Nutritional Studies in Cholera

Influence of Nutritional Status on Susceptibility to Infection

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IT HAS often been suggested that malnutrition enhances susceptibility to infection. Although this hypothesis has been supported by studies of a variety of infections in animals, observations in human subjects have been largely limited to undocumented clinical impressions. For example, clinicians have often observed a high incidence of acute enteric infections in malnourished patients. It has also been suggested that in epidemics of cholera the disease tends to select undernourished members of the population.[1]

In 1963 and 1964 we had an opportunity to study patients with cholera in East Pakistan, where the disease is endemic and data from a recent survey of the nutritional status of the general population[2] were available for comparison. We attempted to determine whether deficiency of any of four nutrients selected for study enhanced susceptibility to cholera.

Ascorbic acid nutriture was investigated since deficiency of this factor is widespread in the population in which cholera exists endemically, and it appeared that the incidence of cholera was at a peak in the spring and late fall, periods roughly coinciding with the seasons of greatest vitamin C deficiency in the population. Although thiamine deficiency is rare in the same population, studies of thiamine metabolism in patients with cholera were undertaken because preliminary clinical observations suggested that high output cardiac failure occurs in the acute phase of cholera.[3] Vitamin B_{12} and folic acid levels were studied since deficiency of one or both of these factors is commonly associated with gastrointestinal disturbances.

MATERIALS AND METHODS

Patients with cholera were studied on the ward of the Pakistan-SEATO Cholera Research Laboratory. A positive diagnosis of cholera was made only when confirmed bacteriologically.

The control group consisted of patients with diarrheal disease from whom no bacterial pathogens were isolated on repeated stool cultures. Blood samples were obtained on admission to the cholera ward for ascorbic acid and erythrocyte transketolase assays. Serum samples were separated and precipitated with trichloracetic acid for subsequent measurement of ascorbic acid content by the dinitrophenylhydrazone method.[4] The trichloracetic acid filtrate was assayed immediately or was stored frozen for later assay. Since the tetracycline preparation used in the therapeutic regimen contained ascorbic acid, only concentrations of ascorbic acid determined on admission, before treatment, are reported.

For the transketolase assay, erythrocytes from

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blood samples obtained on admission and subsequently were separated, washed twice with 0.9 per cent sodium chloride solution, and hemolyzed in distilled water. The hemolysate was assayed for transketolase activity according to the method of Brin et al.[5] This method measures consumption of ribose phosphate by erythrocyte hemolysates with and without added thiamine pyrophosphate. A 20 per cent or greater stimulation of ribose consumption after addition of thiamine pyrophosphate is considered to be evidence of thiamine deficiency.

Serum vitamin B_{12} levels were determined by microbiologic assay with Lactobacillus leichmanii, according to the method of Spray.[6] 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 to 900 mμg. per ml. Serum levels of this vitamin have been shown to correlate well with overt deficiency in a variety of clinical situations, and to become abnormal before tissue stores are exhausted or anemia and symptoms appear.[7]

Serum folic acid levels were measured by microbiologic assay with Lactobacillus casei, according to a modification[8] of the method of Baker et al.[9] Serum folate concentrations have been shown to correlate well with clinical folic acid deficiency.[10,11] In this laboratory the range of normal is from 3.5 to 25 mμg. per ml. Folate concentrations below 3 mμg. per ml. are seen in patients with megaloblastic anemias due to folic acid deficiency.

Urine samples were obtained from asymptomatic subjects as part of an epidemiologic study of the family contacts of cholera patients. Concurrent observations demonstrated that such subjects were exposed to large inoculums of vibrios in food and drinking water.* These subjects were followed by taking daily rectal swabs for culture. Occasionally, in a family contact under surveillance an asymptomatic transient carrier state or true cholera requiring admission to the hospital developed.

After admission, urine specimens were obtained daily from patients who were treated without parenterally administered thiamine and who had not yet been started on oral feedings. In all instances urine samples were acidified to pH 3 and stored under refrigeration 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.[12]

* This prospective study was performed in collaboration with the Epidemiology Section of the Laboratory under the direction of Doctors J. L. Stockard and A. Quader Khan.

TABLE I
Erythrocyte Transketolase Activity on Admission

Case No.	Ribose Consumed (μM/cc./hr.)	Increase with Thiamine Pyrophosphate	% 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	4.8
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	6.3
127	2,640	0	0
131	2,815	0	0
Mean	2,633 ± 114†	...	...
	Patients with Noncholera Diarrhea		
107	2,270	210	9.3
114	2,830	0	0
115	3,050	0	0
118	2,440	260	11.0
122	2,400	190	7.9
125	2,950	180	6.1
126	2,460	120	4.9
128	2,460	70	2.8
Mean	2,608 ± 103†	...	...

* Normal ≤20 per cent stimulation with thiamine pyrophosphate.
† Standard error of mean.

RESULTS

Erythrocyte Transketolase Studies

Twenty-two patients were studied for erythrocyte transketolase activity, fourteen with proved cholera and eight with other diarrheal illnesses (Table I). 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 did not exceed 20 per cent, even on the fourth and seventh days of disease (Table II).

TABLE II
Erythrocyte Transketolase Activity Subsequent to Admission*

Case No.	Day of Disease	Ribose Consumed (μM/cc./hr.)	% Stimulation with Thiamine Pyrophosphate
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,
103	4	3,140	1.4
	7	2,560	5.4

* Cultures positive for V. cholerea.

Urinary Thiamine Excretion before Onset of Cholera

Six patients from whom urine had been collected shortly before the onset of symptoms were admitted with clinical cholera. The patients were under surveillance as household contacts of other patients who had cholera. The data on urinary thiamine excretion are presented in Table III. In no patient was thiamine excretion low enough to indicate deficiency status immediately prior to infection.

Urine samples were also available from subjects whose rectal swabs subsequently grew cultures positive for Vibrio cholerae, but who remained clinically well. A number of these people were studied before, during and after their periods of vibrio excretion. The thiamine excretion values obtained before the first positive culture ranged from low (34 μg. per gm. creatinine) to high (7,200 μg. per gm. creatinine). In only one carrier did urinary thiamine excretion appear to decrease after the appearance of the positive culture; in all other instances, only random variations were observed.

TABLE III
Urinary Thiamine Excretion Before Onset of Cholera

Patient No.	Age (yr.) and Sex	Days Prior to Onset	Urinary Thiamine* (μg./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

* Normal range ≥ 65 μg. per gm. creatinine.

Urinary Thiamine Excretion in Patients with Cholera

The pattern of urinary thiamine excretion was determined in patients hospitalized with cholera. Mean values, graphed in Figure 1, demonstrate the progressive decrease in urinary thiamine during the course of disease. By the fourth day all five patients for whom we have data were excreting thiamine at near negligible levels. This pattern may be the result of starvation alone as shown by Miller and his associates in normal subjects.[13] We cannot assess the effect of the large volumes of feces passed by these patients in depleting the extracellular fluid of thiamine, nor can we

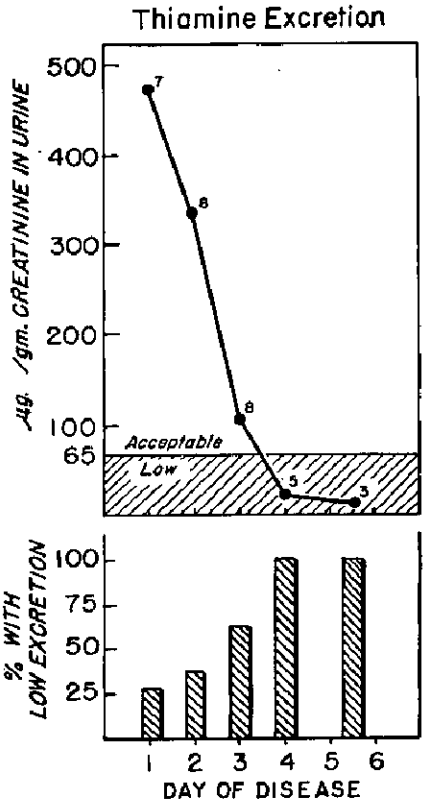

FIG. 1. Decrease in urinary thiamine excretion during the course of cholera. The numbers next to the points in the upper part of the graph repre ent the number of patients studied on that day. The results for days 5 and 6 have been pooled.

exclude with certainty the possibility that the vibrio competes with the host for the available vitamin. It is noteworthy, however, that most studies have failed to demonstrate a requirement for thiamine by vibrios in culture.[1] The transketolase values, which did not decrease even on the seventh day of disease, indicated that functionally significant thiamine deficiency did not develop.

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

Ascorbic Acid

Serum concentrations of ascorbic acid were measured on admission in forty-two patients with cholera and in fifty-five patients with other diarrheal illnesses. Of the forty-two patients with cholera, seven (17 per cent) had initial levels in the deficient range (Table IV). An even larger proportion of patients with noncholera diarrhea had low levels (29 per cent), although the difference did not approach statistical significance. The prevalence of low serum ascorbic acid levels in the general population was found by the nutrition survey to be 26 per cent.[2] Therefore it appears that cholera does not tend specifically to select those members of the population deficient in vitamin C.

TABLE IV
Serum Ascorbic Acid Level

Group	No. of Subjects	% Below 0.2 mg./100 ml.	Mean ± S.E. (mg./100 ml.)
Cholera	42	17	0.85 ± 0.13
Other illness	55	29	0.65 ± 0.08
General population	943	26	0.48 ± 0.02

Serum Vitamin B_{12}

Serum vitamin B_{12} concentrations were assayed in forty-six patients with cholera. The vitamin B_{12} level was subnormal in only two patients, a woman in the third trimester of pregnancy and a twenty year old male subject with malabsorption (Table v). Of the remaining patients, vitamin B_{12} levels were normal in forty-one and elevated in three. In fifteen patients admitted with acute nonspecific enteritides, all vitamin B_{12} levels were within the normal range. The rarity of vitamin B_{12} deficiency in our patient population is consistent with the province-wide nutrition survey in which evidence of vitamin B_{12} deficiency was uncommon.[14]

TABLE V
Serum Vitamin B_{12} Concentration

Group	No. of Subjects	Subnormal* (no.)	Elevated* (no.)	Mean ($\mu\mu$g./ml.)
Cholera	46	2	3	504
Noncholera diarrhea	15	0	0	578

* Normal range 180 to 900 $\mu\mu$g. per ml.

Serum Folate Activity

Serum samples were obtained from seventy-three patients with acute diarrheal disease due to V. cholerae, and from forty-three patients with acute diarrhea from whom pathogenic organisms were not isolated. Samples were taken during the first hospital week, after rehydration had been accomplished, and were assayed for folate activity. The findings are presented in Table VI.

Twelve of seventy-three patients with cholera (16 per cent) and seventeen of forty-three patients with noncholera diarrhea (40 per cent)

TABLE VI
Serum Folate Activity

Group	No. of Subjects	Subnormal* No.	Subnormal* %	Mean Serum Folate (mμg./ml.)
Cholera	73	12	16	7.8
Noncholera diarrhea	43	17	40	9.8

* Normal range 3.5 to 25 mμg. per ml.

had subnormal folate concentrations. The mean folate concentration for the group with cholera was 7.8 mμg. per ml. When the patients with low folate activity were compared with those with normal concentrations, there were no significant differences in age, body weight and hemoglobin and plasma protein levels during convalescence in either the cholera or noncholera groups. Peripheral blood films from twenty-five of the twenty-nine patients with low folate concentrations were reviewed for evidence of folate deficiency (hypersegmented neutrophils with or without macrocytes). In eleven patients these abnormalities were found, although usually they were of mild degree.

There was evidence of folic acid deficiency, then, in only a minority of the patients with cholera. Therefore it is unlikely that either dietary lack or malabsorption of folic acid is a significant factor in the development of cholera in this population.

COMMENTS

"So universal," says Clark in his critical review[15] of the influence of nutrition in experimental infection, "is the belief that deficiencies in diet are important factors in susceptibility to pestilence and so obvious seems the conception that one can improve his well-being and at the same time his resistance to infection by proper eating, that it is difficult to separate fact from fancies." Such is the state of knowledge with respect to cholera where famine and epidemics have always coexisted just as endemic malnutrition coexists today with endemic cholera in the Ganges River basin. In their review of the interactions of nutrition and infection, Scrimshaw and colleagues[16] cite only one study in which the association of diet and cholera has been examined experimentally. This study by Chen and Li in 1930 showed that a vegetarian diet lowered resistance of rats to infection by V. cholerae.

Since comparable studies in man are not feasible, the present study was undertaken to exploit an opportunity to test certain specific, limited hypotheses which might reveal an influence of nutrition on host resistance to infection: (1) Does deficiency of any of the four nutrients under study exist in a high enough percentage of patients with cholera to be considered a necessary or highly predisposing factor? (2) Can one predict, on the basis of information about nutritional status, which subjects exposed to the infectious agent will exhibit the clinical disease, which subjects will carry and shed the organism asymptomatically, and which subjects will reject the organism entirely?

We have been unable to obtain evidence that nutritional status influences host response in the population studied. Deficiencies of thiamine, ascorbic acid, folic acid and vitamin B_{12} are no more prevalent in patients with cholera than in patients with other diseases or in the general population of East Pakistan. Deficiency of none of these nutrients exists in a high enough percentage of patients with cholera to be considered a likely predisposing factor in the disease.

The one prospective aspect of this study was an attempt, using information on thiamine nutrition, to predict the clinical response in persons who were exposed heavily to V. cholerae. Our observations on exposed subjects were limited to urinary excretion of thiamine and subsequent course as determined by daily clinical observation and bacteriologic study of the rectal swab. We failed to demonstrate any value of urinary thiamine excretion which could be used to identify those exposed subjects in whom clinical cholera would develop as opposed to those who would manifest only the carrier state or those who would reject the organism entirely. Subsequent investigations[17] have revealed a satisfactory alternative explanation for the abnormalities of cardiac function that first suggested thiamine deficiency.

Certain limitations of the study are evident. A comparison of the incidence of cholera in a well nourished population and that in a poorly nourished one in the same location would be helpful. However, it is unlikely that factors such as sanitation and previous exposure to antigens of V. cholerae could be shown to be equal in any two such groups. Nutritional factors other than those we have investigated may prove to affect susceptibility to cholera

in man, and other nutrients, individually or in combination, must be studied before final conclusions can be drawn concerning the influence of nutritional status on human susceptibility to cholera.

SUMMARY

The nutritional status of a series of patients admitted to the Pakistan-SEATO Cholera Research Laboratory was studied to evaluate the hypothesis that malnutrition predisposes the exposed subject to infection. These studies demonstrated the following: Thiamine nutriture, as measured by erythrocyte transketolase activity, was normal in patients with cholera and did not differ significantly from that of patients who did not have cholera. Urinary thiamine excretion in subjects exposed to cholera did not indicate thiamine deficiency, nor were such data valuable in predicting the subsequent development of clinical disease. Although urinary excretion of thiamine decreased to nearly negligible levels during the course of cholera, no evidence of the deficiency state was obtained by transketolase assay as late as the seventh day of disease. Serum ascorbic acid concentrations in patients with cholera varied widely. Mean values did not differ significantly from those of patients who did not have cholera or from values obtained for the general population. Serum vitamin B_{12} levels were normal in almost all patients with cholera. Most patients with cholera had normal serum folate levels and the incidence of subnormal levels was less than in a group of patients with nonspecific acute diarrheal illness.

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