+SE	p Ogawa vs Inaba	Cholera Vaccine G.M.T.+ SE	p Ogawa vs.Inaba	p Cholera Vaccine vs.Typhoid Vaccine
0-4	Ogawa	0.85±.18	N.S.[a]	1.22± .15	N.S.	.054
	Inaba	0.72±.12		1.27± .16		.003
5-14	Ogawa	1.48±.17	N.S.	1.97±.19	.045	.025
		1.37±.16		2.36±.14		<.0001
15-29	Ogawa	2.39±.23	N.S.	2.94±.18	.004	.029
	Inaba	2.51±.23		3.61±.18		<.0001
30+	Ogawa	2.73±.17	N.S.	3.11±.18	.012	.060
	Inaba	2.91±.17		3.69±.19		.001

a. N.S. = not significant.

TABLE 6

III(e)

Geometric Mean Vibriocidal Titers by Age in the populations that received Cholera Vaccine, Tetanus Toxoid, and Purified Ogawa Antigen, September-December 1964, and the levels of significance for differences between vaccine group, and between Ogawa and Inaba titers within group.

Age Group	Antigen	Tetanus Toxoid GMT±SE	p Ogawa vs Inaba	Cholera Vaccine GMT±	p Ogawa vs Inaba	Purified Antigen GMT± SE	p Ogawa vs Inaba	p Cholera Vaccine vs Tetanus vaccine	p Purified antigen vs Tetanus vaccine
0-4	Ogawa	0.38±.10	N.S. *	0.64±.12	.020	0.55±.12	N.S.	.045	N.S.
	Inaba	0.22±.21		1.07±.18		0.44±.11		.001	N.S.
5-14	Ogawa	1.88±.17	.019	2.56±.16	N.S.	1.90±.17	.028	.002	N.S.
	Inaba	1.40±.16		2.75±.16		1.44±.16		<<.00001	N.S.
15-29	Ogawa	2.40±.20	N.S.	3.23±.18	.023	2.59±.16	.080	.001	N.S.
	Inaba	2.24±.21		3.75±.18		2.27±.16		<<.00001	N.S.
30+	Ogawa	2.54±.21	N.S.	3.75±.20	N.S.	2.93±.15	N.S.	<.0001	.043
	Inaba	2.55+.21		3.94+.18		3.18+.18		<<.00001	.001

* N.S. = not significant.

TABLE 7

Geometric Mean agglutinating antibody titers by Age in the Vaccine Trial populations.

Age Group	Antigen	Typhoid Vaccine 1963	Cholera Vaccine 1963	Tetanus Toxoid 1964	Cholera Vaccine	Purified Ogawa Antigen 1964.
0-4	Ogawa	0.00	0.06	0.02	0.00	0.05
	Inaba	0.05	0.06	0.00	0.01	0.02
5-14	Ogawa	0.00	0.10	0.08	0.30	0.12
	Inaba	0.00	0.12	0.06	0.29	0.02
15-29	Ogawa	0.26	0.42	0.33	0.72	0.42
	Inaba	0.19	0.35	0.20	0.65	0.23
30+	Ogawa	0.15	0.38	0.55	1.07	0.47
	Inaba	0.21	0.41	0.54	1.11	0.47

TABLE 8

Cholera Cases and Case rates per 1000 persons by age and serotype in each of the vaccine trial populations.

1964-65 Epidemic

Age Group	Serotype of Organism	Typhoid Vaccine 1963 Cases	rate	Cholera Vaccine 1963 Cases	rate	Tetanus Toxoid 1964 Cases	rate	Cholera Vaccine 1964 Cases	rate	Purified Ogawa antigen 1964 Cases	rate
0-4	Inaba	19	14.8	10	8.0	18	11.3	4	2.6	10	6.4
5-14	Inaba	18	7.4	5	2.1	16	5.5	7	2.4	14	5.0
15-29	Inaba	3	2.4	1	0.8	4	2.8	0	-	0	-
30+	Inaba	1	0.5	0	-	4	1.6	1	0.4	1	0.4
0-4	Ogawa	3	2.3	3	2.4	3	1.9	1	0.7	2	1.3
5-14	Ogawa	6	2.5	1	0.4	1	0.4	0	-	1	0.4
15-29	Ogawa	1	0.8	0	-	1	0.7	0	-	0	-
30+	Ogawa	0	-	0	-	0	-	0	-	0	-

III(e)

FIGURE 1

PERCENT DISTRIBUTION OF VIBRIOCIDAL ANTIBODY TITERS BY AGE GROUP IN THE POPULATIONS THAT RECEIVED TYPHOID VACCINE-1963, AND TETANUS TOXOID - 1964

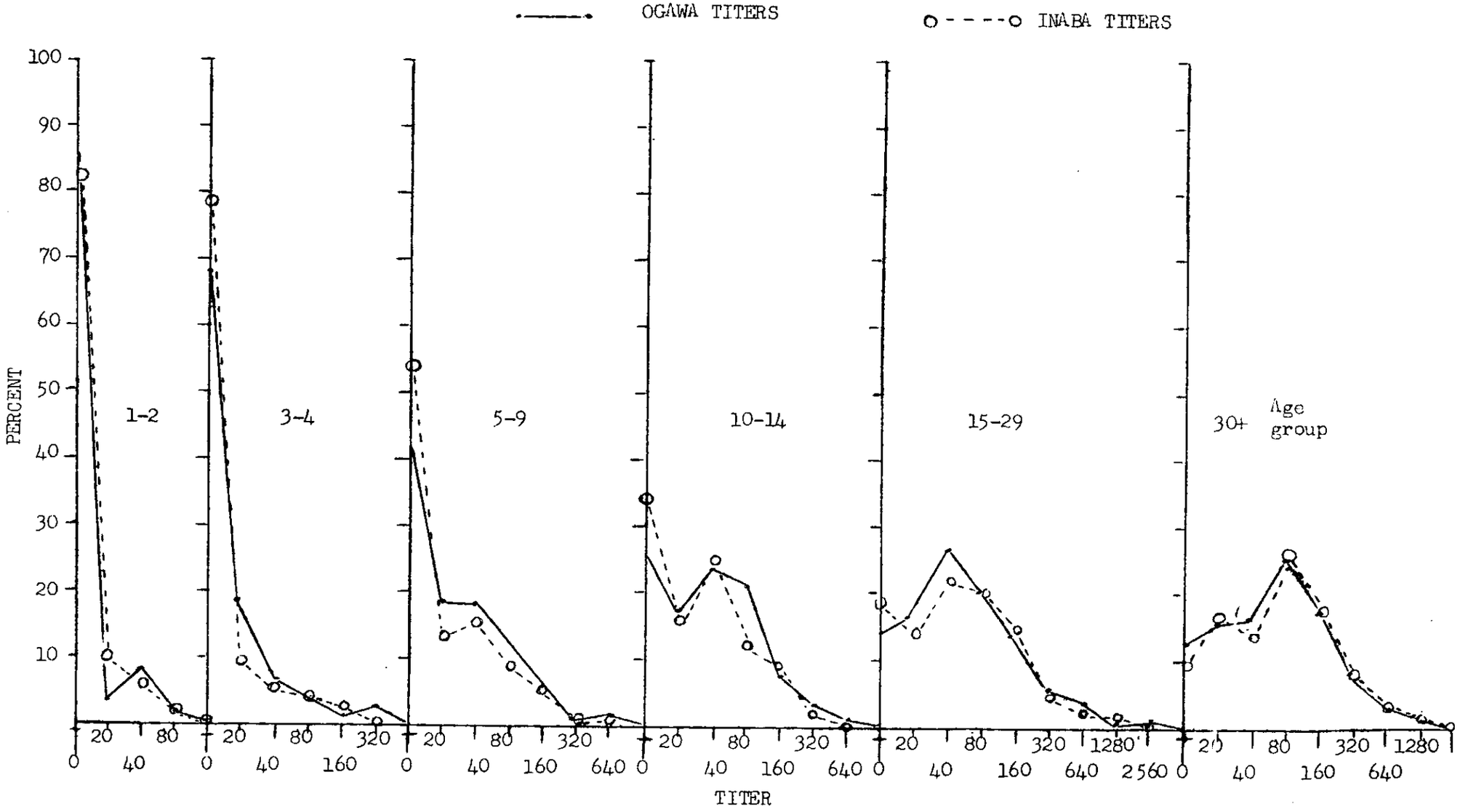
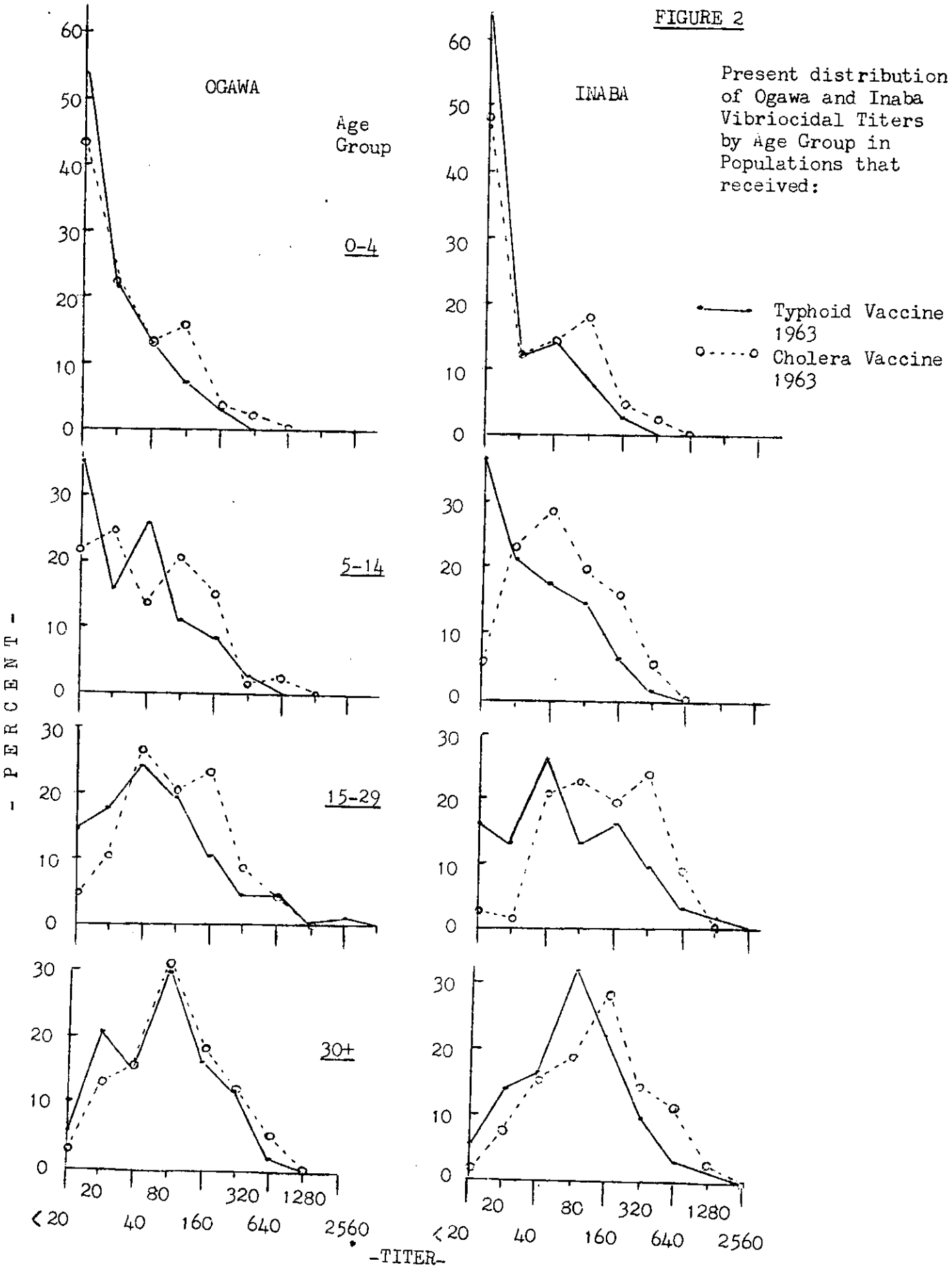
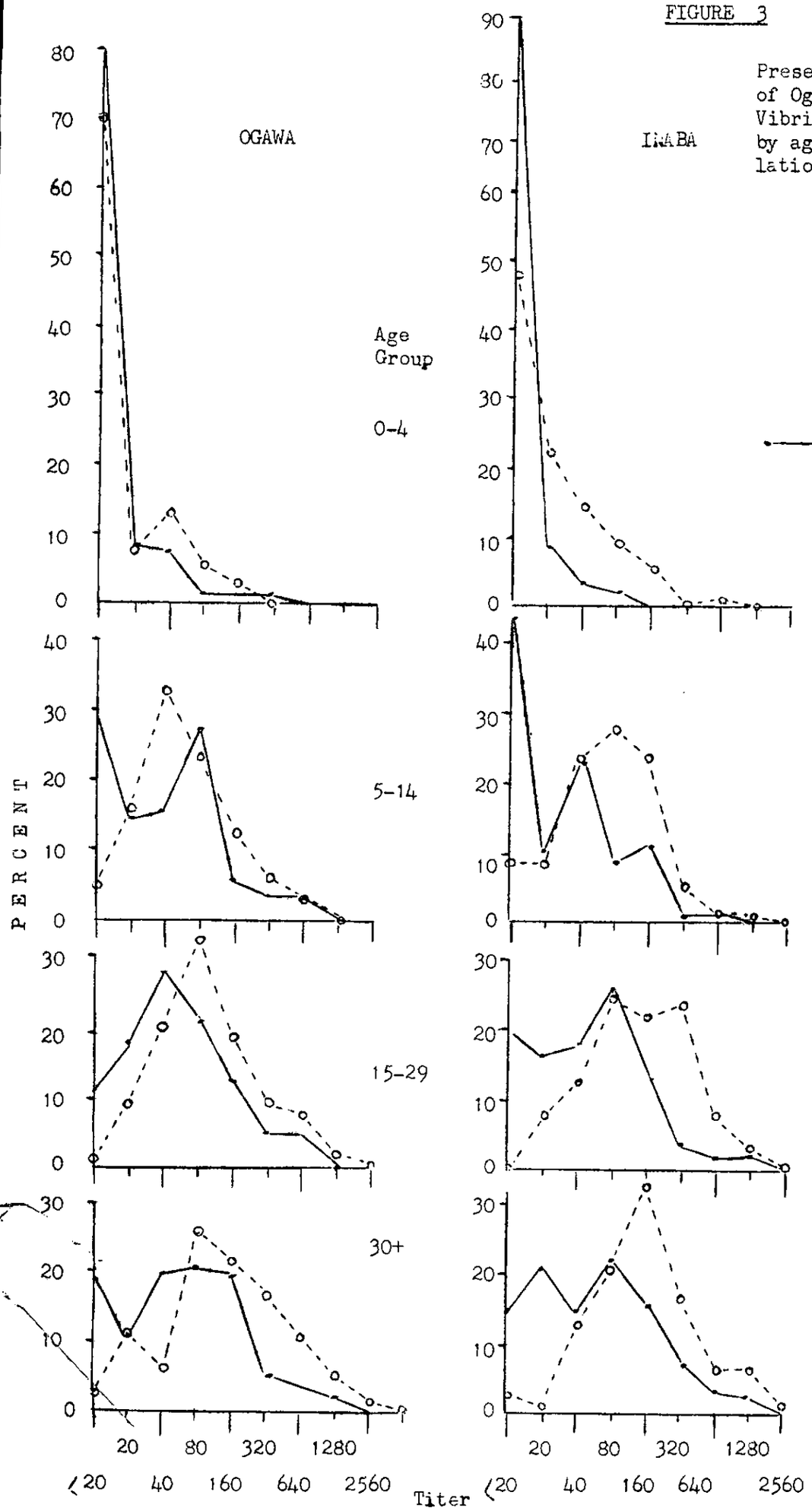


FIGURE 2



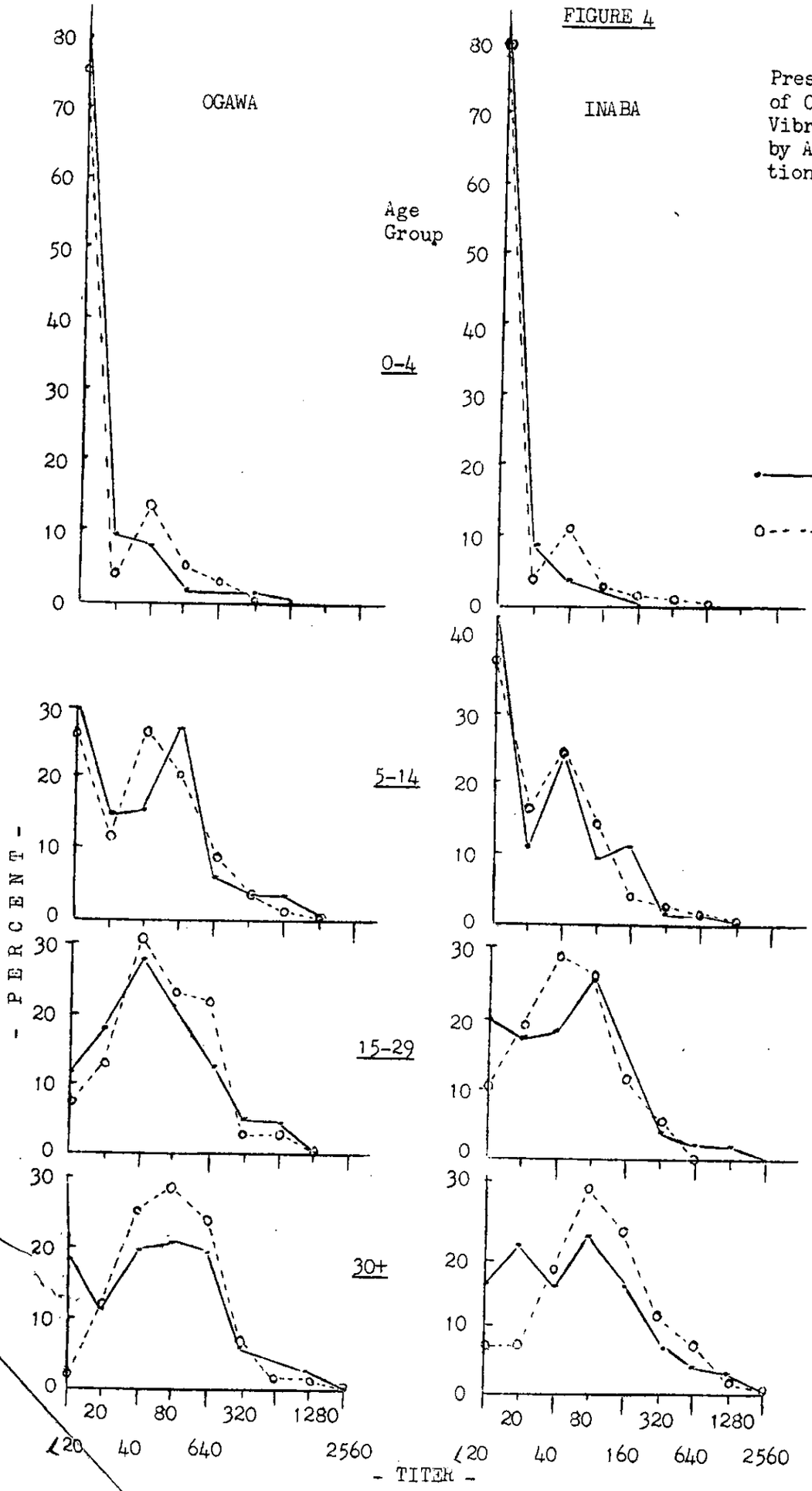
Present distribution of Ogawa and Inaba Vibriocidal Titers by Age Group in Populations that received:

FIGURE 3



Present distribution of Ogawa and Inaba Vibriocidal titers by age group in populations that received.

FIGURE 4



Present distribution of Ogawa and Inaba Vibriocidal Titers by Age Group in Populations that received:

FIGURE 5

GEOMETRIC MEAN VIBRIOCIDAL TITERS BY AGE GROUP IN THE POPULATIONS THAT RECEIVED CHOLERA VACCINE AND TYPHOID VACCINE IN 1963

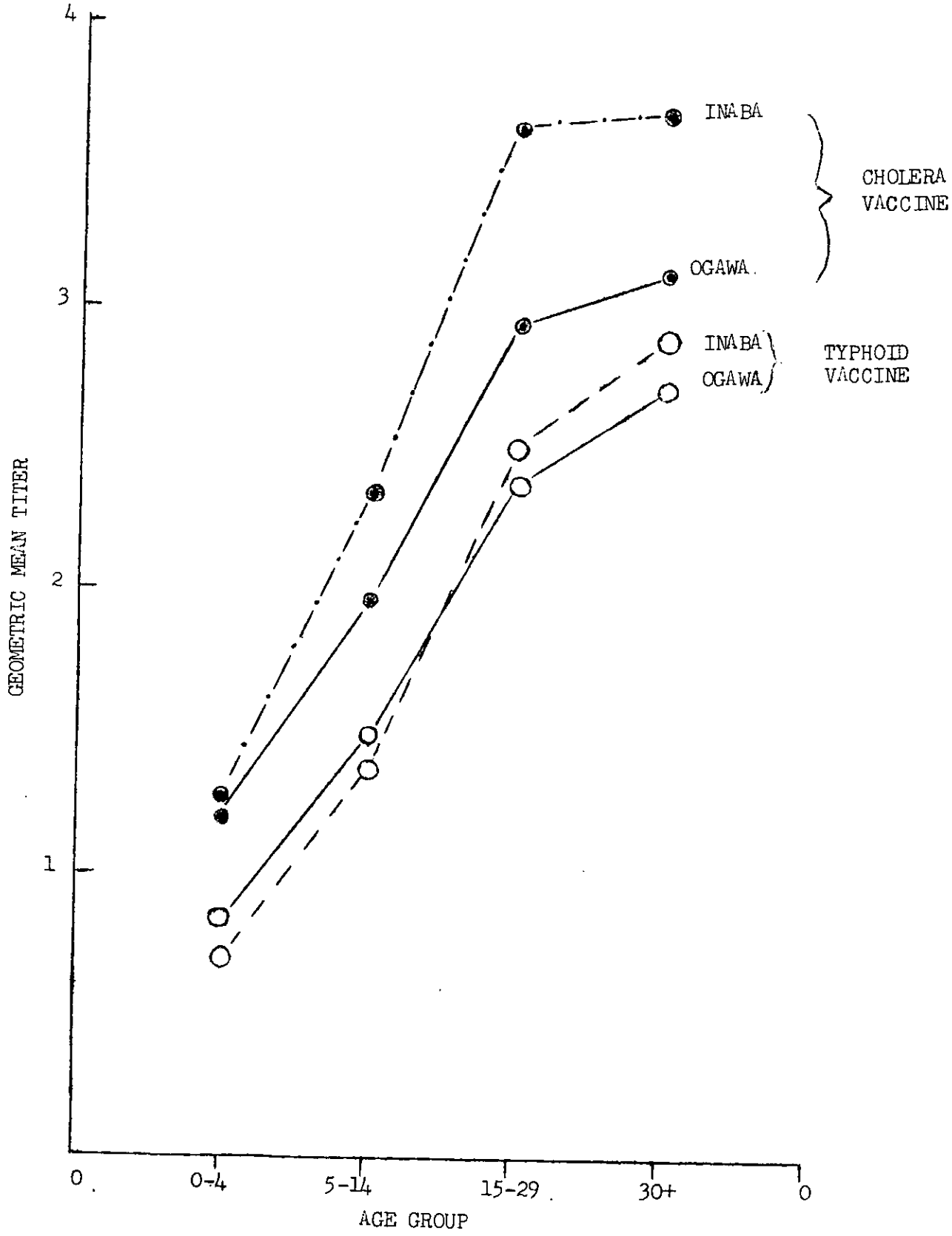


FIGURE 6

GEOMETRIC MEAN VIBRIOCIDAL TITERS BY AGE GROUP IN THE POPULATIONS THAT RECEIVED CHOLERA VACCINE AND TETANUS TOXOID IN 1964

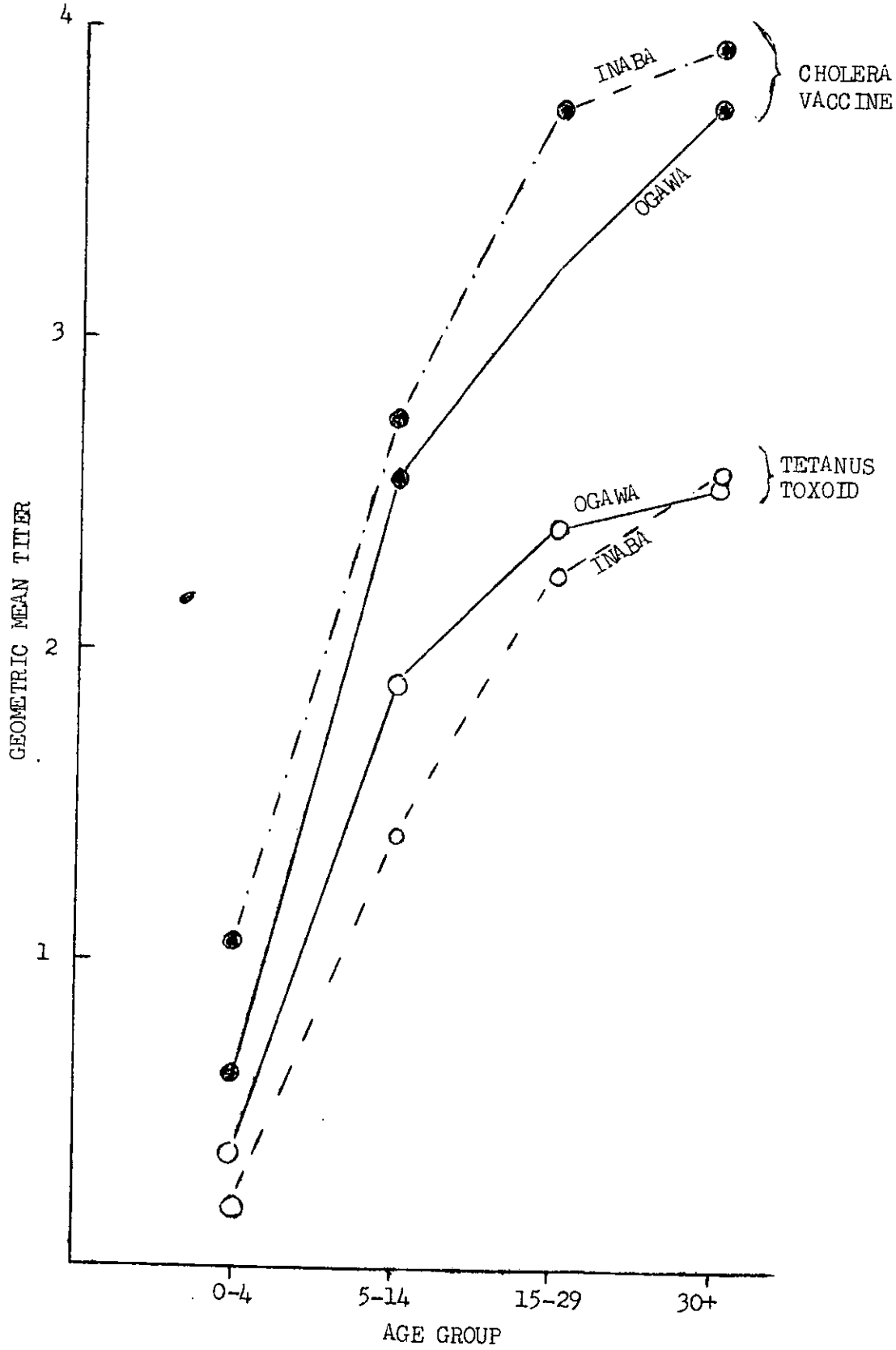


FIGURE 7

GEOMETRIC MEAN VIBRIOCIDAL TITERS BY AGE GROUP IN THE POPULATION THAT RECEIVED PURIFIED OGAWA ANTIGEN AND TETANUS TOXOID IN 1964

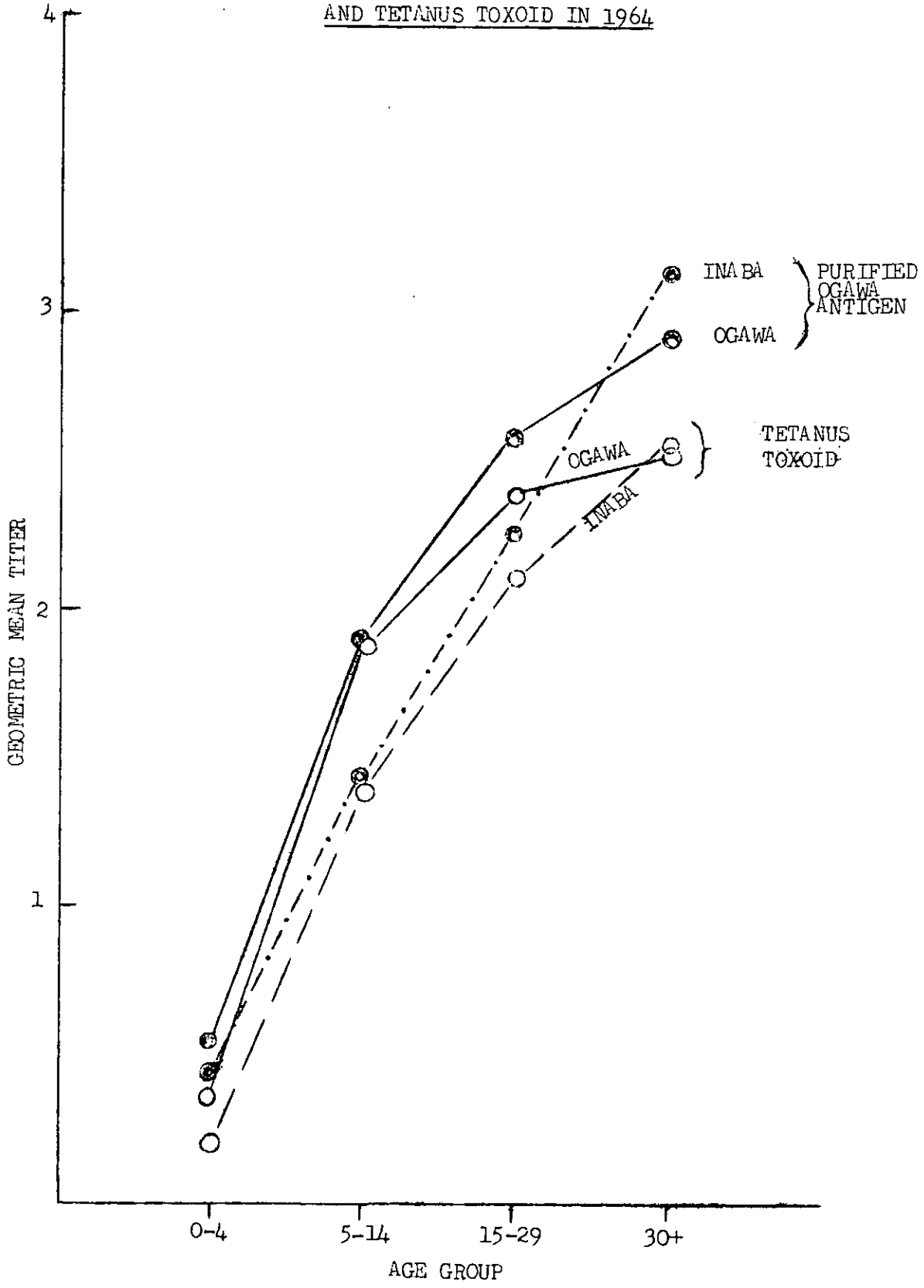


FIGURE 8

RELATIONSHIP BETWEEN INABA CHOLERA CASE RATE BY AGE GROUP TO THE PERCENT OF THE GROUP WITH INABA VIBRIOCIDAL TITERS OF 1:20 OR GREATER IN THE POPULATIONS THAT RECEIVED CHOLERA VACCINE IN 1963 AND TYPHOID VACCINE 1963

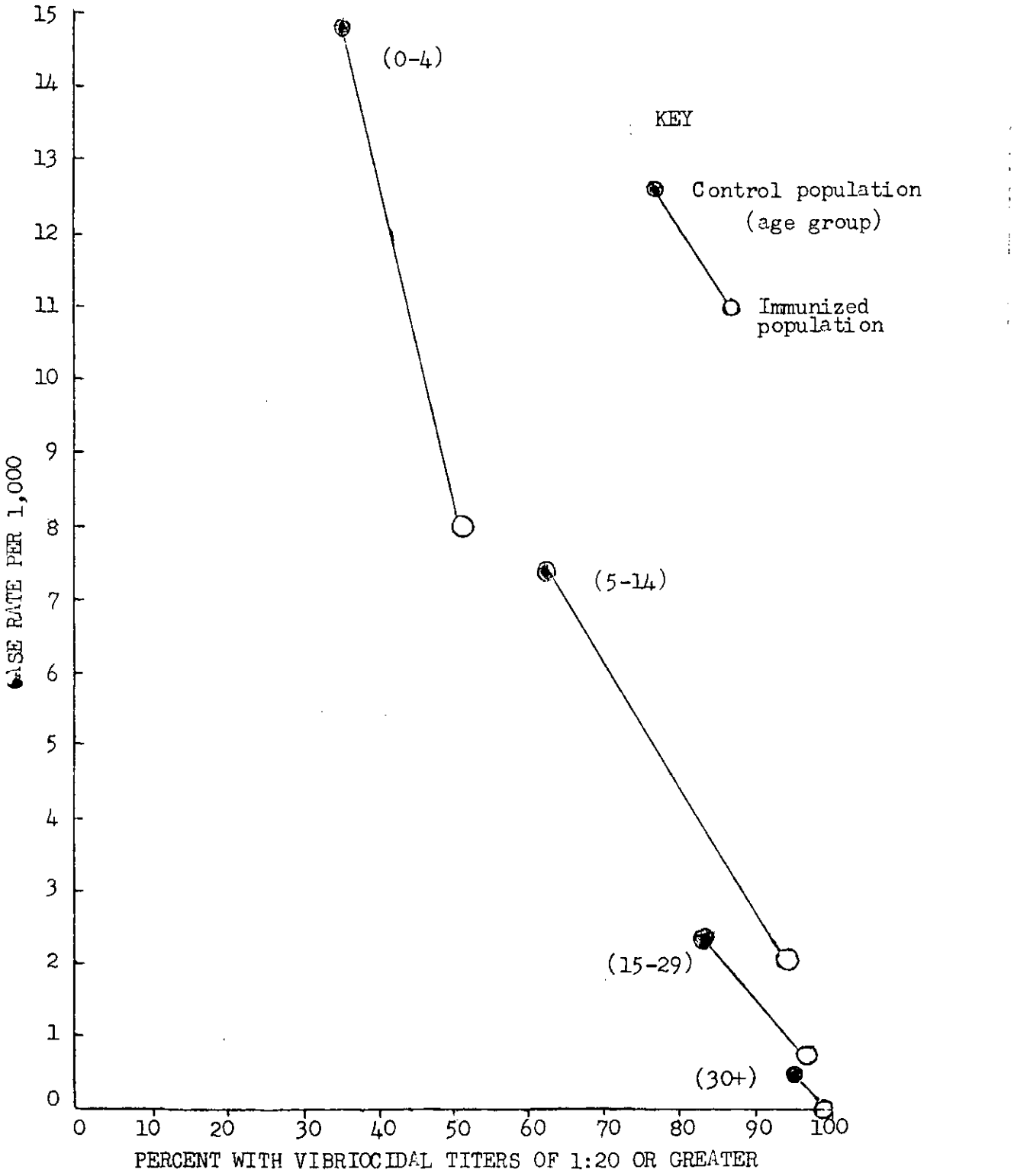


FIGURE 9

RELATIONSHIP BETWEEN INABA CHOLERA CASE RATE BY AGE GROUP TO THE PERCENT OF THE GROUP WITH INABA VIBRIOCIDAL TITERS OF 1:20 OR GREATER IN THE POPULATION THAT RECEIVED CHOLERA VACCINE IN 1964 AND TETANUS TOXOID 1964

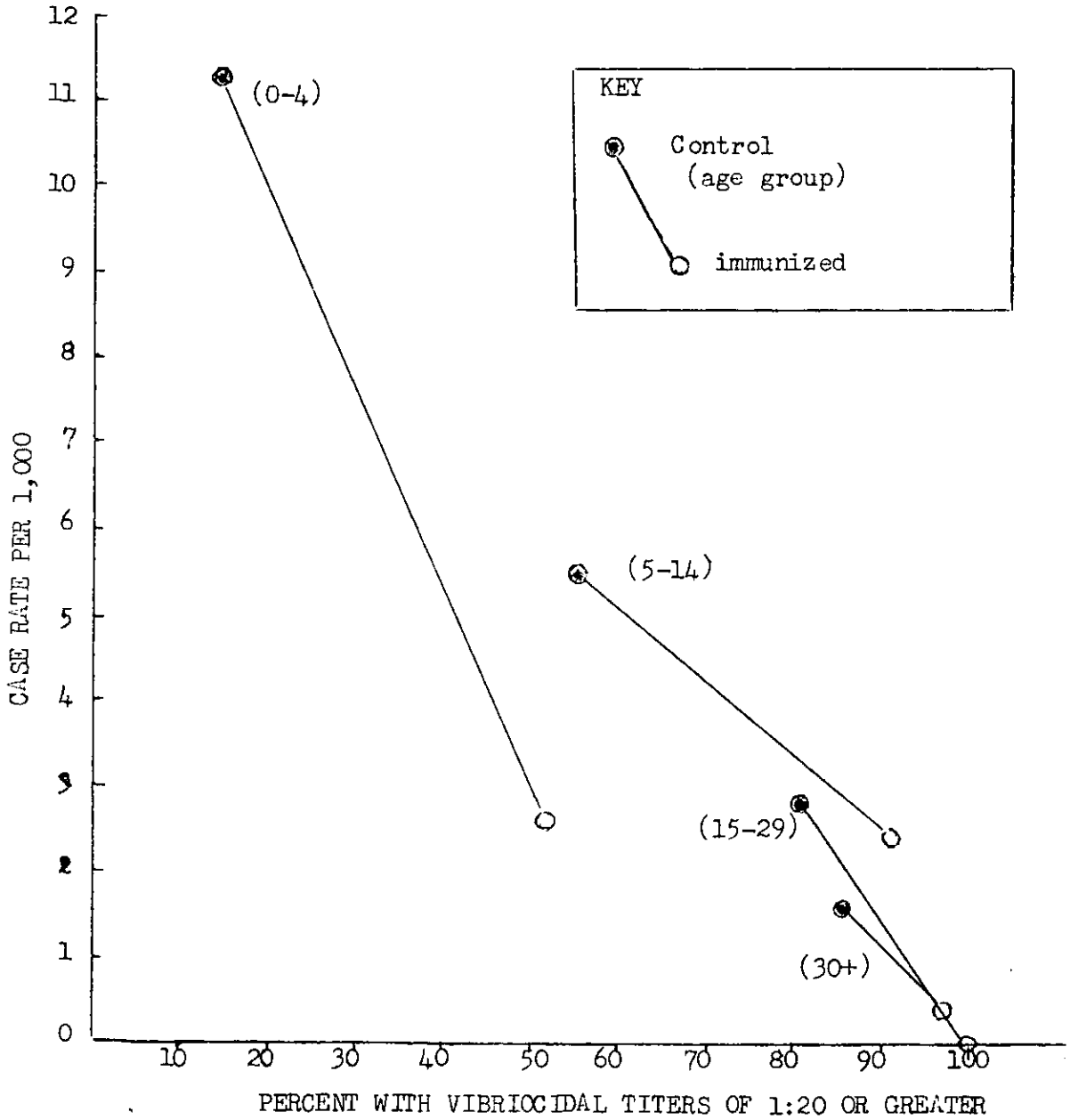
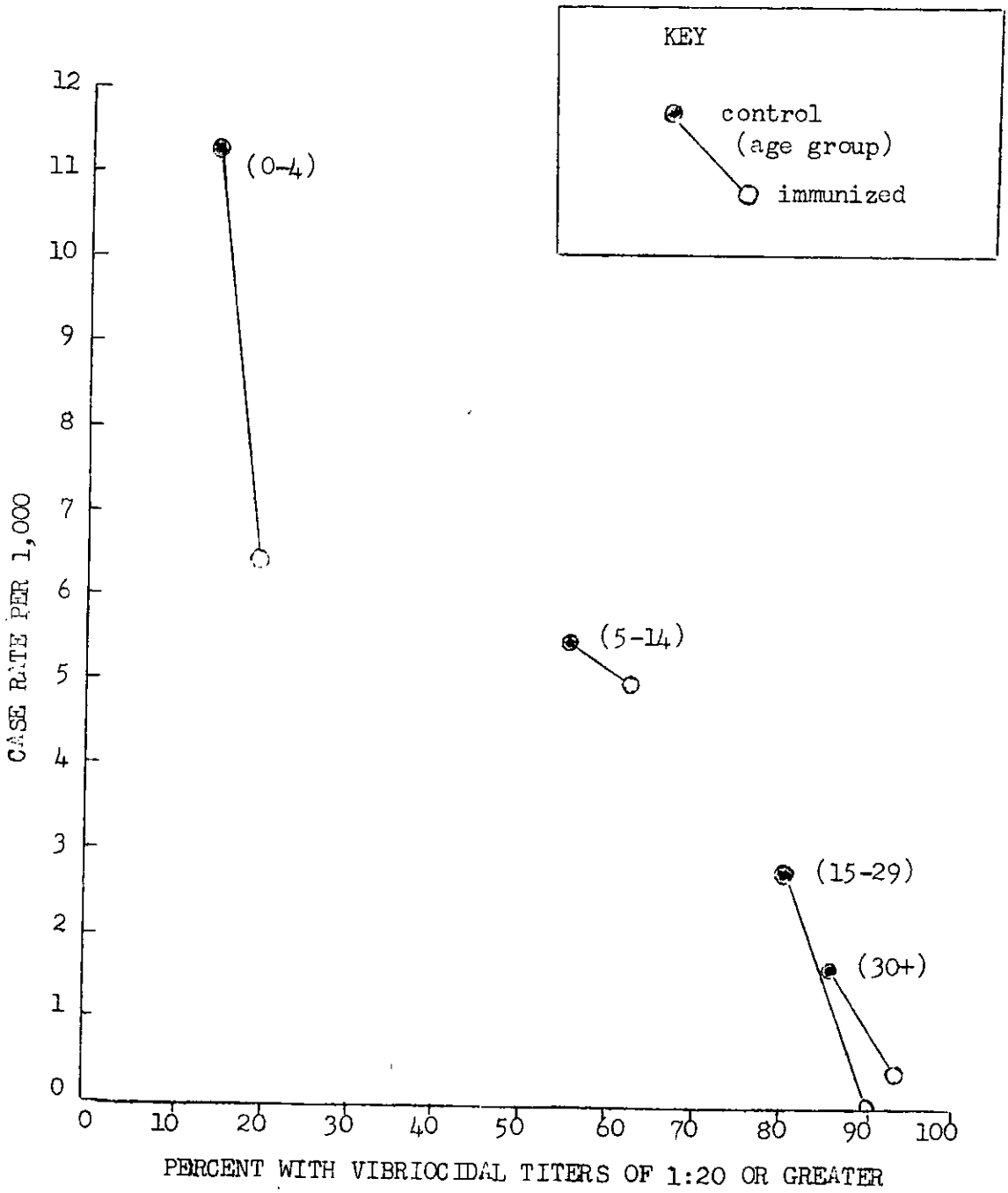


FIGURE 10

RELATIONSHIP BETWEEN INABA CHOLERA CASE RATE BY AGE GROUP TO THE PERCENT OF THE GROUP WITH INABA VIBRIOCIDAL TITERS OF 1:20 OR GREATER IN THE POPULATIONS THAT RECEIVED PURIFIED OGAWA ANTIGEN IN 1964 AND TETANUS TOXOID IN 1964



TRANSPLACENTAL TRANSFER OF VIBRIOCIDAL AND AGGLUTINATING ANTIBODIES

By Ranjit Barui and Wiley H. Mosley,

with technical assistance of Manik Paul, and Mrs. Shushama Pashi

An analysis of the 1964-65 CRL Hospital cholera experience revealed that clinical cholera was rare in children under the age of 1 year as compared to children in older age groups (1). This same pattern has also been found in the hospital experience in the field hospital in Matlab Bazar (2). Evidence that this paucity of infants with cholera does not merely represent the pattern of hospitalization is the fact that infants under 1 year contributed the highest proportion of cases of non-cholera diarrheas seen in the CRL Hospital (1). Further evidence pointing to some factors favoring resistance to clinical cholera in infants rather than merely lack of exposure to the organism is the fact that in the Family Studies conducted in 1964-65, 30% of the infants examined were found bacteriologically positive for V. cholera, however none had enough symptoms to require treatment (3).

The serological survey conducted in Matlab has revealed that over 80% of persons age 15 and over had vibriocidal antibody titers of 1:20 or greater, and further, that there seemed to be a relationship between vibriocidal antibody and protection against cholera (4). Since these findings indicated that the majority of women in the childbearing age would have vibriocidal antibody, it was of interest to determine if there was transplacental transfer of maternal antibody to the infant as a possible factor contributing to the resistance of infants to cholera.

Methods:

Specimens were collected from the maternity ward of Sir Salimullah Medical College Hospital. Only expectant mothers without complications, and who had not received any antibiotics were selected. At the time of delivery, immediately after the cord was cut, approximately 5 cc of cord blood was collected in a sterile tube. Subsequently, 5 cc of venous blood was collected from the mother. The cholera vaccine history of the mother was also determined.

After separation of the cells, agglutinating and vibriocidal titers of the paired maternal and cord sera samples were determined by the microtiter method previously described (5,6). The lowest dilution tested was 1:1.

Results:

Paired maternal and cord blood specimens were collected from 20 individuals. All the mothers had both vibriocidal and agglutinating antibodies. The correlation tables showing the relationship between the maternal and cord antibody titers are given in Table 1. The majority of the cord blood specimens had vibriocidal antibody, generally with titers about 3-fold lower than the maternal specimen. The agglutinating titers in the mothers were much lower, and since the cord blood sample had about a 3-fold lower titer, very few of the infants had detectable agglutinating titers. The maternal, and correspondingly the cord blood titers were not significantly influenced by the cholera vaccine history.

Discussion:

While this study has demonstrated the transplacental transfer of vibriocidal and agglutinating antibodies against V.Cholera, the possible role these antibodies play in protecting the infant against the disease remains undetermined. Evidence that infants do have a resistance to the disease has been presented, however, there are several facts which indicate that maternal antibody could not be the only factor (if a factor at all) in this resistance. For example, the same pattern of a lack of cases in infants in spite of infection being present has been observed in the Philippines in 1962.[7] While antibody studies were not done, that was a non-endemic area, and it is unlikely that there would have been transplacental transfer of protective antibodies in that situation. In addition, this resistance to cholera in infants seems to extend beyond the first year of life, far beyond the period when transplacental antibodies are known to be protective.

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TABLE I

RELATIONSHIP BETWEEN MATERNAL AND CORD BLOOD VIBRIOCIDAL AND AGGLUTINATING TITERS AGAINST OGAWA AND INABA ANTIGENS

A. Vibriocidal Titers

OGAWA

MATERNAL TITER \ CORD TITER	0	1:1	1:2	1:4	1:8	1:16
0						
1:1						
1:2	1	1				
1:4	1	2				
1:8	1		1			
1:16	2		1	1	1	
1:32	1					1
1:64			2	1	1	
1:128 or greater			1	1		

INABA

MATERNAL TITER \ CORD TITER	0	1:1	1:2	1:4	1:8	1:16	1:32
0							
1:1							
1:2							
1:4	2		1				
1:8	1	1					
1:16	1	1					
1:32			1		2		1
1:64	1		2	1			
1:128 or greater			1	1	2	1	

B. Agglutinating Titers

OGAWA

MATERNAL TITER \ CORD TITER	0	1:1	1:2	1:4
0				
1:1				
1:2	1	2		
1:4	6			
1:8	4	1	1	
1:16	1		2	
1:32	2			

INABA

MATERNAL TITER \ CORD TITER	0	1:1	1:2	1:4
0				
1:1				
1:2	1	1		
1:4	9			
1:8	2	1		
1:16	3	1	1	
1:32	1			

III (g)

PRELIMINARY REPORT

THE EFFECT OF DOSAGE AND IMMUNIZATION SCHEDULE ON THE ANTIBODY RESPONSE TO A PROVEN POTENT CHOLERA VACCINE: LUDUA VILLAGE VACCINE TRIAL

by: W. H. Mosley, M. Fahimuddin, R. Barui, with the assistance of S. Islam, M. Chowdhury and K.A. Aziz.

General Purpose

To determine the effect of increasing the dosage and administering two doses of a proven potent cholera vaccine on the duration and persistance of vibriocidal and agglutinating antibodies in children with little or no previous exposure to cholera antigen.

Background Data

A single injection of the cholera vaccine used in this study had been proven effective in protecting against cholera in two controlled field trials in Matlab Bazar (1). In examining the results of the vaccine field trials in detail, however, it becomes apparent that even though the cholera vaccine reduced the attack rate by 70 to 80% in each age group, in the first epidemic year of both field trials, the attack rate in immunized children remained about ten times higher than in unimmunized adults. Furthermore, during the second epidemic year of the first field trial, the effectiveness of the cholera vaccine in the under 5 year age group fell from 80% to 45% while it continued to be effective in the older age groups (1).

A preliminary analysis of the serological survey conducted in the vaccine trial populations has provided a suggestive explanation for the age pattern of cholera seen in both the control and immunized populations(2). This survey demonstrated that in the control population there was an increase in the proportion of the population with vibriocidal antibody which directly correlated with the fall in the attack rate with increasing age. In the immunized populations, while the proportion with vibriocidal antibody was higher in each group than in the corresponding controls, antibody levels remained much lower in children than in adults. Thus the fall in attack rate with age in the immunized population

also correlated with the increase in the antibody titer with age plus the antibody response elicited by the vaccine. Pertinent to this study is the fact that children, who had the lowest mean level of antibody initially, even after immunization, continued to have a much lower mean titer than unimmunized adults, and correspondingly a higher cholera case rate.

The serological survey has thus suggested a direct (inverse) correlation between the cholera case rate of a population, and the vibriocidal antibody level of that population. It has further suggested that to ensure a high level of protection of the population(case rate of less than 1/10,000) over 95% of the population must have a vibriocidal titer of at least 1:20(with the microtiter technique used in this laboratory). The present study is an approach to this practical problem of how to administer a potent cholera vaccine to population so that uniformly high, and presumably protective, vibriocidal antibodies will develop and persist in all age groups.

Vaccine, Dosage, and Immunization Schedules:

The cholera vaccine used in this study is the same vaccine that has been used in the two previous vaccine trials. This vaccine has been selected because of its proven effectiveness in field trials, as well as because of the large amount of supporting laboratory data and field experience with it. Laboratory studies of this cholera vaccine have demonstrated it to be highly antigenic in terms of the mouse protection test. In addition it has been shown to contain from 2 to 3 times the bacterial mass of other standard cholera vaccines, and correspondingly is more toxic in the mouse toxicity test (3).

In the two vaccine field trials, the following dosages were used according to the vaccinee's age:

Age Group	Dosage
0 - 1 year	0.1 ml
2 - 12 years	0.2 ml
13 and over	0.4 ml*

*0.5 ml was initially used, but because of the high frequency of delayed local reactions in adults, this was reduced to 0.4 ml.

Both the results of the vaccine trials, and the serological survey have indicated that these small doses in children have produced a significant degree of protection, and a significant antibody response relative to the controls. The children are however inadequately immunized when their case rates and antibody titers are compared to adults. The reason for the poor response in children is not only due to a lower dosage, but, as the serological survey suggests, the large majority of children were probably receiving their primary challenge with the antigen. For the majority of the adults, this was a "booster" injection. The 0.4 ml dosage was apparently quite adequate as a booster, and recent studies have suggested that a much smaller dose of cholera vaccine would have satisfactorily produced a maximal booster response(5).

In considering the maximum tolerable higher dosage schedule that can be used on children, the following observations made here by Dr. Paul Joseph with this same vaccine in 1963 are pertinent (4). He injected several communities with the following dosage schedule:

Age	Dosage
0 - 1 year	0.25 ml
2 - 13 years	0.5 ml
14 and over	0.5 and1.0 ml

Immediate reactions, including pain and fever, were mild and not considered significant. (It is of interest that the typhoid vaccine control used in this study (0.5 ml to all ages) produced severe immediate reactions, requiring a reduction in dosage for children). A high frequency of severe delayed reactions, including local erythema, induration, pain and incapacitation developed in adults, including 5% of those receiving the 0.5 ml dosage, but none were reported among children. It was these severe reactions in adults that interfered with participation in the subsequent vaccine field trials.

Based on these observations, it was felt that 0.5 ml would be the maximal tolerable single dose for children, and that this would be equivalent to 1 to 1.5 ml of most commercial vaccines. This dose was reduced to 0.25 ml for children under 1 year. For adults, because of the high frequency of delayed reactions that was still observed at the 0.4 ml dosage in the previous trials, a further reduction to 0.25 ml was made to determine if this still elicited a satisfactory antibody response.

Since it is recognised that a single injection, even at a higher dosage is unlikely to give a satisfactory and durable antibody response in persons who have never had a previous exposure to the antigen, both single and divided dosage schedules were set up. The design was as follows:

Age Group	Dosage Category	First Dose	Second Dose
Under 1 year	Single high dose	0.25 ml	Control*
	Divided high dose	0.25 ml	0.25 ml
	Control	Control*	Control
1-9 years	Divided low dose	0.25 ml	0.25 ml
	Single high dose	0.5 ml	Control
	Divided high dose	0.5 ml	0.5 ml
	Control	Control	Control
10 and over	Single dose	0.25 ml	-
	Control	Control	-

* Control vaccine was tetanus toxoid

The response to vaccine is to be determined from paired serological specimens collected from the vaccinees. The first specimen has been collected prior to the first injection, and the second specimen will be collected between 6 and 12 months after the second injection.

Methods:

Ludua village (population 3006), adjacent to the Matlab vaccine trial area was selected for this study. Factors influencing the selection of this village were its size, the assurance of co-operation by the village leaders, and the fact that it could be incorporated into the longitudinal surveillance programme of the vaccine trial area. A census was conducted in this village in September, 1965, and each person assigned a census number.

The population was stratified into the 22 age and sex groups shown in Table I, in order to insure comparability of the vaccine group. An equal number from each of these strata was pre-assigned to each of the vaccine groups in a random manner based on the last digit of their census number. Serological specimens were to be obtained from all the vaccinees under age 5, a 50% random pre-selected sample of children ages 5 - 9, and a 20% stratified, random, preselected sample of all persons age 10 and over. The ditribution of

the population into each of the vaccine groups and the number selected for serology is given in Table II.

The cholera vaccine used in this study was designated Vaccine B, and the Tetanus Toxoid, Vaccine A. The vaccines were administered by jet-injector guns which were pre-set to deliver the required dose of the vaccine. Serological specimens were collected immediately prior to the first injection from a finger-prick according to the method previously described, for the serological survey.(2)

The first round of injections was given during the week of September 20th. through the 25th 1965. The second series was given during the week of October 17th through the 23rd, 1965.

Results:

The participation by the population of Ludua in the trial was good. As Table III indicates, 65% of those selected, completed the immunization schedule, and submitted a serological specimen. Only in the age group under 1 year, where the available population was small, will the number of individuals be inadequate to draw any conclusions following the second bleeding.

There was no immediate reactions of any consequence reported. While no systematic followup was made for delayed reactions, on a visit to the village the week following the first inoculation, 18 persons reported severe delayed local reactions. All 18 had received the cholera vaccine; 16 of the 18 were adults, 2 were children one aged 4 and one aged 8, who had received the 0.5 ml. dosage. Since a total of 784 adults had received the cholera vaccine, and 484 children had received the 0.5 ml dosage, then a minimal estimate of delayed reactions would be 2.2% in adults (to a 0.25 ml dosage) and 0.4% in children (to a 0.5 ml dosage).

On a return visit to the village the week following the second inoculation, only 3 persons reported delayed actions. These three were among the small group of adults that had missed the inoculation on the first round. No reactions were reported among children.

Discussion:

Followup serological examination of this population in 6 months will provide information on the antibody response elicited by a single large dose of cholera vaccine versus the same amount given in divided doses, versus 2 large doses of cholera vaccine. In addition observations on the antibody response of children to cholera vaccine can be determined. Further, the effect of pre-existing antibody on the antibody response of an individual can be

determined. The answers to all of these questions are important, particularly as they relate to cholera immunization practices in endemic versus non-endemic areas, and in adults versus children.

Though the original protocol called for a second serology prior to the second injection in order to study the serological response in more detail, the possibility of poor cooperation for the second injection led to the decision to omit this specimen. This change in the protocol will not effect the primary purpose of the study, which is to evaluate the vaccine dosages and schedules which will produce the most satisfactory serological response over a prolonged period of time. The information obtained from this study will be of value in the design of a future vaccine trial which should incorporate a vaccine schedule designed to give the maximum protection to all segments of the population.

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TABLE I

Ludua Village

Distribution of the population by age and sex.

Age group	Male	Female	Total
Under 1	27	34	61
1	46	53	99
2	55	65	120
3	48	56	104
4	75	50	125
5 - 9	307	289	596
10 - 14	206	164	370
15 - 19	127	100	227
20 - 29	147	268	415
30 - 49	280	323	603
50+	148	137	285
Unknown		1	1
Total	1466	1540	3006

TABLE II

Ludua Village

Distribution of the population by age and vaccine group

Age Group	Number	First Dose	Second Dose	Number for Serology
Under 1 year	20	B2	B2	20
	20	B2	A	20
	21	A	A	21
1 - 4 years	112	B2	B2	112
	112	B5	A	112
	112	B5	B5	112
	112	A	A	112
5 - 9 years	149	B2	B2	75
	149	B5	A	75
	149	B5	B5	75
	149	A	A	75
10+	950	B2	-	197
	951	A	-	187
Totals:	3006	(3006)	(1105)	1193

B2 = 0.25 ml cholera vaccine
B5 = 0.50 ml cholera vaccine
A = 0.50 ml tetanus toxoid

TABLE III

Ludua Village

Number of persons who completed immunization schedule and submitted first serological specimen.

Age Group	Dosage Schedule		Number of Persons	% participation
Under 1 year	B2	B2	3	15.0
	B2	A	8	40.0
	A	A	12	60.0
1 - 4 years	B2	B2	66	58.9
	B5	A	71	63.6
	B5	B5	76	67.8
	A	A	64	57.1
5 - 9 years	B2	B2	53	70.6
	B5	A	51	68.0
	B5	B5	48	64.0
	A	A	59	78.6
10+	A	-	131	66.4
	B2	-	135	72.1
Total:			777	65.1

B2 = 0.25 ml cholera vaccine
B5 = 0.50 ml cholera vaccine
A = 0.50 ml tetanus toxoid

PRELIMINARY REPORT RAYER BAZAR

COMMUNITY STUDY

by Wiley H. Mosley, Muslimuddin Khan, Ranjit K. Barui and Yousuf A. Saeed.

General Purpose:

Prospective observations on the patterns of cholera, diarrhea, births, deaths and migration in 1200 families in a suburban, low economic community.

Introduction:

Rayer Bazar located at the periphery of Dacca, about six miles from the Cholera Research Laboratory, is a low economic community, consisting of a mixed population of potters, laborers, hawkers, service workers, with a considerable number of transitory families. Studies in Rayer Bazar began in January, 1964 during a sharp outbreak of cholera in a refugee camp set up in the community during the communal disturbances. Those studies which consisted in collecting weekly rectal swabs, and observing the diarrhea morbidity in the cholera affected families, and in the control families for 17 months have been reported in the 1964 and 1965 Technical Committee Report (1,2). Altogether, 244 families, totaling 1744 persons were followed for some period during the 17 months of study. Though 24 subsequent cases of cholera occurred in Rayer Bazar during this time, none occurred in the families under previous surveillance, and thus one of the aims of the family studies, to detect the pattern of cholera and inapparent infection could not be fulfilled. With this background, it was decided to modify the Rayer Bazar study to include prospective observations on the entire community in an effort to further define the patterns of cholera cases and inapparent infections, and the relationship with various environmental factors.

Background:

Rayer Bazar extends for approximately 2 miles along a single paved road and is approximately ¼ mile in width (Map, Figure 1). There are over 1100 families living in this area, with a total population of 6,500 persons. The majority of the houses are constructed with mud walls and mud floors and a tile or sheetmetal roof and usually consist of 1 or 2 small rooms. Most families have an individual dug well near the house for drinking water and for cooking. For bathing and washing purposes, the people along the western side of the road use the adjacent canal, tanks and ditches indicated on the map. Most of the population on the eastern side of the road use the individual dug wells. Only the most primitive privies are available for waste disposal.

Rayer Bazar is surrounded by low lying areas, much of which is flooded during the rainy season. During the dry season, water remains only in the tanks shown in the map and in two canals which arise from the Buriganga River and come to the western margin of the village. The lowland through which the canals run, virtually turns into a brick field every winter, with 20 to 30 brick fields starting production each employing 50 to 150 workers. These workers come from surrounding districts; many stay in Rayer Bazar during the dry season, and most do their daily marketing in Rayer Bazar.

There is also a considerable amount of boat traffic to Rayer Bazar during all seasons of the year. The boats, come from the surrounding districts to purchase the pottery made in Rayer Bazar for distribution throughout the province, as well as to bring in wood and mud for the kilns.

Methods:

The entire community was censused during August, 1965. In preparation for the census, the community was divided into 20 areas designated alphabetically A to T (Map, Figure 1). Each household was then identified by the Area letter, given a serial number, and located on a detailed map. At the time of the 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. 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 x 7 inch card giving the household number, location, and name, age and sex, and individual number of each member. These cards are used by the field teams and clinic personnel in identifying each individual for coding purposes.

Field surveillance is carried out by 2 teams, each consisting of a sanitary inspector and a female field assistant, who visit each house in the community once weekly. If a diarrhea is reported, a rectal swab is collected and information regarding the individual's census number, age, sex, date and time of onset, and date of a culture, and a check list of symptoms is recorded on a standard form The individual is then treated in the field with Kaolin-bismuth mixture, or referred to the field clinic or 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, as well as to provide some basic demographic information

Beginning on calendar week 40 surveillance for inapparent infections was begun by the collection of rectal swabs from all the children below the age of 6 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 the surveillance. This insures cross-sectional coverage of the entire community each week, and coverage on every household with children every ten weeks.

The centrally located clinic, staffed by a physician and aide, which has been in operation over the past 12 months for the family studies is open 7 days a week for the treatment of diarrhea cases. Patients are encourged 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.

Routine water collections for bacteriology are made 3 times weekly from the tanks and various points along the canal which are used by large segments of the population. The water surveillance studies are reported in more detail in a separate report (3).

When a cholera infection is identified, all the members of the affected household as well as all members in the adjacent households

are cultured daily for 5 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 Methods:

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 6 hours incubation 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 agar, and placed in selenite broth.

Results:

Demographic Data.

The population of the study area at the time of the census in August, 1965 was 6440. The distribution of the population by age and sex is given in Table 1. Fifty percent of the population is under the age of 15 years. There were 1105 households, giving an average household size of 5.8 persons. Since the majority of the houses had only 1 or 2 rooms, the degree of household congestion can easily be imagined.

The immunization status of the population by age is given in Table 2. The low rate of immunization among young children, which increases with age, reflects the increasing probability of being inoculated by vaccinators in their annual epidemic control programs.

Table 3 gives the number of births, deaths and migrations by week since the beginning of surveillance. The fifty births during the first 15 weeks of observation cannot be extrapolated for the entire year, since births tend to be seasonal, and this represents the peak season. The distribution of deaths by age is given in Table 4, revealing that 6 of the 13 deaths are in children under the age of 1 year, with 2 additional deaths in children under the age of 5.

Diarrhea Surveillance Data:

Table 5 summarizes the results of the weekly diarrhea surveillance and culturing of children for the inapparent infections

carried out by the field teams. During the 18 weeks from September through December, 1965, 733 diarrhea cases were seen, of whom 727 were cultured (99.2%). From this group, 3 cholera cases were detected, two of the cases were family contacts of a known case and both required hospitalization. Over the last 12 weeks of the same period, 1262 rectal swab cultures were collected from approximately 1000 different asymptomatic children. All were negative even though during the same interval, there were a total of 10 cases of cholera in the community.

Table 6 summarizes the weekly experience in the field clinic. Among the 517 diarrhea cases treated there, cultures were obtained from 396 (76.6%). About 20% of those not cultured represented follow-up cases or persons cultured in the field. The remainder were cases reported by relatives coming for medication. Seven cases of cholera were detected by the clinic; four required hospitalization, the remainder responded to outpatient treatment. Shigella were isolated from 4.5% of the diarrheas cultured at the clinic.

Cholera in Rayer Bazar:

During the 18 weeks of study in Rayer Bazar, there have been 10 confirmed cases of cholera, one death clinically compatible with cholera and 3 individuals with inapparent infection. A detailed look at these cases provides some information on the factors involved in the spread of cholera in this community. The cases have been listed by day of onset and by family number in Table 7.

The first positive isolation of V.cholerae in the community in week 36, was not from a study family, but from a 15 year old boy who had been traced to Rayer Bazar from Dhamrai where his family had a severe cholera outbreak with 2 deaths and 4 cases. He came to stay with a study family in Rayer Bazar, and though asymptomatic, was found bacteriologically positive. Serial cultures of all the members of the household where he was staying, plus examination of the water sources failed to reveal any spread of infection, and the boy subsequently left the family and was lost to followup.

The first case was a 5 year old boy in House K-37 adjacent to the canal with onset of cholera on 24th October 1965. Subsequently 2 additional members of his family developed cholera on 29th October and 30 October 1965. Serial culturing of 18 persons in the adjacent households was negative. The family dug well was negative, however,

the ditch(which was connected to the canal at Y point (Map)) used for washing and bathing was positive.

During the same time that the above cases were occurring, a boatman was hospitalized with cholera on 26th October 1965. He had come from Zinzera, a known infected area, and developed diarrhea while travelling to Rayer Bazar. He came to Rayer Bazar by canal to Y point (see Map, Figure 1) on the evening of 25th October 1965 and remained there through the night with diarrhea, then came to the hospital. Y point on the canal and the connecting ditch were subsequently found bacteriologically positive for V. cholera intermittently from 29th November through 9 December 1965.

A 4 year old child in family Q-51 developed mild cholera on 29th October 1965. Though this family lived near the main road, and had a tube well available for drinking, they used the canal, at Y point mentioned above, for washing and bathing. Serial cultures revealed no other infections in the family or in 19 members of 3 adjacent families.

The next case in a study family was in a 7 year old girl in family O-87 with onset of cholera on 30 October 1965. Investigation revealed that this family had stayed with family K-37 mentioned above, eating and sleeping there in the preceding 2 days. In addition they used water from the canal at Y point for bathing and washing. The child's father was not available for follow-up until 3rd November 1965 when he came home late in the evening developed severe diarrhea and vomiting and expired in a few hours before medical attention was requested.

On 31st October 1965 a 7 year old girl in family M-72 had onset of diarrhea. When reported on 5th November 1965 a culture was positive for V.cholera. This family was adjacent to canal point Y, and used that water for washing and bathing. Serial cultures revealed no additional infection in this family or in 12 members of 4 adjacent families.

The next case was a 7 year old boy in family P-19 with onset on 3rd November 1965. Although this family had originally lived in Area P (see Map), they had moved to Area M and were obtaining water from the ditch connecting to the canal at Y point, where the boatman mentioned above had been. Serial cultures revealed two additional

infections in the family, however 8 members of the adjacent family were negative.

Finally, on 8th November 1964, a 16 year old laborer who had moved into Area M next to the canal, developed cholera requiring hospitalization. He and 3 other laborers in the same house had used the canal at point Y for drinking as well as bathing and washing. His three companions left with the onset of his illness and were not available for study.

There were no further cases of cholera or inapparent infections associated with this outbreak. To reiterate the water studies, cultures of the canal from Y point next to Area O and the connecting ditch extending past Areas M and K were positive for V.cholerae on the 29th and 30th of October and the 3rd through the 9th of November at one or more locations. All subsequent cultures in this area have been negative. through the last week in December.

On the 28th December 1965 a 1½ year old girl in family Q-115 developed diarrhea and was bacteriologically positive for V.cholerae. Intensive investigation revealed no likely source of infection, and serial cultures of the family members, and 27 persons in the 6 surrounding households, as well as the water sources were entirely negative.

Discussion:

Prospective observations in Rayer Bazar have provided interesting clues regarding the pattern of cholera in the community. The data available to date indicates that the canal is a prime source of infection to the population. The large amount of boat traffic from all areas of the province provides ample opportunity for repeated introductions of the infection. While the hospitalized boatman mentioned above could not account for the first case in family K-37, he obviously contributed to the contamination of the canal, and serves to illustrate the mode of introduction of infection. It is of interest in this regard that in March, 1965 there was a well documented outbreak of Ogawa cholera in the brickfields served by this same canal due to a boatman introducing the infection from the outside (3,4).

An examination of last years cholera experience in Rayer Bazar in light of these observations, reveals that all of the cholera cases occurred on the western canal side of the road, with 22 of the 24 cases occurring in Areas K,M and O. The majority of these families lived in houses adjacent to canal point Y, and the ditch mentioned above. This aggregation suggests that the same factors involved in the transmission of cholera this year were operative last year.

The prospective observations have provided some valuable negative information regarding the spread of cholera. Infection was found only in the households of the cases who had contact with the infected water source. In spite of intensive surveillance for other inapparent or mild cholera cases by culturing all mild diarrheas, all contacts in adjacent houses to the cases, and 10% of the children below the age of 6 years in the community every week, none was found. Since the Family Studies comparing the serological response with rectal swabs indicate that rectal swabs are a relative sensitive method for detecting inapparent infection,(5) this study provides strong evidence against a high level of inapparent infection existing in the community. In addition, it also indicates that there is essentially no "contact spread" of infection outside of the family, in spite of intense crowding and bad sanitation.

In regards to how water used for "bathing" can transmit infection, it should be noted that bathing in Bengal characteristically involves washing out ones mouth and gargling. Thus while the people are careful to distinguish between the water used for "drinking" and for "bathing", from the epidemiological standpoint this distinction is entirely artificial;

The Rayer Bazar study has been designed to provide a broad based observation of the entire community from an epidemiologic and public health standpoint. The collection of demographic data is one aspect of this program which has been easily incorporated into the diarrhea surveillance routine. The newborns in Rayer Bazar were used for preliminary observations on transplacental transfer of vibriocidal antibody, and recently surveillance for enteropathogenic E.coli has been initiated in conjunction with the bacteriology section. It is envisioned that Rayer Bazar will provide a "field laboratory" incorporating more detailed studies on diarrheal diseases in general, as well as other endemic and epidemic diseases as they affect the community.

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TABLE I

RAYER BAZAR

DISTRIBUTION OF THE POPULATION BY AGE AND SEX

Age	M	F	Total	Percent	Cumulative percent
Under 1	91	97	188	2.9	2.9
1	124	111	235	3.6	6.5
2	107	113	220	3.4	9.9
3	118	109	227	3.5	13.4
4	117	127	244	3.8	17.2
5-9	610	630	1240	19.3	36.5
10-14	481	380	861	13.4	49.9
15-19	248	263	511	7.9	57.8
20-29	396	555	951	14.9	72.7
30-49	736	484	1220	18.9	91.6
50+	339	204	543	8.4	100.0
Total:	3367	3073	6440		

TABLE II

RAYER BAZAR

CHOLERA AND SMALLPOX VACCINE HISTORY BY AGE

Age group	Number of persons	Cholera Immunization: Immunization[a] percent.	Cholera Immunization: Never immunized percent.	Cholera Immunization: Unknown percent.	Smallpox vaccine: Vaccinated[b] percent.	Smallpox vaccine: Never vaccinated percent	Smallpox vaccine: Unknown percent
Under 1	188	0.5	98.4	1.1	2.2	96.8	1.1
1	235	9.4	90.2	0.4	13.2	86.4	0.4
2	220	27.7	69.5	2.3	27.7	70.9	1.4
3	227	46.3	52.9	0.9	52.9	45.8	1.3
4	244	59.0	39.8	1.2	65.6	32.0	2.5
5-9	1240	76.3	19.9	3.8	83.8	14.6	1.7
10-14	861	83.5	10.1	6.4	92.1	5.9	2.0
15-19	511	82.0	9.0	9.0	92.6	4.7	2.7
20-29	951	78.1	8.8	13.1	91.2	5.4	3.4
30-49	1220	79.0	6.5	14.5	93.7	3.4	2.9
50+	543	79.2	5.7	15.1	90.2	3.5	6.3
Total:	6440	71.8	20.8	8.4	80.5	16.9	2.6

a. 75.6% of those immunized received their most recent injection in the vaccine program following the epidemic of January, 1964.

b. 84.9% of those vaccinated received their most recent injection in the preceding 5 years.

TABLE III

RAYER BAZAR

NUMBER OF BIRTHS, DEATHS AND INDIVIDUAL MOVING IN AND OUT OF RAYER BAZAR BY CALENDAR WEEK OF SURVEILLANCE.

Week	Births	Deaths	Moved In	Moved Out
31	1			
32	2	2		
33	1			
34	1			
35	2	1	44	1
36	2	1	44	5
37	3	3	4	23
38	1		18	27
39	3		113	
40	1	1	17	11
41	3	2	14	13
42	13		97	17
43	8	2	66	63
44	5	1	19	27
45	3		32	8
46	1		67	
Total:	50	13	535	195

TABLE IV

RAYER BAZAR

DISTRIBUTION OF DEATHS BY AGE AND SEX

Age	Male	Female	Totals
1	2	4	6
1-4	2	-	2
5-9	-	-	-
10-19	-	-	-
20-29	-	-	-
30-49	2	-	2
50+	1	2	3
Totals:	7	6	13

TABLE V

RAYER BAZAR

SUMMARY OF WEEKLY DIARRHEA SURVEILLANCE AND CULTURE FOR INAPPARENT INFECTION PERFORMED BY FIELD TEAMS

Calendar Week No,	Diarrhea Cases			Number of asymptomatic children cultured
	Number of cases	Number cultured	Number positive for cholera	
35	72	72		
36	75	74		
37	59	58		
38	31	31		
39	72	71		
40	43	43		72
41	17	16		89
42	53	51		115
43	38	38	3[a]	112
44	55	55		119
45	40	40		61
46	20	20		69
47	38	38		87
48	35	35		101
49	26	26		110
50	18	18		117
51	17	17		100
52	24	24		110
Total:	733	727	3[a]	1262[b]

a. 2 were family contacts of a known case.
b. All cultures negative for V.cholerae

TABLE VI

WEEKLY SUMMARY OF DIARRHEA CASES SEEN IN THE FIELD CLINIC

	Diarrheas			
Calendar week No.	Number of cases	Number cultured	Number V.cholerae	Number Shigella
36	18	16		2
37	25	20		
38	63	49		1
39	55	40		3
40	50	34		2
41	30	20		2
42	21	18		1
43	39	22	2	
44	27	26	3	1
45	15	15	1	
46	25	21		
47	23	21		
48	31	21		2
49	27	24		1
50	30	19		3
51	21	18	1	
52	17	12		
Total:	517	396	7	18

TABLE VII

RAYER BAZAR

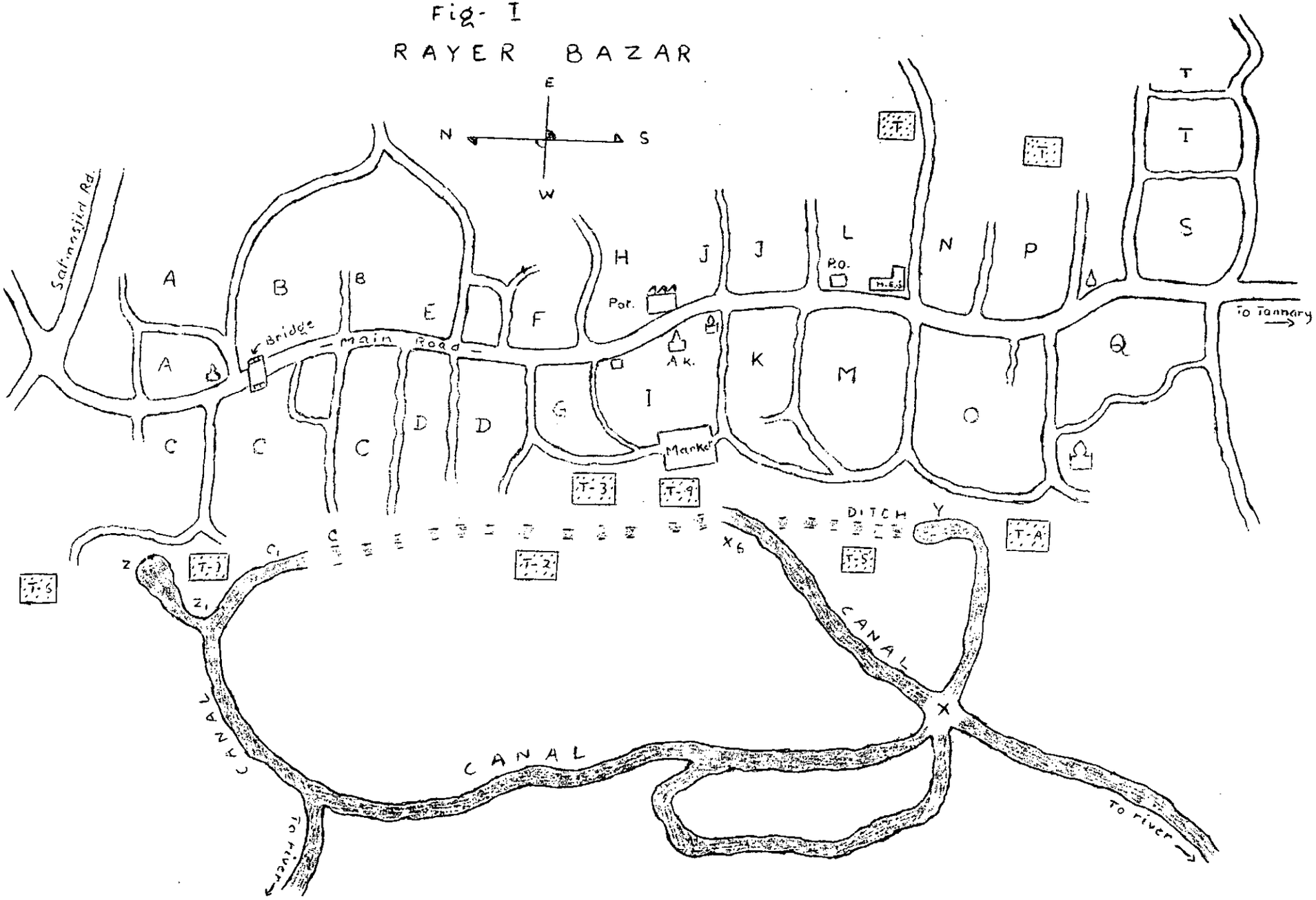
CHOLERA CASES BY DAY OF ONSET

Date	Case (Family Number)
October 24	(K-37)
25	
26	(Boatman)
27	
28	
29	(K-37) (Q-51)
30	(K-37) (O-87)
31	(M-72)
November 1	
2	
3	(O-87)* (P-19)
4	
5	
6	
7	
8	(Laborer)
December 26	(Q-115)

* Died of diarrhea and vomiting. No culture obtained.

Fig- I

RAYER BAZAR



III(i)

PATTERN OF DIARRHEAL DISEASES IN RURAL EAST PAKISTAN

by: Md. Fahimmudin.

Diarrheal diseases are of wide world distribution particularly in the underdeveloped tropical and sub-tropical regions. East Pakistan is no exception and is perhaps the worst sufferer as its climatic and socio-economic environments favour growth and persistance of almost all kinds of disease-producing micro-organisms. Statistics of diarrheal mortality in other countries are not available as most of the advanced countries no longer record diarrhea as a cause of death and the World Health Organization has also dropped diarrhea from the International list of causes of deaths. A comparative study of the incidence of the disease in East Pakistan with those of other countries is not therefore possible. Nevertheless, diarrheal disease is a leading cause of deaths in East Pakistan and is only second to Malaria, which, however, can be controlled and prevented. It causes more deaths, much more illness and disability and loss of working hours even than cholera. Yet diarrhea has not attracted much attention either from the patient himself or his relatives, or the attending physician, if one is consulted at all. In fact it is a neglected disease in East Pakistan to kill the 'unwanted' and the poor. During the last World War, the number of deaths from diarrhea in East Pakistan was greater than the entire war casualty in the Far East. In a refugee camp where I had the misfortune to work, more than 50% of the inmates died of diarrhea and perhaps none escaped the attack; yet better preventive measures and treatment of communicable diseases and environmental sanitation than those available in rural areas had been provided for the victims. The greatest danger of the disease is that in most cases, it is of a mild nature and rarely causes any panic either to the patient or to his relatives as does small-pox or cholera. Its course is insidious and it does not cause much pain; it appears mostly in sporadic form thoughunrecognised epidemic is known to occur occasionally.

Cholera and Diarrhea Mortality in Pre-Partition India and Bengal:

In the Indo-Pakistan sub-continent, before the Second World War, the diarrhoeal diseases(which included dysentry and diarrhea due to difficulties in making differential diagnoses) caused on the average more than 7% of the total deaths per annum. This was more than .. 2Rs

times higher than deaths from cholera. As seen in Table I, in pre-partition Bengal during the same period, diarrhoeal diseases claimed an equal a number of deaths as cholera did. The average annual death rate from diarrhea was 1.02 against 1.06 from cholera.

TABLE I

CHOLERA AND DIARRHEA MORTALITY IN PRE-PARTITION BENGAL

1930 - 39

Year	Cholera	Rate per mile	Diarrhea	Rate per mile
1930	54963	1.2	39367	.84
1931	79073	1.6	42764	.86
1932	33910	0.7	39562	.80
1933	29242	0.6	46697	.90
1934	50742	1.0	53947	1.0
1935	59605	1.2	51930	1.0
1936	76100	1.5	57212	1.1
1937	32710	0.7	56800	1.1
1938	71133	1.4	69232	1.4
1939	33221	0.7	54453	1.1
Average-	52070	1.06	51196	1.02

Pattern of Cholera and Diarrhea Mortality in East Pakistan:

Since World War II, the position in East Pakistan has slightly improved. The incidence of cholera seems to have been declining and so is the case with diarrheal diseases as can be seen from Table II and Graph I.

TABLE II

CHOLERA AND DIARRHEA MORTALITY IN EAST PAKISTAN

1950 - 1959

Year	Cholera	Rate per mile	Diarrhea	Rate per mile.
1950	24911	.56	19965	.48
1951	17817	.42	17868	.42
1952	19411	.46	16840	.40
1953	27132	.64	19590	.46
1954	12473	.25	19530	.48
1955	14096	.33	20425	.48
1956	18471	.44	24390	.58
1957	7002	.16	18839	.44
1958	15655	.37	20868	.49
1959	22685	.54	33612	.80
Average:-	17965	0.40	21192	0.50

Unlike cholera which shows much fluctuation from year to year, the mortality from diarrheal diseases is comparatively steady as seen from the Graph I.

Present Trend of Diarrhea Mortality in East Pakistan:

In recent years diarrhea mortality has been showing an upward trend as can be seen from the diarrhea mortality figures of Comilla and Bakherganj districts in Table III.

TABLE III

DIARRHOEAL MORTALITY RATE PER MILE IN COMILLA AND BAKHERGANJ DISTRICTS

	1950	51	52	53	54	55	56	57	58	59	60
Comilla	.45	.40	.54	.46	.46	.41	.49	.37	.70	.77	.60
Bakherganj	.91	.80	1.06	.89	1.12	.79	1.58	1.56	1.52	2.40	2.53

Seasonal Variation of Diarrhea Mortality in East Pakistan.

Diarrheal disease seems to prevail in East Pakistan almost evenly throughout the year. There is a slight rise in December and again in April as can be seen from Table IV. But there is no sharp seasonal rise as in the case of cholera. Distribution of cholera mortality by month is shown in Table V for comparison.

TABLE IV

DISTRIBUTION OF DIARRHEAL MORTALITY BY MONTH

IN EAST PAKISTAN

(1950 - 1951 to 1954 - 1955)

	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
1950-51	1614	1517	1383	1162	1620	1886	1536	1265	1546	2278	2029	1801
1951-52	1082	932	969	1210	1554	2166	1587	1419	1585	2076	1979	1328
1952-53	1092	935	925	1137	1170	1607	1282	957	1307	1867	1359	1140
1953-54	1180	1075	1460	2214	2539	2513	2277	1592	2241	2569	1947	1347
1954-55	1098	866	1075	1232	1562	1703	1407	1322	1548	2297	2128	1617
Average:	1216	1065	1162	1391	1688	1975	1618	1311	1645	2217	1988	1347
Monthly percentage:	6.5	5.7	6.2	7.5	9.0	10.7	8.7	7.0	8.8	11.7	10.5	7.2

TABLE V

DISTRIBUTION OF CHOLERA MORTALITY IN EAST PAKISTAN

BY MONTH

	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
1950-51	1153	1094	619	671	1527	2774	1536	916	1570	2830	1806	968
1951-52	552	302	235	559	1754	4222	2500	2263	2953	4204	2244	737
1952-53	737	457	210	278	672	948	1262	736	1294	2079	1378	518
1953-54	389	363	2660	6682	5283	4488	2716	1543	2713	2285	1314	441
1954-55	272	85	67	52	236	748	657	556	1063	2581	2155	1143
Average:	621	460	758	1648	1899	2540	1734	1203	1919	2796	1779	761
Mothly percentage:	3.4	2.5	4.2	9.1	10.4	14.0	9.5	6.6	10.6	15.4	9.8	4.1

For easy comparison the monthly percentage distribution of diarrhea and cholera mortality in East Pakistan is shown in Graph II.

From Table IV, V and VI it will be seen that diarrheal mortality in East Pakistan shows two seasonal peaks like cholera; but on a scrutiny of the diarrhea mortality figures in different districts, it appears that these peaks are due to local epidemics in different areas at different periods of the year. The peak in the district of Dinajpur is in March-April while that in Comilla is in July-August and in Bakarganj in December-January as seen in Table VII.

TABLE VII

PERCENTAGE DISTRIBUTION OF DIARRHEAL DEATHS BY MONTH IN DINAJPUR (EXTREME NORTH) AND IN BAKERGANJ (EXTREME SOUTH) OF EAST PAKISTAN

	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Dinajpur	8.0	6.0	7.0	8.5	7.2	9.2	8.8	9.2	11.2	11.3	8.5	6.0
Bakerganj	6.5	5.00	4.9	5.7	7.1	11.8	11.1	8.8	10.5	11.4	10.4	6.7

Influence of Climatic Factors on Diarrheal Mortality:

There seems to be very little relationship between the incidence of diarrhea and the temperature and/or humidity as can be seen from the Table VIII below:-

TABLE VIII

MEAN MONTHLY TEMPERATURE, MEAN RELATIVE HUMIDITY AND PERCENTAGE DISTRIBUTION OF MORTALITY FROM DIARRHEA BY MONTH - COMILLA DISTRICT.

(1950 - 1958)

	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Mean Temperature:-	82.4	82.5	83.3	82.1	75.5	66.6	64.5	69.6	77.7	83.3	83.9	83.3
Mean Relative Humidity:-	85	84	80	76	75	77	75	72	72	71	75	82
Diarrheal Mortality % distrib.	5.5	4.9	4.9	6.0	8.0	11.4	11.4	8.2	10.9	15.5	9.5	6.4

Influence of Rainfall on Diarrheal Mortality:

Annual rainfall also does not seem to have any influence on the occurrence of diarrheal disease in East Pakistan. While the annual rainfall in Comilla district varied widely from 117.8 inches in 1951 to 50.1 inches in 1957, the annual mortality rate from diarrhea remained almost steady as seen in Table IX.

TABLE IX

RAINFALL AND DIARRHEAL MORTALITY - COMILLA DISTRICT

	1950	1951	1952	1953	1954	1955	1956	1957	1958
Diarrhea Mortality:	0.45	0.40	0.54	0.46	0.46	0.41	0.49	0.37	0.70
Rainfall in inches:	89.8	117.8	103.0	109.0	77.0	102.0	93.0	50.1	75.1

Monthly Distribution of Rainfall and Diarrhea:

Unlike the case of annual rainfall, monthly distribution of rainfall seems to have some marked influence on the incidence of diarrhea. The less the rainfall the more are the deaths from diarrhea as may be seen from Table X.

TABLE X

MONTHLY RAINFALL AND DIARRHEAL DEATHS IN COMILLA DISTRICT

	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Rainfall in inches:	16.4	17.2	11.4	6.7	1.3	0.3	0.3	1.2	3.0	5.9	12.4	18.3
Diarrhea Mortality:	5.5	4.9	4.7	6.0	8.0	11.4	11.4	8.2	10.9	15.5	9.5	6.4
% Distribution:												

The above presumptions are based on the statistics of vital events collected by our village chowkidars. Their sources of information are mostly 'hearsay' and not supported by certificates from a physician. How far they can be accepted as correct, one can only guess. A unique opportunity was, however, available to the writer to study the epidemiological aspect of diarrheal disease in East Pakistan under the auspices of Pakistan-SEATO Cholera Research Organization which made an intensive study of the incidence of the disease among the vaccinated population in the Cholera Vaccine Trial area in Matlab Thana of Comilla district. The area selected represents typical characteristics of rural East Pakistan. Medical care for the vaccinated persons was provided in a field hospital under the supervision of a Pakistani medical graduate. The patients suffering from diarrhea with or without vomitting or with or without mucous and/or blood came to the hospital voluntarily. Intensive surveillance was also undertaken by trained field workers to locate diarrhea cases among the vaccinated population. Field workers visited each family at least twice weekly to enquire about diarrheal disease and whenever an episode of diarrhea occurred, details of illness were recorded on a simple record form. The vaccination of the population started on the 23rd September,1963 and ended on 23rd December, 1963. Of a total of 14,725 persons, 6956 received a commercial cholera vaccine and the remaining7103 received TAB vaccine for control. The surveillance was started immediately after vaccination. The control group was taken for the present study.

Composition of Control Group Population:

The composition of the control group population of the Matlab Cholera Vaccine Trial Project is as follows:-

TABLE XI

COMPOSITION OF THE CONTROL GROUP POPULATION

Age	Number	Percentage
1	50	0.9
1 -4	1238	17.4
5 -9	1546	21.7
10 -14	869	12.2
15+	3396	47.8
Total :-	7103	100%

Discussions:

Episodes of diarrheal disease were studied in a rural set-up of East Pakistan. This study shows that the incidence of this disease is very high in comparison with cholera. While the admission rate of confirmed cases of cholera in the Matlab Field Hospital from the control group was 3.6 per thousand during the first year of the trial, that from severe diarrhea was over 13.5 per thousand. The field surveillance shows that more than 51% of the population in the control group had an attack of diarrhea during the year. On an average each of the affected persons got more than one attack. Severe cases of diarrhea were more frequent among the adults than among the children while mild cases were more frequent among the infants and the toddlers than among the higher age groups. Mortality from diarrhea in the vaccine trial area was higher (2.4 per mille) than that of the Comilla district (0.5 per mille) Diarrhea caused more than 27.4 percent of total deaths in the vaccine trial area.

Conclusions:

Epidemiological study of diarrheal disease in the vaccine trial area in Matlab indicates that diarrhea is a greater menace than cholera. It causes more illness and perhaps more loss of working hours and efficiency than cholera, diarrhea persists for a longer period of time and is not easily cured like cholera. In East Pakistan no relationship seems to exist between the incidence of diarrhea and local climatic conditions. Incidence of diarrhea seems to precede and follow an outbreak of cholera epidemic.

Admission of Diarrheal Cases in Matlab Field Hospital:

As stated earlier, patients suffering from diarrhea with or without vomitting or with or without mucous and/or blood, attended the hospital voluntarily. Table XII shows the total number of admissions of patients suffering from diarrheal disease in the Matlab Field Hospital from December, 1963 to December, 1964.

TABLE XII

ADMISSION OF DIARRHEAL CASES IN MATLAB FIELD HOSPITAL

	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Total cases:	50	48	55	109	93	65	66	64	69	50	40	45	95
Confirmed cholera:	40	34	25	22	3	5	-	-	1	1	10	11	48
Percentage to monthly total:	80.0	71.0	45.0	20.0	3.3	8.0	0.0	0.0	1.4	2.0	25.0	24.4	82.1
Non-vibrio diarrhea:	10	14	30	87	90	60	66	64	68	49	30	34	57
Percentage to monthly total:	20.0	29.0	55.0	80.0	96.7	92.0	100.00	100.0	98.6	98.0	75.0	74.4	17.9

It will be observed that though the number of admisssions of cholera cases in December, 1963 was smaller in comparison to that of December 1964, the percentage of confirmed cases of cholera to total admissions was almost the same. There was marked seasonal fluctuation of cholera cases but the number of admissions of non-vibrio diarrheal cases remained almost steady throughout the period.

Diarrheal Cases admitted in the Field Hospital from the Control Group:

The patients admitted into the Field Hospital came not only from Vaccine Trial Area but also from the surrounding villages. The number of diarrhea cases which came from the control group is shown in Table XIII.

TABLE XIII

NUMBER OF ADMISSIONS IN THE FIELD HOSPITAL FROM THE CONTROL GROUP SHOWN ON QUARTERLY BASIS.- DECEMBER 1963 to NOVEMBER 1964.

	Total admissions.	Confirmed cholera.	Negative cases.
December-February	29	16	13
March-May	54	5	49
June-August	21	-	21
Sept-November'64.	18	5	13
Total:	122	26	96
Rate per thousand	17.2	3.6	13.5

Diarrheal Cases detected by Surveillance Staff:

Apart from the admissions of patients suffering from diarrhea to the Field Hospital, the surveillance staff detected quite a large number of diarrheal cases in the vaccine trial area during their regular round of visits to each house. The number of cases detected by them among the control group is shown in Table XIV.

TABLE XIV

DIARRHEAL CASES AMONG CONTROL GROUP DETECTED IN THE FIELD

DECEMBER 1963 TO NOVEMBER 1964.

	Total Cases	Confirmed Cholera	Diarrhea Cases
Dec. 1963 to Feb. 1964	718	10	708
March-May	1514	6	1508
June-August	2738	-	2738
Sept-November 1964	2380	3	2377
Total:	7350	19	7331

Table XV below combines the diarrheal and cholera cases admitted to the Field Hospital and detected during field surveillance from among the control group from December, 1963 to November 1964.

TABLE XV

TOTAL DIARRHEAL CASES DETECTED IN THE FIELD HOSPITAL AND IN THE FIELD

DECEMBER 1963 - NOVEMBER 1964.

	Hospital		Field		Total		
	Cholera	Diarrhea	Cholera	Diarrhea	Cholera	Diarrhea	No of persons effected
Dec'63 to Feb'64	16	13	10	708	26	721	656
March-May	5	49	6	1508	11	1557	1028
June-August	-	21	-	2738	-	2759	1265
Sept-Nov'64	5	13	3	2377	8	2390	670
Total:-	26	96	19	7331	45	7427	3619
Rate per 1000	3.6	13.5	2.7	1046	6.3	1060	5100

Admission of diarrhea cases to the Field Hospital was 4 times greater than the admission of cholera cases and in the field the number of diarrhea cases was almost500 times higher than cholera. 51% of the population suffered from at least one attack. On an average each patient had more than two attacks.

Seasonal Distribution of Diarrheal Cases among the Control Group:

The Table XVI below shows the distribution of diarrheal cases among the control group by month. It will be observed that there is an increase of diarrheal cases in July - August which continues through September and October

TABLE XVI

DISTRIBUTION OF DIARRHEAL CASES BY MONTH AMONG THE CONTROL GROUP

	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Total
Persons affected.	108	267	281	419	256	353	389	474	402	276	253	141	3619
No. of cases.	112	282	327	563	406	588	741	1008	1010	927	959	504	7427
Percentage distribution of cases by month.	1.5	3.8	4.5	7.5	5.4	7.9	9.9	13.5	13.6	12.4	13.1	6.8	100.00

Relationship between Diarrhea and Cholera Mortality:

The diarrheal diseases among the control group in 1964 showed a rise in July which continued through August, September and October while cholera cases in the Field Hospital reached the peak in December and continued through January. The pattern of distribution of cholera cases in the vaccine trial area is almost identical to the distribution of cholera mortality in Comilla district as can be seen from Table XVII.

TABLE XVII

PERCENTAGE OF DISTRIBUTION OF DIARRHEAL AND CHOLERA CASES BY MONTH IN VACCINE TRIAL AREA AND CHOLERA MORTALITY OF COMILLA DISTRICT.

	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Percentage distrib.of diarrhea cases by month.	13.5	13.6	12.4	13.1	6.8	1.5	3.8	4.5	7.5	5.4	7.9	9.9
Percentage distrib.of cholera	0.0	0.0	0.0	6.5	7.2	26.3	22.2	16.4	14.5	2.0	3.0	0.0
Percentage distrib.of cholera mortality in Comilla by month.	2.1	.8	.2	.9	4.2	20.0	20.0	11.6	16.0	14.00	7.5	3.0

Distribution of Diarrheal Cases among the Control Group according to Age:

The incidence of diarrhea in the control group according to age is shown in the Table XVIII. It will be seen among the hospitalized diarrhea cases, that the adults formed the majority while among the field cases the younger age group predominated.

TABLE XVIII

DISTRIBUTION OF DIARRHEAL CASES IN CONTROL GROUP

ACCORDING TO AGE.

JANUARY 1964 - DECEMBER 1964

Age	Population	Hospital cases	Rate per 1,000	Field cases	Rate per 1,000
1	50	4	80.0	144	2880.0
1-4	1238	16	12.9	2461	2000.0
5-9	1546	6	3.9	1639	1058.0
10-14	868	4	4.6	658	758.0
15+	3396	64	18.3	3136	923.0

N.B. For the sake of comparison with the district vital statistical figures, only cases for the complete year, January to December, have been included.

Mortality from Diarrhea among the Control Group during the Period December 1963 to November 1964.

All deaths within the vaccine trial area, including the cause of death as far as could be ascertained from local enquiry, were recorded by the local surveillance staff. The number of deaths among the control group is shown in Table XVI. The mortality rate from diarrheal disease in Comilla district during the last decennium was 0.50 per thousand. During the period of surveillance from December, 1963 to November, 1964 among the control group

in the cholera vaccine trial area, the number of deaths associated with diarrhea was recorded to be over 2.4 per thousand, i.e. five times higher than the district diarrheal death rate and six times higher than the cholera death rate. About 27.4 percent of total deaths were due to diarrhea.

TABLE XVIII

DEATHS AMONG THE CONTROL GROUP-DECEMBER 1963

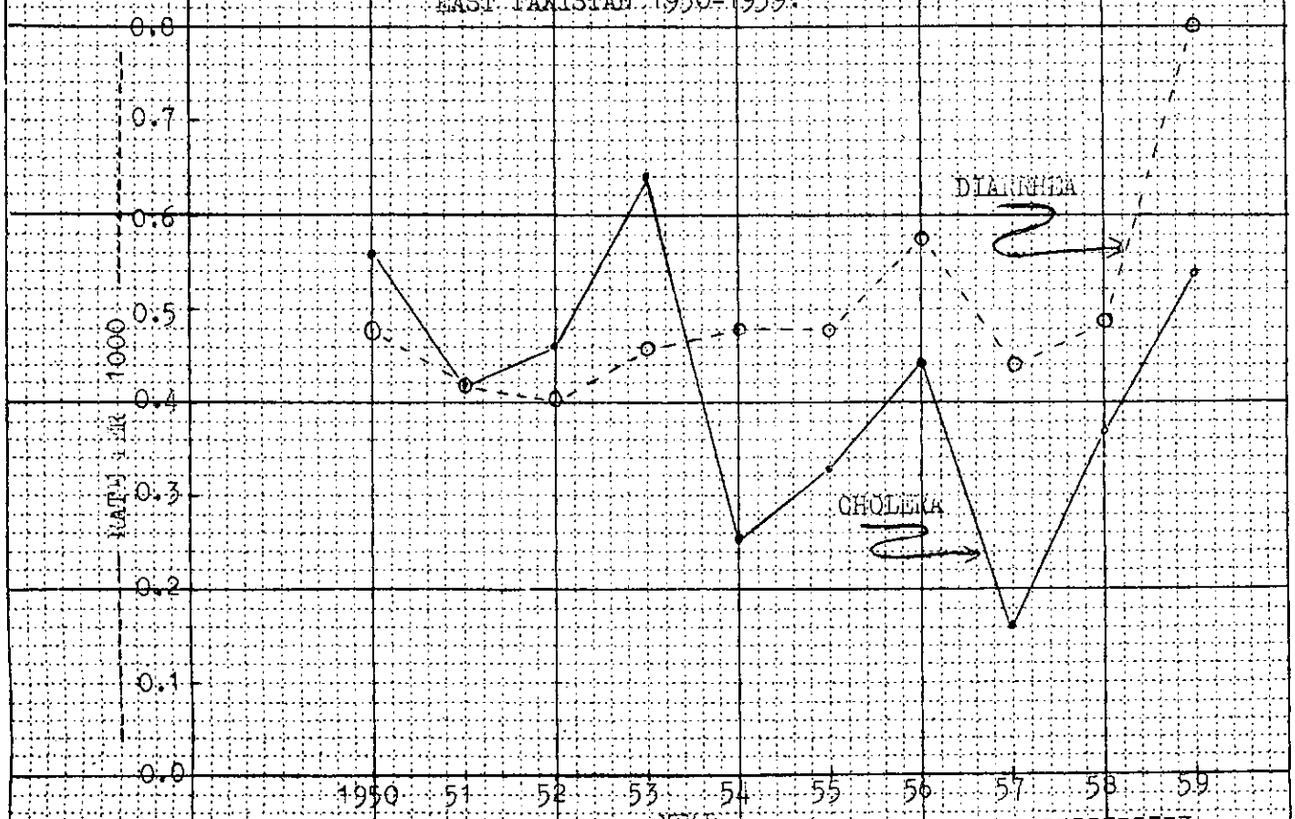
TO NOVEMBER 1964

	Deaths associated with diarrhea	Deaths due to other causes	Total deaths
Dec'63 - Feb'64	4	11	15
Mar-May	4	15	19
June-Aug	2	5	7
Sept-Nov'64	7	14	21
Total:	17	45	62
Rate per thousand	2.4	6.3	8.7
Percent to total deaths.	27.4	72.6	100.00

GRAPH-I III(1)

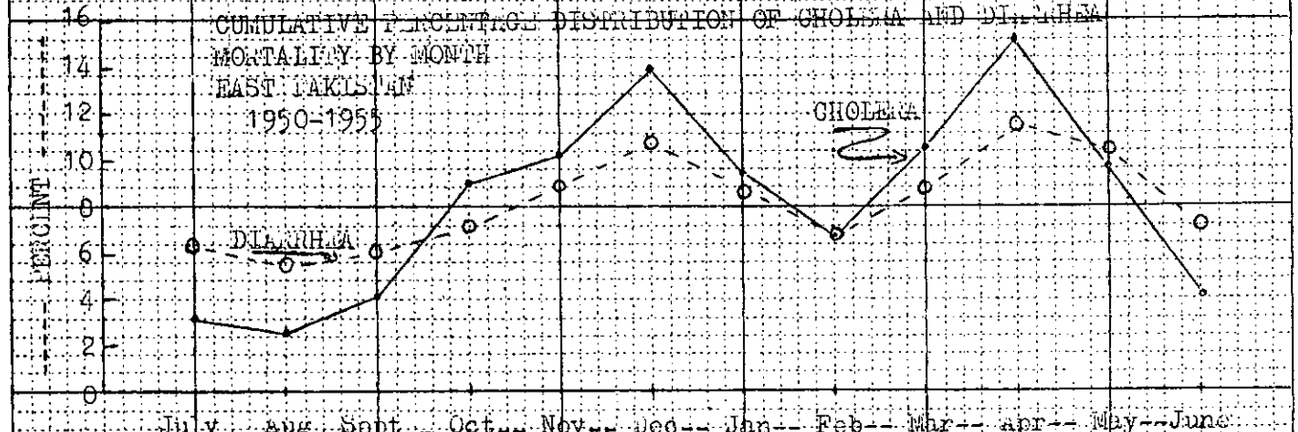
ANNUAL CHOLERA AND DIARRHEA MORTALITY RATES.

EAST PAKISTAN 1950-1959.



GRAPH - II

CUMULATIVE PERCENTAGE DISTRIBUTION OF CHOLERA AND DIARRHEA MORTALITY BY MONTH EAST PAKISTAN 1950-1955



INFANT CARE IN RURAL EAST PAKISTAN

by: (Mrs) S. Lindenbaum

Studies of nutrition and child health often fail to perceive that customs and behavior differing from the observer's are part of an intricate plan of care. A recent report of nutrition in two Kushtia villages comments that:

"Baby and child care is a haphazard affair... Babies and children up to 6 years old are generally ill-clad or utterly naked, but have some pieces of jewelry adorning their necks, noses or waistlines. Babies were seen sporting a peculiar eye make-up in the form of black paint all around the eyelids which made them look grotesque at their very tender age... It is He (God) who can save them from the many evil spirits abounding here, they felt, and therefore the care of the children is left in the hands of God who 'would save them if He wants to'". (1)

Discussing nutrient intake, the survey notes that:

"The diet of the population has too much of cereals, too little of fats, and too little of protective foods - meat, fish, eggs, milk, fruits and vegetables, which is typical of diets in Asia. A too high cereal diet would have little room for other foods, as was found in the survey population where standard of living was low, and poverty, ignorance, illiteracy and utter disregard for any change in usual habits and customs existed."(2)

While the study contains much useful information, its observations about child care, and in some respects nutrition in relation to child care, are misleading. The very facts selected to justify the view that children are treated in a haphazard way are in fact evidence of concern. When mothers allow their children to play in dirty ill-fitting clothes, or at times even deprive them of the 'protective foods, meat, fish, eggs, milk, fruit and vegetables', they may be activated by the highest regard for the child's health and welfare. Ignorance of the protein-vitamin value of foods does not leave mothers operating in a nutritional vacuum. It is important to understand beliefs about the causation of disease and locally accepted food ideologies to appreciate that village mothers are choosing from a large range of accepted activities concerned with the care of children. It also becomes evident that belief in God's will does not preclude active striving to avert disaster or change fate.

1. Nutritional Evaluation of the Food Intake of 91 Households in Kushtia Villages East Pakistan 1962. Report for FAO by Elena S. Quisgue.p.10.
2. Ibid p.15.

The present account is based on fieldwork in several villages of Shaitnal Union, Matlab Thana, an area situated about 20 miles south of Dacca city. These are predominantly Muslim communities, but the area also includes a small number of Hindus whose traditional caste occupations are fishing, weaving and gardening. The women of both communities hold similar beliefs about the foods with which to regulate and maintain the good health of their children, and about the need to protect infants from the predations of the spirit world.

Mothers do not look first to the physician when their infants fall ill.[3] Present day medical belief, a compound of Greek, Hindu and Arabic knowledge and indigenous lore, allows women a wide field of magical activity to care for themselves and their children. In the earliest Greek, Hindu and Arabic literature, women figure largely in the field of magic. Women are mentioned repeatedly in the Odyssey as magicians who have an expert knowledge of drugs, poisons and philters.[4] The Arthevada has a number of hymns andcharms to ensure conception, to make one sterile, to conceive a son, to prevent miscarriage and to secure easy parturition.[5] Hindu medicine on the other hand is concerned with men. Women and children are of minor importance except for pregnant women because they bear male offspring. Pediatrics defined in an early Hindu medical work as 'children's diseases caused by demons'.[6] Finally an early Persian writer, Ali ibn Rabban, devotes one section of his book to pregnancy, the evil eye and dietics,[7] and here is the second most important element of behaviour associated with child care dietary control.

Foods are regarded as 'hot' and 'cold' according to allegedly inherent properties.[8] This appears to have no connection with actual temperature or spiciness, but relates rather to believed effects of food on the body. Illnesses and body states are also classified as 'hot' and 'cold', the distinction providing a framework of do's and don'ts for the maintenance of good health. A 'hot' illness or body state should be treated with 'cold' foods, and a cold illness with 'hot'

3. For confirmation of this see "Medical Care in Fatal Illnesses of a Rural Punjab Population; Some Biological and Cultural Factors and their Ecological Implications" by Sohan Singh, John E. Gordon, John B. Wyon in Indian J. Med. Res. 50:6: 1962, p.865 ff.
4. Henry E. Sigirist, "A History of Medicine" OUP 1961, p.159.
5. Ibid p.159
6. Henry R. Zimmer "Hindu Medicine" Johns Hopkins Press, 1948.p.90.
7. E.G. Browne "Arabian Medicine" OUP 1962, p.38, from "The Paradise of Wisdom" ad. 850.
8. The belief dates back to the humoral theory of disease expounded by Hippocrates and Galen. Incorporated into Ayurvedic and Arabic medicine, it was transmitted by the Arabs to Spain and by the Spaniards to the New World. It is still said to form an important element in Latin American folk belief. As it travelled it underwent modification so the present form of the theory in Latin America is not identical to the one in Asia. Within Asia too, there are interesting divergencies. In Thailand, for instance, eggs are regarded as 'cooling' and guava fruit 'heating', while in E. Pakistan all fruits are 'cooling' and eggs one of the 'hottest' foods.

During pregnancy, for instance, a woman's body is in a hot condition, and she craves the 'coolest' foods - sour fruit, unripe mango, tamarind, lemon, borɔi(9)kamranga(10)kau(11)chaulta,(12)foli,(13)and dewa.(14) The newborn child's diet too is carefully regulated. For 3 or 4 days until the mother's breastmilk comes, the baby is given a little honey and mustard seed oil, fed to it on the tip of the mother's finger. These are both 'hot foods which clean the child of mucous and meconium and prevent it from catching cold. Hindu mothers mention giving the child, in addition, a little cow's milk diluted with water. There is a fear of over-heating or cooling the child's system, so pure cow's milk, considered too 'hot', is never fed to the child. Nor should an infant be given plain cold water which might chill it. An infant suffering from loose motions is believed to have a hot stomach, so it is given a diet of 'cool' foods which lessen the heat and allow the stool to harden. It is therefore given barley water or glucose water with a little lemon, and if the mother is still breast-feeding the child, she too restricts her diet to vegetarian non-heating foods.

Some foodstuffs are considered to have additional specific effects on the body. After delivery, the mother's diet is strictly limited to foods believed to 'dry out' the stomach and womb. She is also considered to be in a delicate state, and for 5 days avoids all foods difficult to digest, and which therefore may cause stomach trouble. She takes no meat, fish, eggs or curried food, but eats sparingly of tea, bread, potato and plain rice with a little black pepper or one chilli, and powdered cumin(15) seed. These are all foods that 'dry' the stomach, the last thought also to produce good breastmilk. Women make their own 'drying' medicine from the stems of banana and arum leaf, dried in the sun, ground to powder and mixed with the spices coriander, cumin, bayleaf and cloves.

It is not typical of women's behaviour, however, that they rely on nutritional control alone to achieve results. The infant is also protected against catching cold by tying a small bundle of cumin seed to its wrist and by hanging an amulet on its neck enclosing part of the withered umbilical cord. Magic and nutrition are dual themes in maternal and child health behaviour.

On the eve of the 5th day after delivery, for instance, the mother, and generally her mother or mother-in-law, remain awake all night, holding the baby, and praying for its health. On the 5th day, five dishes are cooked for the mother to eat - chicken or fish curry, brinjal chutney, fried banana(16) pulse and eggs. The meal is taken with the thought that the child may be blessed with a choice of five or more dishes all its life.

9. Zyzyphus mauritiana
10. Averrhea carambola
11. Carcinia cawa
12. Dilenia indica
13. Unidentified
14. Unidentified
15. Bengali 'kali jira' - Nigella sativa
16. Especially of the variety called 'katcha kola' or 'anaja kola' - Musa paradisiaca.

Beliefs about food therefore act as a control over the dietary intake of mothers and infants. Foods are classified and selected for their believed specific effects on the body. It is not questioned here whether these beliefs are objectively verifiable. However, there are times when nutritional intake is restricted on a magical basis. It has been said that mothers restrict their own diet by eating no meat or eggs or fish, and sometimes in addition, no milk for the period in which a breastfed child suffers from diarrhea. They observe this restriction not only during the days of the illness, but for three days again at the full moon and the dark of the moon, times when spirits are believed to be at large. Some women extend this food restriction beyond the period in which they suckle the child in the belief that the activity itself has some efficacy to protect the child's health. One Hindu woman eats vegetarian, that is 'cold' foods, every Thursday, and never takes gajar fish[17] or pomegranate, a diet prescribed for her by a Kobiraj[18] after the death of her first three children. The son she protects in this way is now 17 years old.

The following description of two treatments for childlessness illustrates the characteristic blend of nutritional - magical behaviour.

In the first treatment the girl bathes at mid-day, and in her wet sari returns to the house, face towards the West and drinks medicine prepared by a Fakir,[19] over which he has said a spell seven times. The medicine contains ingredients believed to be both 'cooling' and effective against the spirits which are preventing conception, for example fermented ricewater[20] and gila seed[21] the mixture being stirred with stems of the plant 'bis katali'.[22] The girl is told to eat no eggs, fish or meat for 30 days.

In the second treatment, the Kobiraji, a Muslim woman about 70 years of age, slaps the girl lightly on the forehead and prays to God and the seven 'pari'.[23] She rubs her hands three times down the girl's body, and blows three times into her face, then tells her to take a step backwards, thrusting forward each leg and arm. She gives the girl three tiny gold pills to eat on the first three days of menstruation, and instructs her to sleep with her husband on the seventh day. The prescription is to be repeated for three months. Amulets, one for the neck and one for the stomach, are to be worn, commencing on a Friday,[24] the latter amulet to be later transferred to the baby for its protection. Both women are assured of the success of treatment within three months.

17. Gajar fish - Ophicephalus marulius
18. Kobiraj - Ayurvedic physician. Loosely applied in the village to any practitioner, Muslim or Hindu, who gives treatment with indigenous medicines and has power to control the spirits.
19. Fakir - Muslim religious mendicant.
20. Fermented ricewater - Bengali 'kanzir jau'. Often used ritually to prevent entry of spirits into the house.
21. Gila seed - Entada pursetha. This seed is also hung on the neck of a cow in calf to prevent attack by spirits.
22. 'Bis Katali' - Polygonum hydropiper. 'Bis' - poison. 'Kata' prickle. Compare the use of thorny plants as a protection on the walls of the delivery room.
23. 'Pari' - a fairy. A benevolent female supernatural being of entrancing beauty.
24. A Muslim holy day.

The practioners consulted are in the first case, a Fakir, and in the second, a female Kobiraji, both believed to have power to control spirits. Consultation and treatment are characterised by ritual behaviour.[25] There is a mystical belief in the efficacy of numbers, especially the numbers 3 and 7. The women are restricted to a diet of 'cooling' foods or treated with 'cold' medicines, for 'hot' food and 'hot' medicines are believed to cause abortion. The formula for behaviour is considered as important as the medicine. This became more apparent when in two consultations, the Kobiraji gave identical medicine to two women, one wishing to conceive and the other wishing to abort, the difference provided only by accompanying spells and activities.

Preoccupation with magical activity increases with the birth of a child.[26] The first month of life is a precarious time. Infant mortality is high and mothers attribute all deaths in the early period of a child's life to attack by spirits and demons. During the first week of life, a baby taken by demons is said to suffer from 'Thakuria'. It changes colour rapidly - black, red, yellow, white, blue - the blood rushes to its head, it refuses to eat, cries like a crow. Only a person or object with power to counteract attack by spirits can cure it. A Kobiraji is hastily called to perform a ritual or utter spells, but since many children die during the first weeks of life, preventive health measures are considered more effective.

The midwife pierces the right earlobe of a newborn boy, or the left of a girl, and inserts a small iron ear-ring.[27] Or she burns the earlobe with a hot needle, thus protecting it with the mark of iron and also slightly damaging the child, making it a less desirable object of attack. A woman whose children continually die during the period of seclusion in the delivery room, may place in each of two iron amulets one hair of her own head and a bone which she fetches from a cow slaughtered at Bakr Eid.[28] The amulets are hung in the house. When the baby is born, the midwife instantly places one amulet on the child and one on the mother. The mother is then considered free to leave the delivery room without fear of demons attacking her.

25. Ritual behaviour - "Any behaviour that is accounted for by mystical notions. There is no objective nexus between the behaviour and the event it is intended to cause. Such behaviour is usually intelligible to us only when we know the mystical notions associated with it". E.E. Evans-Pritchard; "Witchcraft, Oracles and Magic among the Azande". OUP 1937, p.9.
26. In a sample of women whose reproductive cycles were completed, eleven women gave birth to 85 children, of whom 45 died and 40 survived.
27. Iron, fire, the colours blue and black, fermented ricewater, fishnets, brooms, prickles and thorns are believed to be weapons to frighten the spirits.
28. Bakr Eid - A Muslim festival which commemorates the sacrifice of Abraham. It follows the Haj in Mecca where pilgrims gather from all over the Muslim world.

The delivery room too, is armed against spirit intrusion. One Hindu house set aside for childbirth had a temporary low bamboo fence built around it. The mud base of the house was smeared with a line of cowdung [29] and four globs of dung were placed one at each corner of the house. Only one door remained for access to the room where the mother and child were secluded, the other being closed off with a mesh of jute and bamboo. The exterior walls of the house were stuck with the branches of thorny bushes, [30] and a broken water pot, an iron knife blade and a tortoise shell were placed at the entrance door. In a Muslim delivery room, the women had placed by the baby's pillow for its protection, an old broom, a piece of fishnet and an iron scythe.

To safeguard the baby at the time of the first feeding, one Muslim mother says she rubs her hand under her left foot and three times between her breasts. She then expresses a little colostrum and rubs this on the child's left foot. Care should be taken that none of her milk falls on dry ground, for her milk supply would similarly dry. It is believed by Muslim women that angels dwell on the right side and devils on the left, so mothers should commence feeding with the right breast at the time of birth, and during the period of seclusion at least, at the beginning of the day.

Even before the end of the seclusion period mothers begin to guard their infants against another danger - the evil eye. This is basically a protection against covetousness and envy. A child sickens, and its mother and perhaps also the Fakir or Kobiraj diagnose it as the effects of an evil glance or of spoken admiration. Mothers never openly admire a child's health or beauty. To comment favourably about another person's child is regarded as a form of aggression. The safest comment to make about one's own is to say with tenderness 'He is very naughty' .

Belief in the 'evil eye assumes that all people are subject to envy. Beyond diagnosis, there is no attempt to seek out the originator of the evil. Unlike sorcery in some New Guninea and African societies, it is a non-specific charge. The possessor may herself be unaware of the power. It is involuntarilyinflicted, and may even be transmitted through a dog or cat. It is invisible, intangible evil, and like spirits and demons, is more frequently combatted by protective than curative measures.

29. Cowdung is believed by Hindus to be purifying. Spirits frequent dirty, contaminated places such as graveyards, latrines and delivery rooms.

30. For example 'Bet gatch' - Calamus, or 'Kumri lota' - Smilax macrophylla.

On a thread at the child's neck or waist, the mother hangs a small cowrie shell, a paisa coin, seeds of 'rudrake' or 'rita'[31] or a bright blue bead, all worthless in themselves, but designed to deflect the evil glance by attention to the object. A very desirable fair-skinned child will have its eyebrows blackened with mustard oil and soot from a cooking pot, or one large black spot will be placed asymmetrically on its forehead. This gives the illusion that the child is unattractive, slightly disfigured, just as it is if its earlobe is burnt or pierced. Similarly, the child may be given a foul name for people to call it by, its true name being kept hidden - for example, 'rotten one', 'rubbish dump', 'toad', 'cripple', 'bad luck', 'crybaby', 'one who doesn't wash after defecating', or, for a Hindu or a higher caste, 'fisherman'. If the mother feels that someone is watching her feed her child, she may first eat a little of the food herself and spit it out, suggesting it is unpalatable. Some women rely on ritual protection; taking a little mustard oil in the left hand, they spit into it and rub it three times anti-clockwise around the baby's head.

Another ruse is to pretend to sell the child to a lowcaste Hindu woman,[32] often a widow. This is done especially with the 'molli' child, the first child to survive after the death of one or more siblings. A 'molli' child is said to be born 'molli', angry at its sibling's death. It is bad-tempered, un-cooperative and eats poorly.[33] The 'molli' child may be 'sold' soon after birth, if there has been a procession of preceding deaths, or perhaps later, at the age of 1½ to 2 years, when it shows symptoms of some ailment.

One or two women accompany the mother as she visits the Hindu woman's home. The real mother gives the child for a moment to the adopting mother to hold. She carries it around like her own child, rubs it with oil, talks to it. The two mothers call each other 'sister' and sit together and eat betel. The adopting mother gives in payment 2 paisa coins which are kept wrapped in cloth in the true mother's house from this moment until the day the child marries.

31. 'Rudrake' - Elaeocarpus ganitrus, a sacred Hindu tree. 'Rita' Sapindus mukorosi.
32. For example gardeners, crematers, swineherds, leatherworkers, fishermen.
33. The first survivor is often spoiled and granted every whim. Some women equate this with its later petulant, selfish behaviour.

On the marriage day the adopting mother is called to visit and receive a sari and a small gift of puffed rice[34] and beaten rice.[35] The child sits for a moment in her lap, the two coins are returned to her, and the transaction is completed. For Hindu neighbours, the relationship may develop into one of elaborate adopted kinship, with constant visiting, gift and food exchanges, the child calling the woman 'mother' and by extension, her sister, 'mother's sister'. Between Muslim and Hindu women who live some distance apart, it is symbolic relationship only.

If a mother gives birth to infants that continually die, some women say it is possible for her to consult a Kobiraj to change fate. The Kobiraj gives her a metal charm enclosing a particle, such as hair or nail clippings, from the child of the woman whose offspring never die, and the fates of the infants are reversed. If a child falls ill and sorcery is suspected, a Fakir or Kobiraj will be called to locate the hidden charm, which is generally detected on a path the mother frequents, or in the earthen floor of her kitchen. Beyond detecting the charm, which is proof of diagnosis, there is again no attempt to accuse a sorcerer. There may be slanderous comment about the source of the evil, and a tense antipathetic relationship between two women or two kin groups may increase, but no charge is openly made.

While women assume that all illness in infants is supernaturally caused, it must be noted that not all maternal and child care is non-rational. Children with fever are kept to a diet of sage and easily digestible foods, while the temperature is lowered by pouring cold water over the head, the hair often being shaved for the purpose. During childbirth, the emphasis is on practical measures with very little ritual behaviour. A skilled midwife, for instance, rubs oil on her hands and makes an exploratory investigation to see if the child is arriving head first, if it is not, she inserts her hand and gently turns the child. Where it is possible to visibly effect a result, women do so. They say that their ideal of physical beauty is for a girl to have a full round face and straight nose. The bones of a newborn child are 'soft like wet clay', so with a heated cloth and daily pressure, they say they can mould and shape the baby's head, rounding it, and raising the bridge of a flat nose.

But in situations where there is less demonstrable success, they resort to mystical behaviour. For prolonged labour, women may call in a Kobiraj who draws a representation on paper of a womb and fetus, and saying spells, folds the paper and places it in an amulet which he ties to the mother. When the baby emerges, he swiftly removes the amulet and throws it away, lest the mother's entire insides fall out. More commonly,

34. Bengali 'muri'
35. Bengali 'cira'

Muslim women hasten birth by drinking a few drops of water from the pond by a holy man's tomb, or the water in which a flower from Mecca has been floated.

Mystical behaviour is most predominant in situations of greatest anxiety - for the fair skinned child, the 'molli' survivor, the firstborn, the male child, or at times of special danger, at midnight, during seclusion, at phases of the moon or when travelling. Things most prized may be taken away. One woman observed"Children fall ill when parents love them too much". Mothers combat the spirits with objects that frighten, but also mislead them by concealing the work of things. Adherence to formula-like activity inspires confidence in the outcome.

When infants reach one year of age or more, mothers are more inclined to seek advice of a medical practitioner if the child falls ill. They prefer to consult a Homeopath rather than to seek Allepathic cures. Homeopathic medicine suscribes to the same 'law of similarity' which forms the basis of magical thinking in indigenous cures[36] "A homeopath, for example, face to face with a case of cholera, will select a very small dose of arsenic as soon as he meets with the symptoms - incessant vomiting, purging and insatiable thirst".[37] that is, he uses a medicine which produces symptoms similar to those of the disease. Imitative magic too assumed that things that resemble each other have a significant association. Morning libations to a milk-exuding tree will increase the milk supply of a breastfeeding mother. Also, the Homeopath gives tiny medicinal doses, thought to be safe for infants who are protected against the strength of strong substances like pure cow's milk. The medicines are given in a sugar syrup, which small children find palatable, and the fees are modest.

Women also seek medical help from holy men. They visit country fairs and saint's tombs to ask the Fakir or Pir[38] to pray for the health of the child. Ritual activity is again an important part of this behaviour. Thursday night, but especially the first Thursday of the new moon, is considered an auspicious time to visit a saint's tomb. The visitor presents a gift of sweets, candles and incense sticks. The Pir may give petals from flowers scattered on the tomb for the child to eat. He also gives an amulet enclosing a holy inscription. The emphasis of this treatment is on emotional comfort for the patient and family - the amulet having some efficacy in itself, but serving too as a sign of the continuing efforts of the Pir to intercede with God on the child's behalf. If the parayers are answered and the child recovers, the mother makes a second visit to the tomb with a thanksgiving offering of a chicken or goat, a male animal for a male child, female for a female.

36. See "Attitudes to Health and Disease in Rural East Pakistan"; Report to the Technical Committee, 1964, by S.Glasse, p.3.
37. "Homeopathic Family Practice". H.C. Bhattacharyya; p.72. Economic Press, Cal. 1961.
38. Pir - Muslim spiritual guide for whom there is a great reverence.

Emotional peace is a major part of all curative treatment. Much of the behaviour of a Fakir is considered evidence of his contact with the supernatural powers which cause illhealth - his trance-like detachment from the world around him, the spasmodic shaking of his body. In consultation he 'transfers' a little of his influence by blowing on the patient, and rubbing his hands over the patient's body. He also blesses and blows on grains of salt, sand or oil seeds which become enchanted and may be left with the family to use against future attacks by spirits. He prescribes a complex of permissive and taboo actions which give women the feeling that they are participating in the protection or cure of the child. This feeling of reassurance is heightened by adherence to formalised ritual behaviour. The formula becomes part of the efficacy of the medicine. Treatment for scabies may be with an ointment compounded of 7 ingredients,[39] or the ceremonial swallowing of seeds,[40] under water on the last day of the Bengali year, a day on which behaviour and omens are considered significant for the coming year.

Rules and formulas provide emotional security and confidence, but it is a deceptive satisfaction based on logical consistency, the harmony of numbers, the balance of characteristics and corresponding patterns. This predilection for classification and for equating things which are similar gives an illusion of real insight into hidden connections and causations of facts.[41] The itinerant medicine seller's or the Fakir's powers of attraction depend in part on magical tricks and the suggestion of contact with the spirit world, but also on an acceptance by the audience of an analogy as a demonstration.

Village women are secluded from empirical or scientific education. Faced with the day to day emergencies of individual health and welfare they seek aid where they have access and where they derive most assurance. Modern scientific medicine which does not offer equal assurance may be disregarded. Dr. G.M. Carstairs, a psychiatrist with training in social anthropology, reports of his fieldwork in Rajasthan:

> "Throughout my stay both villagers and country folk remained very skeptical of the quality of my medicine. They had three grounds for distrusting it. First, I did not describe their illnesses in the terms they had come to expect from their own healers. Second, I failed to prescribe elaborate dietary restrictions as did the practitioners of classical Hindu medicine. Finally, they were dismayed to find that I did not invariably, and in dogmatice terms, assure them that my medicine would immediately cure them. In their eyes, my failure to do so amounted to malpractice. As many of them pointed out to me, it is not so much the ingredients of the prescription which effect the cure as the patient's unhesitating belief in its efficacy".[42]

39. Bis Katali, Nim leaves (Azardirachta indica), camphor, alum, copper sulphate, mustard oil and lime.
40. Seeds of the 'Kapila' tree - unidentified.
41. H.R. Zimmer; "Hindu Medicine", Johns Hopkins Press 1948, p.183.
42. G.M. Carstairs; "Medicine and Faith in Rural Rajasthan" in Health, Culture and Community, edited by B.D. Paul, Russell Sage Foundation, N.Y. 1955, p.122

Summary:

Muslim and Hindu mothers are actively concerned with the health of their children. The beliefs they hold about the cause of illness and the functions of the body lead them into two main areas of health behaviour - nutrition and magic. Adherence to formula and ritualised activity heightens the sense of security provided by a deceptively satisfying classification of phenomena. Rural women in general are secluded from education or experience which might challenge mystical thought.

VACCINE FIELD TRIALS -

A - B STUDY

by Abram S. Benenson., W.H. Mosley

Md. Fahimuddin and R.O. Oseasohn.

Surveillance has been maintained on the group immunized during November - December 1963, to follow the duration of effectiveness. Results through one year were presented at Honolulu, but since the disease has proven to be so sharply seasonal in Matlab, this presentation will be based on cholera seasons. The period December, 1963 to June, 1964 constitutes the first season after the vaccine was administered, and the second season covered the period August, 1964 to April, 1965, chronologically about one to one and one half years after vaccine administration.

The population has remianed relatively static. Recensus was started in April but was interrupted by the cyclone; a pre-selected sample chosen for the serological survey (See Report III e) revealed less than 10% attrition.

Recasting the cholera experience in these two groups by cholera season, reveals a 78% "effectiveness" of the whole cell vaccine during the first season, with 7 vs 32 cases in the control group, and a 60% effectiveness during the second season with 19 vs 50 cases. (Table I). In the second cholera season, the most significant protection is seen in the group with mild diarrhea(field), over four years of age (12 vs 0, $p \sim .00024$). (Table of Binomial Probability Distribution, National Bureau of Standards, U.S. Dept. of Commerce).

Including the hospitalized cholera cases, the difference between the vaccinated and control groups in ages over 4 (6 vs 29) is highly significant ($p \sim .0009$), but in the 0 - 4 group, the experience of 13 vs 2 cases is not outside the likelihood of chance ($p \sim .074$)

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13 Ogawa cases are included,, 10 in the control group and 3 in the vaccine group. Considering test subjects over four years of age, 7 Ogawa infections occurred in the control group and none among the recipients of the cholera vaccine; (this difference is significant p ~ .0078). Among the Ogawa strains, 7 were El tors; five in the control group, and two in the vaccine group. Only two occurred among those over 4, both in the control group.

There was no significant difference in the vibrio negative diarrhea experience, or in the death experience in the two groups. There is a suggestion in the greater number of field diarrheas reported during the second year that the quality of surveillance has improved with experience. (Table II).

Thus, significant immunity has persisted over a second year; this is primarily found in those over four years of age (which had contributed 58% of the cases occurring in the control group). While the percent protectiveness among those under 5 years old is 36%, in those older it is 78%, and highly significant. Comparing the experience over the two years it is only the young age group in which the immunity has been lost, raising the question whether their experience might reflect that which would pertain in non-endemic areas, while the long lasting protection of the older individual is an attribute of the immuniologically oriented individual who has grown up in a cholera endemic area. Serological studies should shed light on the age differenees

TABLE I

PROVEN CHOLERA CASES OVER TWO CHOLERA SEASONS AMONG VACCINATED INDIVIDUAL. A - B VACCINE STUDY

Age Group	Number of Positive Cases: Vaccine A Hosp.	Field	Total	Vaccine B Hosp.	Field	Total	Rates per 1000: Vaccine A Hosp.	Field	Total	Vaccine B Hosp.	Field	Total
FIRST CHOLERA SEASON DEC. 1963 - JUNE 1964.												
0 - 4	6	8	14	2	0	2	4.7	6.2	10.9	1.6	0	1.6
5 -14	9	3	12	1	3	4	3.7	1.2	4.9	0.4	1.3	1.7
15 & over	4	2	6	0	1	1	1.2	0.6	1.8	0	0.3	0.3
Total:	19	13	32	3	4	7	2.7	1.8	4.5	0.4	0.6	1.0
SECOND CHOLERA SEASON Aug. 1964 - April 1965.												
0 - 4	9(1*)	12(2*)	21(3)	5	8(1.2*)	13	7.0	9.3	16.3	4.0	6.4	10.4
5 -14	14(3)	10(1.2*)	24(6)	5	0	5	5.8	4.1	9.9	2.1	0.0	2.1
15&over	3	2(1)	5(1)	1	0	1	0.9	0.6	1.5	0.3	0.0	0.3
Total:	26(4)	24(6)	50(10)	11	8(3)	19(3)	3.7	3.4	7.1	1.6	1.2	2.8

PERCENT EFFECTIVENESS OF VACCINE B

	1st Cholera Season (0-6mos) Hosp.	Field	Total	Second Cholera Season (9-16 mos) Hosp.	Field	Total
0--4	66	100	85	43	31	36
5 -14	89	0	65	64	100	79
15&over	100	50	83	67	100	80
Total:	85	67	78	57	65	60

Asterisk numbers indicate number of El tor Ogawa strains.
One underlined = p .05 to .01, two underlined = .009 - .001, three underlined = .0009 to .0001.

TABLE II

DIARRHEA AND DEATH EXPERIENCE IN VACCINATED INDIVIDUALS

Age Group	Original Number in Test Groups		1st. Cholera Season (Dec 63 - June 64) Vibrio-Negative Diarrhea				2nd. Cholera Season (Aug 64 - April 65) Vibrio-Negative Diarrhea			
	Vaccine A	Vaccine B	Vaccine A		Vaccine B		Vaccine A		Vaccine B	
			Hosp.	Field	Hosp.	Field	Hosp.	Field	Hosp.	Field
0 - 4	1288	1248	17	1024	13	983	10	2384	5	2243
5 -14	2414	2384	8	756	15	763	10	2196	17	2078
15&over	3396	3324	43	1180	32	1120	36	2924	30	2816
Total:	7098	6956	68	2960	60	2866	56	7504	52	7137

	Deaths							
	Vaccine A		Vaccine B		Vaccine A		Vaccine B	
	Total	with diarr.	Total	with diarr.	Total	with diarr.	Total	with diarr.
0 - 4	10	6^{0-2*}	10	6	25	11	18	6
5 -14	5	2^{-1}	1	0	5	0	9	5^{0-4}
15&over	14	2	13	2^{1-0}	30	5^{0-1}	24	6
Total:	29	10^{-3}	24	8^{1-0}	60	16^{0-1}	51	17^{0-4}

* 6^{0-2} indicates 6 diarrhea cases, none confirmed cholera, 2 with symptoms of possible cholera, either vibrio negative or non-cultured.

VACCINE FIELD TRIAL
R-U-T Study.

by: Abram S. Benenson, M.D.

Studies reported last year showed that a whole cell cholera vaccine can indeed confer significant protection against cholera and raise the question whether specific antigenic factors might be responsible for this protection. Watanabe and Verwey had succeeded in isolating from an Ogawa cholera vibrio a factor which gave a single peak on ultracentrifugation and diffused through agar as a single band. The active ingredient had been isolated by fractionating culture filtrate, using the mouse protective potency as the selecting factor. In a limited number of humans this material had been shown to elicit vibriocidal antibody. They had not yet been able to isolate a similar "purified" factor from Inaba strains.

Verwey and Watanabe have proposed that a "practical" antigen, which contains 10% "inactive" material, could be used as an immunizing preparation. However, it was felt that a field trial could be justified only if purified material could be tested. Testing the Ogawa antigen in an area in which essentially all disease the previous cholera season had been caused by the Inaba serotype involved the risk inherent in all field trials that the particular disease might not appear during the study period. However, if the design of the field trial included a second study group given the whole cell vaccine previously tested, the likelihood was greatly increased that the results would justify the effort.

There followed an unusual example of international cooperation almost world wide in its geographic involvement. Dr. Watanabe had prepared a batch of crude material at the Kitasato Institute in Tokyo but he was now in Geneva. By telegraphic, telephonic and letter communication, arrangements were made for the Kitasato Institute to airship this frozen crude material to Galveston where Dr. Verwey carried it through to the purified antigen. This was then flown to West Point, Pennsylvania, for freeze drying; by Merke, Sharpe and Dohne. Drs. Pittman and Feeley at DBS, NIH, collaborated with Dr. Verwey in performing the potency and safety tests necessary. The dried material, together with reconstituting fluid, was flown to Dacca in time for administration before the end of the rainy season would make travel of the immunizing teams difficult. All of this was accomplished in just about a two months !

Since this type of soluable antigen had never been administered to residents of a cholera endemic area, an initial pilot study was carried out on 26 of the laboratory staff. Fingertip samples were collected periodically, and from all on the fourteenth day after the vaccine was given for later serological studies. The 0.4 ml dose projected for the field trial was administered. One physician followed the reactions. Essentially all had erythema around the site of the injection. A few individuals were febrile on the day following injection but this was suspected of being coincidental.

(more)

Alum precipitated tetanus toxoid was procured to serve as the control preparation; the same whole cell vaccine previously tested was used as the third preparations. Matched labels were obtained to mark the three products as vaccine R. U. or T.

Meanwhile census data was being developed on a group of villages which had not been included in the first trial. One of these was selected as a pilot village which was immunized using the three vaccine R.U.T., the vaccine selection being based on the terminal digit of the number preassigned on the census list. Jet injectors (twolent by the Medical Equipment Development Laboratory, Ft. Totten, N.Y.) were used to administer 0.4 ml of the three preparations. 143 individuals received vaccine R; 139 received vaccine U, and 140 vaccine T.

The vaccinated individuals were examined daily by a physician who recorded reactions as follows:

0 - No reaction
± - Doubtful reaction, including slight local redness and tenderness. The individual has complaints but carries out usual activity or work.
± - Local swelling, redness, with pain and tenderness. There might be slight fever and malaise.
++ - Individual is able to work but the reaction interferes with work. Usually marked local swelling, pain and tenderness; generally a rise in temperature.
+++ - Unable to carry out usual activity or work.
++++ - Pt. is bedridden. Localized swelling affects whole arm; high rise in temperature.

These criteria were applied without clinical interpretation, so that one individual is recorded as a ++++ reactor with typical clinical findings of epidemic parotitis.

It is evident from the Tables I, II, III that vaccine R produced a significant number of delayed reactions occurring between the 6th and the 9th day, almost entirely in those 15 years of age or older. Vaccine U. was interestingly devoid of reactions, while vaccine T did indeed produce an appreciable amount of reaction on the day after vaccination among those 15 years or older. However, the total reaction picture was well within acceptable limits of tolerance.

35 villages were then immunized between 23 September and 22 November 1964. Surveillance, as previously described, was established over these new villages.

The total population of the villages included was 32, 448; 25,267 were vaccinated a participation of 78% (Table 4). This is in sharp contrast to the experience in the first trial, with only 51% participation. Again working males are least well represented; during the rice harvest season, they were loath to risk a reaction which might interfere with work. Almost half of the recipients (47.4%) are 15 years old or over. The three vaccine groups are comparable in terms of age and sex (Table 5).

Cholera appeared in the study area during October, with isolation of Ogawa El Tor strains. On 27 October, an Ogawa field infection occured in a recipient of Vaccine T, three days after immunization.* On 9 December, an Ogawa case was hospitalized six weeks after Vaccine R had been given. However, from 17 December until 12 February there was sharp outbreak of cholera, all Inaba. From 12 February to 1 May, cases occurred sporadically among the vaccinees, with 8 detected infections, six Ogawa. Thus, despite the presence of the strain in the area, the hoped-for El Tor Ogawa outbreak did not appear, and the homologous challenge of the lipopolysaccharide antigen did not occur. However, 88 cases of proven vibrio infection did occur among vaccine recipients, 9 with Ogawa and 79 with Inaba serotype. Of the nine Ogawa, three were El Tor type with the remainder classical organisms.

The distribution of cases into vaccine groups is shown in Table VI. The whole cell vaccine is again effective. While numbers are small, there is significant protection afforded in the 0-4 and 15 or over age groups; and in both the field cases and the hospitalized group. The overall protective ratio is 73%.

The results with the purified lipopolysaccharide vaccine are most interesting. Although the outbreak is predominantly due to the Inaba subtype, significant protection is afforded. This is found to be confined to the over 15 group suggesting that the vaccine might be exerting booster effect. Two cases of Ogawa cholera, one mild (field) and one severe (both classical strains) occurred. Overall protection appears to be better in the field, but the numbers are too small significance. Analysis of the appearance of cases by time after immunization reveals no suggestion that an early immunity to cholera is being lost when the 15 and over age group is not included,(Fig.1). A cumulative curve of the vibrio positive diarrhea cases in the field (Fig.2) is highly suggestive that protection is being afforded by the lipopolysaccharide antigen; however the difference from the control group (8 vs 19) is not statistically significant which that afforded by the whole cell antigen (6 vs 19) is significant.

Analysis of the vibrio negative diarrhea experience in the group reveals no significant difference in hospitalized or field diarrheal experience in the three groups (Table VII),and no difference in death experience, either total or with diarrhea.

Thus, this study has served to corroborate the protective influence of the whole cell vaccine against Inaba disease. Despite the absence of enough Ogawa disease for a challenge, the purified Ogawa lipopolysaccharide preparation conferred significant protection to the adults and suggestive protection against mild infection (field cases). This suggest a crossing effect and weak antigenicity so that it is acting on a bed of basic immunity. This postulation is supported by the serological survey of the basic population (q.v.), and suggests the necessity to study the antibody responses among vaccine recipients. Since this was a soluble antigen in contrast to the whole cell material, it may be necessary for the antigen to be held by adsorption on a mineral adjuvant for full effectiveness. Such studies are under way.

*

Antibody Response to Lipopolysaccharide Vaccine.

Finger tip samples obtained from 26 CRL employees before and 14 days after immunization with Vaccine T were titered by the agglutination and vibriocidal techniques. Twenty three were tested by the agglutination test, starting at 1:40, and 21 by the vibriocidal test, starting at 1:50 using both Ogawa and Inaba antigens. All but two showed a rise in titer in the vibriocidal test with both antigens. Rises occurred in the agglutination test with both antigens; they were made marked with the Inaba antigen.

	Agglutination				Vibriocidal Test			
	Ogawa Antigen		Inaba Antigen		Ogawa Antigen		Inaba Antigen	
Tubes Pos.	Pre	14 days	Pre	14 days	Pre	14days	Pre	14 days
0	19	16	19	11	13	1	13	3
1	1	3	1	8	3	4	3	3
2		2		2	1	4	4	4
3					3	6	-	7
4					1	4	1	3
5					-	2		1
Mean	0.05	0.33	0.05	0.57	0.86	2.67	0.71	2.33

Intermediate sera of 10 showing a rise in titer were tested seeking the time interval for the antibody rise; this occurred as early as the 6th day in all of three with sera at this time interval.

=

Day After Injection

	1	2	3	4	5	6	7	8	9	10
++++	1		2		2	1	1			
+++	4	1				2	2	2		
++	5	1	1	2	1	1	5	5		
+	22	5	4	2		2	10	10	9	2
±	25	14	7	8	5	5	1	9	7	5
None	84	120	127	129	133	130	122	115	123	136

All Ages

++++	1		1		1	1	1			
+++	4	1				1	2	2		
++	4		1	1	1	1	4	5		
+	22	4	3	2		2	8	8	9	2
±	21	14	7	7	5	4	1	9	5	5
None	19	52	59	61	64	62	54	47	58	66

Ages 15 and over

++++										
+++										
++	1									
+		1	1				1			
±	2			1		1		1		
None	49	51	51	51	52	51	51	51	1 51	52

Ages 5-14

++++			1		1					
+++						1				
++		1		1						
+							1	1		
±	2								1	
None	16	17	17	17	17	17	17	17	17	18

Ages 0-4

TABLE I

REACTION AFTER ADMINISTRATION OF VACCINE R

Day After Injection

All Ages

	1	2	3	4	5	6	7	8	9	10
++++			1	1	1					
+++						1				
++							1			
+	4	1						1	1	1
±	12	1	1				1			
None	123	136	136	138	138	138	137	138	138	138

Ages 15 and over

++++			1	1	1					
+++						1				
++							1			
+	2							1	1	1
±	10		1				1			
None	56	68	66	67	67	67	66	67	67	67

Ages 5-14

++++										
+++										
++										
+										
±	2									
None	46	48	48	48	48	48	48	48	48	48

Ages 0-4

++++										
+++										
++										
+	2	1								
±		1								
None	21	21	23	23	23	23	23	23	23	23

TABLE II

REACTION AFTER ADMINISTRATION OF VACCINE U

Day After Injection

	1	2	3	4	5	6	7	8	9	10
++++										
+++	4	1	1							
++	3	2						1		
+	22	2	3	1	1	2			2	1
±	35	7	2	4	2	1	3	2	1	1
None	76	129	135	136	138	138	138	138	138	139

All Ages

++++										
+++	4	1	1							
++	3	2						1		
+	14	2	2	1	1	2			2	1
±	18	5	1	3	2	1	3	2	1	1
None	26	56	62	62	63	63	63	63	63	64

Ages 15 and over

++++										
+++										
++										
+	7									
±	16	1	1							
None	24	46	46	47	47	47	47	47	47	47

Ages 5-14

++++										
+++										
++										
+	1		1							
±	1	1		1						
None	26	27	27	27	28	28	28	28	28	28

Ages 0-4

TABLE III

REACTION AFTER ADMINISTRATION OF VACCINE T

TABLE IV

PARTICIPANTS IN R-U-T VACCINE STUDY BY AGE AND SEX

Age Group (year)	MALE			FEMALE			TOTAL		
	No. of inhabi-tants	Vaccinated		No. of inhabi-tants	Vaccinated		No. of inhabi-tants	Vaccinated	
		No.	%		No.	%		No.	%
< 5	2800	2345	83.8	2760	2330	84.4	5560	4675	84.1
5 - 14	5331	4436	83.2	4768	4173	87.5	10099	8609	85.2
15 +	8113	5209	64.2	8676	6774	78.1	16789	11983	71.4
Total :	16244	11990	73.8	16204	13277	81.9	32448	25267	77.9

Male : Female = 100.2 : 100.0

TABLE V

SIMILARITY OF SUBJECTS IN R-U-T VACCINE STUDY BY AGE AND SEX WITHIN VACCINE GROUPS

		VACCINE R		VACCINE U		VACCINE T	
		Number	% of Total	Number	% of Total	Number	% of Total
Total Subjects		8358	100%	8453	100%	8456	100%
Age (yrs)							
<5	M	718	8.6	791	9.4	836	9.9
	F	796	9.5	797	9.4	737	8.7
	Total :	1514	18.1	1588	18.8	1573	18.6
5 - 14	M	1484	17.8	1487	17.6	1465	17.3
	F	1397	16.7	1411	16.7	1365	16.1
	Total :	2881	34.5	2898	34.3	2830	33.4
15 +	M	1704	20.4	1746	20.7	1759	20.8
	F	2259	27.0	2221	26.3	22.94	27.1
	Total:	3963	47.4	3967	47.0	4053	47.9

TABLE VI

PROVEN CHOLERA CASES AMONG VACCINATED INDIVIDUALS
R-U-T VACCINE STUDY

OCTOBER 1964 - APRIL 1965.

Age Groups	R			U			T		
	Hospital	Field	Total	Hospital	Field	Total	Hospital	Field	Total
0-4	2(2)*	3	5(2)	9	12(3)	21(1+2)*	6	5(1)	12(1)
5-14	4	3	7	10	7(1)	17(1)	12(1)	3	15(1)
15+	1	0	1	8(1)	1	9(1)	0	1	1
Total:	7(2)	6	13(2)	27(1)	20(4)	47(5)	18(1)	9(1)	28(2)
	Rates per 1000								
0-4	1.3	2.0	3.3	5.7	7.6	13.3	3.8	3.2	7.0
5-14	1.4	1.0	2.4	3.5	2.4	5.9	4.2	1.1	5.3
15+	0.2	0	0.2	2.0	0.3	2.3	0	0.2	0.2
Total:	0.8	0.7	1.5	3.2	2.4	5.6	2.1	1.1	3.3
	Protective Ratios (Against Comparative Cell Vaccine U)								
0-4	77	74	75	-	-	-	33	58	43
5-14	60	58	59	-	-	-	-20	54	10
15+	90	100	91	-	-	-	100	33	91
Total:	75	71	73	-	-	-	34	54	41

( ) Number in parenthesis gives number of Ogawa cases included in the total. Asterisk number indicates number of El Tor Ogawa strains

Underlined numbers indicate significant differences from experience of control group - one underline : p~.05 to .01, two : .009-.001, three : .0009 to .0001.

T A B L E VII.

DIARRHEA AND DEATH EXPERIENCE IN VACCINATED INDIVIDUALS OCTOBER 1964 - APRIL 1965.

	D I A R R H E A					
	Vaccine R		Vaccine U		Vaccine T	
	Hospital	Field	Hospital	Field	Hospital	Field
0-4	17	2646	11	2711	18	2819
5-14	5	1683	11	1642	3	1674
15+	32	2243	19	2215	23	2321
Total:	54	6572	41	6568	44	6814

	D E A T H S														
	Vaccine R					Vaccine U					Vaccine T				
	Total		With	Cholera		Total		With	Cholera		Total		With	Cholera	
	No.	Rate per 1000	Diarr. hea.	+	Poss-.ible.	No.	Rate per 1000	Diarr-hea	+	Possi-ble.	No.	Rate per 1000	Diarr-hea.	+	Poss-ible
0-4	30	19.8	14	0	1	29	18.3	9	0	0	26	16.5	9	0	0
5-14	4	1.0	1	0	1	8	2.8	5	0	2	7	2.5	4	1	0
15+	22	5.6	5	0	1	20	5.0	4	1	0	23	5.7	3	0	1
Total	56	6.7	20	0	3	57	6.7	18	1	2	56	6.6	16	1	1

***

Figure- 1. IIIK(b)

HOSPITALIZED CHOLERA CASES UNDER 15YEARS OLD.

R - U- T VACCINES

1965

By interval since vaccination

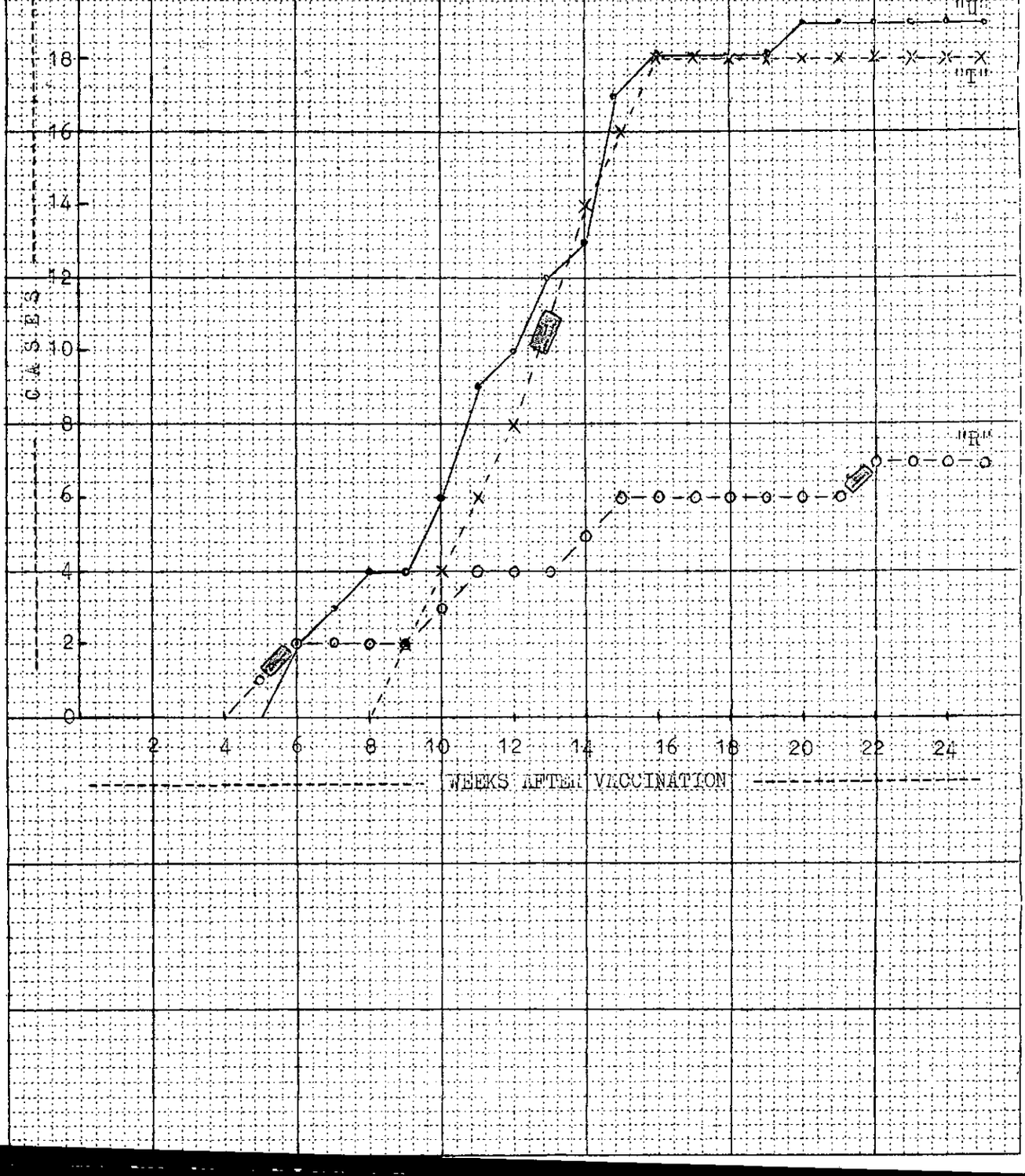
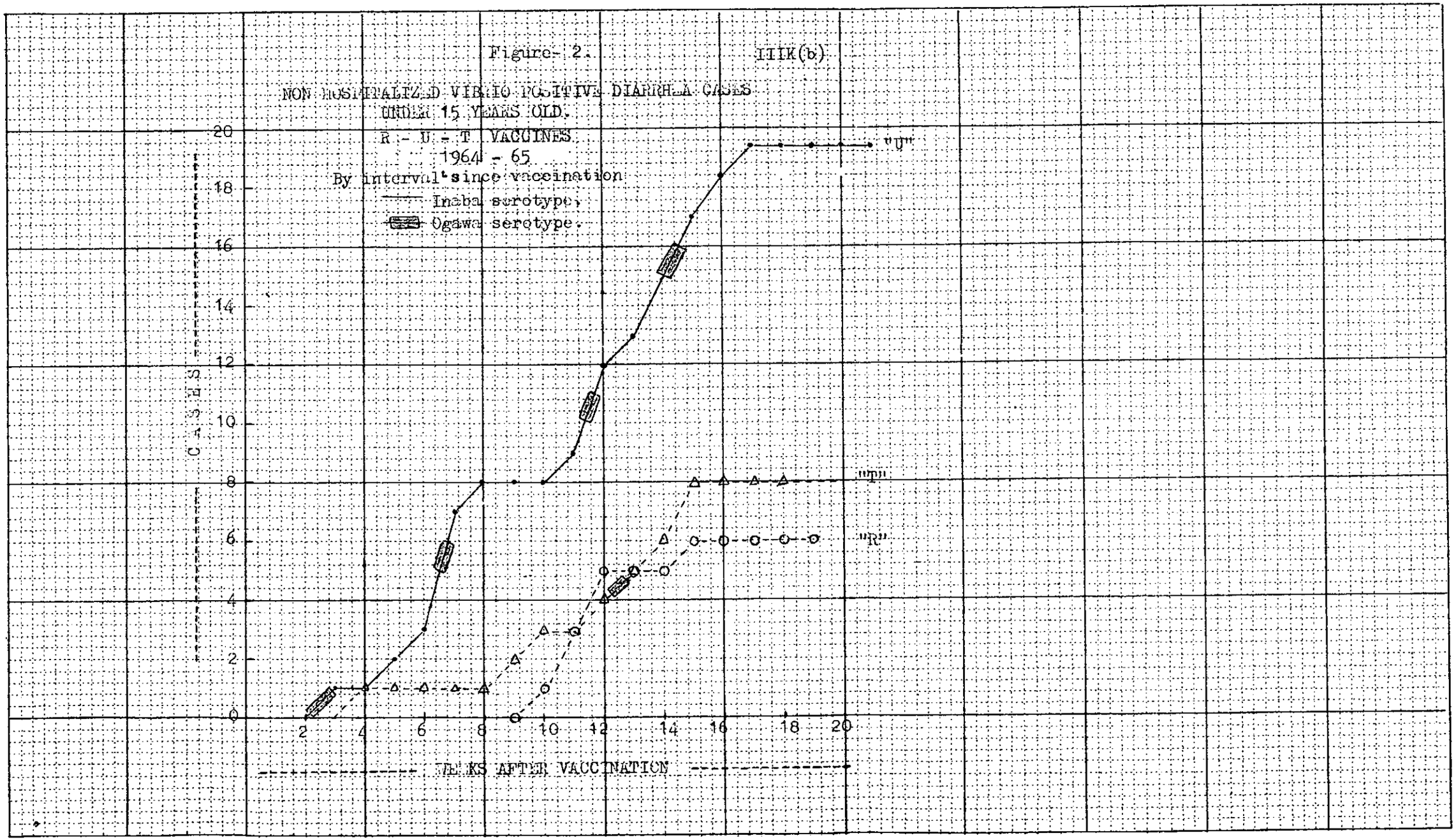


Figure- 2. IIIK(b)



III K(c)

EFFECT OF VACCINATION ON VIBRIO INFECTION AMONG HOUSEHOLD CONTACTS OF PROVEN CHOLERA CASES

by Abram S. Benenson., W.H. Mosley., Md. Fahimuddin and R.O. Oseasohn

Vaccination against cholera is the principal international quarantine measure depended on to prevent the importation of cholera from an infected area. To test whether vaccine does in fact have any influence on the asymptomatic carrier of vibrios, rectal swabs were obtained over a four day period, from the households of all hospitalized or incapacitated individuals among our study families, and evidence of secondary disease sought.

When the bacteriological and clinical experience of the family contacts of index cases found to be positive for V.cholerae are compared, no protective effect of vaccination is evident in terms of clinical or subclinical (carriers) infection. (Table I). The A-B study experience covers both cholera seasons; the R-O-T.data for the one season. Although the numbers are relatively small, with only 573 exposed family contacts in all, 10.8% of the contacts who had received control vaccine were infected, against 9.2% of those who had received the whole cell vaccine. Including the purified lipopolysaccharide antigen recipients, 9.9% of those who had received a cholera vaccine were infected. Carrier rates (asymptomatic infections) were 6.9% among those receiving a control preparation and 6.5% among those who had received the whole cell vaccine.

Of interest when dealing with a disease with the traditional explosive epidemic reputation of cholera, is the finding that only 2.6% of unprotected household contacts of a case develop a disease requiring hospitalization (6 of 231); among the 0,4 age group, only 2 of 37 contacts (5.4%) developed cholera. Considering the vibrio positive mild diarrhea found in the field, in the most susceptible age group, only 4 of 37 (10.8%) developed symptoms; another 7,however, have asymptomatic infections. Among all contacts in this age group (110), 8.2% have symptomatic and 17.3% asymptomatic infection, for a total of 25.5% infected. In the 5-14 age.group, a total of 10.8% of 195 are infected, and 3.7% of the 268 in the group are vibrio positive.

These observations indicate that any assumption that the person immunized against cholera with a good vaccine cannot carry vibrios is fallacious; indeed, in this group, there is only a slight suggestion that the vaccinee is less likely to become infected. The paucity of secondary cases of cholera among household contacts is in sharp contrast to experience with other infectious diseases, and raises again the fascinating questions of the conditions of the environment and/or the host which are necessary for the diesease to supervene.

TABLE I

"SECONDARY" INFECTIONS AND CASES AMONG HOUSEHOLD CONTACTS OF CHOLERA CASES. III K (c)

CONTROL GROUPS

Cg Group	No. Exposed	VACCINE A No. Infected Hospitalized	Field Diarrhea	Asymp-tomatic	Total Infected	No. Exposed	VACCINE B Hospitalized	Field Diarrhea	Asymp-tomatic	Total Infected
0-4	27	1	-	4	5	37	1	1	6	8
5-14	50	-	1	2	3	56	-	-	2	2
15.+	76	-	-	1	1	81	-	-	3	3
Total:	153	1	1	7	9	174	1	1	11	13
		VACCINE U						VACCINE R		
0-4	10	1	2	3	6	17	1	1	3	5
5-14	27	4	-	4	8	38	3	-	2	5
15.+	41	-	-	2	2	31	-	-	1	1
Total:	78	5	2	9	16	86	4	1	6	11
		TOTAL CONTROL GROUPS						VACCINE T		
0-4	37	2	2	7	11	19	1	-	3	4
5-14	77	4	1	6	11	24	2	-	1	3
15+	117	-	-	3	3	39	-	-	3	3
Total:	231	6	3	16	25	82	3	0	7	10

% INFECTED (TOTALS)

Vaccine Groups	Hospitalized	Field Diarrhea	Total Symptomatic	Asymptomatic	All Infectious
A + U (Controls)	2.6	1.3	3.9	6.9	10.8
B + R (Whole cell)	1.9	0.8	2.7	6.5	9.2
B+R+T (All Chol.V)	2.3	0.6	2.9	7.0	9.9
All Groups	2.4	0.9	3.3	7.0	10.3

SUMMARY OF WARD ACTIVITIES

from 1st. October 1964 to 31st. December 1965.

by Miss Dorothy Torrance.

The activities of the Cholera Research Laboratory Ward have continued to increase. There has been a steady flow of cholera patients each month with the exception of August, when no cholera patients were admitted (Table I). The peak months were December, 1964, when 150 cases of cholera were admitted and November, 1965, when 131 cases were admitted.

The months of March and April were the peak months for non-cholera cases, 115 cases were admitted in March and 121 in April. Thus a wider variety of clinical entities presenting with diarrhea in addition to cholera have been investigated and treated.

Forty-nine per cent of the cholera cases were children of 10 years of age and under. The youngest patient with cholera was a one month infant. Only 26% of the cases without cholera were 10 years of age or less. The majority of non-cholera cases admitted were adult males. Causes of death in cholera and non-cholera are given in Table II.

As the ward was built to accommodate 20 patients for research purposes, the increase in the number of patients and in research activities has made it necessary to utilize every available space. The wards and corridors during the cholera season are packed to capacity and the congestion makes working conditions for all staff very difficult indeed. Patients are moved over to the convalescent ward as soon as possible, leaving room for new acute cases.

An ambulance is sent daily to the Salimullah Hospital (formerly called Mitford Hospital). Any patient who arrives at this hospital with severe diarrhea is now automatically sent to the CRL in the ambulance. Approximately 250 cases have been admitted this way, and 50% had cholera. No medical or nursing staff are in attendance with this ambulance.

In January, 1965, 11 prisonaers were admitted in one week from the Central Jail, Dacca. Ten had Inaba Cholera, and one had acute amebiasis with ulcerative colitis and died 48 hours after admission. Staff from the Clinical and Epidemiology sections visited the Jail, but no further cases occured.

On the 1st. February one patient with acute cholera was brought by taxi from the village of Baushia, about 30 miles from Dacca, four

hours round trip by car. A few days later the District Council at Baushia reported that there were many people dying of cholera in that area and they appealed for help. An ambulance and a male nurse were dispatched daily, with a supply of I.V. fluid for infusion (Serofusine, in disposable plastic packs, similar in composition to the solution used on the CRL ward). The nurse began intravenous fluids when necessary and probably saved a number of lives.

A total of 54 cases were brought to the CRL, 35 with cholera and 19 without. Nearly all patients were included in various Clinical Research studies. This year the District Council at Baushia have been told to take any cases to the CRL unit at Matlab, which is only 10 miles away.

In March, 1965, a sharp outbreak of cholera occured at Mirzapur, a township about 45 miles from Dacca. At the request of the SDO, Tangail, and with permission from the Hospital authorities at Mirzapur, a team consisting of one doctor and three nurses demonstrated to the Medical and nursing staff of this hospital the CRL methods of treating and nursing cholera.

In October, 1965, an outbreak of cholera occured at Dhamri, a small town 30 miles from Dacca. An ambulance and one male nurse went out daily for several weeks to this area. More than 90 cases were admitted, the majority with cholera. Intensive studies were made in this village by the staff of Epidemiology Section.

Twenty patients were transferred from the Crl unit at Matlab for further investigations and treatment. Only one case had cholera, a child of 4 years of age with severe acidosis. He subsequently did well and was discharged in seven days. The other cases suffered from mixed syndromes of malnutrition, hypovitaminosis and kwashiorkor; some had amebiasis. Four patients died; the final diagnoses of these four patients are recorded separately on Table II. The others responded satisfactorily to good nursing care, diet, and appropriate medical treatment.

The following studies have been carried out or are going on:

a) Antibiotic Study
b) Cardiac Catheterization Study by Dr. Harvey and colleagues
c) Intestinal Absorption Study
d) Studies on Jejunal Biopsies (morphology, enzyme analysis).
e) Chromium 51-Albumin Study.
f) Studies on Hypoglycemia.
g) Intubation Studies with Miller Abbott tube.
h) Blood volume and electrolyte changes during intravenous therapy.

The nursing staff has enthusiastically responded to the stimulus of the various studies and has co-operated to the full. The measurement of urine and stool are done by the nurses, intake and output totals recorded and all charting done by them. Meticulous care and a high degree of accuracy are responsible for the successful treatment of patients and enable research work to be carried out smoothly.

Medical and Nursing Staff.

Doctors	8
Hospital Supervisor	1
Senior Staff Nurse	1) for follow
Male Aid Nurse	1) up work.
Senior Staff Nurse for Medical records and coding	1
Male Staff for special studies, etc.	1

Ward Duties.

Male Staff nurse	1
Male Aid nurse	1
Senior Staff nurses	4
Female Aid nurses	7

X-ray and ECG

Technician	1

Pharmacy

Male nurse	1.

X-ray Department.

As the number of patients has increased so the work of this department has increased considerably. X-ray examinations from 1st. October, 1964 to 31st. December, 1965 are shown on Table III.

Pharmacy.

From the 1st. October, 1964 to 31st. December, 1965, 13,256 litres of I.V. solution for fluid replacement were made in the Pharmacy.

During the peak month of December, 1964, 1921 litres were used.

Six grams sodium chloride and 4 grams sodium bicarbonate in one litre of distilled water (6:4) had been used successfully for several months. Green coconut water was used as a source of potassium, if the patient could tolerate fluids by mouth. As the load of patients increased in November, green coconut water was discontinued as the method of supplying potassium and a solution

of 5 grams sodium chloride, 4 grams sodium bicarbonate and 1 gram potassium chloride was started (5:4:1). This solution has now replaced 6:4 solution for initial hydration and maintenance of uncomplicated cases.

For 11 months, when bicarbonate was not available, 50 mEq/litre of sodium lactate was used and was quite satisfactory.

The proceedure for filling and making solutions is as follows:-

One litre glass bottles with screw caps are washed in hot water, rinsed twice in distilled water and hung to drain. They are sent to Bacteriology department for drying in the oven (170 C dry heat). The bottles are then returned to the Pharmacy. When cool they are filled with distilled water directly from the Menstry Still. Five grams sodium chloride and 1 gram potassium chloride are weighed and added to each bottle of fluid which is then sent to be autoclaved. After autoclaving the caps are screwed on tightly, sealed and labelled and the bottles are stored.

Four grams sodium bicarbonate is weighed out and stored in small clean bottles labelled 5:4:1. When a bottle of replacement fluid is required the contents of one of these small bottles is added to the bottle of autoclaved fluid containing 5 grams sodium chloride and 1 gram potassium chloride, making a solution of 5:4:1. The label from the small bottle is then attached to the litre bottle, the date and time of mixing are written on the bottle with a black marking pencil. The fluid is ready for infusion.

Darrows solution in disposable packs has been used as an intravenous replacement fluid in a few children with severe hypokalemia. This solution contains 4 grams sodium chloride, 2.67 grams potassium chloride, and 53.3 mEq sodium lactate in 1 litre of water for injection.

Serofusine in disposable packs is used in field work. This solution contains 133 mEq/L sodium chloride, 12 mEq/L potassium, 3 mEq/L calcium, 4 mEq/L magnesium, 103 mEq/L chloride, 49 mEq/L acid lactate.

The use of a single intravenous solution for fluid replacement has simplified the work of the pharmacy and of the nurses in charting the intravenous fluids.

TABLE I.

ADMISSIONS 1964 /1965.

	CHOLERA			NON-CHOLERA		
	Admissions	Deaths	Autopsies	Admissions	Deaths	Autopsies
Children 10 years and under	417	3	1	281	15	4
Males over 10 years	272	1	0	548	10	5
Females over 10 years	152	2	1	263	3	0

Total Admissions 1964 / 1965 = 1933

Total Admissions 1963 / 1964 = 865

Total Admissions 1962 / 1963 = 488.

TABLE II.(a)

DEATHS - CHOLERA AND NON-CHOLERA CASES.

CHOLERA

CRL Case number	Age in Years	Sex	Final diagnosis or cause of death	No.of hours or days after hospitalization	Autopsy	Transfd. from Matlab
1371	2½	M	Acute cholera with severe dehydration	15 hrs.	nil	-
1505	3½	M	Ac. Cholera Inaba with severe acidosis, hypoglycemia with neurological damage	2 hrs.	done	-
2943	2	F	Ac.Cholera Inaba, severe acidosis	8 hrs.	nil	-
2887	18	M	Ac.Cholera Inaba, severe acidosis	40 mins.	nil	-
1725	22	F	Ac.Cholera Inaba. ? Cardiac disease. Leprosy.	29 hrs.	done	-
1819	55	F	Ac.Cholera Inaba with hypertensive cerebral stroke	105 hrs.	nil	-

cont. . . . .

TABLE II (b).

DEATHS - CHOLERA AND NON-CHOLERA CASES.

NON-CHOLERA.
under 10 years.

CRL case number	Age in Years	Sex	Final diagnosis or cause of death	No.of hours or days after hospitalization	Autopsy	Transfer from Matlab.
1353	8/12	F	Ac. and chr. diarrhea with shock and acidosis	15 mins.	nil	-
1383	7	M	Ac. gastroenteritis	28 hrs.	nil	-
1458	2½	F	Ac. gastroenteritis,Encephalopathy with fatty liver, hypoglycemia	25 mins.	done	-
1775	1	F	Ac. gastroenteritis	91 hrs.	nil	-
1925	1½	M	Salmonella, septicemia with hypoglycemia	38 hrs.	done	-
2106	8/12	M	Severe dehydration, chr. and ac. diarrhea	30 mins.	nil	-
2321	1	M	Brain abscess,L.mastoid abscess	118 hrs.	nil	-
2355	3	F	Hypoglycemia with brain damage	12 hrs.	done	-
2381	11/12	M	Pneumococcal septicemia, bilateral pneumonia, severe malnutrition, hypoglycemia	8 hrs.	done	-
2401	6/12	M	Kwashiorkor	6 days	nil	-
2582	1½	F	Kwashiorkor with severe hypokalemia. Fatty liver	21 hrs.	nil	-
2585	4	F	Amebic colitis,? perforation	6 days	nil	Yes.
2662	1½	F	Ac.gastroenteritis with shock and acidosis	1 hr.	nil	-
2691	4	F	Severe malnutrition,NAG diarrhea	8 days	nil	--
2815	10/12	M	Paralytic Ileus with septicemia	2 hrs.	nil	-

cont . . . . .

TABLE II (b) continued.

DEATHS - CHOLERA AND NON-CHOLERA CASES.

NON-CHOLERA
over 10 years.

CRL case number	Age in Years	Sex	Final diagnosis or cause of death	No. of hours or days after hospitalization	Autopsy	Transfer from Matlab.
1212	75	M	Amebic colitis, ? amebic liver abscess	29 days	done	-
1748	40	M	Amebic colitis	72 hrs.	done	-
1752	45	M	Hepatic failure, ? viral hepatitis, renal failure	20 days	done	yes
1796	30	M	Amebic colitis with heamorrhage, anemia, ? cirrhosis liver	10 days	done	yes
1798	48	M	Congen. heart failure	31 hrs.	nil	-
1857	35	M	Pulmonary T.B.	54 hrs.	nil	-
2055	70	M	Amebic dysentry	68 hrs.	nil	-
2281	55	M	Amebic dysentry	82 hrs.	nil	-
2350	27	M	Anuria, uremia and acidosis. Uremic lungs with pulm. hemorrhage	9 days	nil	yes
2522	55	M	? Arsenic poisoning	81 hrs.	done	-
2041	80	F	Hypertensive stroke	35 mins.	nil	-
2233	40	F	Anemia, pneumonia, ? pulmonary T.B.	15 mins.	nil	-
2956	23	F	Death from unknown cause. Vomiting, diarrhea, anuria. r/o poisoning	1 hr.	nil	-

TABLE III.

X-RAY EXAMINATIONS -

1st. October, 1964 to 31st. December, 1965.

Upper Gastrointestinal series	17
Barium Enemas	11
Small bowel series	75
Cholecystogram	1
I.V. Pylogram	1
Total opaque media	105
Chest and others	1835
TOTAL	1940

Photocopy of interview with Neogi

WATER STUDIES

by: Syed Zafar Ahmed, Yousuf A. Saeed, Syed Iltafat Ahmad and P.K. Bose Neogi.

uction

The seasonal appearance of cholera year after year in this part globe has made this zone to be classified as an endemic area. This a delta region, water seems to be the logical factor to look into. ast histories of cholera epidemics have proven beyond doubt the part played by water as being the principal agent in disseminating the disease together with some environmental factor.

Abundance of surface water sources within easy reach of the dwellers make water more suspicious to be probed into for the endemicity of the disease either as a reservoir for the causative organism, as a vehicle for the transmission, or as an agent to harbour the pathogen. The climatic conditions described in literature favourable for the propagation of the germ and its longer survival also prevail in this region and have some influence on the surface water sources. For example, heavy rainfalls dilute the surface waters and change the chemical as well as bacterial quality. High humidity and sub-tropical climate have definite influence on the micro-organisms living in waters.

With all these factors in mind and in the absence of any detailed study on waters of this area, it was necessary to study the waters to find some clue to all these riddles. The facilities at this laboratory provided an opportunity to take up the issues confronting the role of waters. A programme was drawn up to study the waters both chemically and bacteriologically to determine the quality of different waters and the incidence or prevalence of cholera organisms. The technicians in the Water Studies Section of this Laboratory had been doing complete chemical analyses and sanitary bacteriology analyses of some waters on a separate project before joining the CRL. Chemical analyses, consisting of determinations like pH, conductance, alkalinity, salinity, hardness, nitrogens and other minerals, were performed routinely. On the bacteriology side, determinations of the Coliforms MPN by tube fermentation technique and also by the Millipore filter technique were carried out regularly. The isolation of vibrios from water was done by a new technique which was set up according to the treatment and procedures employed for isolation of these organisms from stool specimens in the Bacteriology Section of this laboratory with some modifications suitable to waters. The methods for culturing water for vibrios are described partly in the 1962 report and in an appendix in the 1963 report.

Surveillance Studies

1962: The study on waters started with surveillance of an endemic locality, Kaliganj, across the river in the suburbs of Dacca city. Eight tanks, three dug-wells and two canal points were sampled every week and tested for temperature, pH, alkalinity, chlorides and nitrogens on the chemical side and for coliforms and vibrios on the bacteriological side. The results of a nine-month study were reported in 1962 in the Technical Committee Meeting.

The findings did not show any significant relationship with any of the factors, physical, chemical or bacteriological. There was wide fluctuation in the chemical constituents. The level of pollution as indicated by coliform organisms remained high and no pattern could be visualised with seasonal changes. The most important factor was that no classical cholera organisms were ever isolated from any source during this nine months' surveillance. However, non-cholera vibrios were isolated time and again; this isolation could not be related to the water quality or quantity.

1963: The surveillance study continued and some new areas where cholera recently appeared were included. The same type of determinations were carried out. The areas covered were Kushiarbagh, Aganagar across the river Buriganga, Mudafa, an agricultural community living in Tongi area and another refugee settlement, Bander colony, in Narayanganj area. Kushiarbagh and Aganagar had cholera cases and the main tanks of these two villages were found positive for Vibrio cholerae; but when the epidemic was over the tanks did not show any positivity for classical organisms and also no cholera cases were reported during the entire period of our study there.

In some case investigation studies it was found that water storage jars gave positive indication of contamination with the same strain infecting the case. This suggested transmission of cholera germs from humans to water. To confirm this hypothesis, a surveillance study was conducted on the water-storage jars in Kushiarbagh area. The stored water in the household as well as the sources of water which filled these jars were cultured for vibrios and coliforms. This study showed contamination of jars with coliforms as well as non-cholera vibrios irrespective of the contamination of the water sources. Unfortunately, studies on persons using such storage jars were not conducted to establish true relationship between the findings in waters.

With studies under surveillance or studies conducted in connection with cholera cases on tanks and ponds, we were able to classify the tanks according to their use by the populace and their chemical nature and bacteriological quality. The deductions were reported in detail in the report to the Technical Committee in the year 1963.

Two consecutive years of study on tanks, dug wells etc. indicate that the appearance of classical Vibrio cholerae in these waters is not an environmental, climatic or seasonal phenomenon but is solely dependent on the contamination by a cholera case, or carrier.

Rayer Bazar Surveillance, 1964-1965

In early 1964, Ogawa cholera broke out in an epidemic form in a relief camp in Rayer Bazar, a locality in Dacca Town. This locality had a past history of cholera and in 1963 a small Ogawa outbreak was witnessed there. The re-appearance of the disease with the same serotype organism suggested the possibility of an endemic focus somewhere either in the population or in the environment. The waters studied in the 1963 outbreak had shown positivity in tanks and dug-wells used by the affected families. The special feature as regards the water source in this locality is that the majority of the population has its private source of water which is dug-well. This offered a good opportunity to do a prospective study on waters as well as the people to establish any relationship between the vibrio situation in the waters and the diarrheal incidents in the people. A comprehensive plan was made and protocol for prospective sampling of the water sources and rectal swabs of the population was set up together with the Epidemiology Section under the supervision of Dr. John P. Craig.

On an average, seventy to eighty dug-wells, six tanks and seven to eight tube-wells were examined every week for vibrios for a period of sixteen months from April 1964 to July 1965. During this sixteen month period, all the waters remained free from classical cholera organisms except in three instances where the dug-well showed positivity after the families using that water had apparent or inapparent infection with Vibrio cholerae.

The apparent relationship of findings in waters and in the people are to be discussed in detail by Dr. Craig. The results of the vibrio findings in waters are shown in Tables 1, 2 and 3.

It is evident from the results that in dug-wells the vibrio population has been influenced by two factors; the rainfall and the water level. When the rains came the water rose in the wells and so the occurrence of non-cholera vibrios increased. When the water level dropped the occurrence of non-cholera vibrios also dropped.

The tanks followed a similar pattern to some extent. After the rains raised the water level in the tanks, the frequency of non-cholera vibrios increased and when the rains stopped this frequency dropped. The The relationship of the finding of non-cholera vibrios in tanks and dug-wells to seasonal factors such as rainfall and humidity are shown in Graph number 3.

But as far as the isolation of classical cholera is concerned it is subject to human positivity and subsequent contamination of the water sources. In the absence of human cases, tank and well water does not show signs of Vibrio cholerae.

However, one trend evident from the study of tanks and wells is that during the period when cholera subsides the incidence of non-cholera vibrios increases and during the cholera period it drops down.

TABLE I - TABLE SHOWING FREQUENCY OF OCCURENCE OF NON-CHOLERA VIBRIOS IN TUBE-WELLS OF RAYER BAZAR, APRIL 1964 - JULY, 1965

No. of Tube-well studied	Month 28-35 day period	Weeks of study	No. of samples examined	No. of positive for NCV	% positive for NCV
	1964				
7	APR	12-15	23	0	0
6	MAY	16-20	12	0	0
6	JUNE	21-24	18	2	11.1
7	JULY	25-29	28	3	10.7
7	AUG	30-33	18	1	5.5
11	SEPT	34-37	29	4	13.7
12	OCT	38-42	47	2	4.2
12	NOV	43-46	36	0	0
9	DEC	47-50	32	0	0
	1965				
21	JAN	51-55	41	0	0
11	FEB	56-59	40	2	5.0
13	MAR	60-64	56	2	3.5
14	APR	65-68	47	1	2.1
12	MAY	69-72	56	3	5.4
14	JUNE	73-77	65	5	7.6
14	JULY	78-81	37	2	5.4
Total :			585	27	4.6

TABLE II - TABLE SHOWING FREQUENCY OF VIBRIO CHOLERAE & NON-CHOLERA VIBRIOS IN TANKS OF RAYER BAZAR APRIL 1964-JULY 1965

Month 28-35 day period		Weeks of Study	Number of Samples examined	Number positive for NCV	Percent of samples positive for NCV
1964	APR	12-15	26	21	80.8
	MAY	16-20	24	15	62.5
	JUN	21-24	25	18	72.0
	JUL	25-29	33	23	70.0
	AUG	30-33	24	10	41.6
	SEPT	34-37	20	10	50.0
	OCT	38-42	28	14	50.0
	NOV	43-46	19	7	36.8
	DEC	47-50	18	6	33.0
1965	JAN	51-55	25	7	28.0
	FEB	56-59	19	8	42.0
	MAR	60-64	20	13	65.0
	APR	65-68	16	11	70.0
	MAR	69-72	16	9	56.2
	JUN	73-77	20	13	65.0
	JUL	78-81	16	13	81.2
Total :			349	198	56.7

TABLE III

Frequency of Isolation of *V. cholerae* and non-cholera Vibrios from Dug wells, pH of Dug well Water, and Depth of water surface below ground level, by month*, Rayer Bazar, Dacca, April 1964-July, 1965.

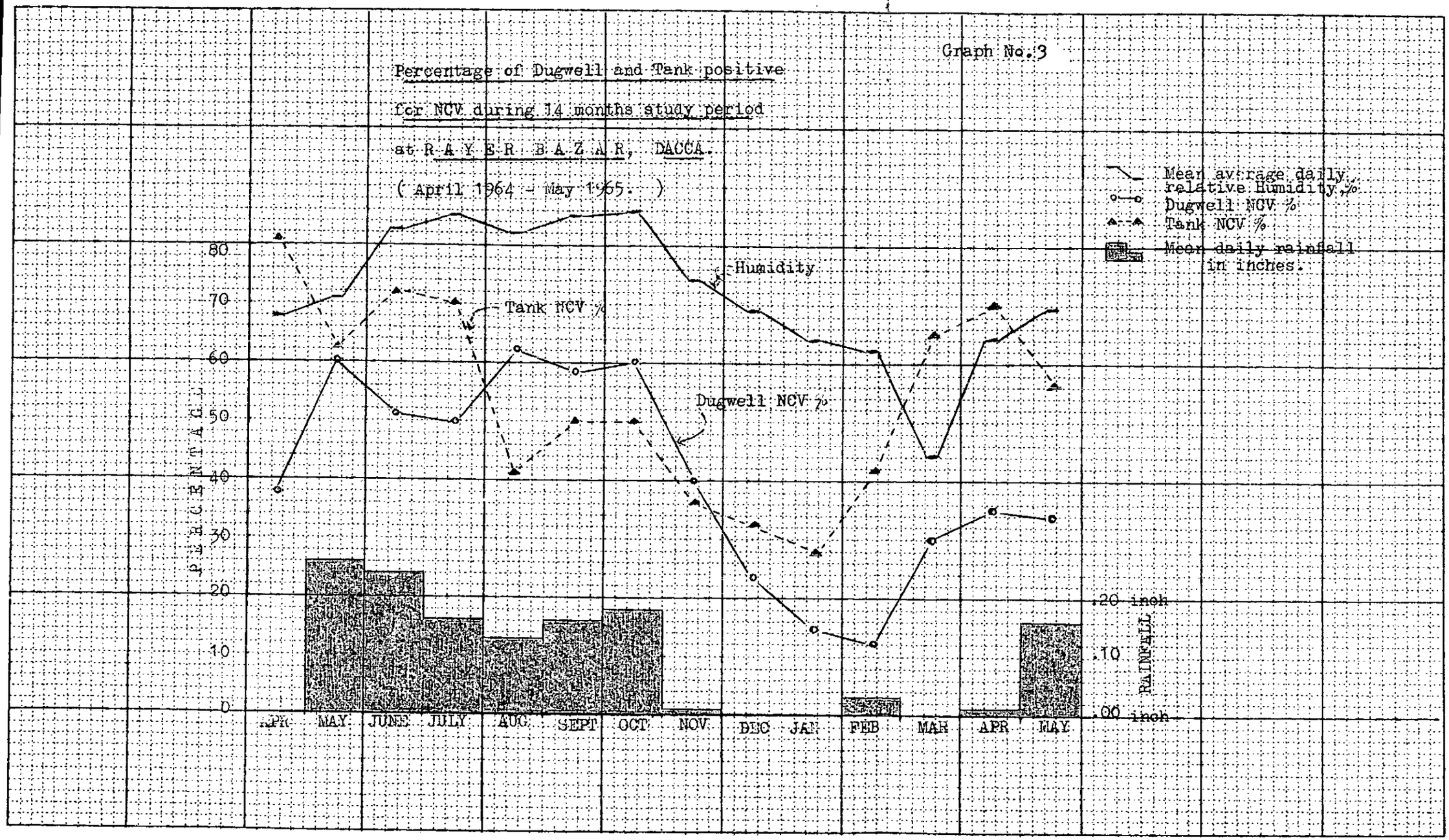
Month (28-35 day period)	Weeks of Study	Number of samples examined	Number Positive for NCV	Percent of samples Positive for NCV	Number of samples Positive for *V. cholerae*	Mean Dug Well water level below Surface	Mean pH of all Samples
1964 APR	12-15	152	58	38.2	-	24.9	6.43
MAY	16-20	247	149	60.3	-	18.5	6.34
JUN	21-24	251	130	51.8	-	18.9	6.33
JUL	25-29	348	174	50.0	-	10.9	6.27
AUG	30-33	293	184	62.8	-	7.3	6.25
SEPT	34-37	308	181	58.8	-	9.9	6.30
OCT	38-42	413	249	60.3	2	10.5	6.31
NOV	43-46	329	133	40.4	-	17.1	6.37
DEC	47-50	321	77	24.0	1	23.0	6.50
1965 JAN	51-55	382	58	15.2	1	26.4	6.47
FEB	56-59	335	43	12.8	-	29.0	6.50
MAR	60-64	425	129	30.4	-	28.4	6.48
APR	65-68	340	122	35.9	-	29.7	6.44
MAY	69-72	302	103	34.1	-	26.4	6.39
JUN	73-77	360	198	55.0	-	19.6	6.47
JUL	78-81	283	167	58.5	-	12.4	6.47
TOTAL :		5089	2155	40.3	4		

* Based on Weekly sampling.

Graph No. 3

Percentage of Dugwell and Tank positive for NCV during 14 months study period at R A Y E R B A Z A R, DACCA.

(April 1964 - May 1965.)



River Surveillance in Dacca and Narayanganj

In addition to the studies on tanks, dug-wells and tube-wells, two major rivers, Sitalakhya and Buriganga, one flowing through Narayanganj about ten miles south of Dacca and the other flowing through Dacca Town have been studied simultaneously since 1962 and 1963. The findings on these two rivers have been reported each year to the Technical Committee Meeting (Report of 1962, 1963 and 1964). The data for 1965 on these two rivers has been shown in separate graphs (graphs I and II).

Except for occasional isolation of classical cholera organisms in the absence of any known human cases in the vicinity, the rivers have remained negative throughout the year on surveillance. However, in early 1964 when cholera broke out in epidemic form in a relief camp at one of the jute mills on the bank of Sitalakhya river, the river was found positive for classical cholera and a surveillance set up after the epidemic was over showed that the river remained positive up to three months following the outbreak. The findings were reported to the Technical Committee Meeting in 1964. The surveillance shows that rivers follow a similar trend each year as far as the chemical and physical aspects are concerned. During the dry period, October - March, the pH, alkalinity and specific conductance are higher. But with the rains, water becomes diluted and these factors drop down then again start increasing when the monsoon is over. A comparative study of these constituents by years on the two rivers has been shown in Tables 1 to X.

The fecal contamination in these rivers does not show such a fall or rise during the monsoon and dry periods. The same seems to be the case with vibrio population. Though there is some fluctuation in the percentage of non-cholera vibrio isolations, that does not seem to follow a fixed trend as in the case of chemical and physical factors involved.

These findings again suggest that no possible relation seems to exist between the chemistry and bacteriology of water.

TABLE I

LAKHYA RIVER SURVEILLANCE

SHOWING ISOLATION OF NON CHOLERA VIBRIOS/TOTAL NUMBER OF SAMPLES TESTED. ISOLATION OF VIBRIO CHOLERAE IS SHOWN WITHIN BRACKETS.

YEAR	JAN-MAR	APR-JUN	JUL-SEP	OCT-DEC
1962	3/8	1/7	2/9	-
1963	2/5	4/7	1/7	2/4
	(2/5 INABA)			
1964	3/6	2/5	3/6	4/7
1965	2/6	3/6	2/7	-

TABLE II

BURIGANGA RIVER SURVEILLANCE

SHOWING ISOLATION OF NON CHOLERA VIBRIOS/TOTAL NUMBER OF SAMPLES TESTED. ISOLATION OF VIBRIO CHOLERAE IS SHOWN WITHIN BRACKETS.

YEAR	JAN-MAR	APR-JUN	JUL-SEP	OCT-DEC
1963	4/4	5/6	4/8	4/5
1964	3/5	4/4	4/6	3/6
	(1/5 INABA)		(1/6 INABA)	(1/6 INABA)
1965	4/5	6/6	3/7	
	(1/5 INABA)	(1/6 OGAWA)		

TABLE III

Average pH of Lakhya River

	Jan-Mar.	Apr-June.	Jul-Sept.	Oct-Dec.
1962	8.3	8.0	7.4	-
1963	7.9	7.7	7.2	7.8
1964	8.1	7.5	7.5	7.4
1965	8.1	7.7	7.4	-

TABLE IV

Average Alkalinity mg/l in Lakhya River

	Jan-Mar.	Apr-June	Jul-Sept	Oct-Dec.
1962	168	87	51	-
1963	165	107	49	93
1964	157	57	47	64
1965	145.2	76.5	41.4	-

TABLE V

Average Specific Conductance of Lakhya River

	Jan-Mar.	Apr-June	Jul-Sept.	Oct-Dec.
1962	338	180	118	-
1963	316	223	113	202
1964	328	167	109	149
1965	302	180	108	-

TABLE VI

Average E. coli/100 ml of Lakhya River

	Jan-Mar.	Apr-June	Jul-Sept	Oct-Dec
* 1962				
1963	6.2×10^2	1.9×10^3	2×10^3	6×10^2
1964	5.6×10^2	2.5×10^3	3.6×10^3	2.8×10^3
1965	1.7×10^3	1.9×10^3	1.7×10^3	-

* Coliforms instead of E. Coli were estimated.

TABLE VII

Average pH of Buriganga River during the following years

	Jan-Mar.	Apr-Jun.	Jul-Sept.	Oct-Dec.
1962	8.0	7.7	7.7	7.4
1963	8.0	7.3	7.1	7.32
1964	8.0	7.8	7.5	7.4
1965	8.5	7.9	7.5	-

TABLE VIII

Average total alkalinity Mg/l of Buriganga River.

	Jan-Mar.	Apr-Jun	Jul-Sept	Oct-Dec.
1962	136	97	54	86 mg/l
1963	155	94	52	78
1964	162	82.5	45	64
1965	165.5	102.3	51.7	-

TABLE IX

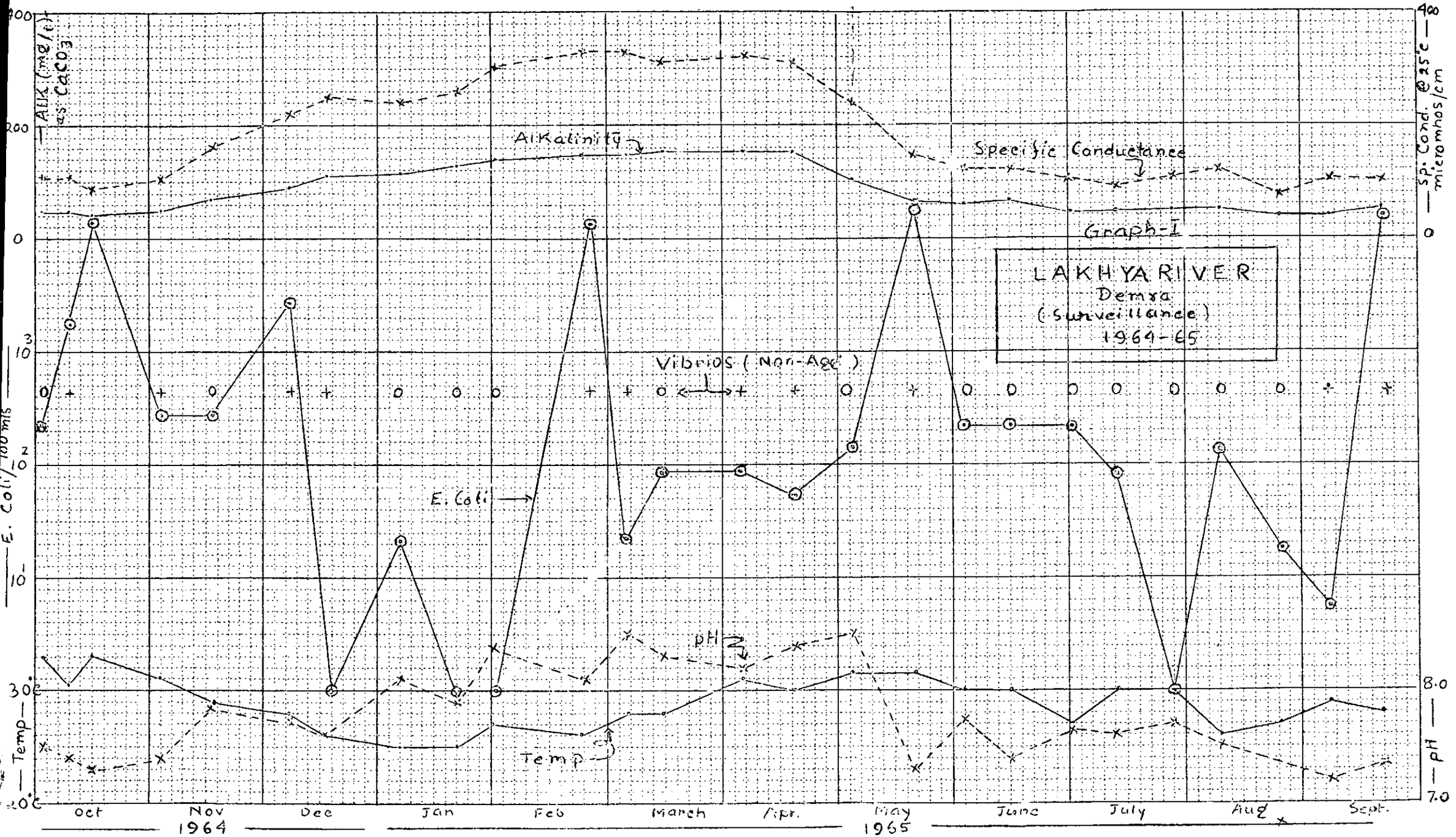
Average Specific Conductance Micromhos/cm of Buriganga River.

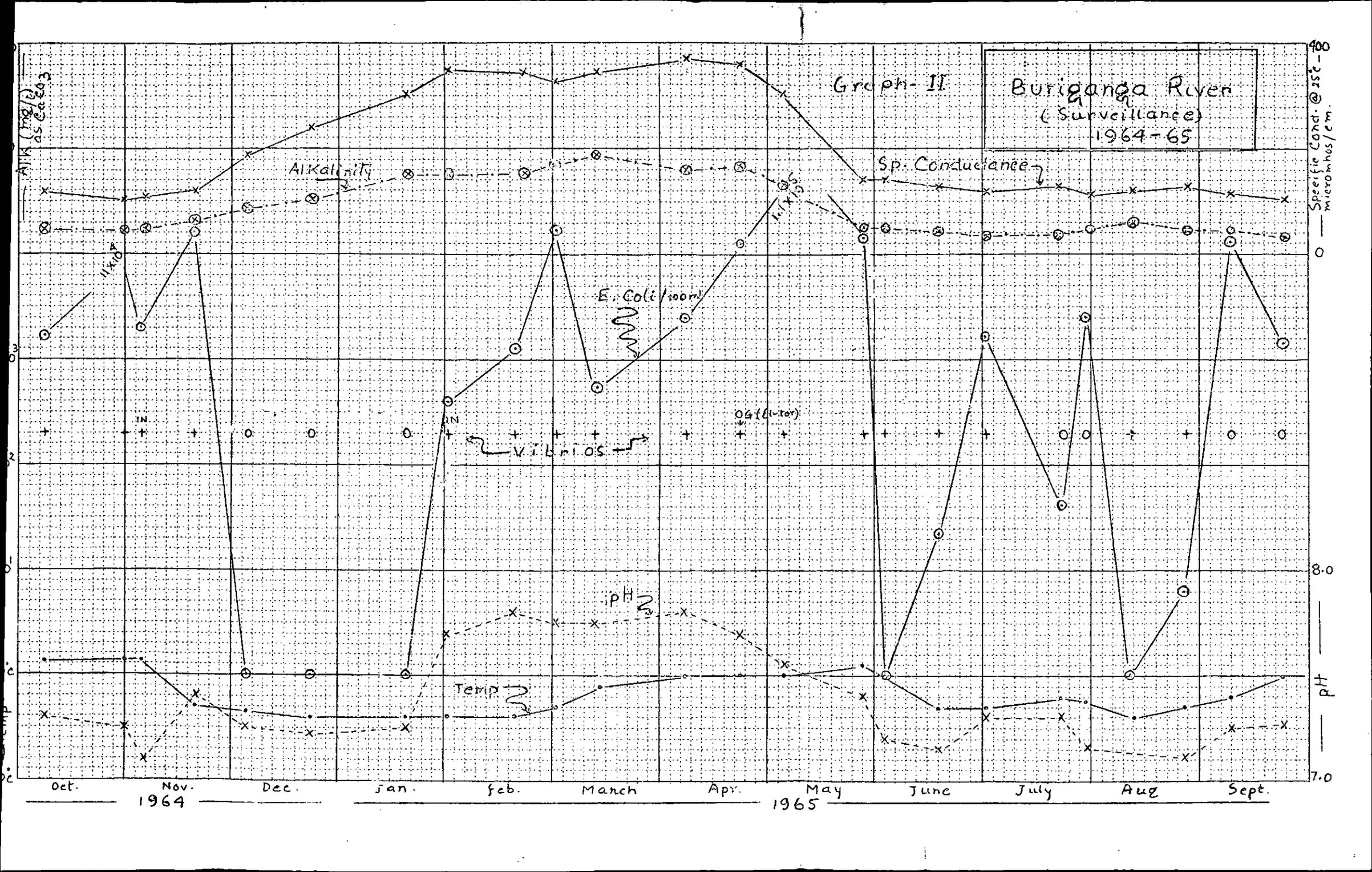
	Jan-Mar.	Apr-Jun	Jul-Sept	Oct-Dec.
1962	290	235	123	220
1963	295	190	122	178
1964	346	202.5	110	145
1965	344.5	242	120.7	-

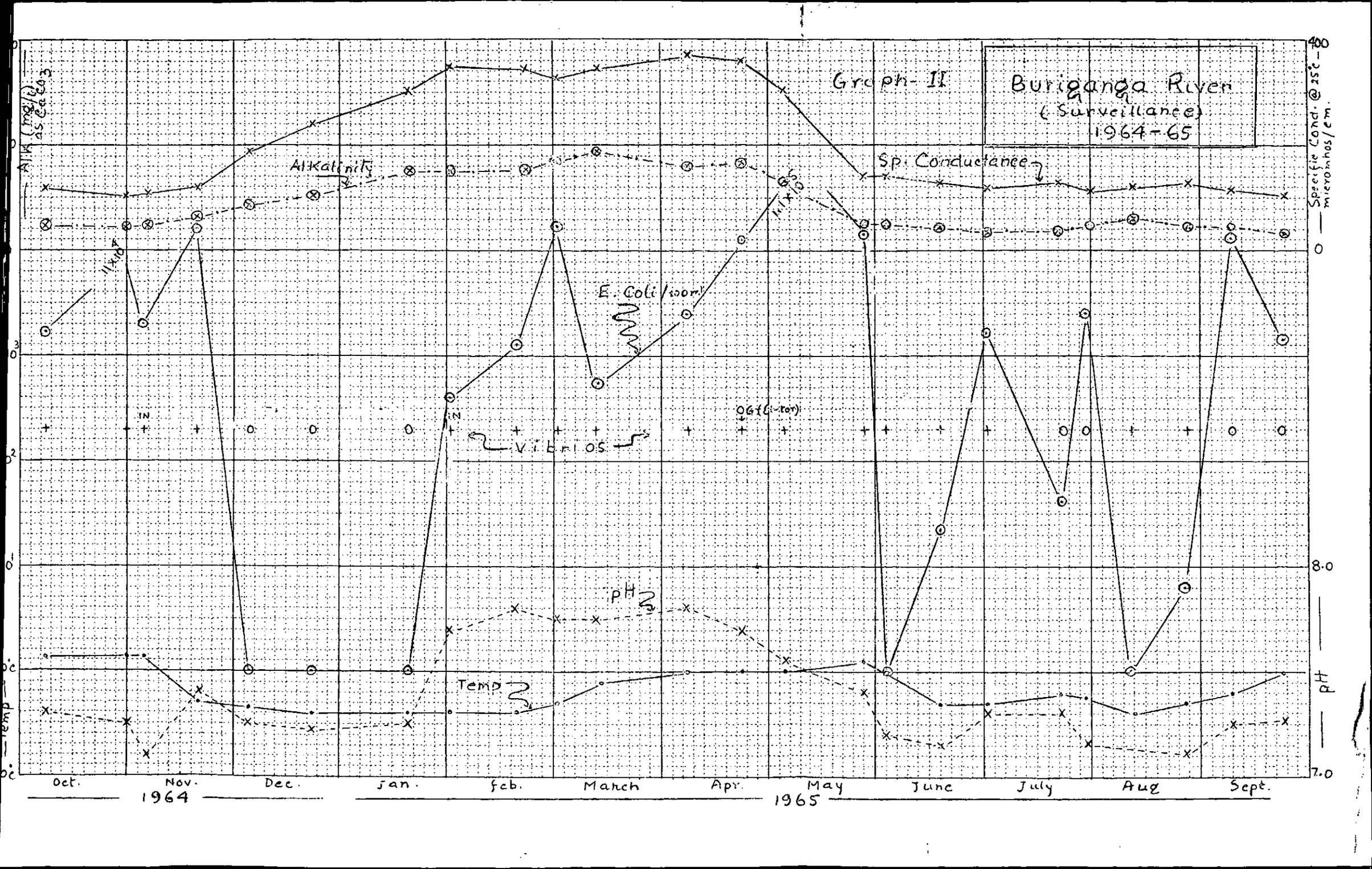
TABLE X

Average E. Coli/100 ml. of Buriganga River

	Jan-Mar.	Apr-Jun.	Jul-Sept.	Oct-Dec.
1963	6.6×10^3	4.9×10^3	1.3×10^3	10^3
1964	5.2×10^3	8.4×10^3	4.8×10^3	6.8×10^3
1965	4×10^4	4.2×10^4	8×10^3	-


Graph-I
LAKHYA RIVER
Demra
(Surveillance)
1964-65
Alkalinity
Specific Conductance
Vibrios (Non-Agg)
E. Coli
pH
Temp
Alk. (mg/l) as CaCO3
Sp. Cond. @ 25°C micromhos/cm
E. Coli/100 mls
Temp °C
pH
Oct
Nov
Dec
Jan
Feb
March
Apr.
May
June
July
Aug
Sept.
1964
1965


Graph- II
Buriganga River
(Surveillance)
1964-65
Alkalinity
Sp. Conductance
E. Coli/100ml
Vibrios
pH
Temp
Alk (mg/l) as CaCO3
Specific Cond. @ 25°C micromhos/cm.
pH
Oct.
Nov.
Dec.
Jan.
Feb.
March
Apr.
May
June
July
Aug
Sept.
1964
1965


Graph- II
Buriganga River
(Surveillance)
1964-65
Alkalinity
Sp. Conductance
E. Coli/100ml
Vibrios
pH
Temp
IN
IN
Oct.
Nov.
Dec.
Jan.
Feb.
March
Apr.
May
June
July
Aug
Sept.
1964
1965
Specific Cond. @ 25°C — micromhos/cm.
pH
Temp — °C

Matlab Bazar Surveillance Study

From Matlab Bazar, the vaccine trial area, we have gathered helpful information available on the disease trend for the last 18 months. The people and the disease have been studied very closely. The majority of the people inhabit the banks of the rivers and canals. With the facilities available, it was considered significant to study the water of this region for vibrio before any cholera case was reported. Matlab Bazar was surveyed and 62 points in 41 different villages were fixed for the purpose of a surveillance study (please refer to map and report on fixation of points for water collection in Matlab).

Cotton swab technique (described elsewhere) for collecting water was applied and the swabs were collected every third day, transferred into a plastic bag, sealed and transported to the laboratory in an ice-chest, within 24 hours of collection. This study was started on 18.9.65 and since then over 1500 samples have been examined. Only once from one of our study points along the river in village No. V-14, Inaba El-tor organism was isolated. The water was sampled again and was found negative. Since there were no cholera cases around that area and no more organisms could be isolated, it was suspected to be a laboratory contamination.

Recently there was a small outbreak in Udamdi (in Vaccine Trial Area) and one of the four tanks used by the affected families was found to be contaminated with Inaba, only on the third day of the occurence of the last case in that locality.

More data is to be collected before any definite pattern can be visualized to establish a relationship between the positivity of water and occurence of endemic outbreak.

Report on Fixation of Points for Water Collection at Matlab

Block No. I

Village : THATALIA (G)

G. - 1 Tank No. 1 is owned by Hakim Ali Beopari. It is used for drinking, cooking, bathing and washing by about 100 people. In the rainy season, it is connected with the canal.

G. - 2 Tank - opposite Syed Ali Hazi's house. More than 200 people use this point for drinking, cooking, washing and bathing.

Village : SEPOYKANDI (F).

F. - 3 River - opposite Ali Nawaz Beopari's house. This is being used for drinking, cooking, bathing and washing by more than 150 people. Two fly latrines are nearby this point.

F. - 4 Canal - This is in front of the house of Abid Reza (North Sepoykandi), and is used by 4 families for drinking, cooking, bathing and washing. Two fly latrines are nearby this point.

Village : BINAND (V)16

V 16 - 5 Canal - In front of the house of Abdul Qader Dhali(Dhalibari). About 125 people use this point for drinking, cooking,bathing and washing. This point is also used by the people of Bhati-Rasulpur as it is in the middle of these two villages.

Village : ENAYET NAGAR (V 14)

V 14 - 6 River - In front of Adam Ali Dewan's house - rice mill. More than 200 people use this point for drinking, cooking, bathing and washing.

Village : GAZIPUR (V 5)

V 5 - 7 Tank - owned by Swab Ali Prodhan - used by more than two hundred people for drinking, cooking, bathing and washing.

V 5 - 8 River - In front of Zafar Ali Khan's house. This is being used by 300 people for drinking, cooking, bathing and washing. A fly latrine is nearby this point.

Village : TORKEY (V 18)

V 18 - 9 Canal - In front of the house of Madhu Sarkar. More than 100 people use this point for drinking, cooking, bathing and washing. Village Torkey is just opposite to this point; its people also use this point. There is one fly latrine near this point.

V 18 - 10 Canal - In front of the house of Yakub Ali Sarkar (Sarkar Bari). Used by more than 150 people for all purposes, i.e. drinking, cooking, bathing and washing. Village Hathigatta is opposite to this point. A fly latrine is nearby this point.

V 18 - 11 Canal - In front of the house of Bagh Bari. About 150 people use this point. A fly latrine is nearby. Is used for drinking, cooking, bathing and washing.

Village : HATHIGATTA (V 17)

V 17 - 12 Canal - In front of the house of Abdul Aziz Prodhan. About three hundred people use this point for drinking, cooking, bathing, etc.

V 17 - 13 Canal - In front of the house of Hazi Md. Prodhania. More than 100 people use this for drinking, cooking, bathing and washing. There is a mosque nearby this point.

Village : BARLAKSHIMIPUR (I)

I - 14 Canal - In front of the house of Ali Hussain Beopari. About 300 people use this for drinking, cooking, bathing and washing.

I - 15 River - In front of the house of Abdul Majid Prodhan (Chota-Lakshimipur). Near about 350 people drink, cook, bathe & wash in it. There is a cow-shed nearby this point.

Village : CHAR LAKSHIMIPUR (I)

I - 16 River - In front of the house of Lalu Prodhania (Char Lakshimipur). A group of 150 people drink, cook, bathe and wash in it.

Village : NABUR KANDI (V 9)

V 9 - 17 Canal - In front of the house of Afazuddin Master. Used by 150 people for drinking, cooking, bathing and washing.

Village : GOALBHAR (V 8)

V 8 - 18 Canal - In front of the house of Swab Ali Majee. About 300 people use this for drinking, cooking, bathing and washing.

Village : NAYA KANDI (V 7)

V 7 - 19 River - In front of the house of Abdus Samad Patawari. About 200 people drink, cook, bathe and wash at this point.

Block No. 3

Village : KHADERGAON (V 21)

V 21 - 20 River - In front of a Union Council Office. There is a market nearby this point. Opposite to it is a primary school.

Village : LAMCHARI (H)

H - 21 River - In front of the house of Sarat Ch. Mallick. About 300 people drink, cook, bathe and wash in this point.

Village : CHAR PATHALIA (V 25)

V 25 - 22 River - In front of the house of Dip Charan Das - used by more than 100 people for drinking, cooking, bathing and washing. A fly latrine is nearby this point.

Village : AMUA KANDA (T)

T - 23 River - In front of the house of Solaiman Pradhan - used by nearly 150 people for drinking, cooking, bathing & washing.

Village : MACHUA KHAL (V 24)

V 24 - 24 River - In front of the Machua Khal Launch Ghat. Nearly 500 people drink, cook, bathe and wash in this point.

V 24 - 25 Canal - A moscue in the house of Jamal Pradhania faces this point. It is used by about 100 people for all purposes.

Village : Durgapur (V 35)

V 35 - 27 River - This point is in front of Indra Pradhania's house. About 100 people use this for drinking, cooking, bathing and washing.

V 35 - 28 Canal - Abdul Habib Majhi's house faces this point. 100 people drink, cook, bathe and wash in this point. Two fly latrines are nearby this point.

V 35 - 29 River - This point is near Durga Bazar - in front of A. Salam's shop - used by local people for all purposes.

Village : NANDALALPUR (R)

R - 30 Canal - In front of the house of Karim Beopari & Mrs. Fazilaten Nessa (Dhai). About 200 people use this point for drinking, cooking, bathing and washing. A fly latrine and one cow-shed are nearby this point.

R - 31 River - In front of the Nandalal Bazar. Is used by the people of the bazar, school students' etc. for all purposes. This point is just near the Tea Stall of Ismail Mia.

VILLAGE : NAYAR GAON (O).

O. - 32 River - Just near a goldsmith shop of Kamana Sagar of Nayargoan bazaar. This is used by the people of the bazaar, school students etc. for all purposes.

VILLAGE : SHIBPUR SOUTH (V29).

V.29 - 33 River - In front of the house of Bipin Pradhania. Many people use this point for all purposes.

VILLAGE: SHAHPUR(K)

K. 34 River - In front of the house of Ali Hossain. About 100 people use this point for drinking, cooking, bathing and washing.

K. 34 - 35 River - In front of the house of Ayub Ali Beopari. More than 80 people use this point for drinking, cooking, bathing and washing.

VILLAGE : TAT KHANA (L).

L. - 36 River - Just opposite to the launch ghat of Machua Khal and in front of the house of Dai (CRL). About 150 people use this point for drinking, cooking, bathing and washing.

VILLAGE : NARAYANPUR (V 26).

V 26 - 37 Canal - Just in front of the house of CRL H.A. & Mohan Doctor. This point is used by the people of the bazaar. It is the main ghat of Narayanpur Bazaar. There is a High School opposite to this point.

V 26 - 38 Tank - In front of the Mosque of Narayanpur Bazaar. Many people use this tank for drinking, cooking, bathing and washing.

VILLAGE : PANCH GHARIA (V27).

V 27 - 39 Canal - In front of the house of Waresh Majhi (Majhi Bari). About 100 people use this point for all purposes.

VILLAGE : KHIDIR PUR (V 28).

V 28 - 40 Canal - In front of the house of Jaladhar Das (Das Bari). About 100 people use this point for all purposes.

B L O C K N O. 2.

VILLAGE : CHAR PATHALIA (V 4).

V 4 - 41 River-In front of the house of Mr. Fazlul Huq. About 100 people use this point for all purposes.

VILLAGE : CHAR NILOKHI (V 3).

V 3 - 42 Tank - Just behind the house of Nawab Ali Sardar. About 100 people use this point for all purposes. Indiscriminate human excreta is visible around the tank. This is connected with flood water during the rainy season.

VILLAGE : NILIKHI (V 2).

V 2 - 43 Canal - Situated behind the house of Zafar Ali Sardar. About 150 people use this point for all purposes.

VILLAGE : UDHAMDI (SOUTH) A.

A. - 44 Canal - Located in the house of Mr. Sabar Ali Pradhania. About 200 people use this point for all purposes.

VILLAGE : KADAMTALI (V 1).

V 1 - 45 Tank - Situated beside the house of Mr. Muzaffer Hussain Dewan. About 200 people use this point for all purposes. This is connected with a canal in the rainy season.

VILLAGE : KADAMTALI E.

E. - 46 River - In front of the house of Abul Khair. Nearly 200 people use this point for drinking, cooking, bathing & washing.

VILLAGE : KADAMTALI W.

W. - 47 Canal - In front of the house of the late Hamendra Chandra Ghosh (Sadhu Ghosh), and near the barge (Matlab Hospital). Many people use this point for all purposes i.e. for drinking, cooking, bathing and washing.

VILLAGE : MATLAB BAZAAR X.

X. - 48 Tank - In front of the house of Chinta Haran Banil. This tank is called 'Maszed Tank'. Seven or eight families use this tank for washing, bathing and cooking. Sometimes they use it for drinking sources too when the tubewell fails to supply water. Muslims use this water for ablution purposes.

X. - 49 Canal - In front of the house of the Sardar of Badias and just opposite the Dak Bungalow. The badias who live on boats in this place use this point for all purposes.

X. - 50 River - This point is the launch ghat of Matlab Bazaar. Bazaar people bathe,wash, drink and cook with this water.

VILLAGE : MATLAB SCHOOL Z.

Z. - 51 Tank - In front of the Matlab school,and other offices at Matlab. Generally used for bathing, washing and cooking etc.

VILLAGE : BAISPUR (DASPARA) U.

U. - 52 Canal - On the west side of the house of Dr. Gopal Das. This is used for all purposes by many people of the locality.

U. - 53 Canal - In front of the house of Mvi. Monoruddin Pradhania. Nearly 200 people use this for bathing, washing, drinking and cooking.

VILLAGE : CHAR MUKUNDI D.

D. - 56 River - In front of the house of Mvi. Dudud Mia. About 100 people use this for all purposes.

BLOCK NO. 1.

VILLAGE : LUDHUA V 36.

V 36 - 57 Tank - Belongs to Mvi. Abdul Latif Sarkar. About 150 village people use this point for all purposes.

V 36 - 58 Canal - In front of the house of Mr. Belayet Mijhi. About 150 people of the para use this point for all purposes.

V 36 - 59 Canal - In front of the house of Idris Ali Pradhania. About 200 people use this for drinking, cooking, bathing & washing. There is a cowshed near this point.

V 36 - 61 Tank - In front of the Ludhua High School. Boys going to school and local people use this tank for all purposes.

V 36 - 60 Canal - Near the fly latrine which is used by the students of the Ludhua High School.

VILLAGE : BINADAPUR (V 16).

V 16 - 54 Canal - In front of the house of Hafiz Ali Beopari. About 200 people use this for drinking, cooking, bathing and washing.

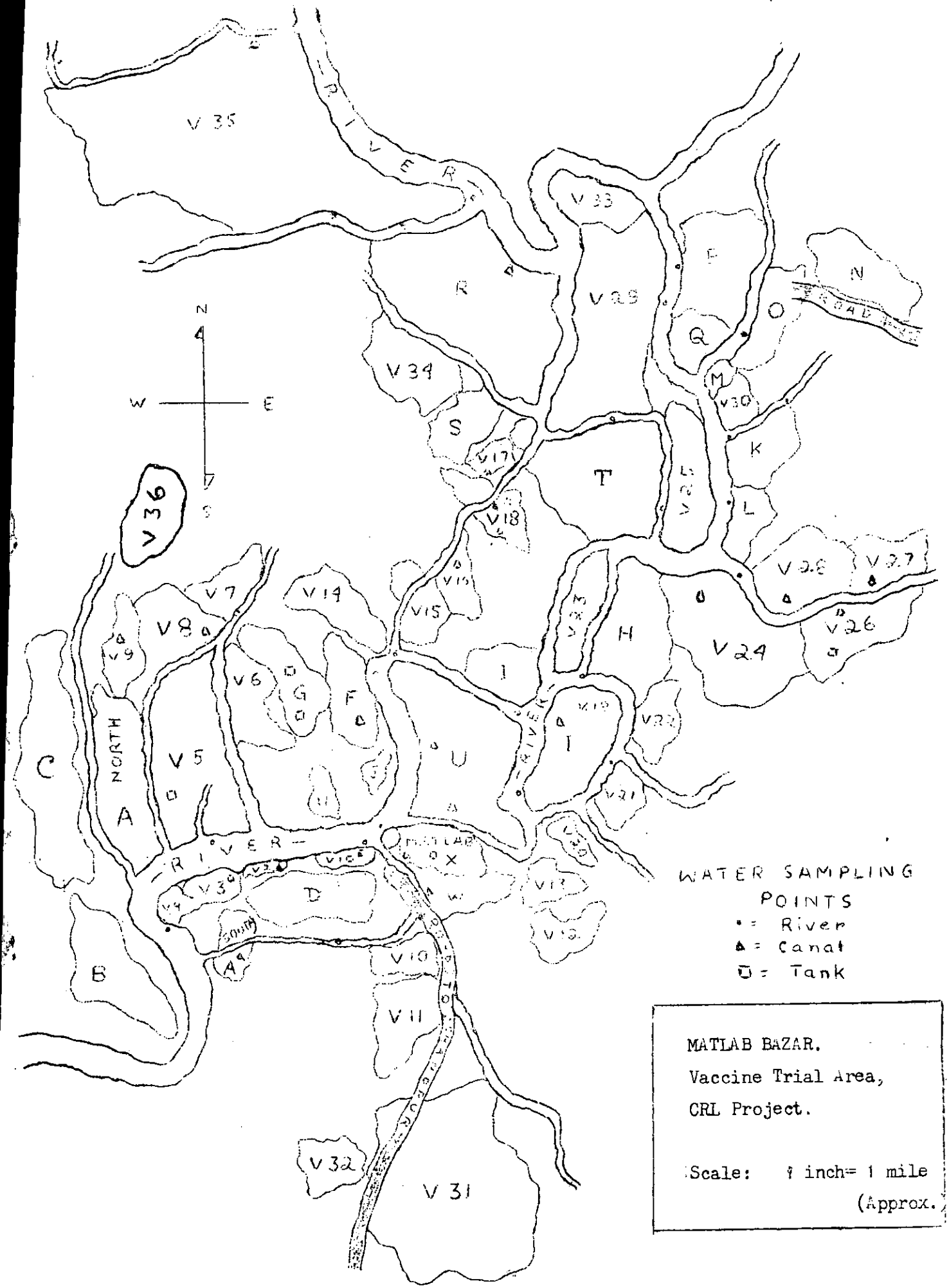
VILLAGE : BHATI RASULPUR (V 15).

V 15 - 55 Canal - In front of the house of Tabdil Hussain Dewan. About 150 people use this point for all purposes.

V 15 - 26 Canal - In front of the house of Abdur Rahman Master. More than 200 people use this point for all purposes.

VILLAGE : SEPOY KANDI F.

F. - 62 Tank - Adjacent to the house of Qader Beopari. About 300 people use this tank for drinking, cooking, bathing and washing.


V 35
RIVER
V 33
R
V 29
P
N
ROAD
Q
O
M
V 30
V 34
S
V 17
T
K
L
V 36
V 18
V 7
V 14
V 15
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V 28
V 27
V 8
V 9
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G
F
H
V 24
V 26
I
V 5
U
V 22
C
A
V 21
MATLAB
BAZAR
V 3
V 4
D
V 12
V 13
B
V 10
V 11
V 32
V 31
WATER SAMPLING POINTS
• = River
▲ = Canal
□ = Tank
MATLAB BAZAR.
Vaccine Trial Area,
CRL Project.
Scale: 1 inch= 1 mile
(Approx.)

SPREAD OF CHOLERA THROUGH WATER

Ahmed, S.Z., Saeed, Y.A., Neogi, P.K.B. and Sohail, S.M.

This study was carried out for finding the relationship between contaminated water and occurence of cases in the cholera affected communities. In 1962 and early '63, households with confirmed cholera were studied for the contamination of water. The findings as reported previously could not clearly define the role of water in the spread of disease either in the households or in the community at large. The common observation was, however, that households with multiple cases had more frequently positive waters than the single case households.

In 1964-65, family studies were conducted with some modification in the protocol. Family water sources and the storage jars were observed for contamination with Vibrio cholerae for a period of ten days, starting within two days of onset of illness in vibrio positive index cases. A total of 92 families have been studied. Findings within these families are summarised in Table I. Initially cholera vibrios were present in water samples from all sources in 52 (56.4%) of the 92 families. With the passage of time the frequency of positive water sources continued to decline for the rest of the study period. The relationship between water findings and appearence of more than one case in the same family is shown in Table II. This table shows that 40 (65.5%) families out of 61 families having contaminated water sources had more than one cases in them.

A comparison between the available types of water sources and the way they are used by the singly and multiply infected families does not show any significant difference as shown in Tables IIIa and IIIb.

However, the difference in the positivity of different sources of water according to their usage between the multiple and single case families as shown in Tables IVa and IVb, is very interesting. Table IVa shows that out of six multiple case families 5 (83%) had used contaminated dugwell water for drinking and cooking; and out of 32 multiple case families 29 (90%) had used contaminated dugwells for other purposes. Three families using contaminated river water for drinking and cooking purposes were all found to be multiple case families. However, 4 of 9 multiple case families (44%) were using the contaminated river water for other purposes. In all multiple case families 25.4% storage jars used for cooking and drinking and 26.4% jars used for other purposes were found positive for Vibrio cholerae.

Table IVb shows considerably lower percentage of positive water sources in single case families in comparison to the multiple case families.

Some of the examples cited below from these family studies, reveal very interesting associations which could be argued in favour of water serving as a vehicle in the transmission of the disease in this endemic setting.

Kafrul Outbreak: September, 1964.

Kafrul village is situated on the north side of the Agricultural College near the Second Capital site. The first case from Kafrul came from an area comprising about 12 houses and having 3 dugwells (Map I). He was a 12 year old boy belonging to Family F-42 which had 8 members living in the

same house and using dug well No. 1 for drinking, cooking and bathing purposes. Dug well was of open type and without any pucca construction or a platform. Second case from the same family also occurred on the same day. The dug well ampled on the 2nd day after the onset of the two cases, was found to be positive. Samples of water lying around the surface of the well, used as a bathing place, also showed Inaba vibrios. Storage jars containing water from this dug well also were positive on the second and third day of the sampling.(see figure 1). Family 42 had 2 more subsequent cases and three carriers for Inaba. Dugwell No. 1 remained positive for Inaba for five consecutive days. On the sixth day the dugwell and the jars were negative but the water lying around the surface of the well was positive.

Another family F-43, having four members, lived nearby F-42 in the same courtyard, ate in the house of F-42 and drank water from dugwell No. 1. Family F-43 had its first case on the same day on which F-42 had its second. Two days later a second case from this family was admitted to Cholera Research Laboratory. Soon after,the second case F-43 stopped eating in the house of F-42 and went to the house of Solaiman near dugwell No. 2. Wells No. 2 and 3 which were used by a number of other families, were also sampled and found to be negative.

A.K. Das Lane Outbreak; November, 1964.

A,K, Das Lane is an extremely congested locality of Gandaria, comprising about 500 to 800 houses and a Girl's School. It has many very narrow by-lanes. The majority of the houses have service type of latrines. Lanes and by-lanes have open surface drains on both sides which are also connected to the latrines. Case family F-69 lived in one of the narrow by-lanes of that locality. There were six members of the family living in a one-roomed house. In this family there was only one case and a carrier nine days later.

Family F-71 who lived in the same compound and shared the same dugwell, had their first case two days after the index of F-69. They had a carrier on the same day who became a case the next day. The third case in Family F-71 occurred after 8 days of the index in this family. Dugwell water was negative on the first two days but became positive on the third and fourth days after the first case in F-69. The open surface drain connected to the latrine was however positive for 8 consecutive days.

Family F-74 which was living in the house opposite to F-69 and F-71 across the by-lane, had its first case of cholera on the day after the second case in F-71. It had two more subsequent cases one day after another. This family also had a dugwell and used it for washing, bathing and cooking purposes. This well sampled one day after the onset of the

first case in F-74 was positive for Inaba for two days.(see figure 3).

Nayatola-Maghbazar Outbreak - November 1964:

An interesting case of the contamination of water from other districts and then spread of the disease through water was observed in Nayatola.

Index case of Family F-75 developed loose motions while coming from Faridpur on a launch, went to Maghbazar and was admitted to CRL the next day. Water from the only dugwell sampled on the day index was admitted, was positive. Water of the well was found contaminated on repeated sampling for 6 days. This family had two carriers and a second case after 8 days of the index.

Another family F-78 living in the same courtyard and sharing the same dugwell, had its first case and two carriers four days after the index of F-75. Water in the lowland was now positive. This family had one more carrier later. No carriers were detected in F-78 after four days of the index but the water was positive. Dugwell was intermittently positive till the fourteenth day of its sampling. On the fourteenth day,however, we had another carrier in F-78.

Dhalkanagar Outbreak - November 1964.

Dhalkanagar is a very congested place near Gandaria, Dacca. An outbreak of cholera was recorded in November, 1964 involving four inter-related families living in the same compound. In all there were twelve families living in the same compound, each family living in a one roomed house made of tin. There were two dugwells used for cooking, washing and bathing purposes. A big open drain passed through the middle of the compound

Family 79 (see sketch map 2), had its first case on the 21st November. On the 23rd November there were two more cases. On the 25th there was a sudden outbreak among the four families, namely Nos. 79,80,81 and 82 having among them 9 cases in one day (see figure 2). Dugwell water sampled on the 25th November was positive for V.cholerae. On the 26th November, the dugwell was chlorinated. There were two more cases on the 26th and one each on the 27th and 28th November. The second dugwell was negative on the 25th and 26th but positive on the 27th November. No more cases were reported after the 28th November, but there were carriers in F-82 until the 3rd. December.

Raza Bazar Outbreak - November to December, 1964:

Three families in this congested community using the common dugwell for washing, cooking and bathing purposes had 7 cases among them. F-53 had 3 confirmed cases out of which two individuals were carrying the germs one day before developing cholera symptoms. It also had 2 carriers with inapparent infection. Family F-54 had 3 confirmed cholera cases and 5 carriers. Family F-55 had only one case. Dugwell water was positive till the tenth day of the study except two days in the middle. Storage jars of F-53 and F-54 were also positive (see figure 3).

Family F-91 and F-92 - A.K.Das Lane, December, 1964.

22 members of the family were living in a nine roomed house. There was one confirmed case and three carriers in this family. Dugwell when sampled after three days of the case, was positive and remained so for three days after which it was chlorinated. Another family living in the same compound and using the same dugwell had one confirmed case and two carriers when the dugwell was still positive.

Family F-110 and 111 - Rajnarayan Dhar Lane - December, 1964.

These families lived in a Bustee near Buriganga River. Sanitary conditions around their house were very bad. Each family had a case on the same day and F-111 had a second case three days later. Water collected from the 'ghat' of the river used by these families, was found to be positive for vibrios on the first two days of sampling.

F-109, Aulad Hussain Market - December, 1964:

This family had 7 members living in a house constructed of tin. Three individuals from this family had cholera. They were using water from the Laldighi tank for washing, bathing and cooking purposes. The tank was positive for 3 days. Two cases occurred on the same day. Water from the tank was positive on the very day and the day after the occurrence of the two cases. The third case was reported two days after the first two cases.

F-11, Shakari Bazar Lane - January to February, 1965.

A very interesting family study in which all the four members of the family were affected. Two of them confirmed cases and the other two inapparent carriers. Water of the dugwell was positive for 9 days. The two storage jars used by the family for drinking and cooking were also positive.

First Dhamrai Outbreak - September, 1965.

Another very interesting case of the spread through water was observed during the first Dhamrai outbreak in September, 1965. In these cases a dugwell which was possibly contaminated by a boy who had probably picked up cholera somewhere near Savar and returned to the house. This boy had loose motions and he died the next day. His body was washed on the platform of the dugwell and his dirty clothes were washed in a nearby canal without the knowledge of the other neighbours. There were in all two deaths and four confirmed cases of cholera in the family of Abdus Salam. Water of the dugwell situated in the house of Salam had 7000 org. per ccm the 16 September, the first day of sampling. The storage jar used for drinking water in this house, was also found to be positive on the same day. On the 16th September, 1965 however, all the members of the affected family were brought to Cholera Research Laboratory and the house was locked up.

Abdul Khaleque who was living across the road with his family in another house, was also using the same dugwell until there was one cholera death and two cases of cholera in his family. One carrier was also found in this family.

A third family that of Subol Chandre Mondal was also using the canal which had been contaminated by the washing of the dirty clothes by the first family. The above family also had one cholera death and one cholera case on the 14th September. This was the same day that the families of Salam and Khaleque had two and one cases respectively Fig.4.

Occurrence of 10 cases and one carrier within five days of the first cholera death and the heavy contamination of dugwell water on the 5th and 6th days suggest a common source of infection which could very well have been the dugwell.

Postogola Outbreak - September to October,1965:

On October 19th.. CRL 2506 was admitted with cholera. He lived in Karimullah Bagh, Postogola, near the railway crossing. The colony of about 200 to 300 houses is surrounded by a low lying area. The fly latrines from most of the houses nearby drain into this area. During the monsoon season this lowland is filled with water and the people living in this place use this water for bathing, cooking and washing purposes. Water from this low lying area was sampled at a number of points and was found to be very heavily positive for Inaba El-tor and the contamination remained at one point or the other for 14 days (see figure 13). There were only three cases altogether in this area although random culturing of 97 children in the area on three occasions discovered 5 carriers. Drinking water storage jars of the two cases were also positive for Inaba EL-tor. The positivity of water for such a long time suggests the constant pollution of water by faeces.

Bouchilla Canal (Rayer Bazar) Outbreak - March , 1965.

On the northern side of Rayer Bazar up to Buriganga River, there is a low lying area of about three to four square miles in which there are about 25 to 30 brickfields, employing about 5000 workers. A network of canals extending from Rayer Bazar to Buriganga River divide the area covered by the brickfields into about five small islands. These canals are the only source of water for drinking, washing, bathing and cooking for the brickfield labourers and there is always a regular traffic of boats plying up and down.

CRL 1940, a boatman from Faridpur visited the "X" point of the canal (sketch map 3) and stayed there for a couple of days before he developed cholera and was admitted to our hospital where he was confirmed to be an Ogawa case. Withing six days following the abovecase of the boatman, three more cases, all of them brickfield labourers, were brought in and confirmed to be Ogawa cases. It was on the seventh day that we took up the study of the canals around the brickfields and fixed three points viz., X,Y and Z (sketch map 3) according to the usage of the population of the brickfields. Point X the meeting point of all the canals, was found to be contaminated with Ogawa on the first three days. There were four more cases from among the brickfield workers and two from Rayer Bazar within the next twelve days. Approximate positions of all these cases are shown

by a dot and a circle on the sketch map 3. Some more sampling points in the canal were selected for the study (e.g. X_1, X_2, X_3, X_4 etc.) to see whether the contamination was coming from the river or if there was a carrier somewhere in the population. The points near the river were found to be negative all the time they were being sampled. X_1 was positive for Ogawa on the third day after the last case. It is important to note that there were no Ogawa cases in the town at that time of the year.

Discussions:

The above family case studies have shown two types of pattern:

(1) -single or multiple cases within the same family and(2)-two or more families having a common source of water. Some of the examples cited above, show that while the water remained positive a group of affected families having a common source of water recorded a sudden occurrence of three or four cases on the same day, then a gradual decrease in the number of cases took place in the next two or three days; or an occurrence of fresh case or cases after three or four days of the first case (in one case even after 8 days) was noted. Consistant with this was the fact that 90% of the dugwells used by the multiple case families were positive for Vibrio cholerae, whereas only 42% of the single case families were using the contaminated dugwells.

It is difficult to say whether the family having both a dugwell and a tubewell would always use the tubewell for drinking purposes. They have in fact a preference for dugwell, tank or river water because of its taste and also because they have had this habit for centuries.

Contamination of the dugwell could be due to several possibilities. Carriers bathing near the well or washing soiled clothes could contaminate the stagnant water around the well and this in turn would contaminate the dugwell. Besides this, Indo-Pakistan custom requires people to wash the anus clean with water by the hand after excretion, this necessitates the washing of the hands afterwards. Thus carriers after excreting may go to the dugwell, hold the bucket in their hand, put it into the water, pull it out and wash their hands either in the bucket or near the well.

TABLE I

WATER FINDINGS IN 92 CHOLERA FAMILIES

Vibrio Culture	Days after onset of illness in Index case				
	1-2	3-4	5-6	7-8	9-10
Positive	52	26	17	12	6
Negative	40	66	75	80	86

TABLE II

WATER FINDINGS AND MULTIPLE INFECTION IN 92 CHOLERA FAMILIES

Cholera Vibrios in Water	Multiple Infection		
	Present	Absent	Total
Present	40	21	61
Absent	9	22	31
Total:	49	43	92

TABLE III a

SHOWING WATER SOURCES AND THEIR USAGE BY MULTIPLE CASE FAMILIES

(49 FAMILIES)

Sources	Water Used		
	Drinking	Cooking	Other Uses
MS	34/34	27/34	6/34
DW	3/32	6/32	32/32
TW	12/14	13/14	7/14
Tanks, Rivers etc.,	0/9	3/9	9/9

TABLE IIIb

SHOWING WATER SOURCES AND THEIR USAGE BY THE SINGLE CASE FAMILIES

(43 FAMILIES)

Sources	Water Used		
	Drinking	Cooking	Other Uses
M.S.	26/26	24/26	10/26
D.W.	3/26	9/26	26/26
T.W.	18/21	14/21	6/21
Tanks, Rivers etc.	0/15	3/15	15/15

TABLE I V a

SHOWING RELATIONSHIP BETWEEN POSITIVE WATER SOURCE AND ITS USAGE IN MULTIPLE CASE FAMILIES.

(MULTIPLE CASE FAMILIES-49)

Water Sources	Number of Water Sources for: Cooking & Drinking			Other Purposes		
	No.tested	No.positive	%positive	No. tested	No.positive	%posit
M.S.	34	0	0	34	0	0
T.W	14	0	0	14	0	0
D.W.	6	5	83	32	29	90
Tanks,Rivers etc.,	3	3	100	9	4	44
Jars	67	17	25.4	72	19	26.4

TABLE 1 V b

SHOWING RELATIONSHIP BETWEEN POSITIVE WATER SOURCE AND ITS USAGE IN 43 SINGLE CASE FAMILIES.

Water Sources	Number of Water Sources for: Cooking and Drinking			Other Purposes		
	No.tested	No. positive	%positive	No.tested	No. posit.	%posit.
M.S.	26	0	0	26	0	0
T.W.	21	0	0	21	0	0
D.W.	9	3	33	26	11	42
Tanks,Rivers etc.	3	1	33	15	6	40
Storage Jars	60	4	6.6	67	4	6

FIGURE - 1.

KAFRUL VILLAGE OUTBREAK.

(Showing relationship between appearance of cases and positivity of water.)

Cases by day of onset.

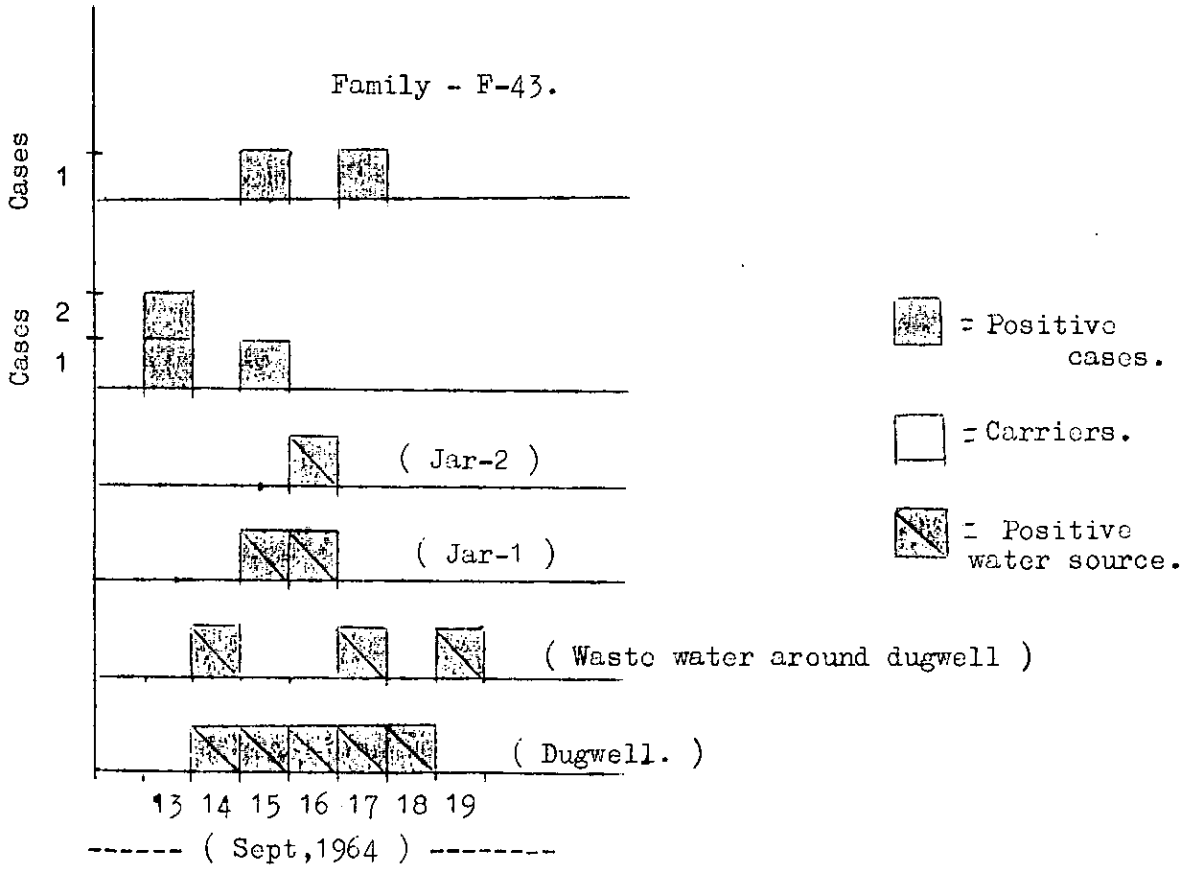


FIGURE - 2.

DHALKANAGAR LANE OUTBREAK.

(Showing relationship between appearance of cases and positivity of water.)

Cases by day of onset.

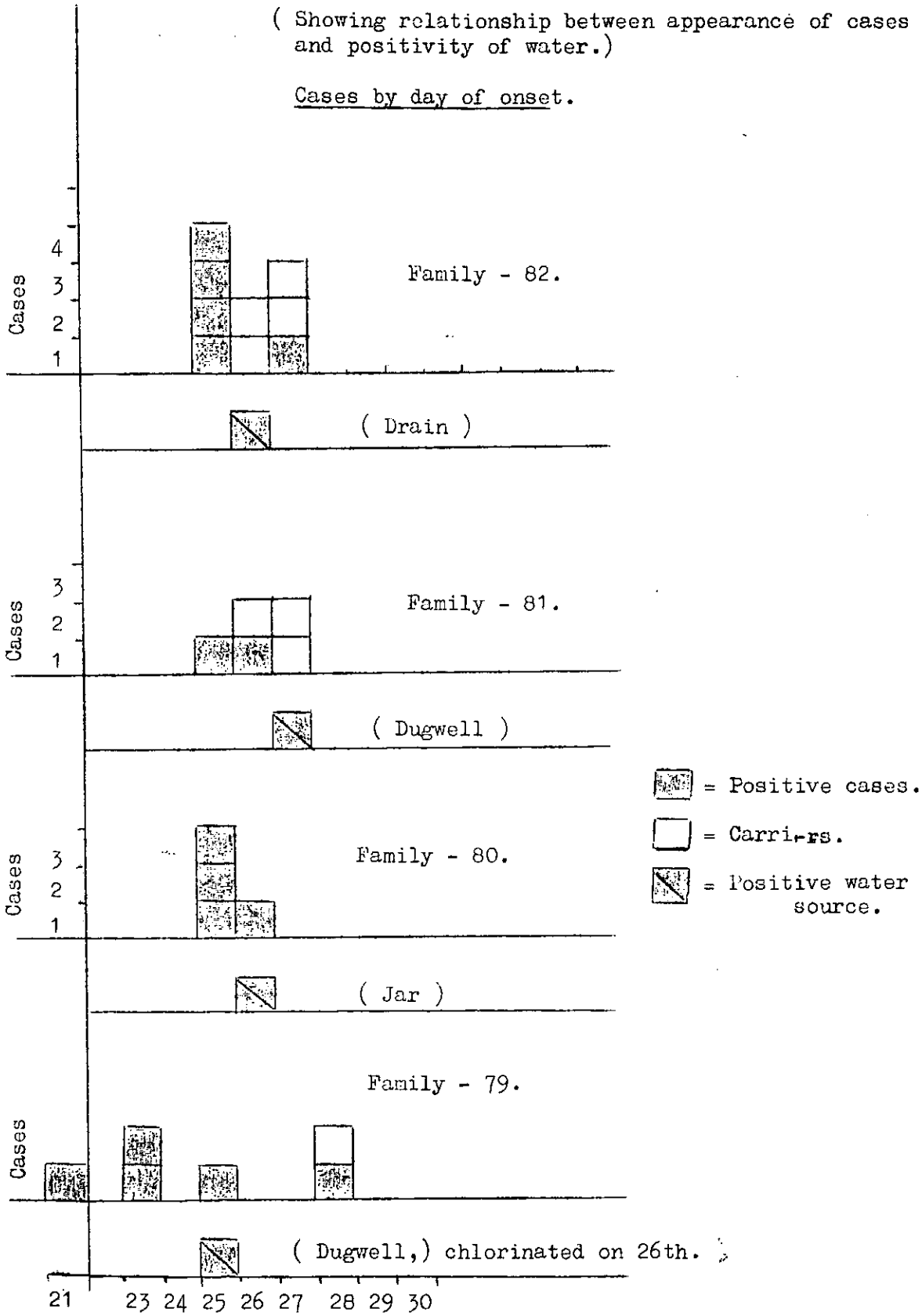


FIGURE - 3

R A Z A B A Z A R O U T B R E A K .

(Cases by day of onset)

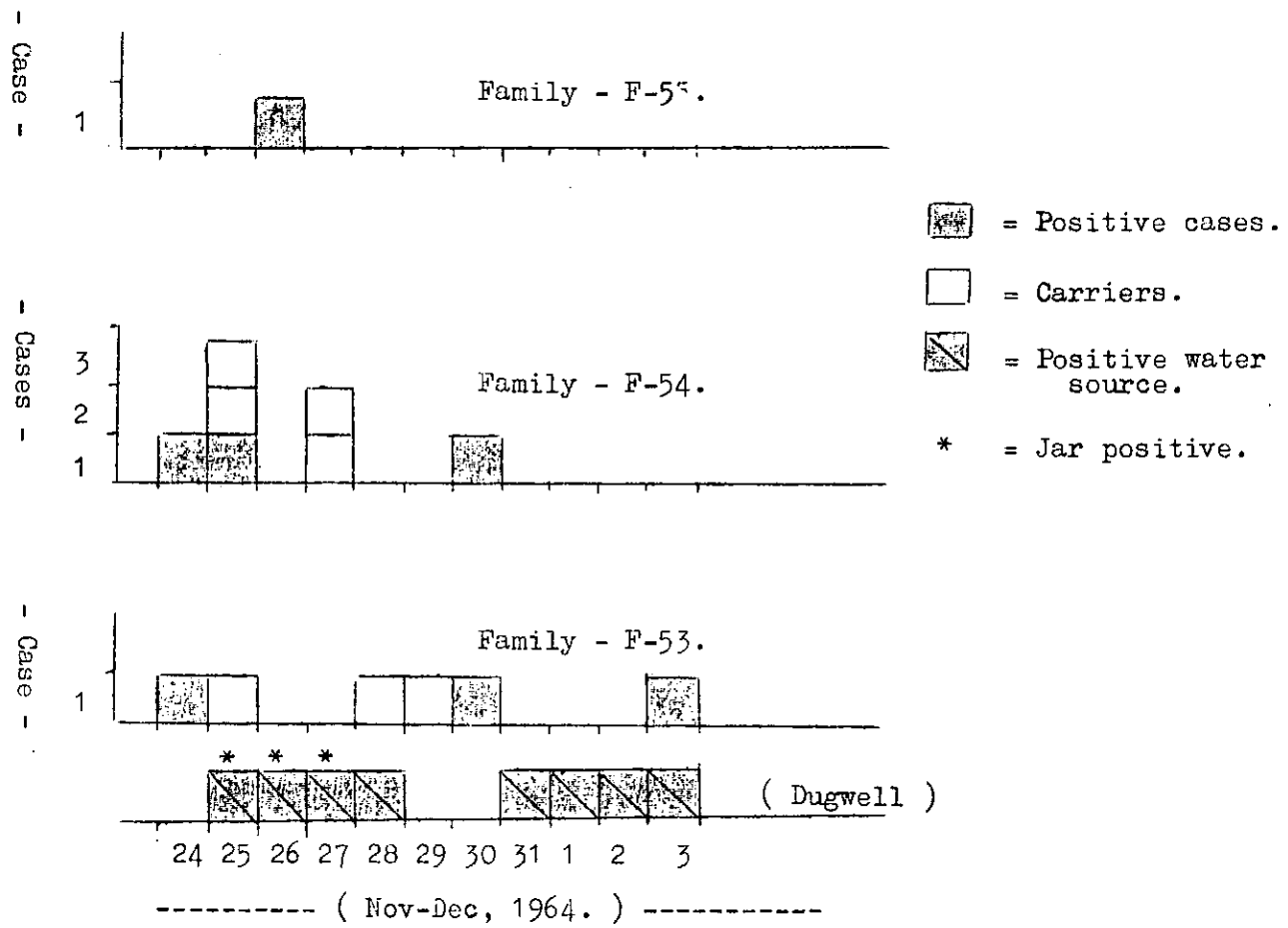
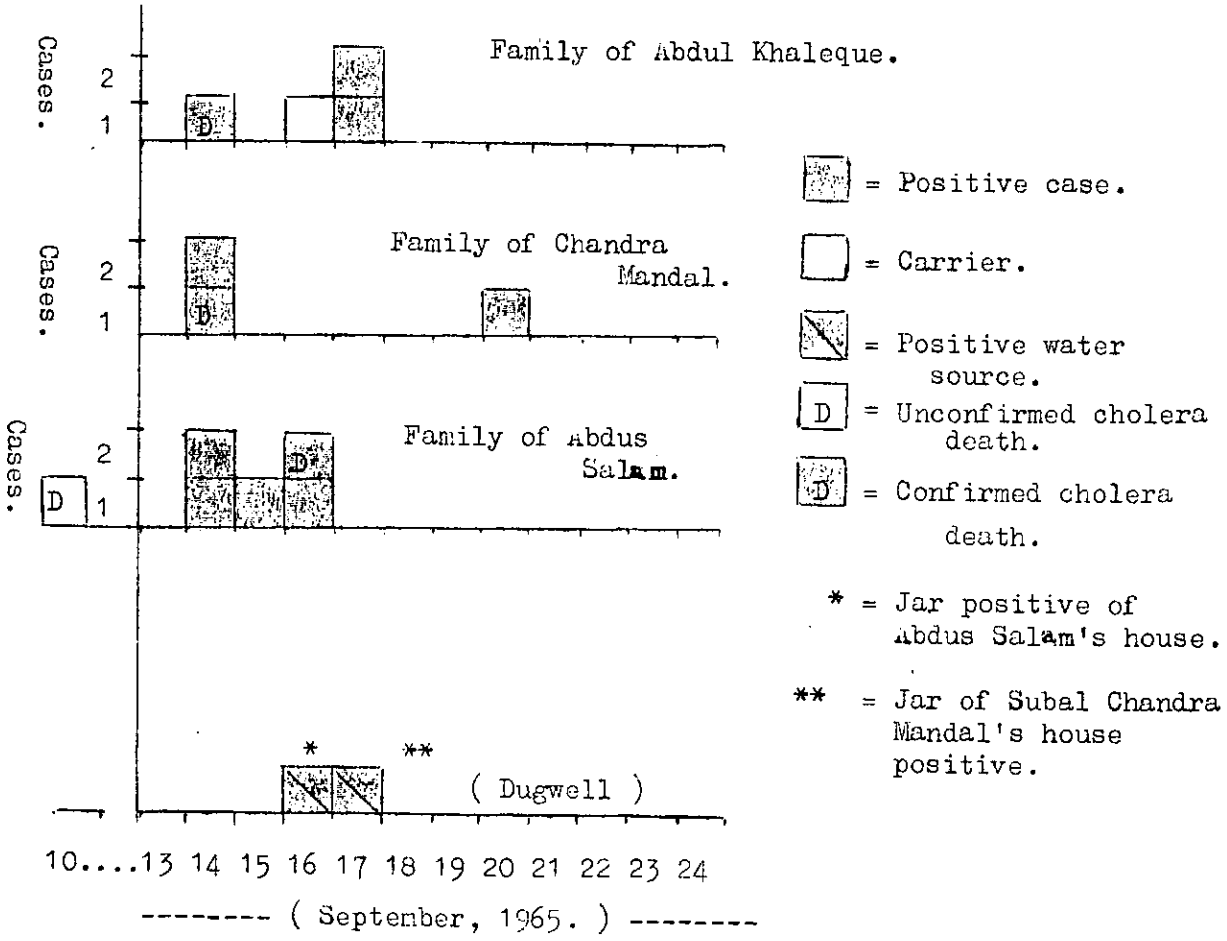


FIGURE - 4.

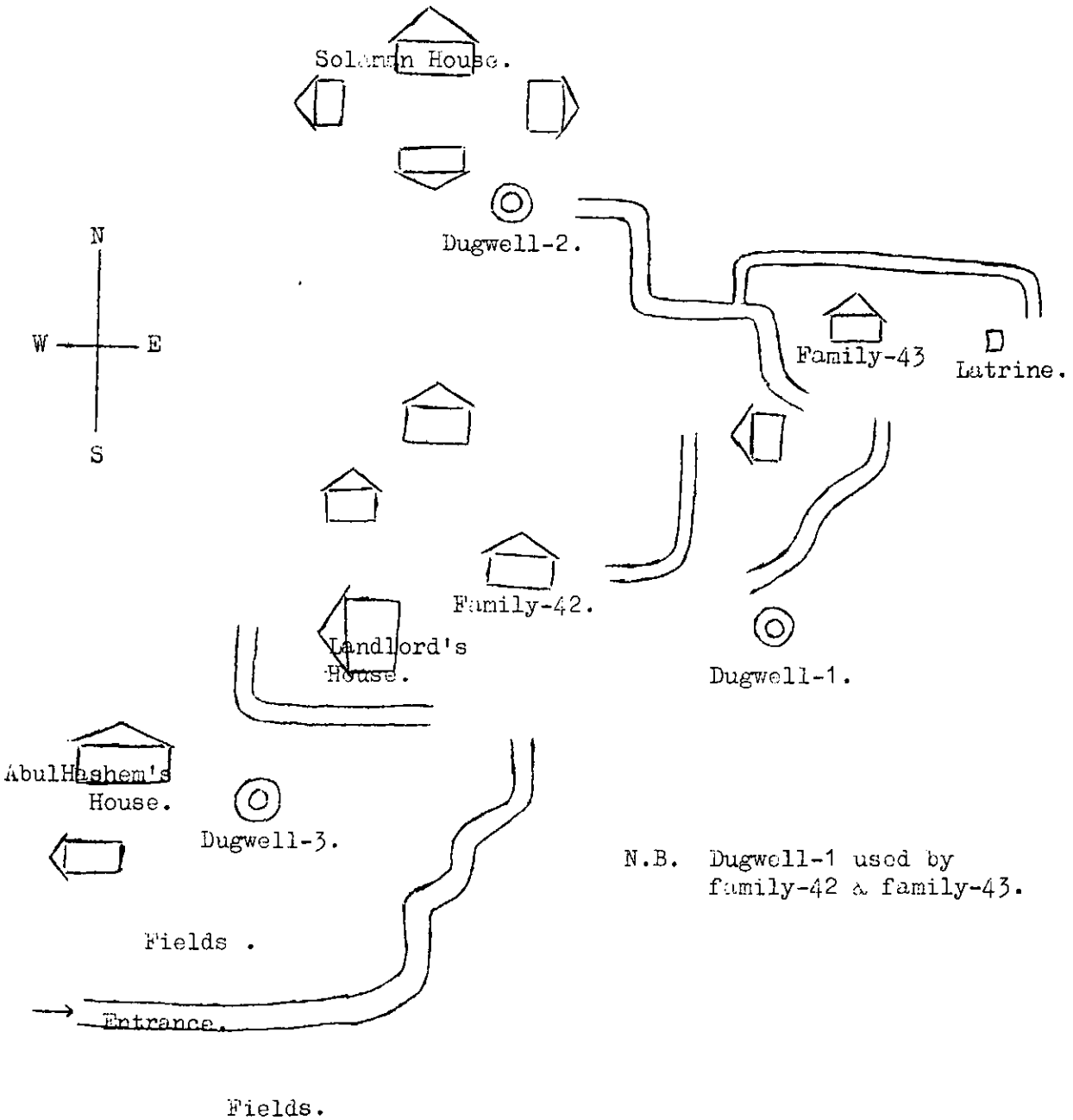
FIRST DHAMRAI OUTBREAK.

(Possible spread of disease among three different families using common source of water.)



Sketch Map- 1.

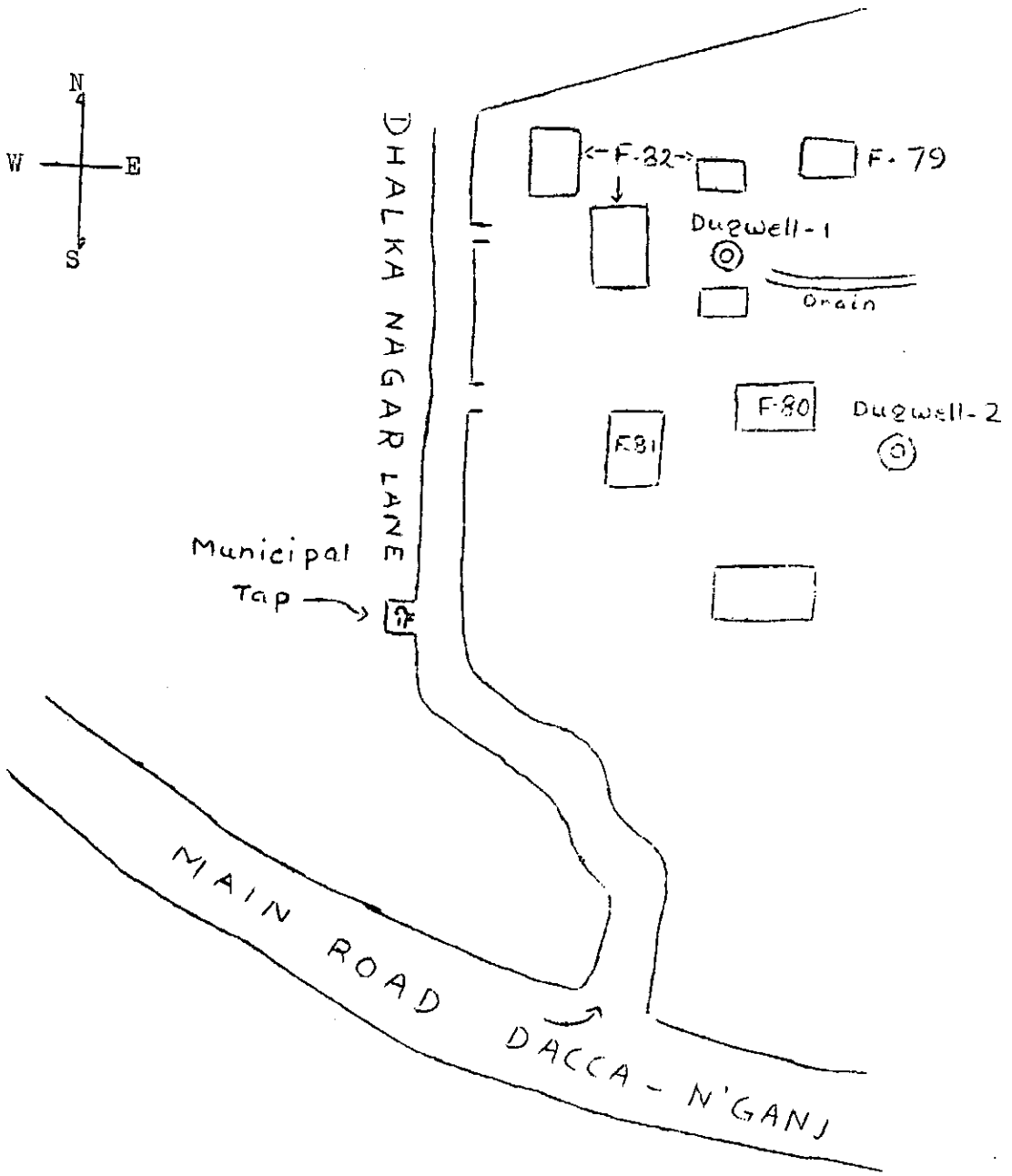
KAFRUL VILLAGE

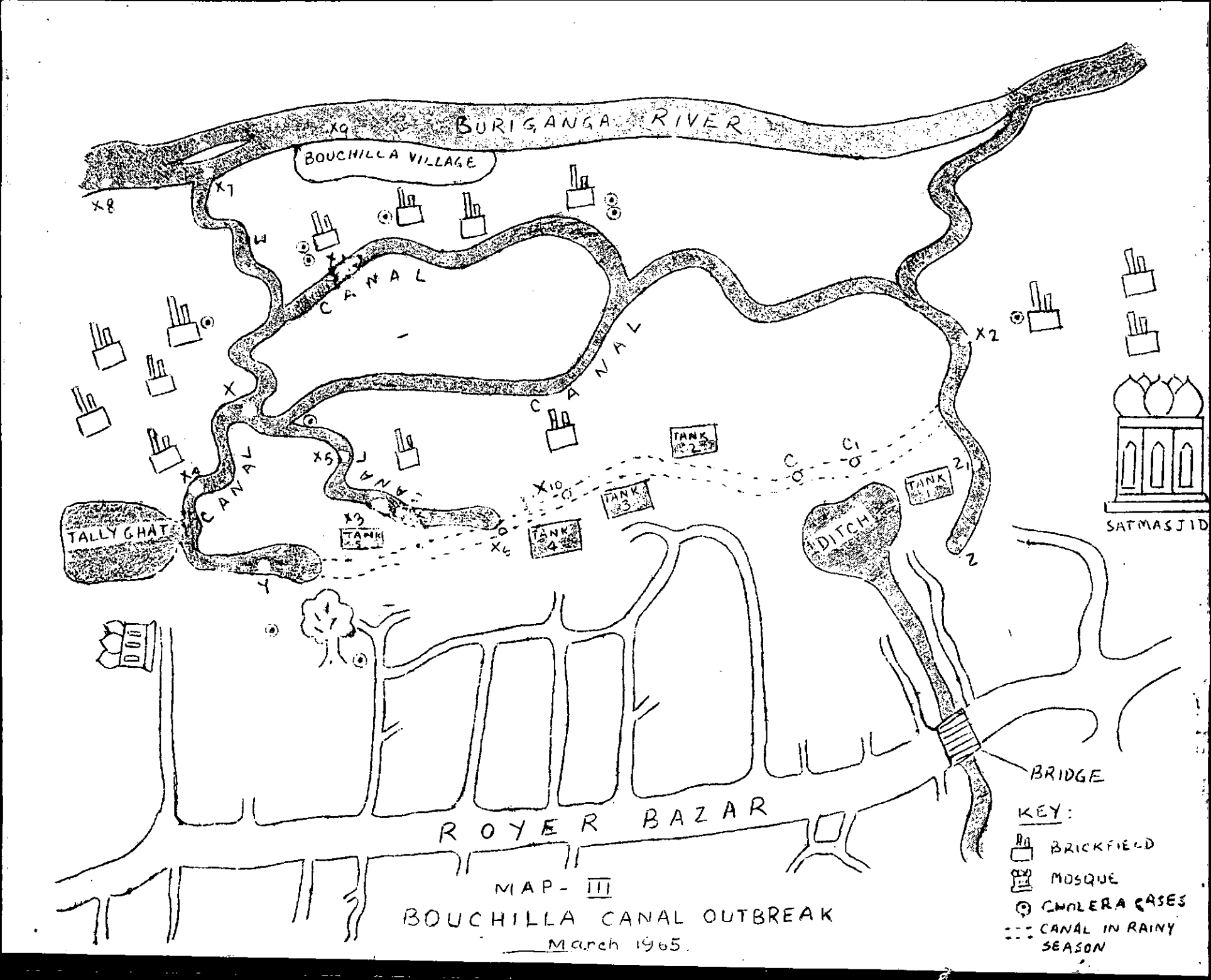


N.B. Dugwell-1 used by family-42 & family-43.

Sketch Map-2.

D H A L K A N A G A R .





MAP - III

BOUCHILLA CANAL OUTBREAK

March 1965.

Ahmed, S.Z., Saeed, Y.A., Ahmad, S.I., and Neogi, P.K.B.

Study of Rice 1963 - 1965.

Study on a special type of rice cookery of East Pakistan, "Panta Bhat", was first started in 1963 when most of the multiple cases had occurred within the family. The custom of storing left-over rice that was cooked for the evening meal and then eating this rice the next day offered a possible source of spread. Preliminary experiments on the survival of vibrio in stored rice showed considerable multiplication of vibrios, a rise of two to four logs within twenty-four hours. Aus variety of rice, which is more commonly eaten by the poor people, showed a log rise of three to four log, whereas Boro variety which is not so staple showed a log rise of two to three within twenty-four hours. The temperature range in which maximum multiplication occurred was 29°C to 33°C. The addition of 0.1% of salt showed a considerable increase in titer rise of vibrio which was between three to four log. In some cases Boro rice seemed to kill the organism. A third variety of rice, Aman, which is seeded in spring and harvested in winter and is the most staple rice along the coastal districts, supported the growth of vibrio very well. (Reported in 1963 and 1964 Technical Committee Meetings).

1964-65.

Due to the fact that the coastal districts of East Pakistan generally show higher cholera incidence, and also the fact that the addition of very little amount of salt gives very good results, it was thought that some varieties of rice from high salinity areas like Barisal may have a higher amount of NaCl in the grains than a northern district such as Dinajpur. Results of chemical analysis of the seed ash of about 8 varieties of rice are shown in table(IV). There is not an appreciable difference in sodium, potassium and phosphate contents of the different varieties of Aus and Aman rice. Sodium is present in a very minute quantity, phosphate and potassium are slightly higher than the sodium. About 8 varieties both from Dinajpur and Barisal were studied for their capacity to support the multiplication of vibrios. Using Millipore filtered tap water for inoculation and storing the cooked rice, it was seen that Barisal Aus showed better results than the Dinajpur Aus. Similarly, Barisal aman showed better results than the Dinajpur Aman. Collectively taken Aman varieties of Dinajpur and Barisal show better results than the Aus varieties from Dinajpur andBarisal. (Table I)

To see the effect of fecal organisms on vibrio, if present in the water used, it was decided to inoculate the Millipore filtered (0.45m) tap water with vibrio cholera and vibrio free stool from patients in CRL Ward. In both Barisal and Dinajpur Aus and Aman the titer rise of vibrio overnight was Three to five log with salt and two to three log without salt.(Table II) Presence of enteric organism does not in any way seem to retard the growth of Vibrio cholerae in rice. The rice was also inoculated with unfiltered vibrio free tank water and contaminated with Vibrio cholerae. Log rise of vibrio was found to be four to five. In Dinajpur Aus and Aman the rise was four log with salt and three log without salt, whereas in Barisal Aus and Aman the rise was four to five log with salt and four log without salt.(TableIII) The presence of other organisms in the tank water also does not seem to inhibit the growth of

vibrio. In fact the raw tank water seemed to help in the rise of vibrio.

It is therefore evident from the above study that if water containing about 20 to 80 organisms (Vibrio cholerae)/c.c. is used for the storage of rice overnight, a titer rise of four to five log is obtained, which is probably a very good effective dose for cholerae.

Methodology for the growing of vibrios in rice from Coastal & Northern regions.

Strain: 7634, Stock No. 325 A/64, Inaba.

Water: Millipore filtered laboratory tap water.

Inoculum: 0.1 ml of 10^{-4} dilution.

Control: a) 100 mls. of Millipore filtered laboratory tap water + NaCl (0.1%).

Method: Equal quantity (approx. weight) of the same variety of rice from Barisal and Dinajpur were taken into two sterilized Handis (earthen pots) and cooked in about 500 mls of tap water until boiled to the desired softening. The excess water after cooking was thrown away and half of the rice from each Handi was transferred into two more sterilized Handis and properly labelled. Four sterilized measuring conical flasks with 100 mls of (0.45M) M.F. Lab. tap water in each were taken and 0.1 gm. of NaCl was added into two of the flasks. Each flask was now inoculated with 0.1 ml of 10^{-4} dilution of overnight grown Inaba culture. Contents of one flask were transferred to one Handi in such a way that Barisal rice got one with salt and one without salt. So was the case with the Dinajpur rice. Two additional flasks one with salt and one without salt, having the same inoculum, served as controls. All Handis and the control flasks were left overnight inside the laboratory at 25°C. 0 hour count of the organism present in the mixture was taken by pipetting out 0.1 ml of the same from each Handi and placing it over a completely dried S.P. plate in about 20-25 drops. 0.1 ml of 10^{-6} dilution of the inoculum was also plated on S.P. to have a count of the inoculum and to find out theoretical distribution of organism/ml in rice. Plates were read next morning after overnight incubation at 37°C. After overnight incubation of the Handis a 10-fold serial

dilution of the rice-water mixture from each Handi was made and 0.1 ml. of 10^{-1}, 10^{-2} and 10^{-3} dilutions were plated on completely dried S.P. plates. 0.1 ml.direct from control flasks was also plated, overnight count was taken the next morning and the rise in titer was recorded.

Methodology for the study of growth of Vibrio in Barisal rice in presence of other enteric organisms.

Strain: a) #7634, Stock #325A/64 - Inaba

b) Vibrio free stool from CRL Ward patients.

Water: Millipore filtered laboratory tap water.

Inoculum: 0.1 ml. of 10^{-4} dil.

Control: 1) 100 mls. of Millipore filtered lab. tap water + Vibrio + 0.1% NaCl.

2) 100 mls. of M.F. Lab.tap water + Vibrio

3) 100 mls.of M.F. Lab.tap water + Stool + Vibrio + 0.1% NaCl

4) 100 mls. of M.F. Lab.tap water + Stool + Vibrio

Method: Two sets of experiments were run simultaneously, one with Barisal Aus and the other with Barisal Aman. Rice was cooked as described in the previous experiment. Six sterilized Handis were used for Aus rice and six for Aman rice. Rice after cooking was equally distributed in the two sets of six Handis each. After the rice was cooled down, 100 mls. of Millipore filtered lab. tap water contaminated with 0.1 ml of 10^{-4} dil. of Inaba and 0.1 ml of 10^{-4} dil. of the vibrio free stool from CRL Ward patients was added to the four Handis of each set. Two of these with 0.1% NaCl and two without. The other two Handis of each set were contaminated with 100 mls. of M.F. Lab. tap water + 0.1 ml of 10^{-4} dil. of Inaba culture, one of them having 0.1% of NaCl and the other without. O hour count was taken in the same way as described earlier except that Mannose agar plates were used for plating and the transparent yellow colonies (confirmed by agglutination) of vibrio were counted after overnight incubation. Initial count of 10^{-6} dil. of Inaba culture and the stool was also taken. Overnight count of vibrio from each Handi was taken by making 10-fold serial dilutions of the rice-water mixture and plating 0.1 ml from 10^{-2} to 10^{-4} dilutions of the same on Mannose agar plates.

Rise in titer of vibrio in the presence of other enteric organisms was recorded.

V (d)

TABLE I

EFFECT OF SALT AND RICE VARIETY ON VIBRIO MULTIPLICATION IN PRESENCE OF OTHER ORGANISMS IN 'PANTA BHAT'

Quality of Rice	Changes in Titer after overnight Incubation	SALT ADDED (0.1%)				NO SALT ADDED			
		Millipore Filtered (M.F) Tap Water + V.C.	M.F. Tap Water + V.C. + Stool	V.C. + M.F. Tank Water	V.C. + Tank Water	M.F. Tap Water + V.C.	M.F. Tap Water + V.C. + Stool	V.C. + M.F. Tank Water	V.C. + Tank Water
Dinajpur AUS	Fall	-	-	-	-	-	-	-	-
	Rise x 10^1					1			
	Rise x 10^2	1				4			
	Rise x 10^3	3		1				1	1
	Rise x 10^4	1			1				
Barisal AUS	Fall	-	-	-	-	-	-	-	-
	Rise x 10^2					7	2		
	Rise x 10^3	5	1			2	2		
	Rise x 10^4	3	2	1	1			1	2
	Rise x 10^5	1	1	1	1			1	
Dinajpur AMMON	Fall	-	-	-	-	-	-	-	-
	Rise x 10^2					2			
	Rise x 10^3			1				1	1
	Rise x 10^4	2			1				
Barisal AMMON	Fall	-	-	-	-	-	-	-	-
	Rise x 10^2					4	3		
	Rise x 10^3	1	1			2	1	2	
	Rise x 10^4	4	2	2	1				2
	Rise x 10^5	1	1		1				

TABLE II - TITER RISE IN AUS AND AMMON VARITIES OF RICE WITH AND WITHOUT ADDITION OF SALT

Quality of Rice	M.F. Tap Water + V.C. + Rice		M.F. Tap Water + V.C. + Stool + Rice		V.C. + M.F. Tank Water + Rice		V.C. + Raw Tank Water + Rice	
	With salt	Without salt	With salt	Without salt	With salt	Without salt	With salt	Without salt
AUS	2 to 5 log	1 to 3 log	3 to 5 log	2 to 3 log	3 to 5 log	3 log	4 to 5 log	3 to 4 log
AMMON	3 to 5 log	2 to 3 log	3 to 5 log	2 to 3 log	3 to 4 log	3 to 4 log	4 to 5 log	3 to 4 log

TABLE III - TITER RISE IN DINAJPUR AND BARISAL VARITIES OF AUS AND AMMON - WITH AND WITHOUT SALT

Dinajpur AUS	2 to 4 log	1 to 2 log	-	-	3 log	3 log	4 log	3 log
Barisal AUS	3 to 5 log	2 to 3 log	3 to 5 log	2 to 3 log	4 to 5 log	3 log	4 to 5 log	4 log
Dinajpur AMMON	4 log	2 log	-	-	3 log	3 log	4 log	3 log
Barisal AMMON	3 to 5 log	2 to 3 log	3 to 5 log	2 to 3 log	4 log	3 to 4 log	4 to 5 log	4 log

V(d)

TABLE IV

CHEMICAL ANALYSIS ON RICE

Varities	BARISAL					DINAJPUR				
	Na^+ %	K^+ %	PO_4^{-3} %	Total Iron %	Total Nitrogen %	Na^+ %	K^+ %	PO_4^{-3} %	Total Iron %	Total Nitrogen %
Kataktara)	0.006	0.226	0.88	0.002	0.91	0.005	0.296	1.0	0.0002	0.46
)										
Dharial)	0.008	0.236	-	-	-	0.004	0.280	1.08	0.0004	-
) AUS										
Dhalai Saita)	0 003	0.250	-	-	-	0.004	0.248	0.88	0.0007	-
)										
Hashikalmi)	0.006	0.288	0.6	0.002	0.84	0.005	0.272	0.8	0.003	1.0
Latisail - AMMON	0.003	0.152	0.86	-	0.8	0.003	0.120	0.8	-	0.84

OTHER PLACES

Barisal Ammon	0.007	0.2	0.64	0.0046	-
Sylhet Aus	0.004	0.17	0.4	0.0041	-
Kataribhog Ammon	0.006	0.64	0.36	0.0036	-

Study on Congregation of Quaran Society.

Religious gatherings and congregations have given rise to cholera epidemics as reported in literature, particularly in India. Such a gathering took place in Dacca in the month of February, 1965, sponsored by a religious society called "TABLIGE JAMAET" of the QUARAN SOCIETY. Delegates from all over the province of East Pakistan as well as West Pakistan and some from India were invited to attend. The majority of the participants were, however, from East Pakistan. The organizers of the congregation set a sort of campus for the delegates.(See map) The following arrangements were made:

Accommodation:- A huge 'Shamiana' (open ground covered with a canopy), was erected. The participants, about 12,000 daily, attended the congregation in the morning and slept at night stretching their own mats on the ground. They rested anywhere they could get a space. Next morning they would go away and a new batch of about 12,000 would come. This continued for four days and about 50,000 people attended the congregation during this time.

Food Supply:- A central kitchen was set up known as 'Lungree'. Food was cooked and distributed twice a day consisting of baked bread and one curry (meat and one vegetable) or bread and brown sugar. The food was given in earthen receptacles. No prepared rice was served from the lungree. The management, however, had allowed some private food shops within the premises where rice, meat, fish and other food stuffs were available. The amount of food supplied was just equal to the minimum requirements of an individual, so the wastage and left-over was practically nil. The food was served within an hour of its cooking.

Water Supply:- The mosque had intermittent Municipal Water supply, but for this occasion 5 overhead tanks were supplied by the Municipal Authority and 25 taps were connected to these tanks to secure water. In addition, 8 tubewells were sunk (65 - 70ft deep), for washing, ablution and drinking etc. One tank (size 70 x 50ft. approx.) outside the mosque area across the road, served as a bath-tub for the total gathering estimated to be more than 50,000.

Sanitation Arrangements:- i) Urinals - Bricks laid longitudinally on the ground with bamboo mat partitions and a drain dug along the line, served as a urinal. Bleaching powder was sprinkled twice a day in the drains. At the end of the drain, a large hole was dug in the ground serving as a collection pit.

(ii) Latrines - A seating place for about 300 people was made on a bamboo raft raised from the ground, with earthen pots beneath for collection of the discharges. These were again partitioned with bamboo mats and had gunny-bag curtains in the door. The fecal matter thus collected in the earthen receptacles was emptied in the dumping pit dug in the ground at one end of the enclosure. There were 10 such holes dug in the ground. Bleaching powder was spread all over twice daily.

(iii) Flies - Fly traps were set up, but no flies could be caught. At some places the fly traps were removed by the people.

The lay-out of the campus is shown in the sketch.(Map).

The congregation was to deliberate for three days from 14th February, 1965 to 16th February, 1965. The delegates started arriving from the 12th February. We surveyed the area on the 13th and started collecting samples from tank, tubewells and food articles both from the lungree and foodshops and the fecal dumping pits.

Methods:- 50 mls. of water samples were collected in 25 mls. of triple strength bile peptone media, incubated overnight and streaked into solid TTGA plates, incubated overnight. Typical colonies of Vibrio cholerae were detected, isolated and identified. Food samples were collected in single strength T_1N_1, media (liquid media for enrichment) and streaked on TTGA plates, incubated and identified in the normal laboratory procedure. Fecal matter was collected with sticks, having a cotton swab at one end, which were put into the dumping pits and then placed in T_1N_1 liquid media and processed in routine way for isolation of Vibrio cholerae

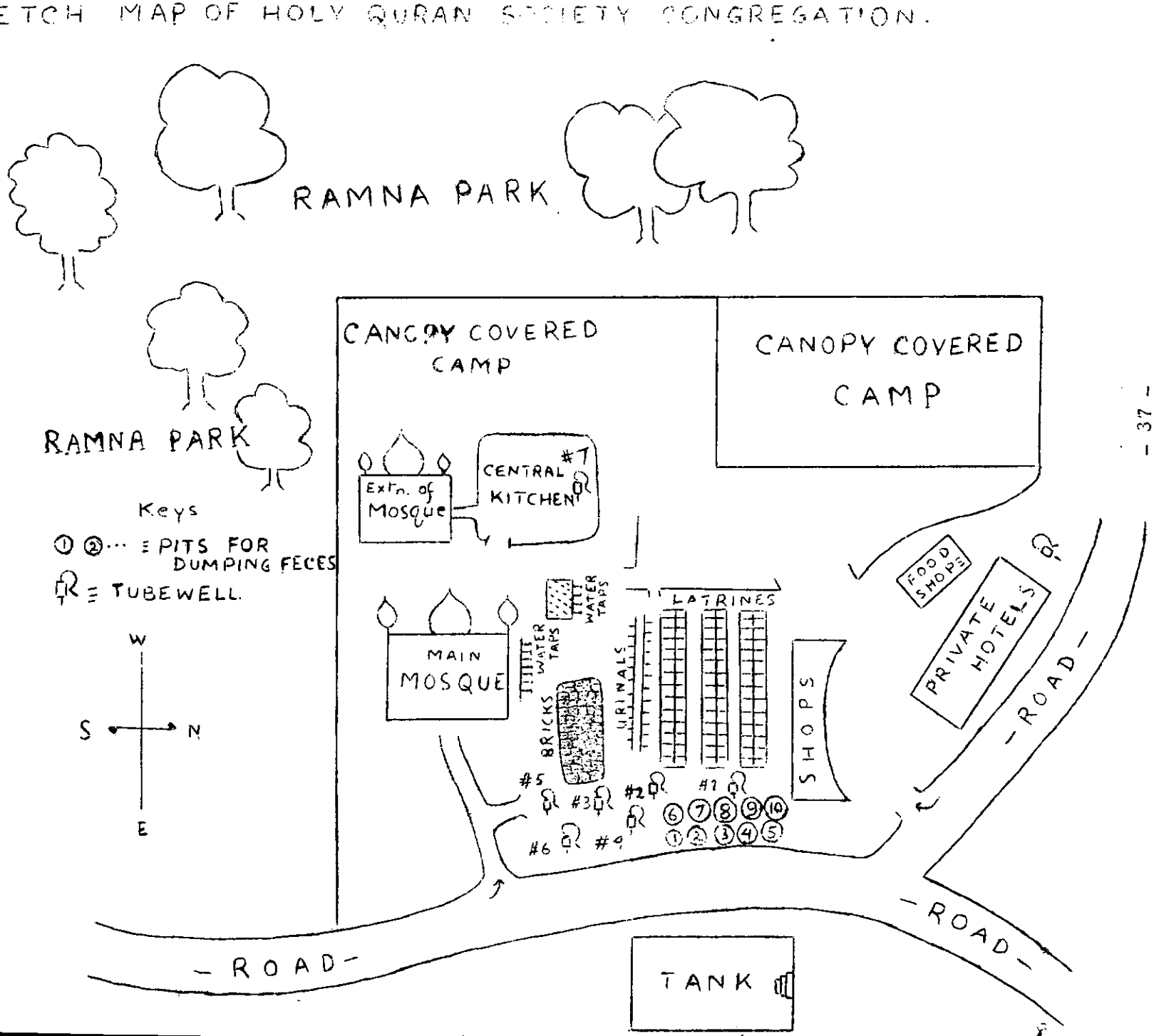
Results:- Out of one tank, 7 tube wells and municipal supply sampled each day from 13th to 18th February, only the tank showed positively for Vibrio cholerae Inaba on the 15th and 16th inst.

Sixteen food samples comprising various items both from the lungree and food-shops collected over three days from the 13th to 15th, were all negative.

Faeces:- Out of ten dumping pits, all of which were not used at one time, the feces being thrown into one pit and as this filled into the next, samples were obtained for three days from the 14th to the 16th. One pit was positive for Inaba on the 14th and the remaining nine pits were positive on the 15th. On the 16th, only six pits were in use and again all six were positive. The pits were finally filled up and closed on the 17th. February.

Discussions:- Positive isolation from feces suggested definite infection in some of the participants. This infection spread and the organisms were isolated from the tank. It was a situation where there was so much congestion, more personal contact and inadequate sanitary facilities. In order to find out the source of infection, enquiries were made of the management as to whether there were any complaints of diarrhea or any mild symptoms. There was no affirmative answer. However we apprised the management of our findings and suggested swabbing of the participants to locate the excretor. The management refused to co-operate. We therefore tried on our own and were able to obtain swabs from 136 people. The people swabbed were from various districts, towns and villages of the province. The swabs were processed by routine methods of isolation and one rectal swab gave positive result. The organism was Inaba as were the water and feces isolates. The carrier was reported to have left the campus on the 17th February for his village. The entire campus was dispersed by 18th February 1965, and the studies were stopped.

V(d)


SKETCH MAP OF HOLY QURAN SOCIETY CONGREGATION.
RAMNA PARK
RAMNA PARK
CANOPY COVERED CAMP
CANOPY COVERED CAMP
Keys
① ② ··· = PITS FOR DUMPING FECES
= TUBEWELL.
W
S
N
E
Extn. of Mosque
CENTRAL KITCHEN
#7
MAIN MOSQUE
WATER TAPS
WATER TAPS
BRICKS
URINALS
LATRINES
SHOPS
FOOD SHOPS
PRIVATE HOTELS
#5
#3
#2
#1
#6
#4
① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩
ROAD
ROAD
ROAD
TANK

The fish has been claimed as carrying the germs and spreading the disease and causing epidemics. Much work on fish has been reviewed by Pollitzer. More recent epidemic caused by the fish has come to our knowledge in Phillipines. The work was done mainly on sea fishes and the theories formulated have been conflicting and no conclusive role of fish in the endemicity and epedemiocity of cholera has been demonstrated so far. We have tried to study the smaller species of fish which abound in the small riverlets, canals and tanks. A small aquarium was set up in the laboratory. The fish used for study came from tanks and comprised of small species like "Put hi", "Taki" and "Magur". Aquarium was filled with lab. tap water (drawn from the tube well). The catch soon after arrival in the lab. was checked for the presence of vibrio. In no case vibrios were isolated from the fish brought in for aquarium. The expriment was divided in four phases:-

Expriment No. 1 : Aquarium water was contaminated with a fresh culture of V. Cholerae so that the water contained about 10^3 to 10^5 org/c.c. and the viable count was taken every day directly from the water - Some amont of water was also transferred into enrichment media to see the presence of vibrio in water if the direct count was negative. Simultaneously the fish was alsotransferred into enrichment media to see the presence of vibrio in water if the count was negative. Simultaneously the fish was also cultured after 2 hours, 24 hours, 4 days and 8 days to see if vibrios take shelter into the fish body and are so harboured. Results of this expriment are shown in the Table No. 1.

Experiment No.2 : In this experiment the fish was directly fed through a tube, a measured quantity of broth culture of vibrio cholerae, and then was left into the vibrio free water. After fixed time intervals one of the contaminated fish was killed and its intestine cultured to test for vibrios. This experiment showed the period for which vibrios survived in the intestine of the fish. Table No. II.

Experiment No.3 : This experiment was done to see how long vibrios survive in meshed uncooked and boiled fish meal. It was done in the following ways:-

a) Raw meshed fish with Nacl
b) Raw meshed fish with Nacl filtered through M.F. 4.5 ml.
c) Fish meshed with Nacl and boiled.

Each was inoculated with a measured amount of vibrio cholerae and viable count was taken after 4,6,24,48, 72 and 96 hours. Results are shown in table No. III.

Discussions: This study shows that Vibrio cholerae does not survive more than a few hours inside the body of the fish. The reason for the death of Vibrio inside the fish body could be due to the presence of Pyocynea, which has a vibriocidal action. Experiment No.4 shows that vibrio, however, multiply very happily in the boiled fish meal or millipore filtered meshed raw fish meal.

TABLE I

SHOWING SURVIVAL OF VIBRIO IN AQUARIUM WATER AND IN FISH BODY

Volume of water in Aquarium.	'Org' put in	WATER — Count of the Organism								FISH — Time Fish cultured after infection	FISH — Results	
		½ Hr.	1 Hr.	20 Hrs	24 Hrs	48 Hrs	72 Hrs	96 Hrs	6 days		Outside washing	Whole fish
60 liters	OG / 3×10^5	3.5×10^4/cc	10^4/cc	2×10^2/cc	30/cc	10/cc	+ in enrichment.	+ in enrichment.	0	8 days	0	0
79 liters	OG / 8×10^4/cc	2.5×10^4/cc	1.2×10^4/cc	-	60/cc	+ in enrichment.	0	0	0	4 days	0	0
		½ Hr.	1 Hr.	5 Hrs.						2 Hrs	+	+
70 liters	OG / 10^2/cc	-	40/cc	+++ enrichment.	+ enrichment.	0	0	0	0	24 Hrs	0	0

TABLE II

SHOWING THE CONTAMINATION OF WATER BY FISH AND SURVIVAL OF VIBRIOS IN THE INTESTINE OF FISH

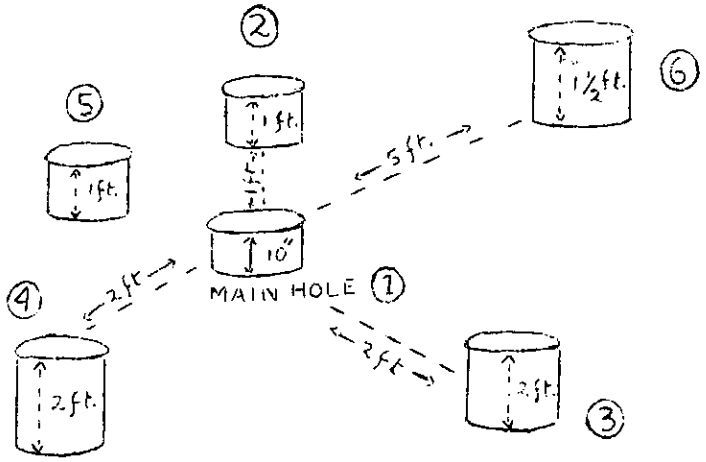
Species of Fish	Quantity of culture fed thru	Type of Water fish put in after feed	WATER POSITIVITY 0 Hr.	4 Hrs.	24 Hrs.	48 Hrs.	72 Hrs.	96 Hrs.	Time-fish killed after feed	Cultures made	Results	Remarks
Singhi	0.2 cc INABA	Tap Water 1 liter	+ Heavy	0	0	-	-	-	24 hrs	1. Stomach 2. Purified fish 24 hrs after killing.	Negative (Direct, Enrich) Negative (Direct, Enrichment	
Taki	.2 mls "OGAWA"	Filtered well water 100 mls pH 8.4	++++ V. Heavy growth	++++	+++	++ 10^{-2} dilution	+	+	96 hrs	Stomach meshed with 20 mls. N.S. - diluted 10 times	Negative (Direct, Enrich.	Pyocyanea inhibiting growth of vibrios
Manguri (Shingi)	1st Dose .2 mls. OGAWA	100 mls Sterile N.S.	+++ Heavy	-	+++ Heavy	++	+	+	-	Nothing done		
	2nd Dose .2 cc of 10^{-1} dilu.	Tap Water 100 mls.	$2x10^2$/cc	-	0	0	-	-	24 hrs	Stomach in 20 mls N. Saline	Direct $1.4x10^4$ Enrich. - Negative	pyocyanea inhibiting growth of vibrios
Taki	.1 cc of 10^{-2} dilu. OGAWA	Filtered River Water 50 mls pH 7.0	2 $2x10$/cc	++ Enrich.	++ Enr.	++ Enr.	0	0	-	Anus culture 1 hr. after feed 6 hrs " " 24 " " "	+ OG 4 colonies Negative	
	2nd Dose .1 cc of OGAWA	"	6 10	4 10	+ in enrichment only	0	0	0	24 hrs	Stomach mashed 20 mls N.S. filtered, plated after dilution	Director - + Enrich. - Negative	

TABLE III

MULTIPLICATION OF VIBRIO COMMA IN FISH MEALS

Species	Weight of whole fish	Quantity of N.S. used for homogenizing.	Inoculum culture	Fish Meals 5 cc	Inoculum culture distribution	Counts at atmospheric temp. - 26-32°C						
						0 Hr.	4 Hrs.	6 Hrs.	24 Hrs	48 Hrs	72 Hrs	96 Hrs
Puthi	3.5 gms	100 mls	INABA	RAW	$6x10^4$/cc	$5x10^4$	$1.4x10^6$	$2x10^7$	$6x10^7$	$8.8x10^7$	$1.5x10^7$	$5x10^5$
			.1 cc of 10^{-6}	Filtered through M.F. .45 m/y	6/cc	$3x10^2$	$2x10^3$	$8.7x10^6$	$1.6x10^8$	$5.3x10^8$	$6x10^7$	$6x10^7$
			.1 cc of 10^{-2}	Boiled	6/cc	0	0	$2x10^3$	$2.1x10^8$	$7.6x10^8$	$4.6x10^9$	$9.4x10^8$
Meni	9.6 gms	100 mls	INABA	RAW	10^5/cc	10^4	$3x10^6$	$2x10^7$	10^8	10^6	-	4.0^4
			0.1 cc of 10^{-2}	Filtered through M.F. .45 m/y	10/cc	3x10	0	10^5	10^7	10^7	-	$7.5x10^5$
			0.1 cc of 10^{-6}									
			0.1 cc of 10^{-6}	Boiled	10/cc	3x10	$3.5x10^3$	$6x10^4$	$1.6x10^7$	$1.3x10^8$	-	pyocyanea over growth
Dori	3.8 gms	100 mls	0.1 cc of 10^{-2}	RAW	10/cc	4x10	0	$<10^2$	$<10^4$	$<10^6$	-	0 in direct plate
			0.1 cc of 10^{-6}	Filtered through M.F. .45 m/y	10/cc	4x10	0	10^4	$2x10^7$	10^7	-	$4x10^6$
			0.1 cc of 10^{-6}	Boiled	10^5/cc	$2x10^4$	$2x10^5$	10^7	$2x10^8$	$4.5x10^8$	-	2x10

Study of Seepage and Survival of V. cholerae through Soil:



In order to see how far can the vibrio travel and how long they can survive in the soil a very crude experiment was devised.

One hole about 10" deep was dug into the soil and numbered as Main Hole No. 1. Holes No.. 2,3,4, 5 and 6 were dug around No. 1 at a distance of 1ft, 2ft, 2ft, 1ft and 1½ft respectively (See figure). The central hole was then completely filled with water and about 1 ml of culture of V.cholerae Ogawa was inoculated into it. Water from the central hole seeped through the soil into holes No. 2,3 and 4. Samples of water from all the 4 holes were transferred to enrichment media and tested for vibrios. Samples were taken after 15 minutes 1 day, 2 day, 3 ay and 4 day (See table). Water was added intermittently to the main hole so that it was always completely filled.

Vibrio cholerae seemed to have seeped through the soil along with water to a distance of about 2ft. and survived for 3 days.

TABLE I

OGAWA

No. of holes	Depth of distance of holes from Main Hole No. 1	Count 15 minutes	1 day	2 day	3 day	4 day
No. 1	Main hole 10" deep	++++	0	+	+	0
No. 2	2ft deep, 1ft away	++++	0	0	0	0
No. 3	2ft deep, 2ft away	+++	+	+	+	0
No. 4	2ft deep, 2ft away	++	+	+	+	0
No. 5	1ft deep, 1½ft away	+	+	0	0	0
No. 6	1½ft deep, 5ft away	0	0	0	0	0

Courtesy Analysis:

The set-up of the laboratory being the most modern and well equipped in this wing, it is serving as a reference laboratory for the analysis of water both chemically and bacteriologically applying the most modern techniques and procedures.

With the industrial development of the country, the different industrial concerns find it necessary to test their water for human as well as industrial consumption. Nevertheless the government, semi-government and other international agencies, serving as consultants to the government in different capacities, view with importance the availability of good water which necessitates primarily the testing of the quality of water. In view of the growing need and in the absence of any satisfactory laboratory, they requested the help of this laboratory to achieve their objective and occasionally sent their water for testing. The laboratory responded to the request and has been analysing the water since 1961 and suggesting ways and means to consume water after the proper treatment both chemically and bacteriologically, whenever this is necessary. The total number of samples for courtesy analysis so far completed comes to 1451 for chemical and 29 for bacteriological.

TABLE

Number of samples analysed.

Year	Chemical	Bacteriological
1961 - 62	32	4
1963	11	5
1964	26	9
1965 (15 th Dec.65)	1087 + 295	7 + 4
	1156 + 295	25 + 4
Total:	1451	29

Training Facilities

The Water and Sewage Authority, Chittagong, sent its trainee in May, 1964 to attend the laboratory for about ten months. During his stay in the laboratory, he was properly trained to undertake his assignment in the agency for the purpose for which he was deputized.

The other trainee who has been working in the laboratory since January, 1965 has been deputed by Messers Camp, Dresser and Mackee, a Consulting Engineering firm of Massachusetts(U.S.A.) under the Directorate of Public Health Engineering, Government of East Pakistan. The trainee has been working mainly on the samples referred to the laboratory by the said concern. The average pour of samples to this laboratory from the above team is 75 per month. They collect samples from every district of East Pakistan with a view to finding out the quality of the water.

Evaluation of Methods for Sanitary Bacteriology of Water:

Tube Fermentation Method.

The standard Tube Fermentation method to determine the sanitary quality of water (using Lactose broth for Presumptive Test and Brilliant Green Bile for confirmation of Coliform organism), was found to be too time consuming. It was also found to be not suitable for the water sources such as dug wells, tanks, ditches etc., which are always contaminated by faecal and non-faecal matter carrying enough Coliform organisms. The use of Membrane Filter technique for determining the Coliform organisms(though it reduced the time in routine analysis), was also found to be not very helpful as the dilution factor was always a matter of guess work, also the clogging of membrane pores frequently confronted us. Moreover,the desire to know the actual faecal contamination made determination of faecal coli imperative. This all necessitated a change in the existing methods.

Fortunately, we had at the time an Australian expert, Mr. A.A. Crispe from the Sydney Metropolitan Water Board, deputed by Dr. Flynn to work here for four weeks. With the help of Mr. Crispe we modified our methods and started using McConkey broth for Coliform density. This reduced the time factor and also gave comparable results to those obtained by Membrane Filter technique.

The find incidence of E.Coli was also studied and Eijkman test was performed. Incubation temperature for optimum temperature for faecal E. Coli was studied under the supervision of Mr. Crispe and E.C. Test at 45°C was found to give satisfactory result than at 44°C. The routine adopted was plantation of tube straight for E.C. media at 45.5 and making E.Coli count with the same M.P.N Table. Later on it was suspected that the above procedure was giving high results and this was later confirmed when typical Coli colonies streaked on E.M.B. McConkey Agar plates and were finally put to IMVIC differential test. Apart from this the laboratory ran short of bile salt No. 3 a component of E.C. media; therefore, from the 26th August 1964 to the present date the whole procedure was revised and rearranged in the following manner. Initial inoculation is made in Lactose broth (3 tube series). After 24 hours of incubation at 37°C gas forming tubes are picked out. From Positive Lactose broth tube inoculation is made in two sets of Brilliant Green Bile. One set goes to incubator (35-37°C) and the other in the incubator (45.5-46°C).

Thus the former confirms the presence of Coliform group expressed as MPN/100 mls and the latter indicates the presence of fecal Coliform group mostly heat resistant E.Coli expressed as MPN/100 mls.

Millipone Filter Technique:

It is confined to less polluted or better water viz; tube well and Municipal water supply. The aliquot varies from 5 to 50 mls and the results expressed as Coliform count/100 mls. The colonies with the golden sheen, found on the filter, were inoculated in BG BL and incubated at 45.5°C in the water jacketed incubator for 24 hours and in some cases for 48 hours to watch any gas formation. If there is any formation of gas in a tube or tubes that is taken as an indication for the presence of fecal Coliform organism, most probably E.Coli, which is confirmed by IMVIC differential test. This is practised only when there is any suspicion regarding these colonies. The parallel test procedure however, is not carried out on routine basis because the load of bacteria of a particular water is not known. As a result sometimes the colonies do not grow or have a confluent growth but in some special cases more attention is needed. Therefore parallel plantation in Lactose broth is also practised to correlate the result and to avoid any suspicion.

Selection of method depends on the water sources subjected to such analysis and is usually as follows:-

When the water comes from river, tank, canal, dug well, drains, sewers etc., it is analyzed by Tube fermentation technique. But when the source is Municipal supply, tube well or drinking water storage jar contamination, it is subjected to Membrane Filter technique.

Medias used for tube fermentation and Millipore Filter procedures are tested as shown hereunder:-

1. Lactose broth as supplied by Difco Laboratory 13 gram/liters. The media is prepared in two strengths, one single and one double. Then 10 mls media is distributed in each tube which have a Durhams tube inside.

Double strength media is used when 10 mls sample is seeded into it and single strength for 1 ml or lower dilution.

2. Brilliant Green Bile 2% as supplied by Difco Laboratory in 40 gram/liters.

Distributed 10 mls media in each tube having fermentation tube inside.

3. McConkey Broth:

2 strengths: (i) Triple strength

(ii) Single strength

10 mls samples are seeded in triple strength whereas single is used for 1 ml or lower dilution.

5 mls media were distributed in each tube which had a fermentation tube inside.

Ingredients of the Media:

Peptone	10 grams
Lactose	10 grams
NaCl	5 grams
Bile Salts No. 3	5 grams
H_2O	1000 mls
Phenol red	10 mls (0.1% alcoholic)
N NaOH	2.5 mls to adjust pH 7.4

4. E.C. Medium (Hajua and Perry Am. J. Public Health 33:550 1943)

Double and single strength media for the aliquot of water already mentioned in connection with L.Broth and McConkey Broth.

5 mls media were distributed in each tube which chad a fermentation tube inside.

Ingredients:

Tryptose	20 grams
Lactose	5 grams
Bile Salt No. 3	1.5 grams
K_2HPo_4	4 grams
KH_2Po_4	1.5 grams
NaCl	5 grams
H_2O	1000 mls

M.F. Media

1) MHENDO 57 gram/liter
as supplied by Difco Laboratory.

2) MENDO MF(R) 48 gram/liter
as supplied by Difco Laboratory.

Reservoir of Cholera Vibrio.

Search for the reservoir of Vibrio cholerae is underway, and the obrious suspicion goes to water where there are so many cholera like vibrio inhabiting. The duration of survival of Vibrio cholerae was the subject of this study.

Survival in raw water.

Routine technique was applied in this experiment. The water was inoculated with a known number of vibric cholerae and then counts were taken at different time intervals till direct count was negative (less than 10 organism per c.c. of water). That water (in portions) was transferred to enrichment medium and agglutinable vibrio were isolated. Positive results meant vibrio surviving in that water. When a portion of infected water was found negative, it was transferred into enrichment; if the quantity of water was too large, it was passed through membrane filter and the filter pad was transferred into the enrichment and the vibric were isolated as usual. If there was no isolation at this stage, it was concluded that the Vibrio cholerae were deed. The whole duration of positive isolation was counted as survival period.

Survival in millipore filtered water (.45 m):

In addition to this direct inoculation and isolation method, another method was used involving filtration of water through millipore filter and then inoculating it with vibrio and the subsequent study of their survival as before. This was done with an aim to study whatever effect this treatment had on vibrio in a water freed from most of the natural bacterial flora, minimising the adverse of antagonistic influence of other microorganism on it and also allowing them to derive nutrition without any compitition.

Survival in boiled water:

In a third procedure, water was sterilized by boiling. This had the usual effect ofkilling the naturally present microorganism in water and also some change in the chemical quality of water. Dissolved gases were evolved raising the pH, shifting the alkalinity level of the sample and increasing the concentration of other constituents. Water was inoculated with Vibrio cholerae as usual and their period of survival was observed.

The results (Table I & II) confirmed the observation of previous workers that Vibrio cholerae survives for a longer duration in a water free from other microorganism. In boiled water vibrio survive longer than in the filtered or raw water and the reason being that the organism which die as a result of boiling provide additional food or source of nutrition for the vibrio.

Beside the difference in survival period, in the raw, the filtered and the boiled water, the raw water did not help the vibrio to grow where as the same water, if filtered, helped the vibrio to propagate to a greater extent and the boiled water served as an enrichment medium sometimes.

T A B L E 1

Survival Period

	Raw	Filtered	Boiled
Dug well	3-9 days	7-12 days	12-16 days
Tanks	2-5 days	-	11-13 days
Rivers	3-9 days	6-10 days	-
Tube well	1	-	1 day
Rainwater	4-6 hours	-	-
Sewage effe.	1-2 days	-	26 days

T A B L E II

Growth

	Raw	Filtered	Boiled
Dug well	No rise	1 - 1½ log.	3-4 log.
Tank	No rise	-	4 long rise
River	No rise	-	-
Tube well	No rise	-	No rise
Sewage effe.	No rise	-	3-4 log rise

LIST OF PAKISTAN-SEATO CHOLERA
RESEARCH LABORATORY STAFF

November 30, 1965.

DIRECTOR'S OFFICE

		Joined
Dr. Abram S. Benenson	Director	28 June 1962
Dr. Robert A. Phillips	(Will assume Directorship on December 1, 1965)	
Mr. Robert E. Freise	Executive Officer	29 January 1964
	2 Steno/Secretaries	

CLINICAL RESEARCH SECTION

Dr. John Lindenbaum	Chief, Clinical Research & Deputy Director, CRL	19 August 1963
Dr. Norbert Hirschhorn	Assistant Chief	3 December 1964
Dr. David B. Sachar	Research Physician	23 July 1965
Dr. James O. Taylor	Research Physician	12 July 1965
Dr. Md. Rafiqul Islam	Senior Physician	13 February 1963
Dr. A.K.M. Jamiul Alam	Junior Physician	21 August 1963
Dr.(Mrs.) Rezia Akbar	Junior Physician	1 February 1963
Dr. A.S.M. Mizanur Rahman	Junior Physician	29 April 1963
Dr. Zahidul Hoque	Junior Physician	25 June 1964
Dr.(Mrs.) K.M. Ally	Junior Physician	29 April 1964
Dr. Azad Firoze	Junior Physician	22 July 1965
Dr. Wali Imam	Junior Physician	31 July 1965
Dr. A. Mazid Mollah	Junior Physician	20 September 1965
	3 Senior Research Assistants	
	4 Senior Laboratory Technicians	
	2 Senior Research Nurse	
	1 Radiology Technician	
	1 Medical Record Secretary	
	4 Junior Labotarory Technicians	
	1 Laboratory Attendant	

(more)

CRL WARD

			Joined
Miss Dorothy G. Torrance, M.B.E.		Supervisor	16 July 1963
	8	Senior Nurses	
	3	Junior Nurses	
	10	Aid Nurses (Two seasonal)	
	1	House Keeper	
	2	Cooks	
	2	Cook-helpers (One seasonal)	
	4	Wordboys (One seasonal)	
	8	Orderlies	
	3	Laundrymen	
	2	Glass Washers	

BACTERIOLOGY SECTION

Dr. Saiyid Sibte Hasan Rizvi		Chief	27 August 1963
Mr. Imdadul Huq		Deputy Chief	16 November 1962
Dr. Ansaruddin Ahmed		Clinical Bacteriologist	17 February 1965
	3	Senior Res. Assistants	
	3	Bacteriological Assistants	
	1	Veterinarian	
	3	Laboratory Assistants	
	2	Junior Lab. Technicians	
	3	Media Makers	
	8	Lab. Attendants(Glass Wahers)	

EPIDEMIOLOGY SECTION

Dr. Wiley H. Mosley		Chief	18 June 1965
Dr. Moslemuddin Khan		Assistant Chief	3 April 1964
Dr.(Mrs.) Shamsa Ahmed		Deputy Asstt. Chief	1 May 1964
Mrs. Shirley Lindenbaum		Anthropologist	18 Sept. 1963
Dr. Ranjit Kumar Barui		Physician	11 November 1963
	3	Senior Res.Asstts(Sociologists)	
	1	Statistical Supervisor	
	2	Statistical Assistants	
	1	Field Sociologist	
	1	Nurse Field Investigator	
	2	Sanitary Inspectors	
	6	Field Assistants	
	1	Interpreter	

WATER STUDIES SECTION

			Joined	
Mr. Yousuf A. Saieed		Acting Chief	9 July	1964
	1	Senior Res. Assistant		
	1	Junior Res. Assistant		
	1	Senior Lab. Technician		
	2.	Sanitary Inspectors		
	1	Laboratory Assistant		
	1	Junior Lab. Technician		
	1	Sample Taker		
	1	Bottle Washer		
	1	Laboratory Attendant		

ADMINISTRATIVE & GENERAL SERVICES SEC:

Mr. Robert E. Freise		Chief	29 January	1964
Mr. N.H. Choudhuri		Administrative Officer	11 July	1963
	1	Administrative Assistant		
	1	Accountant		
	1	Maintenance Supervisor		
	1	Head Mechanic		
	2	Supply Assistants		
	2	Accounts Assistants		
	2	Typist/Clerks		
	2	Maintenance Assistants		
	1	Electrician		
	1	Plumber		
	1	Electronic Technician		
	1	Telephone-Operator-cum-Receptionist		
	1	Librarian		
	1	Dispatcher		
	18	Drivers		
	2	Mechanic Helpers		
	1	Tailor		
	6	Darwans		
	1	Night Guard		
	2	Messengers		
	1	Animal Caretaker		
	3	Sweepes		
	1	Gardener		

FOREIGN TRAINING PROGRAM

Mr. S. Zafar Ahmad	Chief, Water Studies.	London School of Hygiene & Tropical Medicine.
Mr. Syed Ahmed Khan	Sociologist	Western Reserve University Cleveland, OHIO
Mr. Syed Iqbalul Hasan	Research Assistant	Jefferson Medical College, Philadelphia
Mr. S.O.Q. Haider Ali	Sociologist	Western Reserve University, Cleveland, OHIO.

MATLAB VACCINE TRIAL

EPIDEMIOLOGY SECTION:		Joined
Khawja M. Ashraful Aziz	Field Surveillance Supervisor	25 August 1964
	10 Sanitary Inspectors	
	38 Field Assistants	
	76 Dais	

WARD:	
One Physician	Remains on duty alternately.
	1 Ward Master
	1 Junior Nurse
	2 Aid Nurse
	1 Dai
	1 Washerwoman
	2 Sweeper

GENERAL SERVICES:	
	1 Barge Caretaker
	1 Sarang
	1 Driver
	1 Greaser
	1 Sukani
	2 Khalashis
	5 Outboat Drivers.

	PROFESSIONAL & EXECUTIVE	TECHNICAL	SUPPORTING	TOTAL
DIRECTOR'S OFFICE	2	-	2	4
CLINICAL RESEARCH SECTION	13	14	2	29
CRL WARD	1	21	22	44
BACTERIOLOGY SECTION	3	15	8	26
EPIDEMIOLOGY SECTION	5	10	7	22
WATER STUDIES SECTION	-	9	2	11
ADMINISTRATIVE & GENERAL SERVICES	-	-	57	57
MATLAB	-	10	122	132
FOREIGN TRAINING PROGRAM	1	3	-	4
GRAND TOTAL:	25	82	222	329

CONSULTANTS:

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