Intestinal Dipeptide Hydrolase Activities in Undernourished Children*

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Kumar, V., Ghai, O. P., and Chase, H. P. (1971). *Archives of Disease in Childhood*, 46, 801. **Intestinal dipeptide hydrolase activities in undernourished children.** Intestinal dipeptide hydrolase enzymes cleave dipeptides into amino acids. In 8 undernourished children with diarrhoea, mean intestinal mucosal glycyl-proline hydrolase activity was reduced 40%, valyl-proline hydrolase activity 38%, and alkaline phosphatase activity 54%, as compared with 4 controls. The use of hydrolysed proteins or simple amino acid mixtures in the initial treatment of undernourished children is suggested.

Undernutrition is the most common disease to affect the health of children in the world. Diarrhoea is a frequent complication of undernutrition (Wharton, Howells, and Phillips, 1968) and often prevents effective rehabilitation. Causes of diarrhoea in poorly nourished children include carbohydrate malabsorption (Bowie, Brinkman, and Hansen, 1965), intestinal infections, and parasitic infestations. Exclusion of disaccharides from the diet is sometimes helpful (Prinsloo *et al.*, 1969), but frequently the cause of diarrhoea in undernourished children cannot be determined.

Nutritional deprivation in childhood has been shown in microscopical changes in the microvillous membrane of the small intestine (Stanfield, Hutt, and Tunnicliffe, 1965) and in alterations of carbohydrate (Bowie *et al.*, 1965) and lipid absorption (Dutra de Oliveira and Rolando, 1964). Dipeptide hydrolase enzymes are located in the intestinal microvillous membrane in a similar location to disaccharidases (Lindberg and Karlsson, 1970), and are believed to be responsible for splitting dipeptide molecules into component amino acids (Peters, 1970) for absorption and utilization in the body. As disaccharidase activities are known to be diminished in malnourished children (Bowie,

Barbezat, and Hansen, 1967) and dipeptide hydrolase activity is reduced in undernourished animals (Solimano, Burgess, and Levin, 1967), it is likely that dipeptide hydrolase activity is also reduced in undernourished children. Malabsorption of dipeptides might then be responsible for intractable diarrhoea and growth failure in some undernourished children.

In this study the intestinal dipeptide hydrolase activities for valyl-proline and glycyl-proline [iminopeptide hydrolases (EC.3.4.3.7)] (International Union of Biochemistry, Standing Committee on Enzymes, 1965) as well as the activity of alkaline phosphatase have been determined in 8 undernourished children and 4 controls.

Material and Methods

Eight undernourished children with diarrhoea, 6 months to 7 years of age were studied in the paediatric wards of the All India Institute of Medical Sciences in New Delhi, and the PBM Hospital in Bikaner, India. All 8 children had diarrhoea ranging in duration from 15 days to 2 years (Table 1). The heights and weights were below the third centile in all patients (Ghai and Sandhu, 1968) and head circumferences were low in 7 of the 8 patients. Cases 1, 2, and 3 are classified as having marasmic kwashiorkor because the body weights of these children were below 60% of the 50th centile for age (*Lancet*, 1970). These 3 patients all had hepatomegaly, pitting oedema, total serum proteins between 3.9 and 5.0 g/100 ml and serum albumins from 1.8 to 2.6 g/100 ml. Hair changes characteristic of protein deprivation were present in 2 of the 3 children. The other 5 undernourished children were classified as marasmic because of body weight less than 60% of the

Received 11 May 1971.

*This work was supported in part by Grant Number NS 07537-04 from the National Institute of Health, Bethesda, Maryland, U.S.A.

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‡Dr. Chase is a recipient of a Career Research Development Award from the National Institute of Child Health and Human Development, K4-HD-38, 839-03.

TABLE I
Clinical Features of 8 Undernourished Children Studied

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8
Diagnosis*	MK	MK	MK	M	M	M	M	M
Age (yr)	3	3	7	6/12	8/12	5	1	4
Sex	M	F	F	M	F	M	F	F
Duration of diarrhoea (mth)	0.5	0.5	24	1	1.5	18	1	6
Length (cm)	67	74	84	58	56	75	60	65
Weight (kg)	7.0	6.5	9.5	4.0	4.0	9.0	4.5	5.5
Oedema	Present	Present	Present	Nil	Nil	Nil	Nil	Nil
Liver size (cm)†	6	3	4	2	2	6	Not palpable	3
Tongue papillae	Atrophy	Atrophy	Hypertrophy	Normal	Normal	Hypertrophy	Hypertrophy	Atrophy
Wrinkled conjunctiva	Nil	Nil	Nil	Nil	Nil	Present	Present	Present
Hb (g/100 ml)	6.5	9.8	8.6	9.9	8.6	10.0	7.8	9.8

*MK, marasmic kwashiorkor; M, marasmus (*Lancet*, 1970).

†The number of cm the liver was palpable below the right costal margin in the right nipple line.

Note: Case numbers on this table correspond to Case numbers in Table II.

50th centile (*Lancet*, 1970). These 5 children also had gross wasting of muscles and loss of subcutaneous fat. Oedema was not present. 2 of the children classified as having marasmus (Cases 6 and 8) had hair changes suggestive of protein malnutrition. All 8 patients had microcytic-hypochromic anaemia, with haemoglobins ranging from 6 to 10 g/100 ml. Clinical signs of vitamin A deficiency were present in at least 4 children: Case 2 had lost her vision after keratomalacia, Case 3 had Bitot's spots, Cases 6, 7, and 8 had wrinkled dry conjunctiva, and Cases 3, 6, and 7 had hypertrophied tongue papillae. Rickets was present in Case 3. No evidence of protein, calorie, iron, or vitamin deficiencies was seen in any of the control patients.

A Carey capsule (Carey, 1964) was passed in the fasting state in the control and undernourished subjects. Controls were selected as a part of another study to determine normal intestinal disaccharidase activities. The position of the capsule was confirmed to be in the region of the ligament of Treitz by fluoroscopy, and a suction biopsy of the mucosa obtained. The biopsy specimens for enzyme analyses were frozen and kept in this state until analysis.

The jejunal biopsy specimens were weighed and homogenized in a 1:30 dilution of 0.15 M sodium chloride for both the valyl-proline and glycyl-proline hydrolase assays. Activities for dipeptide hydrolases were measured by the method of Josefsson and Lindberg (1965). The dipeptides were obtained from Schwarz/Mann Research Laboratories, Orangeburg, New York, 10962, and purity was verified by paper chromatography.

Alkaline phosphatase assays were performed by the method of Bessey, Lowry, and Brock (1946) using a 1:180 dilution of 0.15 M sodium chloride.

Protein was measured by the method of Lowry *et al.* (1951).

Result

Intestinal dipeptide hydrolase activities for valyl-proline and glycyl-proline in 8 undernourished children and 4 controls are shown in Table II.

The dipeptide hydrolase activities for both dipeptides were significantly reduced ($P < 0.05$ for valyl-proline hydrolase and $P < 0.02$ for glycyl-proline hydrolase) in the biopsies from the undernourished children compared to the controls. No significant

TABLE II
Dipeptide Hydrolase and Alkaline Phosphatase Activities in Jejunal Biopsies of Malnourished and Control Children

	Jejunal Enzyme Activities (units/mg protein)		
	Glycyl-proline*	Valyl-proline*	Alkaline Phosphatase†
Controls			
A	2.5	3.8	8.6
B	3.3	3.9	7.4
C	2.3	4.5	10.6
D	2.9	6.4	13.1
Mean and range	2.7 (2.3-3.3)	4.7 (3.8-6.4)	9.90 (7.4-13.1)
Case No.			
Marasmic kwashiorkor			
1	2.3	2.5	3.7
2	1.2	2.9	6.7
3	1.4	2.4	3.0
Marasmus			
4	0.9	2.3	8.2
5	2.8	3.7	7.1
6	1.3	2.7	2.4
7	1.8	3.2	3.0
8	1.4	3.2	2.7
Mean and range	1.6 (0.9-2.8)‡	2.9 (2.3-3.7)§	4.6 (2.4-8.2)¶

*A unit of iminopeptide (glycyl-proline or valyl-proline) hydrolase activity is the amount of enzyme required to catalyse the cleavage of one μ mole substrate in 15 minutes under standard assay conditions.

†A unit of alkaline phosphatase is that amount of enzyme required to catalyse the release of one μ mole of p-nitrophenol in 30 minutes under the standard assay conditions.

‡ $P < 0.02$ § $P < 0.05$ ¶ $P < 0.02$. Significance was determined by Student *t* test.

differences in the activities of glycyl-proline and valyl-proline hydrolases were found in Cases 1 to 3 with marasmic kwashiorkor compared to Cases 4 to 8 with marasmus. Intestinal mucosal alkaline phosphatase activity was also significantly reduced ($P < 0.02$) in the 8 undernourished patients compared to the 4 controls (Table II). There was no significant difference between the mean activity of alkaline phosphatase in children with marasmic kwashiorkor compared to children with marasmus (Table II).

The ratio of intestinal mucosal protein to biopsy wet weight was not different between the test and control patients. It thus appears valid to express enzyme activity as units of activity per mg protein.

Light microscopy of intestinal biopsies was available in Cases 2, 5, and 6. These patients showed subtotal villous atrophy and cellular infiltrations in the lamina propria. In other test patients inadequate mucosa was obtained to do light microscopical studies. Light microscopy was normal in all 4 controls.

Discussion

Intestinal mucosal dipeptide hydrolase and alkaline phosphatase activities were significantly diminished in undernourished children as compared to the controls (Table II). Intestinal mucosal dipeptide hydrolase and alkaline phosphatase activities have been shown to be reduced in patients with coeliac disease (Berg *et al.*, 1970) and in patients with mucosal villous atrophy (Heizer and Laster, 1969). No mention was made of the nutritional status of these patients. As a result of the present finding of reduced dipeptide hydrolase activity in undernourished children, it is important to exclude undernutrition as a responsible factor in the reduction of dipeptide hydrolase activities in patients with mucosal villous disease.

No differences were found in the degree of reduction of dipeptide hydrolase or alkaline phosphatase activities in the 3 children with marasmic kwashiorkor as compared to the 5 children with marasmus (Table II). The diminution of dipeptide hydrolase and alkaline phosphatase activities was also not apparently related to the duration of diarrhoea or to the age of the child. Thus the activity of valyl-proline hydrolase was as low in Case 1 who had diarrhoea for only 15 days as in Case 3 who had diarrhoea for 2 years. With regard to age, Case 4, 6 months old, had the lowest level of valyl-proline hydrolase activity and Case 5, 8 months old, the highest level of activity. It has previously been shown that the level of dipeptide hydrolase activity is as high in the human fetus as in the adult (Lindberg, 1966).

A previous study has shown a decreased number of goblet cells in the intestinal mucosa and decreased intestinal protein synthesis by membrane-bound polyribosomes in vitamin A deficient rats (De Luca, Little, and Wolf, 1969). The levels of dipeptide hydrolase activities in the children of this study, however, did not appear to be related to deficiencies of vitamin A or any other vitamins. Thus Case 2, having keratomalacia as well as the most severe vitamin A deficiency, had higher activity of glycyl-proline hydrolase than did Case 4 with no clinical evidence of vitamin A deficiency. Likewise, Case 3 with vitamin D deficient rickets had higher glycyl-proline hydrolase activity than did Cases 2 or 4 who did not have rickets. Though the number of patients in this study is small, it is of interest that Cases 3, 6, 7, and 8 all showed tongue or eye changes suggestive of vitamin A deficiency and had the lowest levels of alkaline phosphatase activity. Case 3 who had rickets had a similar level of mucosal alkaline phosphatase activity to Cases 6 and 7 who did not have clinical evidence of rickets.

Children with iron deficiency anaemia have been shown to have structural and functional alterations of the small bowel mucosa (Guha *et al.*, 1968). As all of the children in this study had hypochromic microcytic anaemia indicative of iron deficiency anaemia, it is impossible to evaluate the relation between anaemia and depression of dipeptide hydrolase activities. There was, however, no correlation between the degree of anaemia and the activities of dipeptide hydrolase or of alkaline phosphatase.

The mechanism of diminished dipeptide hydrolase activity is unknown. A possible explanation might be a generalized decrease in intestinal protein enzyme synthesis secondary to undernutrition. Studies on rats have also shown that undernutrition results in decreased dipeptide hydrolase activity (Solimano *et al.*, 1967); however, undernutrition in the rat does not decrease intestinal protein synthesis (Hirschfield and Kern, 1969). It is possible that the mechanism of decreased dipeptide hydrolase activity is related to intestinal mucosal damage. The depth of damage of the human intestinal mucosa may be important in determining which enzymes for hydrolysis of disaccharides or dipeptides are reduced.

Little is known about protein absorption and the consequences of protein malabsorption. Reduction in dipeptide hydrolase activity would likely produce malabsorption of dipeptides which could then lead to diarrhoea, either secondary to osmotic absorption of large quantities of fluid in the gut

lumen, or to toxic effects of the unabsorbed dipeptides. Likewise, with decreased intestinal dipeptide hydrolase activities, dipeptides might be absorbed into the blood. It is conceivable that dipeptides absorbed into the blood might have a toxic effect on the brain. An encephalopathy has been described after the feeding of high protein diet to undernourished children (Balmer, Howells, and Wharton, 1968), and the mechanism of the encephalopathy is at present poorly understood. Reduced dipeptide hydrolase activity might also result in decreased availability of amino acids and growth retardation.

Intestinal mucosal alkaline phosphatase activity was reduced by a mean of 54% in the undernourished children compared to the controls. The function of intestinal mucosal alkaline phosphatase is unknown but is believed to be related to hydrolysis of phosphorus-containing compounds. Activity has also been suggested to be related to carbohydrate absorption in some species (Moog and Ortiz, 1960). Alkaline phosphatase activity has previously been shown to be decreased in patients with intestinal villous atrophy (Heizer and Laster, 1969).

It is conceivable that high protein feedings instituted for rehabilitation of undernutrition may aggravate diarrhoea in some undernourished children and result in a delay in recovery. Studies have not as yet compared the effectiveness of protein hydrolysates or amino acid solutions to whole milk protein. Pretorius and De Villiers (1961) added protein hydrolysate or casein to skimmed milk and noted no differences in recovery during the first 12 days of rehabilitation from kwashiorkor. Whole milk protein formulae at the present time are usually used in the therapy of undernourished children. Further trial of protein hydrolysates or of simple amino acid mixtures, particularly during rehabilitation from undernutrition, now seems indicated.

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