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PREFACE

The International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) is an autonomous, international philanthropic and non-profit centre for research, education and training as well as clinical service. The Centre is derived from the Cholera Research Laboratory (CRL). The activities of the institution are to undertake and promote study, research and dissemination of knowledge in diarrhoeal diseases and directly related subjects of nutrition and fertility with a view to develop improved methods of health care and for the prevention and control of diarrhoeal diseases and improvement of public health programmes with special relevance to developing countries. ICDDR,B issues two types of papers: scientific reports and working papers which demonstrate the type of research activity currently in progress at ICDDR,B. The views expressed in these papers are those of authors and do not necessarily represent views of International Centre for Diarrhoeal Disease Research, Bangladesh. They should not be quoted without the permission of the authors.

ABSTRACT

The effect of diarrhoeal illness caused by rotavirus, enterotoxigenic *E. coli* (ETEC) and shigella on coefficients of absorption of nitrogen, fat, calories and carbohydrates were estimated in a group of Children, both in the acute stage and at two and eight weeks after recovery. During the acute stage, absorption of nitrogen was affected in all diarrhoeas being 45, 58 and 41% of the ingested amount for rotavirus, ETEC and shigella respectively. Two weeks after recovery, absorption of nitrogen increased significantly to 69% for rotavirus and 74% for shigella. Absorption of nitrogen for the ETEC group after two weeks was the same as during the acute stage but increased to 73% after eight weeks. Statistically there was no difference in absorption of nitrogen between the three aetiologies eight weeks after recovery. In the acute stage, absorption of fat and calories was comparatively less in rotavirus than in ETEC and shigella. After two weeks, absorption of fat and calories was significantly increased in rotavirus and shigella, and by the eight-week absorption did not vary significantly between the three aetiologies. Absorption of carbohydrate was least affected in any stage of diarrhoea of all aetiologies. In the acute stages, 75% of patients of all aetiologies were xylose malabsorbers compared to only 10% in the recovery period.

INTRODUCTION

Nutritional consequences of diarrhoea are thought to be due to several factors: (a) decreased intake of food resulting from loss of appetite or withholding of food (as practised as a measure to control diarrhoea); (b) loss of major nutrients as well as vitamins and minerals in the faeces due to rapid transit or malabsorption and (c) increased catabolism due to infection.

The main purpose of this presentation is to report our present research on "absorption of macronutrients during and after acute diarrhoeal attacks". To give some information about previous work, first of all, we will summarise the results reported earlier on "loss of enzymatic activity in the gut, secondary to cholera" (1, 2) and then review the work of Chung *et al.* (3, 4) on net absorption of nutrients and the effects of oral feeding and starvation during paediatric diarrhoea.

Two kinds of mucosal enzymes, namely adenosine triphosphatase and disaccharidase in acute cholera and other diarrhoeal disease were studied by Hirschhorn and co-workers (1, 2). The results of ATPase are shown in Table I. The significance of depressed Na-K ATPase in acute diarrhoea is uncertain. Diarrhoea, however, may influence directly the membrane enzyme activity, contributing to the loss of absorptive capacity from the gut. Results on the activity of disaccharidase in acute cholera and other diarrhoeal disease are summarised in Table II. Sucrase, maltase, palatinase activities were low during the acute period. Lactase activity was low during both the acute and convalescent periods compared to healthy North Americans. Enzymatic activities were estimated in serial biopsies on patients. The authors concluded that diarrhoea was probably responsible for the changes in enzymatic activity. However, the significance of the reduction in disaccharidase activities has not been clearly understood. Further research is necessary to correlate these findings with the studies related to nutrient malabsorption in acute diarrhoea.

Net absorption of nutrients in acute diarrhoea was first reported by Chung *et al.* in 1948 (3). The important results are summarised in Tables III and IV. Table III presents the data obtained from a study on four infants who were fed successively three diets; low, medium, and high calorie in the first, second and third 24 hours respectively during the study. Results showed that the absorption of nitrogen and fat was approximately proportional to the amount of intake. Table IV presents the result obtained from a study on two infants treated first with a high calorie diet and then with complete starvation (maintaining a continued I.V. drip). Results showed that during acute diarrhoea, substantial absorption of nitrogen and fat did occur if the food was ingested and no positive absorption occurred during the period of starvation. In a separate report Chung *et al.* (4) studied one hundred and fifteen diarrhoeal patients, fifty-five of whom were starved and sixty fed. They observed that

TABLE I--SODIUM POTASSIUM STIMULATED ADENOSINE TRIPHOSPHATASE OF THE SMALL
INTESTINE OF MAN; STUDIES IN CHOLERA AND OTHER DIARRHOEAL DISEASES

By: Norbert Hirschhorn and Irwin H. Rosenberg, 1968

Results and Comments

- 1) Sodium Potassium stimulated, adenosine Triphosphatase (Na-K ATPase); an enzyme implicated in active sodium transport, was demonstrated in homogenates of human jejunal and ileal mucosa.
- 2) In cholera, acute non-specific gastro-enteritis and bacterial enteritis there was a significant loss of Na-K ATPase activity during the acute phase of the disease compared to the convalescent period.
- 3) Loss of Na-K ATPase in cholera and other enteric disease probably contributes to a worsening of, if not the initiation of, the intestinal fluid loss.

TABLE II--DISACCHARIDASE ACTIVITY IN ACUTE CHOLERA AND OTHER DIARRHOEAL
DISEASES

By: Norbert Hirschhorn and Ayesha Molla, 1969

Results and Comments

- 1) Sucrase, Maltase and Palatinase activities were decreased during acute cholera and enteritis.
- 2) All patients but one had low lactase activity even in convalescence compared to healthy North Americans.
- 3) Serial biopsy studies suggest that diarrhoea and recovery therefrom were responsible for the changes in enzyme activity.

TABLE III--PERCENTAGE OF ABSORPTION OF NITROGEN AND FAT AT DIFFERENT LEVELS OF CALORIE INTAKE DURING ACUTE DIARRHOEA

No. of Cases	Low (30-45)		Moderate (60-69)		High (100-137)	
	N ₂	Fat	N ₂	Fat	N ₂	Fat
12	40	72	50	67	63	60
26	27	48	40	39	74	59
22	38	-27	-	-	60	30
23	-	-	63	70	60	49

Chung *et al.*, 1948.

TABLE IV--PERCENTAGE OF ABSORPTION OF NITROGEN AND FAT DURING A PERIOD OF HIGH CALORIE INTAKE AND STARVATION IN ACUTE DIARRHOEA

No. of Cases	High (100-106)		Starvation	
	N ₂	Fat	N ₂	Fat
27	75	68	-0.30	-2.22
28	65	46	-0.15	-0.51

Chung *et al.*, 1948

the duration of diarrhoea was comparatively longer in the starved group though the statistical difference between the starved and the fed groups was not significant. The observations are listed in Table V.

TABLE V---MEAN OBSERVATIONS ON FIFTY-FIVE DIARRHOEAL PATIENTS TREATED BY STARVATION AND SIXTY BY FEEDING

Observations	Starvation	Feeding
Age (Months)	7.9	7.3
Initial Wt. (Kg)	5.97	5.56
Wt. gain at discharge	Lesser gain	Greater gain
Duration of diarrhoea (days)	7.8	6.1

Chung *et al.*, 1948.

Since the work of Chung *et al.*, no attempt has been made to demonstrate the importance of continuing feeding during acute diarrhoea. Cholera, rotavirus, shigella and ETEC are the major causes of diarrhoeal illness among the under-five population in most developing countries (5, 6). Rotavirus continues to be one of the important causative organisms responsible for childhood diarrhoea even in developed countries (7). The aim of the present study was to determine the effect of diarrhoea caused by rotavirus, shigella and ETEC on net absorption of fat, calories, nitrogen and carbohydrates during acute diarrhoea and two weeks after discharge. The absorption study was repeated eight weeks after discharge to see if there was any difference in the absorption of nutrients between the two stages of recoveries. The absorption data obtained from the above study was analysed to determine if the pattern varies between the three aetiologies. However, before we go into the details of our study we would like to point out an important difference between the study of Chung *et al.* and the present research (Table VI). In this study specific aetiological agents were identified and their relation to nutrient absorption studied, whereas previous work dealt with non-specific diarrhoeas only.

TABLE VI--DIFFERENCES BETWEEN THE STUDY OF CHUNG *ET AL.*, AND THE PRESENT RESEARCH

<u>Chung <i>et al.</i></u>	<u>Molla <i>et al.</i></u>
1. Subjects - Infant, Age 12 - 70 days	Subjects - Children Age 1-4 years
2. Sample size - 6	Sample size - 29
3. Causative organisms - not isolated	Causative organisms - isolated from the stool
4. A liquid formula diet commonly employed for the age group was used	Familiar semi-solid and solid diet was used
5. Three different calorie formulae fed - either low, medium or full	No restriction was applied
6. Study period - 24 hours duration, with each formula diet	Study period - 72 hours duration, with the same food

METHODS AND PATIENTS

Twenty-nine male children below five years of age with a history of acute watery diarrhoea and moderate to severe dehydration were selected at random for the study, those with any obvious complications being excluded. Informed consent was obtained from their parents or guardians. There were 29 patients altogether, 13 of whom had rotavirus infection diagnosed by the enzyme-linked immunosorbent assay, ELISA (8). Ten had *E.coli*: colonies were tested for toxigenicity by infant mouse assay for heat stable toxin (ST) and adrenal cell assay (9) for heat labile toxin (LT). The other six had shigella isolated by standard methods (10). The patients were rehydrated by intravenous route with the Dacca solution* and maintained with the same for 18 to 24 hours.

* Composition: Na^+ 133 mmol/l; K^+ = 13 mmol/l; Cl^- = 99 mmol/l
and HCO_3^- (as acetate) - 48 mmol/l.

After an overnight fast, the patients were fed 5 grams of d-xylose in 100 ml. of water. One hour after ingestion one millilitre of venous blood was drawn to estimate the xylose. Charcoal tablets were fed as markers, the time taken by the charcoal to make its appearance in the stool being taken as the transit time. Four meals were offered daily ad libitum to each patient at regular intervals during the study period. Two types of diet were used: a semi-solid cooked meal containing rice, chicken, lentils, and oil was offered to the patients at lunch and supper. Another cooked meal, consisting of a mixture of rice, egg, milk, butter and sugar was given at breakfast time. Banana, breast-milk, cow's milk and bread were given to the patients at specified intervals during the period of the study. Each item of food was weighed before offering it to the patients and any left over was weighed again to quantify the actual intake. Test weighing was done before and after each breast feeding to measure the amount of milk consumed. Samples from all types of food consumed were saved for analysis of fat, nitrogen and calories. Breastmilk was collected from the mothers, and analysed for calorie, fat and nitrogen. Collection of stools was started after the appearance of the first marker and was continued till the appearance of the second marker fed at 72 hours. The stools collected between markers were pooled and homogenized in a blender. An aliquot from this stool was saved for analyses of fat, nitrogen and calories. Any vomitus collected from the patients during the study was analysed and subtracted. No antibiotics were administered during the study. Patients went home after diarrhoea had stopped. All of the 29 patients were studied two weeks after discharge. Eighteen of them were studied eight weeks after discharge (8 rota, 6 ETEC, 4 shigella). These two recovery stages will be termed respectively R₁ and R₂ in the text.

Serum xylose estimation was done by the Roe and Rice (11) method. Fat was estimated by the Van de Kamer procedure (12). Total nitrogen content was measured according to microkjeldahl procedure (13). Calorie estimation from all samples was performed by using an adiabatic bomb calorimeter, benzoic acid acting as the standard.

Co-efficients of absorption of nutrients at both the acute stage and at recovery were calculated using the following expression: $(\text{Intake} - \text{Output}) \times 100 / \text{Intake}$.

RESULTS

Some physical and clinical features of the study patients are presented in Table VII. Rotavirus patients were comparatively younger than those with ETEC and shigella. Body weight increased between acute and recovery stages 1 and 2 in all groups. All of the patients were anemic.

TABLE VII--CHARACTERISTICS OF THE STUDY PATIENTS (MEAN $\pm$ 1SD)

Particulars	Rotavirus	ETEC	Shigella
No.	13	10	6
Age (months)	23 $\pm$ 14.2	36.3 $\pm$ 10.6	34.1 $\pm$ 8.8
Admission body wt (kg)	8.7 $\pm$ 1.8	9.2 $\pm$ 2	9.4 $\pm$ 1.7
Body wt at recovery 1	9.1 $\pm$ 1.9	9.8 $\pm$ 2	9.9 $\pm$ 2.2
Body wt at recovery 2*	10.2 $\pm$ 2	10.0 $\pm$ 1.8	10.9 $\pm$ 2.4
Serum HCT on admission	31.2 $\pm$ 3.8	32.6 $\pm$ 2.5	33.3 $\pm$ 4.9
1st 24 hr stool volume (litres)	0.70 $\pm$ 0.31	0.68 $\pm$ 0.55	0.93 $\pm$ 0.57

* The number of patients available for the study at stage 2 was 8, 6 and 4 for rotavirus, ETEC and shigella respectively.

The co-efficient of absorption of nitrogen was compared between the three aetiologies of diarrhoea (Table VIII). During the acute stage, absorption of nitrogen was significantly less ($P < .01$) in rotavirus than in ETEC. At recovery 1, absorption of nitrogen did not differ significantly between the three aetiologies. However, even eight weeks after recovery, absorption of nitrogen was comparatively less in the rotavirus group than in the others. Co-efficients of absorption of fat, calories and carbohydrates are shown in Tables IX, X and XI respectively. During the acute stage, absorption of fat and calories was

TABLE VIII--CO-EFFICIENT OF ABSORPTION ($\pm$ 1SD) OF NITROGEN DURING ACUTE (A) DIARRHOEA AND AFTER RECOVERIES (R_1 and R_2)

Aetiology	Co-efficient of Absorption		
	A	R_1	R_2
Rotavirus (13)	43.3 ± 22.3^a	68.5 ± 13.0^b	59.9 ± 28.2^c
ETEC (10)	58.3 ± 13.9^d	54.0 ± 33.3^e	72.8 ± 9.3^f
Shigella (6)	41.3 ± 45.6^g	73.6 ± 4.8^h	72.3 ± 9.7^i

Student's T Test. a vs d : s

Figures in parentheses indicate number of patients.

significantly less ($P < .01$) in rotavirus patients compared to ETEC patients. There was no statistical difference in the absorption of either fat or calories between rotavirus and shigella patients during the acute diarrhoea. In all aetiologies, absorption of carbohydrate was least affected, even during acute diarrhoea (Table XII). In ETEC, absorption of all nutrients was comparatively better in the acute stage compared to recovery stage 1. However, in recovery stage 2, absorption of nutrients in ETEC increased compared to stage 1.

Serum xylose levels were measured during the acute stage and after recovery (Table XII). Patients absorbing less than 20 mg% of xylose were defined as xylose malabsorbers. On an average 75% patients were found to be xylose malabsorbers at the acute stage, which was reduced to 10% at recovery 1. An attempt was made to see the relationship between xylose absorption and the

TABLE IX--CO-EFFICIENT OF ABSORPTION (+1SD) OF FAT DURING ACUTE (A)
DIARRHOEA AND AFTER RECOVERIES (R₁ AND R₂)

Aetiology	Co-efficient of Absorption		
	A	R ₁	R ₂
Rotavirus (13)	42.3 ± 27.6 ^a	79.0 ± 20.0 ^b	81.3 ± 10.1 ^c (8)
ETEC (10)	78 ± 14.2 ^d	80.4 ± 15.3 ^e	88.4 ± 8.3 ^f (6)
Shigella (6)	61.8 ± 34.8 ^g	88.8 ± 3.6 ^h	93.8 ± 2.1 ⁱ (4)

Student's T Test

a vs d : s
c vs i : s

f vs i : s

Figures in parentheses indicate number of patients.

TABLE X--CO-EFFICIENT OF ABSORPTION (+1SD) OF CALORIES DURING ACUTE (A)
DIARRHOEA AND AFTER RECOVERIES (R₁ AND R₂)

Aetiology	Co-efficient of Absorption		
	A	R ₁	R ₂
Rotavirus (13)	55.4 ± 23.7 ^a	91.1 ± 4.6 ^b	81.2 ± 10.1 ^c (8)
ETEC (10)	86.7 ± 7.7 ^d	82.2 ± 10.9 ^e	88.7 ± 6.5 ^f (6)
Shigella (6)	68.1 ± 32.0 ^g	79.7 ± 13.9 ^h	90.4 ± 2.5 ⁱ (4)

Student's T Test

a vs d : s b vs e : s c vs i : s

Figures in parentheses indicate number of patients.

TABLE XI--CO-EFFICIENT OF ABSORPTION (+1SD) OF CARBOHYDRATE DURING ACUTE (A) DIARRHOEA AND AFTER RECOVERIES (R₁ AND R₂)

Aetiology	Co-efficient of Absorption		
	A	R ₁	R ₂
Rotavirus (13)	73.8 ± 26.0 ^a	88.8 ± 5.8 ^b	84.1 ± 11.6 ^c
ETEC (10)	91.7 ± 5.2 ^d	86.8 ± 9.8 ^e	91.0 ± 6.3 ^f
Shigella (6)	77.0 ± 31.7 ^g	78.3 ± 20.8 ^h	91.6 ± 3.7 ⁱ

Student's T Test. a vs d : s

Figures in parentheses indicate number of patients.

TABLE XII--MEAN ONE-HOUR SERUM XYLOSE LEVEL (+1SD) DURING ACUTE (A) AND RECOVERY STAGES (R₁ AND R₂) OF DIARRHOEA DUE TO DIFFERENT AETIOLOGY

Aetiology	Serum Xylose Level (mg%)		
	A	R ₁	R ₂
Rotavirus (13)	13.9 ± 6	26.3 ± 7	33.7 ± 10
ETEC (10)	15.2 ± 6	26.7 ± 8	24.3 ± 5
Shigella (6)	38.6 ± 38	28.6 ± 5	32.1 ± 14

Figures in parentheses indicate number of patients.

absorption of nutrients during acute and recovery stages (R₁ and R₂) of diarrhoea. Figure 1 shows that in rotavirus infection when xylose absorption was poor carbohydrate absorption was as high as 74% even during acute diarrhoea. However, results were not similar for the shigella group. Results in Figure 2 indicate that in shigella, though xylose absorption remained unaffected (39 mg%) in acute diarrhoea, carbohydrate absorption (77%) was not comparatively higher than in rotavirus group (74%).

Stool weight was taken after every 24 hour period during both the acute and recovery stages. Table XIII shows the mean percent change of stool weight in the first and second 24 hour period during acute diarrhoea. A general tendency for stool weight to decrease gradually was noticed between the first and second 24 hour periods at the acute stage of diarrhoea.

TABLE XIII--MEAN PERCENT CHANGE IN STOOL WEIGHT IN THE FIRST AND SECOND 24 HOUR PERIOD DURING ACUTE DIARRHOEA

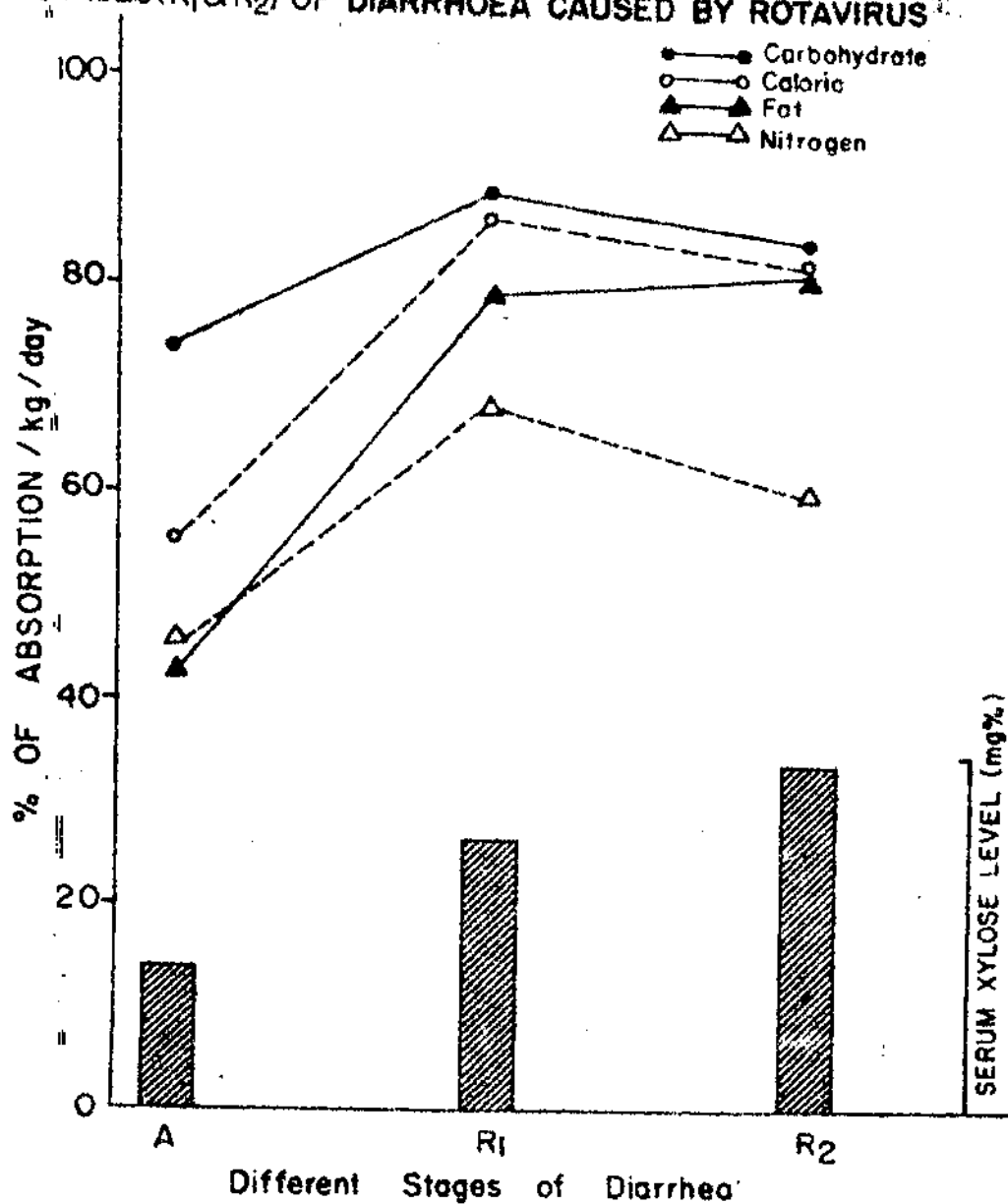
Aetiology	Number	% Change in Stool Wt.	
		1st 24 hour	2nd 24 hour
Rotavirus	13	- 19	- 26
ETEC	10	- 56	- 33
Shigella	6	- 47	- 69

DISCUSSION

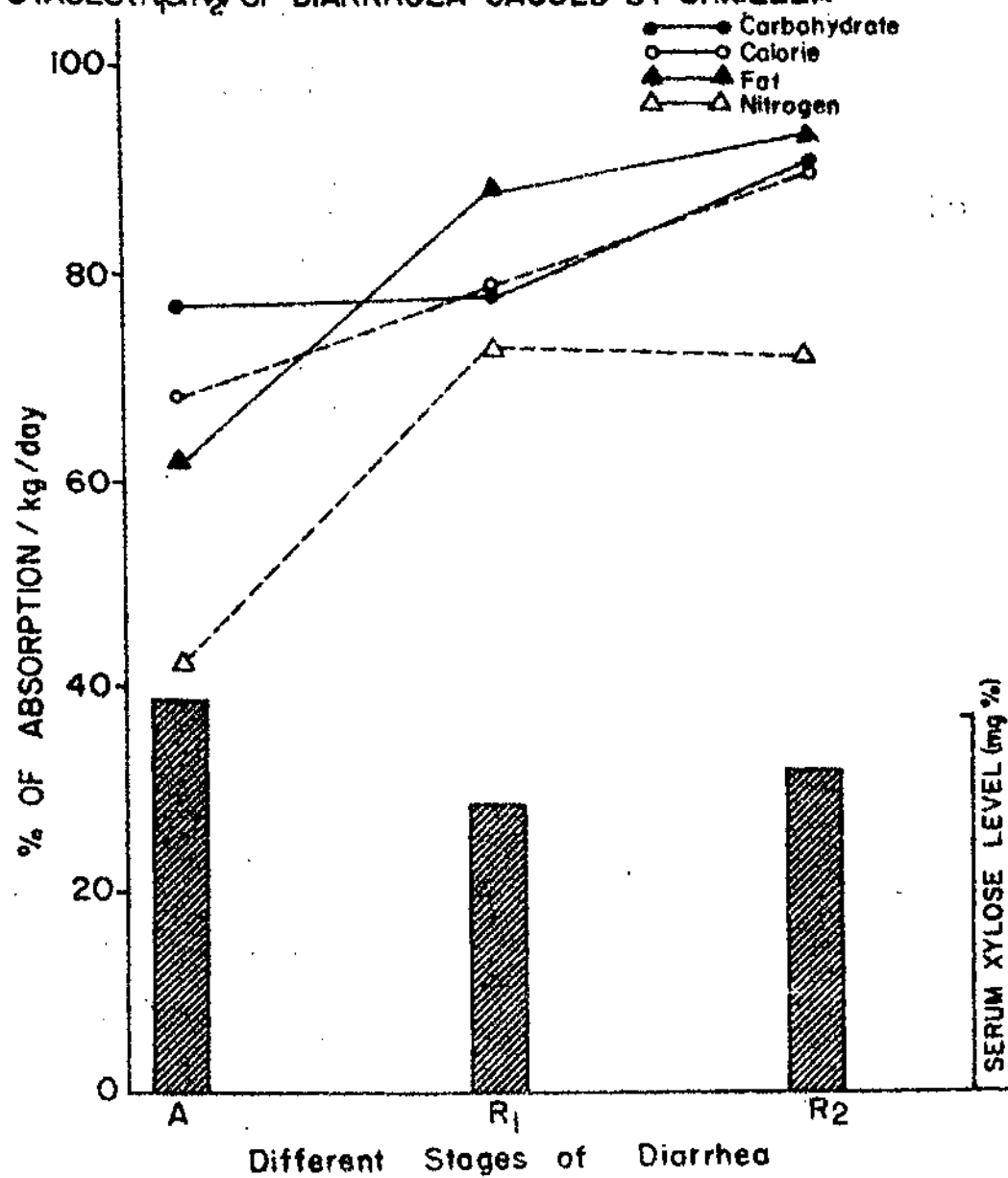
The results of the present research indicate several differences between the aetiologies with regard to absorption of nutrients during acute and recovery stages of diarrhoea.

In the acute stage of rotavirus, absorption of all the nutrients was significantly lower compared to absorption in ETEC diarrhoea. Absorption of nitrogen in rotavirus did not improve even eight weeks after recovery, suggesting the presence of a prolonged malabsorption period. This observation may be explained by the fact that in rotavirus infection villous epithelial cells are specifically affected (14) and the inflammation may continue longer. Previously, it was observed that rotavirus stool contains a large amount of reducing substance

RELATION BETWEEN XYLOSE ABSORPTION AND PERCENTAGE OF NUTRIENT ABSORPTION DURING ACUTE (A) AND RECOVERY STAGES (R₁ & R₂) OF DIARRHOEA CAUSED BY ROTAVIRUS



RELATION BETWEEN XYLOSE ABSORPTION AND PERCENTAGE OF NUTRIENT ABSORPTION DURING ACUTE (A) AND RECOVERY STAGES (R₁ & R₂) OF DIARRHOEA CAUSED BY SHIGELLA



compared to the stool in cholera (15, 16). However, in this study we observed that as much as 74% of the ingested carbohydrate was absorbed in the acute stage compared to 92% absorption in ETEC and 77% in shigella.

During the acute stage of ETEC, absorption of all nutrients was comparatively better than in other aetiologies but failed to show improvement two weeks after recovery. After eight weeks, an increase in absorption of nutrients was clearly evident. The mechanism of mucosal damage by the ST toxin is not yet clearly known. In the present group of ETEC patients, we had four with pure ST, two with pure LT and four with a mixture of ST and LT. The combined presence of ST and LT toxins on the epithelial cells may have affected the absorption of nutrients two weeks after recovery but improved thereafter.

In shigella, during the acute stage, absorption of nitrogen was minimum (41%) compared to the absorption of other nutrients which was in the range of 62-77%. Shigella is a disease of the lower bowel and absorption of nitrogen was probably due to direct loss of protein from the gut rather than failure of intestinal absorption. Absorption of all nutrients was significantly higher two weeks after recovery.

Similarly, the serum xylose level in acute stage of rotavirus and ETEC diarrhoea was below 20 mg% but in shigella it was 37 mg%. Despite the lower xylose level, absorption of carbohydrate was 74% in rotavirus and 92% in ETEC. In shigella during the acute stage it was 77%, though the serum xylose level was much higher. This discrepancy between xylose absorption and carbohydrate absorption may indicate that the absorption status of xylose is not a valid reflection of the absorption of a natural carbohydrate and the other nutrients.

Early feeding of these diarrhoeal patients did not seem to increase the stool volume, which is evident from Table XIII. However, Chung *et al.* (3, 4) showed that in some of their patients feeding during diarrhoea was associated with increase in stool volume though absorption of nutrients was not affected.

Finally, the following conclusions can be derived from this study: Despite reduced absorption of nutrients, feeding of children should be encouraged because substantial absorption does take place in acute diarrhoeal infection. This may help in the prevention of further malnutrition. This observation has profound implications, especially in developing countries where diarrhoea is endemic.

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