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Impact of Zinc Supplementation on Intestinal Permeability in Bangladeshi Children with Acute Diarrhoea and Persistent Diarrhoea Syndrome

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Summary: Zinc has been shown to enhance intestinal mucosal repair in patients suffering from acrodermatitis enteropathica; but the impact on mucosal integrity during acute (AD) or persistent (PD) diarrhoea is unknown. One hundred eleven children with AD and 190 with PD aged between 3 and 24 months received, randomly and blind to the investigators, either an elemental zinc supplement of 5 mg/kg body wt/day or placebo in multivitamin syrup for 2 weeks while intestinal permeability and, biochemical and anthropometric markers were serially monitored. The permeability test was administered as an oral dose of 5 g lactulose/l g mannitol in a 20-ml solution followed by a 5-h urine collection. The ratio of the urinary probe sugars was correlated to clinical, biochemical, and microbiological parameters. At presentation, lactulose excretion was increased and mannitol excretion decreased in both AD and PD as compared with age-matched asymptomatic children. The lactulose/mannitol ratio (L/M) was higher in subjects with mucosal invasive pathogens (rotavirus and enteropathogenic *Escherichia coli*) compared with children excreting *Vibrio cholera* and enterotoxigenic *E. coli*. Two-week zinc supplementation significantly reduced lactulose excretion in both AD and PD, whereas the change in mannitol excretion and L/M was similar between study groups in both studies. Changes in lactulose excretion were significantly influenced by zinc supplementation in children with *E. coli, Shigella* sp., and *Campylobacter jejuni* stool isolates. The greatest reduction in total lactulose excretion was seen in supplemented children who on presentation were lighter (wt/age <80%), thinner (wt/ht <85%), and undernourished [middle upper arm circumference (MUAC) <12.5 cm] or with hypozincaemia (<14 μmol/L). The results suggest zinc supplementation improves intestinal permeability in certain groups of children with AD or PD syndrome and contributes to their recovery. This effect may indirectly reflect enhanced mucosal recovery. Further studies on the mechanisms of mucosal repair following zinc supplementation are recommended. Key Words: Zinc—Intestinal permeability—Acute diarrhoea—Persistent diarrhoea—Lactulose—Mannitol—Bangladesh.

Diarrhoea is one of the most common causes of morbidity and mortality of children in the developing world (1), and in Bangladesh occurs on the average at the rate of two to three attacks per child per year (2,3). Eight to 20% of acute episodes continue and are then classified as persistent diarrhoea (PD) (>14 days) (4). Childhood malnutrition, especially stunting and thinness, may predispose to further attacks of diarrhoea (5–8). PD may then impair nutritional status, creating a vicious cycle of diarrhoea and malnutrition (1,9).

The mechanisms by which diarrhoeal pathogens initiate disease are well described. Subtotal villous atrophy has in part been considered as a determinant of the chronicity of PD syndrome (PDS) (10). Mucosal damage interferes with nutrient absorption, may cause loss of fluids and nutrients, and

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may alter the child's appetite. Some children appear to be unable to repair the intestinal mucosa and have significant histological abnormalities in mucosal morphology (11). Intestinal permeability has been validated as a useful proxy indicator of mucosal damage in children with diarrhoea by small bowel biopsy studies (12–14). Small intestinal morphology may be significantly altered in malnutrition (15,16). Increased intestinal permeability during acute and persistent diarrhoea has been described in studies from the developing and the developed countries (12,13,17–19). The use of ratios of probe markers as indicators of mucosal damage has been accepted as an indirect technique for monitoring intestinal mucosal changes (18). This allows the impact of micronutrient supplements on intestinal morphology and function to be studied indirectly. Significant clinical improvements of diarrhoea in patients with acrodermatitis enteropathica (AE) following treatment with zinc (20) prompted studies on the interrelation of zinc to intestinal mucosal turnover and function. Ultrastructural changes in mucosal morphology of zinc-deficient animals (21,22) have been recorded that respond rapidly to replacement therapy, enhancing enterocyte regeneration and repair of the ultrastructural abnormalities seen in AE patients (23). In the zinc-deficient animal model, zinc supplementation increases enterocyte size and epitheleal growth and improves abnormalities of the intracellular tight junction (24–27). The clinical impact of zinc supplement was studied in two double-blind randomised supplementation studies against placebo in Bangladeshi children presenting with acute diarrhoea (AD) and PD. The aims of the study included determining whether zinc supplementation altered serial measurements of intestinal permeability to lactulose and mannitol and the association between microbial pathogens isolated in faeces and intestinal permeability.

MATERIALS AND METHODS

Clinical Definition

Any child between 3 and 24 months presenting to the International Centre for Diarrhoeal Disease Research, Bangladesh, (ICDDRB) with diarrhoea was recruited in a double-blind fashion by block randomisation to either the PD cohort, where diarrhoea had lasted for >14 days, or to the AD cohort, for acute watery diarrhoea of <3-day duration. The AD cohort was further divided into well nourished children of >75% wt/age and malnourished children of <75% wt/age of the (NCHS) standard. Recovery from illness was defined as passage of a semiformed or normal stool in an afebrile child accepting food.

The controlled clinical trials of zinc supplementation were conducted on 111 children presenting to ICDDRB with AD and 190 children with PD. Dehydration was corrected within 4 h after admission. All patients received daily a multivitamin syrup (vitamin A 3,000 IU, D 600 IU, B_1 1.2 mg, B_6 0.6 mg, riboflavin 2.0 mg, nicotinamide 6.0 mg, and calcium D-pantothenate 6.0 mg in 5 ml) in three divided doses. In each study, half of all participants were blindly assigned to receive the above syrup incorporating zinc acetate providing 15 mg/kg wt/day zinc acetate (3.9 mg elemental Zn mg/kg/day) as a daily dose for the following 14 days. The taste of the two preparations was comparable. The dose supplement was selected to replenish and replace Zn losses during diarrhoea but not disturb other body trace elements including copper and selenium. The study was approved by ethical committees of the ICCDRB and of the London School of Hygiene and Tropical Medicine. Dietary management of PDS children was standardised on a chicken-based diet containing zinc (2.2 mg/L), while the AD cohort received a diet of milk and cereal containing 1.4 mg of zinc per liter.

Permeability Tests

Intestinal permeability was assessed using the disaccharide probe lactulose and the monosaccharide mannitol. A freshly prepared solution with 7.5 ml of Duphalac (Duphar Laboratories) containing 5 g of lactulose and 1 g mannitol was made up to 20 ml with 1% chloroform water. Mothers were supervised as they fed the solution to the baby. Fluid intake, especially breast-feeding, was encouraged for the next 5 h. The perineum was cleaned and dried, and an adhesive paediatric urine collection bag containing a drop of 20% (vol/vol) chlorohexidine gluconate to prevent bacterial degradation of the probes was attached. A complete urine collection over the next 5-h period was made. Whenever urine was obtained, the bag was replaced to reduce spillage; complete collection was obtained in all cases. All urine collections for permeability tests were observed by hospital staff. The permeability test was performed on the day of admission (day 1),

after 1 week (day 8), and at the end of multivitamin supplementation (day 15). Aliquots of urine were collected and stored at −70°C for analysis as a batch. Urinary lactulose and mannitol were analysed using an automated enzyme assay (28,29). Recovery of urinary sugars was estimated as percentage of the initial dose and expressed as a ratio of lactulose to mannitol (L/M). Ten subjects from the PD cohort did not have paired samples, so data presented are for 180 subjects with serial investigations. The percentages of subjects tested on day 15 were in the AD cohort 70% placebo and 68% supplemented and in the PD cohort 75% placebo and 78% supplemented. Serum zinc was analysed from samples collected in a standardised fashion after rehydration on an Atomic Absorption Spectrophotometer (Pye Unicam SP9), while serum vitamin A was estimated using an HPLC technique (Waters 510). Baseline samples were collected after rehydration and before any supplement was administered. Microbiological studies were performed according to the WHO manual (30) including a rotavirus enzyme-linked immunosorbent assay test (31) and stool microscopy for helminths and protozoa. Children were weighed unclothed on admission, after full rehydration, and then every morning between 9 a.m. and 10 a.m. using a weighing scale with a sensitivity of 20 g (Toledo). Their supine lengths were measured with a locally made standardised length board with a sensitivity of 1 mm on admission and every week after their discharge. Mid–upper arm circumference was taken at the midpoint of the hanging left arm by TALC (Teaching Aids at Low Cost) tape, with a sensitivity of 2 mm, on admission and weekly thereafter. All anthropometric instruments were checked and calibrated daily. The weight and height were compared with the NCHS standards.

Statistical Methods

The code for the zinc recipients was broken after analysis. The lactulose and mannitol results were log-transformed before statistical analysis, tested using a Student test, and the anti-log expressed as the geometric mean with 95% confidence interval. Analysis on untransformed data of the change in excretion of markers was undertaken using the Mann-Whitney *U* test, testing for differences between groups. Paired *t* test was used to examine the change over time of anthropometric, biochemical, and permeability data. Analysis of variance was used on stratified or subgroups of data. Statistical significance was accepted at the 5% probability level.

RESULTS

The anthropometric, biochemical, and clinical characteristics of the children at admission are shown in Table 1. Placebo and zinc groups examined after rehydration after admission had comparable anthropometric and biochemical markers in both AD and PD studies. The children presenting with AD were older and more malnourished in comparison with children presenting with PD. Mean serum zinc levels on entry to the studies were at the lower end of the normal range (12–20 μmol/L) and vitamin A levels were well below the internationally accepted normal range (>20 μg/dl).

Clinical Response

Figure 1 shows the pattern of urinary excretion of lactulose, mannitol, and the L/M from the day of admission in children with AD and PD and compares the results with those obtained in well-

TABLE 1. *Cohort description of placebo and zinc-supplemented groups (means ± SD).*

	Acute diarrhoea		Persistent diarrhoea	
	Placebo (n = 54)	Zinc (n = 57)	Placebo (n = 90)	Zinc (n = 90)
Wt/age % median	67 ± 6	67 ± 7	72 ± 12	70 ± 11
Ht/age % median	92 ± 3	91 ± 3	94 ± 4	94 ± 5
Wt/Ht % median	81 ± 7	82 ± 6	82 ± 10	83 ± 8
MUAC (cm)	11.5 ± 1	11.3 ± 1	11.6 ± 1	11.6 ± 1
Age (mos)	11 ± 4	11 ± 4	8 ± 3	8 ± 3
Diarrhoea (days)	2.7 ± 0.7	2.8 ± 1.0	21 ± 10	24 ± 11
Serum Zn (μmol/L)	12.3 ± 4	11.5 ± 3	13.2 ± 5	13.4 ± 5
Serum vitamin A (μg/dl)	14 ± 7.4	15 ± 7.4	21.9 ± 12.9	23.3 ± 12.4

MUAC, middle upper arm circumference.

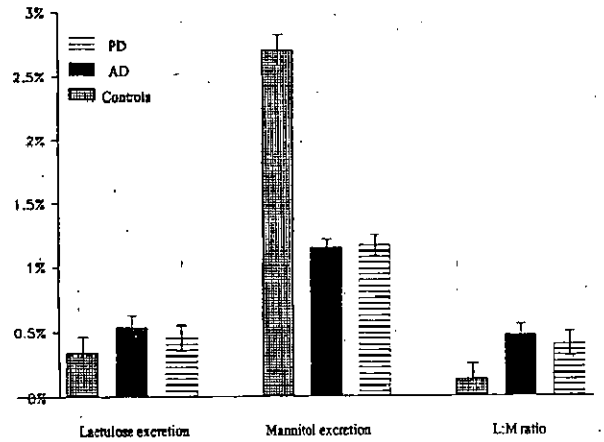

FIG. 1. The mean ± SEM of 5-h urinary lactulose, mannitol, and the ratio of lactulose and mannitol (L:M) in all children with persistent (PD) and acute (AD) diarrhoea at presentation and a control group of 53 children asymptomatic when tested.

nourished Bangladeshi children (>80% weight/age) (mean age 8.2 ± 3.8 months, n = 53; male/female = 30/23). At presentation no differences in probe markers between the two study groups were noted, but levels of all markers were different from those in the healthy control subjects who had a lower mean lactulose (0.35 ± 2.9%) 5-h excretion, giving a significantly lower L/M of 0.13 to the diarrhoea study groups. When grouped according to enteropathogens isolated from the stool, 33% of AD patients and 45% of PDS patients had no pathogens isolated. Permeability was significantly different between groups stratified by enteric isolates (Table 2), with the highest lactulose excretion and L/M found in patients where no pathogens, rotavirus, or enteropathogenic *Escherichia coli* were identified. A similar pattern was also seen in the AD study, where a higher pathogen isolation rate was obtained. The lactulose and L/M values were nearly half those found in children with invasive pathogens. No significant differences in excretion of mannitol were seen in the groups excreting different fecal pathogens.

In the AD study, the total 5-h lactulose excretion at 2 weeks had decreased significantly, but only in the supplemented children (Table 3), while mannitol excretion progressively and significantly increased in both groups. Significant reductions in L/M occurred in both groups by the first week, remaining until day 15. The change was attributable mainly to the two to threefold increase in total mannitol excretion.

In the PD study, the significant reduction in lactulose excretion observed at day 7 (Table 3) in the supplemented children was lost by day 15 when the two groups had similar lactulose excretion. Manni-

TABLE 2. *Intestinal permeability in patients grouped by stool pathogen isolates at admission (geometric mean with 95% confidence interval)*

Pathogen	Lactulose (% dose)	Mannitol (% dose)	Lactulose/mannitol ratio
Acute diarrhoea			
Rotavirus (n = 50)	0.55[a] (0.48–0.87)	0.91 (0.66–1.21)	0.70[a] (0.53–0.92)
EPEC (n = 15)	0.63[a] (0.33–1.08)	1.49 (0.93–2.10)	0.45[a] (0.28–0.72)
Unidentified (n = 68)	0.70[a] (0.55–0.88)	1.30 (1.1–1.55)	0.53 (0.39–0.63)
Cholera + ETEC (n = 12)	0.27 (0.17–0.40)	1.10 (0.70–1.67)	0.24 (0.10–0.27)
Shigella + *C. jejuni* (n = 15)	0.24 (0.12–0.48)	1.10 (1.0–2.55)	0.15 (0.10–0.27)
Persistent diarrhoea			
Rotavirus (n = 10)	0.60 (0.33–1.10)	0.91 (0.72–1.67)	0.70 (0.31–1.16)
EPEC (n = 31)	0.50 (0.34–0.58)	1.00 (0.71–1.42)	0.49 (0.29–0.80)
Unidentified (n = 76)	0.46 (0.24–0.65)	1.23 (0.82–1.94)	0.37 (0.28–0.48)
ETEC (n = 23)	0.48 (0.24–0.65)	1.26 (0.82–1.94)	0.28 (0.18–0.43)
Controls (n = 53)	0.34 (0.26–0.30)	2.67 (2.0–3.50)	0.13 (0.1–0.16)

[a] $p < 0.05$ by analysis of variance of rotavirus enteropathogenic *E. coli* (EPEC) and unidentified against enterotoxigenic *E. coli* (ETEC) and *Shigella* + *C. jejuni*.

TABLE 3. *Change of intestinal permeability in diarrhoea patients (geometric mean with 95% confidence interval)*

	Day 1	Day 8	Day 15
Acute diarrhoea			
Lactulose			
Placebo (n = 34)	0.46 (0.35–0.60)	0.36 (0.29–0.44)	0.36 (0.27–0.47)
Zinc (n = 35)	0.48 (0.36–0.57)	0.35 (0.28–0.43)	0.28[a] (0.23–0.34)
Mannitol			
Placebo (n = 38)	0.96 (0.80–1.15)	1.90[a] (1.54–2.34)	2.84[a] (2.35–3.43)
Zinc (n = 37)	1.21 (0.99–1.47)	1.93[a] (1.50–2.48)	2.20[a] (1.79–2.7)
Lactulose/mannitol			
Placebo (n = 34)	0.50 (0.38–0.66)	0.18[a] (0.14–0.23)	0.13[a] (0.10–0.18)
Zinc (n = 35)	0.41 (0.33–0.51)	0.19[a] (0.14–0.26)	0.13[a] (0.10–0.16)
Persistent diarrhoea			
Lactulose			
Placebo (n = 72)	0.42 (0.33–0.53)	0.33 (0.26–0.42)	0.42 (0.33–0.54)
Zinc (n = 79)	0.46 (0.35–0.59)	0.25[a] (0.19–0.33)	0.41 (0.32–0.52)
Mannitol			
Placebo (n = 71)	1.21 (0.97–1.49)	2.40[a] (2.00–2.85)	2.96[a] (2.50–3.40)
Zinc (n = 74)	1.18 (0.93–1.49)	2.00[a] (1.67–2.40)	2.40[a] (1.90–2.90)
Lactulose/mannitol			
Placebo (n = 71)	0.35 (0.26–0.46)	0.14[a] (0.10–0.18)	0.15[a] (0.12–0.19)
Zinc (n = 74)	0.39 (0.19–0.85)	0.13[a] (0.09–0.17)	0.18[a] (0.14–0.23)

[a] $p < 0.05$ by analysis of variance, day 1 to day 8/day 15.

tol excretion showed a similar progressive increase ($p < 0.05$) as seen in the AD study. The most noticeable reduction of total lactulose excretion was seen in supplemented children between day 1 and 7, in whom no stool pathogens were recovered or where enteropathogenic *E. coli* or *Shigella* sp. was isolated. A reduction in lactulose excretion was also noted in the placebo group excreting enterotoxigenic *E. coli* (Table 4).

In children with PDS who were <80% wt/age (30% of the cohort) or <85% wt/ht (37.9% of the cohort), had MUAC of <12.5 cm (76.3% of the cohort), or had zinc levels at presentation of <14 μmol/L (36.8% of the cohort), supplementation was associated with significant reduction ($p < 0.5$) in total lactulose excretion by the first week.

DISCUSSION

Measurements of intestinal permeability have been used as a proxy indicator of intestinal damage in children with diarrhoea and (18,19) Crohn's and coeliac disease (32). The excretion of lactulose and mannitol has been validated in children with diarrhoea and malnutrition and correlates with abnormalities of small intestinal morphology (12,33). Children with AD and PDS in the present study had significantly higher lactulose and lower mannitol excretion compared with healthy Bangladeshi controls, a finding consistent with other controlled studies of permeability during diarrhoea (13,18). The L/M values were lower than those described in Gambian children with diarrhoea or U.K. children studied with small intestinal pathology (0.40 vs. 1.3 and 1.16) (12,13). The osmolarity of oral dosing solution and concentration of probes are recognised as having a considerable effect on probe kinetics and absorption; therefore, as different dosing solution recipes have been used in the other studies, comparisons of ratios are invalid. Thirty-three percent of AD children and 43% of PD children in our study had no identified stool pathogens; yet, their urinary lactulose and L/M were significantly higher than in children with noninvasive organisms in their stool. This suggests the presence of mucosal damage.

More detailed virological studies may have highlighted the presence of other pathogens such as adenovirus in the "negative" group. Children from both studies excreting rotavirus and enteropathogenic *E. coli* had higher urinary lactulose excretion than children excreting enterotoxigenic *E. coli* (ETEC) or *V. cholera* at admission. This difference suggests that different organisms, depending on their mode of pathology, have a differing impact on enterocyte function. Zinc supplement appeared to have its greatest impact in both studies, as reflected by lactulose excretion, in those children excreting enteropathogenic *E. coli* or in children in whom no pathogens were isolated from stool. Zinc supplementation appeared to reduce lactulose excretion within the first week in PD and by the second week in the AD group. A consistent fall in lactulose excretion was noted in the supplemented AD group (observed only in the first week of the PD group) over 2 weeks of observation. This did not occur in the placebo group.

There was in both studies no difference in mannitol excretion between the pathogen groups. Mannitol absorption is thought to reflect the available mucosal surface area, which may not be functionally altered during an episode of diarrhoea. Nutritional status of all groups was similar although, sur-

TABLE 4. *Impact of zinc supplementation on 5-h lactulose excretion (% oral dose) by enteropathogen (geometric mean and 95% confidence interval)*

Pathogen	Placebo			Zinc		
	n	Day 1	Day 8	n	Day 1	Day 8
Persistent diarrhoea						
Unidentified	33	0.40 (0.29–0.55)	0.39 (0.27–0.55)	35	0.39 (0.27–0.56)	0.2[a] (0.12–0.31)
EPEC	13	0.41 (0.21–0.78)	0.30 (0.15–0.59)	11	0.57 (0.30–1.09)	0.20[a] (0.10–0.38)
Rotavirus	7	0.64 (0.34–1.21)	0.30 (0.11–0.80)	5	0.54 (0.07–0.55)	0.40 (0.17–1.06)
ETEC	13	0.41 (0.24–0.69)	0.21[a] (0.14–0.32)	11	0.38 (0.16–0.8)	0.30 (0.15–0.86)
Shigella and *C. jejuni*	6	0.14 (0.06–0.30)	0.57 (0.41–0.79)	3	0.69 (0.25–1.89)	0.140[a] (0.07–0.26)
Acute diarrhoea						
Unidentified	16	0.60 (0.36–0.98)	0.45 (0.38–0.64)	18	0.7 (0.45–1.08)	0.34[a] (0.22–0.39)
EPEC	2	0.55 (0.24–2.9)	0.51 (0.33–0.74)	2	0.24 (0.30–1.09)	0.08[a] (0.10–0.38)
Rotavirus	6	0.63 (0.30–1.19)	0.21 (0.10–0.36)	12	0.35 (0.19–0.63)	0.30 (0.17–0.53)
ETEC	2	0.10 (0.08–0.11)	0.21 (0.05–0.74)	4	0.3 (0.20–0.44)	0.43 (0.15–0.18)
Shigella and *C. jejuni*	6	0.67 (0.28–1.26)	0.60 (0.43–0.80)		—	—

[a] $p < 0.05$ by analysis of variance, day 1 vs. day 8/day 15.

prisingly, children with AD had a greater weight/age deficit at presentation than did the children with PD; they were, however, on average 3 months older. Malnutrition alone increased the L/M in Gambian children (13); therefore, the higher L/M in the AD group may be rationalised by the group's lower nutritional status and higher prevalence of rotavirus diarrhoea. No attempt was made to assess biochemical zinc status in the groups as it was felt that current laboratory methods for estimating body zinc status were not adequately sensitive. The supplemented group was considered to have received enough zinc by supplement to separate them into zinc-replete and zinc-deficient groups. Children with diarrhoea have been reported to have transient depression of serum zinc levels during diarrhoea (34–37). The impact of zinc supplementation on mucosal cell integrity, especially on the "tight" junction, may be one explanation of the apparent improvement of the barrier function (decreased lactulose excretion) of the mucosa. In experimental studies of severe zinc deficiency (4 μg Zn/kg diet), histological observations have revealed abnormal cell membranes, disruption of desmosomes, increased paracellular space, reduced ribosomes, and degenerate mitochondria (24). Following a 48-h zinc supplementation, repair of many intracellular ultrastructures occurred with increases in size of the enterocytes and repair of intercellular tight junctions. Simultaneous improvements in water and sodium absorption were also noted.

An unexpected rise of lactulose excretion in the second week in children with PD was not easily explained, but may have been subsequent to dietary changes following hospital discharge preceding the 15-day test. The fall in L/M was inversely related to changing pattern of mannitol excretion, and the pattern of increasing mannitol absorption with time was similar in both zinc and placebo groups. The rapid increase in urinary mannitol with recovery from diarrhoea has been reported previously (12,19). The improvement in mannitol excretion may relate to reduced small intestinal transit time, improved function, or regeneration of mucosal surface area.

The most profound reduction of lactulose excretion was observed in the severely malnourished children and those with low serum zinc levels at presentation who received zinc supplement. Hitherto it has not been clear whether zinc status and abnormal mucosal function are interrelated, but the impact of a zinc supplement on improving mucosal permeation of lactulose in malnourished children and those excreting enteropathogenic organisms

suggests an important role for zinc in mucosal regeneration and enhancement of mucosal function.

In summary, the study shows that during diarrhoea, children excreting "mucosal invasive pathogens" had a higher mucosal permeability than children excreting noninvasive organisms. Significant lowering of lactulose excretion after a 2-week zinc supplementation was observed in malnourished children or children presenting with low serum zinc and in children with PD excreting enteroinvasive organisms. This suggests a useful role for zinc in debilitated children with diarrhoea associated with certain enteric pathogens. Further studies are recommended to examine the exact mechanism of action of zinc on intestinal permeability and the relation of permeability to the mucosal lesion in different types of diarrhoea and in children with varied nutritional status.

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REFERENCES

1. Scrimshaw NS, Taylor CE, Gordon JE. Effect of infection on nutritional status. In: Scrimshaw N, ed. *Interaction of nutrition and infection* Monograph Series No. 57. Geneva: WHO, 1968:57.
2. Black RE, Brown KH, Becker S, Alim ARMA, Hug I. Longitudinal studies of infectious diseases and physical growth of children in rural Bangladesh. II. Incidence of diarrhoea and association with known pathogens. *Am J Clin Epidemiol* 1982;115:315–24.
3. Snyder JD, Merson MH. The magnitude of the global problem of diarrhoeal disease: a review of active surveillance data. *Bull WHO* 1982;60:605–13.
4. World Health Organisation. Persistent diarrhoea in children in developing countries: memorandum from a WHO meeting. *Bull WHO* 1988;66:709–17.
5. Rowland MGM, Cole TJ, Whitehead RG. A quantitative study into the role of infection in determining nutritional status in Gambian village children. *Br J Nutr* 1977;37:441–50.
6. Tomkins A. Nutritional status and severity of diarrhoea among preschool children in rural Nigeria. *Lancet* 1981;1:860–2.
7. Tomkins AM, Dunn DT, Hayes RJ. Nutritional status and risk of morbidity among young Gambian children allowing for social and environmental factors. *Trans Roy Soc Trop Med Hyg* 1989;83:282–7.
8. Koster FT, Palmer DL, Chakravarty J, Jackson T, Curlin GT. Cellular immune competence and diarrhoeal morbidity in malnourished Bangladeshi children: a prospective field study. *Am J Clin Nutr* 1987;46:115–20.
9. Mata LJ, Kronmal RA, Urrutia JJ, Gracia B. Effect of infection on food intake and the nutritional state: as viewed from the village. *Am J Clin Nutr* 1977;30:1215–27.
10. Lebenthal E. Prolonged small intestinal injury as a primary cause of intractable diarrhoea. In: Lebenthal E, ed. *Chronic diarrhoea in children*. New York: Raven Press, 1984:5–30.
11. Sullivan PB, Marsh MN, Mirakian R, Hill SM, Milla PJ, Neale G. Chronic diarrhea and malnutrition—histology of the small intestinal lesion. *J Pediatr Gastroenterol Nutr* 1991;12:195–203.
12. Akinbami FO, Brown GA, McNeish AS. Intestinal permeability as a measure of small intestinal mucosal integrity: correlation with jejunal biopsy. *Afr J Med Sci* 1989;18:187–92.
13. Behrens RH, Lunn PG, Northrop CA, Hanlon PW, Neale G. Factors affecting the integrity of the intestinal mucosae of Gambian children. *Am J Clin Nutr* 1987;45:1433–41.
14. Chandra RK, Pawa RR, Ghai O. Sugar intolerance in malnourished infants and children. *Br Med J* 1968;4:611–3.
15. Brunser O, Reid A, Monckeberg F, et al. Jejunal mucosa in malnutrition. *Am J Clin Nutr* 1968;21:976–83.
16. Burman D. Jejunal mucosa in kwashiorkor. *Arch Dis Child* 1965;40:526.
17. Menzies IS. Transmucosal passage of inert molecules in health and disease. In: Skadhauge E, Heintze K, eds. *Intestinal absorption and secretion*. England: MTP Press, 1984:527–42.
18. Ford RPK, Menzies IS, Phillips AD, Walker-Smith JA, Turner MW. Intestinal sugar permeability: relationship to diaarhoeal disease and small bowel morphology. *J Pediatr Gastroenterol Nutr* 1985;4:568–74.
19. Weaver LT, Chapman PD, Madeley CR, Laker MF, Nelson R. Intestinal permeability changes and excretion of microorganisms in stools of infants with diarrhoea and vomiting. *Arch Dis Child* 1985;60:326–32.
20. Moynahan EJ. Acrodermatitis enteropathica: a lethal inherited human zinc deficiency disorder. *Lancet* 1974;2:399–400.
21. Elmes ME, Jones JG. Ultrastructural changes in the small intestine of zinc deficient rats. *J Pathol* 1980;130:37–43.
22. Koo SI, Turk DE. Effect of zinc deficiency on the the ultrastructures of the pancreatic acinar cell and intestinal epithelium in rat. *J Nutr* 1977;107:896–908.
23. Kelly R, Davidson GP, Townley RRW, Campbell PE. Reversible intestinal mucosal abnormality in acrodermatitis enteropathica. *Arch Dis Child* 1976;51:219–22.
24. Roy SK, Drasar BS, Tomkins AM. The impact of zinc deficiency on intestinal response to cholera toxin. *Proc Nutr Soc* 1986;45:39A.
25. Hallbook T, Lanner E. Serum zinc and healing of leg ulcers. *Lancet* 1972;2:780–2.
26. Chvapil M. Effect of zinc on cell and bio-membrane. *Med Clin North Am* 1976;60:799–812.
27. Golden MHN, Golden BE, Jackson AA. Skin breakdown in kwashiorkor responds to zinc [Letter]. *Lancet* 1980;1:1256.
28. Northrop CA, Lunn PG, Behrens RH. Automated enzymatic assays for the determination of intestinal permeability probes in urine. 1. Lactulose and lactose. *Clin Chim Acta* 1990;187:79–88.
29. Lunn PG, Northrup CA, Northrup AJ. Automated enzymatic assays for the determination of intestinal permeability

probes in urine. 2. Mannitol. *Clin Chim Acta* 1989;183:163–70.
30. WHO. Manual for laboratory investigations of acute enteric infections. *Programme for control of diarrhoeal disease* CDD/83.3 rev. 1. Geneva: WHO, 1987.
31. Yolken RH. Enzyme linked immunosorbent assay (ELISA) for detection of human retrovirus-like agents of infantile gastroenteritis. *Lancet* 1977;2:263–7.
32. Pearson AD, Eastham EJ, Laker MF, Craft AW, Nelson R. Intestinal permeability in children with Crohn's disease and coeliac disease. *Br Med J* 1982;285:20–1.
33. Nathavitharana KA, Lloyd DR, Raafat F, Brown GA, McNeish AS. Urinary mannitol:lactulose excretion ratios and jejunal mucosal structure. *Arch Dis Child* 1988;63:1054–9.
34. Sarker SA, Rahaman MM, Ali A, Hossain S, Alam AN. Prolonged depression of serum zinc concentrations in children following post measles diarrhoea. *Hum Nutr Clin Nutr* 1986;39:411–7.
35. Rothbaum RJ, Maur PR, Farrell VK. Serum alkaline phosphatase and zinc undernutrition in infants with chronic diarrhoea. *Am J Clin Nutr* 1982;35:595–8.
36. Naveh Y, Lightman A, Zinder O. Effect of diarrhoea on serum zinc concentrations in infants and children. *J Pediatr* 1979;101:730–2.