REFERENCES

(1) Interdepartmental Committee on Nutrition for National Defense. Manual for Nutrition Surveys. NIAMD, National Institutes of Health, Bethesda, Md., 20014, 1957.
(2) Food and Agriculture Organization (United Nations): *a*, Food Composition Tables for International Use, 1949; *b*, Minerals and Vitamins, Rome, 1954.
(3) National Research Council (U.S.). Food and Nutrition Board, Report No. 115. National Research Council, Washington, D.C., 1958.
(4) Basu, K. P., Basak, M. N., and Rai Sircar, B. C. Indian J. Med. Res., 27: 471, 1939.
(5) British Medical Association. Report of Committee on Nutrition, 1950.

Nutritional Studies in Cholera[1]

The Influence of Nutritional Status on Susceptibility to Infection

Dr. I. H. Rosenberg, Dr. W. B. Greenough III, Dr. J. Lindenbaum, and Dr. R. S. Gordon, Jr.

Pakistan-SEATO Cholera Research Laboratory, Interdepartmental Committee on Nutrition for National Defense (ICNND), National Institutes of Health, Bethesda, Md., U.S.A.

Cholera, while remaining a disease of worldwide interest, exists today as a health problem only in the villages and crowded cities of Asia. Since the end of the last pandemic in 1923, the orbit of cholera's influence has gradually contracted about the Ganges delta region of India and Pakistan, where cholera has existed endemically since earliest times. In the past decade there have been outbreaks in Thailand, the Philippines, Indonesia, and Hong Kong where cholera is associated, as in India and Pakistan, with malnutrition, poor sanitation, and overcrowding. Within such loci of poverty and malnutrition there exists marked individual variation in susceptibility to cholera. Many observations suggest that exposure to large inocula of the *Vibrio cholerae* may be general in a cholera-infected population, yet the disease selects only a fraction of the susceptible population. There may, therefore, be host factors which affect the development of overt disease in the exposed individual.

Malnutrition has long been invoked as a factor contributing to susceptibility to infection, although scientific demonstration of such a causal relationship has seldom been made. Since cholera is prevalent in populations which are also malnourished, malnutrition has been suggested as a predisposing factor. This assumption has not been examined in controlled studies, nor has there been a systematic examination of the effect of nutritional status of the host on the severity or course of the disease.

In East Pakistan a unique opportunity exists for the study of the relationship of malnutrition to cholera. The recent Nutrition Survey of Pakistan provides background information on the nutritional status of the population from which patients with cholera of the Pakistan SEATO Cholera Research Laboratory in Dacca are drawn (1). Thus, it was possible to move from epidemiologic data on malnutrition to studies of nutritional factors in the individual.

We have undertaken a study of the relationship between nutrition and cholera, emphasizing specific nutritional factors which might modify host response to the infective organism, *Vibrio cholerae*. Ascorbic acid was selected for study since deficiency of this factor is widespread in the population in which cholera exists endemically, and since the spring and late fall peaks of cholera incidence appear roughly to coincide with the seasons of greatest vitamin C deficiency in the population (1). Moreover, ascorbic acid has long attracted the interest of investigators concerned with nutrition and infection. Although thiamine deficiency is rare in the same population, studies of thiamine metabolism in cholera patients were undertaken to evaluate the possibility that those patients with severe cholera who exhibit impairment of cardiac function might be suffering from an associated thiamine deficiency. Cardiac, electrolyte, and neurological findings in some patients bear a resemblance to thiamine deficiency (2). Vitamin B_{12} and folic acid were studied since deficiency of one

[1] This work was supported in part by grant No. 196802 between the National Institutes of Health, Bethesda, Md., U.S.A., and the Pakistan-SEATO Cholera Research Laboratory, Dacca, East Pakistan.

or both of these factors is commonly associated with gastrointestinal disturbances. In addition, it has been reported that monkeys fed a folic-acid-deficient diet have increased susceptibility to bacillary dysentery (*3*). Potassium studies were stimulated by the observation that a percentage of the population of East Pakistan were excreting potassium in the urine at levels below 15 meq. per gram of creatinine, suggesting maximal conservation of this cation (*2*). If potassium deficiency exists in this population, as these findings suggest, then it might also affect host response to *Vibrio cholerae*.

Materials and Methods

Patients with bacteriologically proven cholera were selected from the ward of the SEATO Cholera Research Laboratory during 1963 and 1964. Predisease nutritional data were obtained in the course of an epidemiological study of the family contacts of cholera patients.[2] Such contacts were followed with daily rectal swabs for culture. Occasionally, a family contact under surveillance would be found to be an asymptomatic carrier or would require admission for true cholera. In all instances random daily urine samples were collected, acidified to pH 3, and refrigerated in dark bottles until analysis.

Urinary thiamine was determined photofluorometrically by the thiochrome method (*4*). Excretion data were expressed in micrograms per gram of creatinine since collections were random rather than timed. Urinary creatinine was measured by the method of Folin and Wu (*5*).

Blood samples were obtained from patients on admission to the cholera ward for ascorbic acid and erythrocyte transketolase assays. Sera were separated and precipitated with trichloracetic acid for subsequent measurement of ascorbic acid content by the dinitrophenylhydrazone method (*6*). The TCA filtrate was assayed immediately or was stored frozen for later assay. Since tetracycline used in our treatment regimen contains therapeutic amounts of ascorbic acid, only admission, pretreatment levels are reported.

For the transketolase assay erythrocytes from admission and subsequent blood samples were separated, washed twice with 0.9 percent NaCl, and lysed in distilled water. The lysate was assayed for transketolase activity according to the method of Brin *et al.* (*7*). The method measures consumption of ribose phosphate by erythrocyte hemolysates with and without added thiamine pyrophosphate. A 20 percent or greater stimulation of ribose consumption after addition of TPP is considered evidence of thiamine deficiency and is perhaps the most reliable biochemical index of this clinical deficiency state in man.

Vitamin B_{12} levels in serum were determined by microbiological assay with *Lactobacillus leichmanii*, according to the method of Spray (*8*). Serum was obtained during the first week after admission, usually on the second hospital day, after rehydration had been accomplished, but before foods containing Vitamin B_{12} had been added to the diet. The normal range in this laboratory is 180–900 micromicrograms per milliliter. Serum levels of this vitamin have been shown to correlate well with overt deficiency in a variety of clinical situations, and to become abnormal before tissues stores are exhausted or anemia and other symptoms appear (*9*).

Serum folate levels were measured by microbiologic assay with *Lactobacillus casei*, according to a modification of the method of Baker and his colleagues (*10, 11*). Serum folate levels have been shown to correlate well with clinical folic acid deficiency (*10, 12, 13*). In this laboratory the assay has proved reliable and reproducible, though a wider range of normal levels (3.5–25 millimicrograms per ml.) has been found than in other laboratories. Patients with megaloblastic anemias due to folic acid deficiency were found to have folate levels below 3 millimicrogm. per ml.

A 24-hour exchangeable potassium content of patients was studied by the isotope dilution procedure, using K^{42} supplied by the Indian Atomic Energy Authority. Each patient received a tracer dose of 100–200 microcuries by either the oral or the intravenous route. For the following 24 hours all excreta were collected and assayed for radio-potassium. By this means it was possible to determine the fraction of the administered dose remaining in the patient at the end of the first 24-hour period. A urine specimen was then collected between the 24th and 28th hour, and the specific activity of potassium in this specimen determined. Assuming that this specimen reflected the specific activity of radio-potassium in the patient's body as a whole, it was then possible to calculate the total 24-hour exchangeable potassium (*14*).

The radio-isotope assays were carried out with liquid specimens of from 200–900 ml. volume. The samples were counted inside a lead shield over a three-quarter inch thallium-activated sodium iodide crystal scintillation counter. Corrections for physical half-life were eliminated by concurrent counting of known standards and the unknown specimens. All count-rates were high enough to reduce the statistical counting errors to 5 percent or less.

Results

Thiamine nutriture.—In 12 cases it was possible to determine urinary thiamine excretion immediately prior

[2] This prospective study was performed in collaboration with the epidemiology section of the laboratory under the direction of Dr. J. L. Stockard and Dr. A. Quader Khan.

TABLE 1.—Precholera urinary thiamine

Patient number	Age	Sex	Days prior to onset	Urinary thiamine (μgm./gm. creatinine)
96	30	F	2	89
113	12	M	<1	154
149	7	F	1	416
119	6	M	<1	1,214
153	8	F	<1	815
165	8	F	<1	464

to the onset of symptoms, using urine samples obtained in connection with studies of household contacts. As these were casual specimens, we followed the procedure of expressing results as ratios of thiamine to urinary creatinine. The data on the first six of these specimens are presented in table 1. In none of these or the subsequent cases was thiamine excretion low enough to indicate deficiency status.

TABLE 2.—Erythrocyte transketolase activity on admission

Patient number	Ribose consumed (μmole/cc./hr.)	Increase with TPP [1]	Percent stimulation
Patients with cholera			
102	3,000	460	15
103	2,970	0	0
104	2,850	0	0
105	2,440	420	17
106	3,000	0	0
108	2,250	0	0
110	1,880	90	4.7
111	2,300	0	0
113	3,170	320	10
116	2,560	280	11
117	3,090	140	4.5
124	1,900	120	6.4
127	2,640	0	0
131	2,815	0	0
Patients with noncholera diarrheal disease			
107	2,270	210	9.2
114	2,830	0	0
115	3,050	0	0
118	2,440	260	11
122	2,400	190	7.9
125	2,950	180	6.1
126	2,460	120	4.8
128	2,460	70	2.8

[1] Normal = <21 percent stimulation with TPP (thiamine pyrophosphate)

TABLE 3.—Erythrocyte transketolase activity in cholera

Patient	Day of disease	Ribose consumed (μmole/cc./hr.)	Percent stimulation with TPP
102	2	3,340	0
105	3	2,640	0
116	3	2,870	1.4
101	4	1,770	0
	7	2,310	12.4
103	4	3,140	1.4
	7	2,560	5.4

During the course of the family contact study, urine samples were collected from individuals who subsequently developed positive rectal swab cultures for *Vibrio cholerae* but who remained clinically well. A number of these individuals were studied before, during, and after their periods of positive cultures. The vitamin excretion values obtained from persons before the first positive culture ranged from low (34 μgm/gm creatinine) to high (7,200 μgm/gm creatinine). In only one case did urinary thiamine decrease after the appearance of the positive culture; in all other cases there was no correlation with subsequent clinical course.

Erythrocyte transketolase studies.—Twenty-two patients were studied, 14 with proven cholera and 8 with other acute diarrheal illnesses (table 2). In no case was ribose consumption abnormally stimulated by thiamine pyrophosphate in red blood cell hemolysates obtained on admission. In five patients, red blood cell transketolase activity was assayed at later stages of the disease. Stimulation was found not to exceed 20 percent, even on the 4th and 7th day of disease (table 3). Similarities between the noncholera and cholera groups are summarized in table 4.

TABLE 4.—Erythrocyte transketolase activity

Patients studied	Ribose consumed (μmole/cc./hr.) mean ± S.E.	Percent stimulation with TPP [1]	
		Mean	Range
Cholera	2,633 ± 114	4.9	0–17
Noncholera diarrhea	2,608 ± 103	5.2	0–11

[1] Normal = <20 percent stimulation with TPP (thiamine pyrophosphate)

TABLE 5.—Serum ascorbic acid values

	Number	Percent below 0.2 mg./100 ml.	Mean ± standard error (mg./100 ml.)
Patients with cholera	42	16.6	0.85 ± .13
Patients with other illness	55	29	0.65 ± .08
General population	943	26	0.48 ± .02

These observations indicate that thiamine intake prior to onset of disease, and functional status at the time of hospitalization, were normal in the individuals studied and that thiamine deficiency is not an important determinant of cholera susceptibility in this area.

Ascorbic acid.—Serum levels of ascorbic acid were measured on admission in 42 cholera cases and in 55 patients with other diarrheal illnesses. Bloods were not available from household contacts before onset of disease, and the use of ascorbic acid in the therapeutic protocol precluded systematic study of serum levels after rehydration. Of the 42 cholera cases, 7 had initial levels in the deficient range (table 5). An even larger proportion of patients who proved to have noncholera diarrhea had low levels, although the difference did not approach statistical significance. The Pakistan Nutrition Survey found the incidence of deficiency to be 26 percent in fieldwork during the same period. Although we do not have data on the incidence of cholera in vitamin C-deficient individuals versus that in normals, it appears that cholera does not tend specifically to select only those members of the population whose vitamin C nutriture is poor. An attempt was made to evaluate the role of vitamin C in modifying host response to infection by correlating admission vitaman C levels with the severity of disease. No correlation could be demonstrated.

Serum vitamin B_{12}.—As is seen in table 6, the vitamin B_{12} level was subnormal in only two patients, a woman in the third trimester of pregnancy and a 20-year-old male with malabsorption. Of the remaining patients B_{12} levels were normal in 41 and elevated in 3. In 15 patients admitted with acute nonspecific enteritides, the B_{12} levels fell within the normal range. The absence of B_{12} deficiency in our patient population is consistent with the provincewide findings of the Nutrition Survey, where evidence of B_{12} deficiency was uncommon (*15*).

Serum folate activity.—Sera from 73 patients with acute diarrheal disease due to *V. cholerae,* and from 43 patients with acute diarrhea from whom pathogenic organisms were not isolated, were obtained during the first hospital week, after rehydration had been accomplished, and were assayed for folate activity. These data are presented in table 7.

TABLE 6.—Serum vitamin B_{12} levels

Patient group	Number studied	Number subnormal [1]	Number elevated	Mean ($\mu\mu$gm./ml.)
Cholera	46	2	3	504
Noncholera diarrhea	15	0	0	578

[1] Normal range 180–900 $\mu\mu$gm./ml.

Twelve (16 percent) of 73 cholera patients, and 17 (40 percent) of 43 patients with noncholera diarrhea, had subnormal folate levels. The mean folate level for the cholera group was 7.8 millimicrogram per ml. When the patients with low folates were compared with those with normal levels, there were no significant differences in age, body weight, convalescent hemoglobin, and convalescent plasma protein levels in either the cholera or noncholera groups. Peripheral blood smears from 25 of the 29 patients with low folate levels were reviewed by an independent investigator for evidence of folate deficiency (hypersegmented neutrophils with or without macrocytes). In 11 patients these abnormalities were found, though usually of mild degree.

It would appear, then, that folic acid deficiency is present in a minority of patients with diarrheal disease in our population. It is unlikely that either dietary lack or malabsorption of vitamin B_{12} or of folic acid is a major factor in the development of cholera in this population.

Body potassium in cholera patients.—The 24-hour exchangeable potassium was measured in a series of eight cholera patients and nine patients suffering from noncholera diarrhea (table 8). The mean value of exchangeable K, using K^{42} as tracer, in the cholera group was 44 meq./kg. body weight, while the control group with other illnesses had a mean of 42. Since these values compare well with normal values obtained from the literature, the

TABLE 7.—Serum folate levels

Patient group	Number studied	Number subnormal	Mean serum folate ($\mu\mu$gm./ml.)
Cholera	73	12 (16 percent).	7.8
Noncholera diarrhea	43	17 (40 percent)[1].	9.8

[1] Significantly different from cholera group ($p < 0.01$).

TABLE 8.—Mean values of 24-hour exchangeable K

Patient type	Number studied	Mean K_E (mEq/Kg)	Standard error of mean
Cholera	8	43.8	±2.6
Noncholera diarrhea	9	42.0	±1.6
All male patients	13	44.7	±1.4
All female patients	4	36.7	±2.2
Normal (males)	.....	45	.....
Normal (females)	.....	35	.....

results do not support the hypothesis that a preexisting potassium deficiency predisposes to cholera infection in man.

Role of vitamins in therapy of cholera.—Early in the study, cases exhibiting cardiac decompensation improved on a therapeutic regimen including thiamine. However, subsequent analysis has failed to demonstrate any effect of vitamin B or C therapy on the duration of diarrhea in our patients with cholera (table 9).

SUMMARY AND CONCLUSIONS

The nutritional status of patients admitted to the Pakistan SEATO Cholera Research Laboratory was studied to evaluate the hypothesis that malnutrition predisposes the exposed individual to infection. These studies demonstrated that:

1. Thiamine status as measured by erythrocyte transketolase activity was normal in cholera patients, and did not differ significantly from values obtained in patients without cholera.

TABLE 9.—Role of vitamins in therapy of cholera

	Day of cessation of diarrhea, number of cases						Mean duration of diarrhea (days)
	0	1	2	3	4	5	
GROUP A							
No vitamin therapy (16 cases)	5	6	1	3	1	0	1.3
GROUP B							
Vitamin B therapy (11 cases)	0	4	4	1	1	1	2.2
GROUP C							
Vitamin C therapy (11 cases)	3	2	2	0	2	2	2.2

2. Urinary thiamine excretion prior to onset of cholera in exposed individuals did not indicate thiamine deficiency nor were such values related to subsequent development of disease.

3. Serum ascorbic acid levels in cholera patients ranged from low to high, without correlation with severity of infection. These values did not differ significantly from those of noncholera patients or from those in the general population.

4. Levels of serum folate and vitamin B_{12} were normal in cholera patients, with rare exceptions.

5. The 24-hour exchangeable potassium content of patients with cholera was found to be comparable with normal values in the literature. The evidence obtained did not support the hypothesis that potassium deficiency contributes to cholera susceptibility.

6. The use of ascorbic acid alone or in combination with vitamin B was not of value in shortening the diarrheal course of cholera.

The clinical response to proven contact with *V. cholerae* appears not to be conditioned by thiamine, ascorbic acid, vitamin B_{12}, folic acid, or potassium nutriture of the host. Given a population exposed heavily to the organism, it has not been possible to predict, on the basis of these nutritional studies, which individuals would become infected and which would be resistant. Although the hypothesis that dietary deficiency may predispose to cholera is suggested by the prevalence of malnutrition in foci of endemic cholera, these investigations provide no support for a causal relationship.

ACKNOWLEDGMENT

The authors acknowledge with appreciation the technical assistance of Mr. Md. Yasin, Mr. A. Rahman Khan, Mr. S. N. Hasan, and Miss Shirley Wickenden.

REFERENCES

(1) Siddiqui, M. K. R., Ahmad, K., and Rosenberg, I. Nutritional Evaluation of the Population of East Pakistan, I, II, III. Sixth International Congress on Nutrition, 1963.
(2) Report to the Technical Advisory Committee of the Pakistan–SEATO Cholera Research Laboratory 1963.
(3) Janota, M., and Dack, G. M. J. Infect. Dis. 65: 219, 1939.
(4) Consolazio, C. F., Johnson, R. E., and Marek, E. Metabolic Methods. C. V. Mosby and Co., St. Louis, Mo., U.S.A., 1951.
(5) Folin, O., and Wu, H. J. Biol. Chem. 38: 98, 1919.
(6) Shaffert, G. R., and Kingsley, G. R. J. Biol. Chem. 212: 59, 1955.
(7) Brin, B., Tai, M., Ostashever, A. S., and Kalinsky, H. J. Nutr., 71: 273, 1960.
(8) Spray, G. H. Clin. Sci., 14: 661, 1955.

(9) Spray, G. H. Postgrad. Med. J. 38: 35, 1962.
(10) Water, A. H., and Mollin, D. L. J. Clin. Path. 14: 345, 1961.
(11) Baker, H., Herbert, V., Frank, O., Pasher, I., Hutner, S. H., Wasserman L. R., and Sobotka, H. Clin. Chem., 5: 275, 1959.
(12) Herbert, V. Trans. Assn. Amer. Physicians 75: 307, 1962.
(13) Cooper, B. A., and Lowenstein, L. Canad. Med. Assn. J. 85: 987, 1961.
(14) Flear, C. T. G., Cooke, W. T., Sivyer, A., and Domenet, J. Clin. Chim. Acta 8: 768, 1963.
(15) Reiner, M. L., Prince, C., Wickenden, S., and Lindenbaum, J. [Unpublished data].

Malabsorption During and After Recovery From Acute Intestinal Infection[1]

Dr. John Lindenbaum

Pakistan-SEATO Cholera Research Laboratory, Dacca-5, East Pakistan.

In recent years increasing interest has been shown in malabsorption in the small intestine associated with a variety of chronic diarrheal diseases. The effect on absorptive capacity of acute intestinal infection, the most common form of gastrointestinal disease the world over, has received little attention. Many cases of acute enteritis are too mild to require hospital admission, or are discharged from hospital soon after symptomatic recovery. Occasional patients have been reported, however, in whom a sprue-like syndrome associated with malabsorption appeared to follow acute intestinal infection (Achor and Smith, 1955; King and Joske, 1960). In the past year we have also encountered two cases in which diarrhea and weight loss with malabsorption appeared to follow episodes of acute intestinal infection in previously healthy individuals (Lindenbaum, 1965). Interest in these cases, as well as in the possible relationship of previous bouts of acute bowel infection to the asymptomatic malabsorptive state common in tropical countries (Sprinz, Sribhibadh, Gangarosa, Benyajati, Kundel, and Halstead, 1962; Baker, Ignatius, Mathan, Vaish, and Chacko, 1962; Aziz, 1965; Lindenbaum, 1965), prompted a study of absorption during and after recovery from acute intestinal infections in East Pakistan. Preliminary results will be presented at this time.

Methods and Materials

The 95 patients included in this study were Pakistanis admitted to the Pakistan-SEATO Cholera Research Laboratory hospital ward for acute diarrheal illness of less than 2 week's duration. Nearly all patients were admited after only 1 to 2 days of acute illness. While most were young adults of either sex, the entire group ranged in age from 7 to 65 years.

In the majority of cases a bacteriologic diagnosis was established by culture of rectal swabs. In 47 *Vibrio cholerae* was obtained; in 6 Shigellae (types A, B, or D); and in 2 Salmonellae (one type D, one type B). In four patients who had the classifical clinical picture of staphylococcus food-poisoning *Staphylococcus aureus* was isolated from stool cultures and from food recently ingested by all of them. Nine patients, from whom no known pathogens were isolated, had an acute severely dehydrating illness resulting in profound circulatory collapse unassociated with rises in antibody titer against *Vibrio cholerae* ("nonvibrio cholera"). Clinical features of these cases will be reported elsewhere (Lindenbaum, Greenough, Benenson, Oseasohn, Rizvi, and Saad, 1965). In the remaining 27 cases, all of whom had an acute gastrointestinal illness of one to four day's duration not associated with circulatory collapse, no pathogens were isolated despite stool cultures on SS, MacConkey's, gelatin and tellurite-taurocholate-gelatin agars ("acute gastroenteritis").

The 5-hour urinary excretion of D-xylose after a 25-gram oral dose, as well as plasma xylose levels at 2 hours, were measured by the method of Roe and Rice (1948).

[1] This work was supported in part by Research Agreement No. 196802 between the National Institutes of Health, Bethesda, Md., U.S.A., and the Pakistan-SEATO Cholera Research Laboratory. I am grateful to Mrs. Sheila Wickenden for expert technical assistance; to Dr. Robert S. Gordon, Jr., and Dr. Frederick A. Klipstein for advice and criticism; and to Mrs. Madhabi Ghosh, Mrs. Rekha Sarkar, and other members of the nursing staff for their excellent support.

Proceedings of the Cholera Research Symposium

JANUARY 24–29, 1965
HONOLULU, HAWAII

Convened at the East-West Center of the University of Hawaii. Sponsored by the University of Hawaii's Pacific Biomedical Research Center and the Center for Cultural and Technical Interchange between East and West.

Funded by a contractual arrangement with the University of Hawaii, financed by the National Institutes of Health from funds made available to it by the Agency for International Development for the SEATO Cholera Research Program.

For sale by the Superintendent of Documents, U.S. Government Printing Office
Washington, D.C., 20402 - Price $2.25