Intestinal enterokinase deficiency

Occurrence in two sibs and age dependency of clinical expression

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Haworth, J. C., Hadorn, B., Gourley, B., Prasad, A., and Troesch, V. (1975). *Archives of Disease in Childhood,* **50,** 277. **Intestinal enterokinase deficiency: occurrence in two sibs and age dependency of clinical expression.** Intestinal enterokinase deficiency in 2 sibs is described. A boy failed to gain weight and had vomiting, diarrhoea, oedema, hypoproteinaemia, and anaemia in early infancy. His duodenal juice contained very low or absent proteolytic enzyme activity, which increased markedly after addition of enterokinase. He was treated with pancreatic extract and gained weight rapidly. At 44 months of age he is normal, apart from some developmental delay, and no longer needs pancreatic extract. His older sister, who had had similar symptoms in early infancy but then grew normally, had the same abnormality in her duodenal juice when seen at 4 years of age. Enterokinase activity was virtually absent in the duodenal mucosa of both patients. Mucosal morphology was normal. The findings suggest that enterokinase deficiency is an inherited congenital defect and not the result of mucosal damage. Affected patients may show spontaneous improvement and normal growth after the age of 6 to 12 months. This phenomenon may be related to the decreasing growth velocity during the first 2 years of life and the concomitant decrease in protein requirements per unit bodyweight.

Enterokinase (enteropeptidase 3.4.4.8), an enzyme of the small intestinal mucosa, is responsible for the conversion of trypsinogen to active trypsin. Once trypsin is present in duodenal contents it activates other proteolytic pancreatic enzymes (chymotrypsinogens, procarboxypeptidase). Because of the key role of enterokinase in this process deficiency of this enzyme results in severe disturbance of protein digestion. The first case of enterokinase deficiency was described 5 years ago (Hadorn *et al.*, 1969). Since that time, 3 further cases have been reported (Tarlow *et al.*, 1970; Polonovski *et al.*, 1970; Hadorn, Bier, and Polonovski, 1970; Haworth *et al.*, 1971). We now report 2 additional cases in sibs in whom clinical features differ somewhat from the observations made in the previously published cases.

Case reports

Case 1. A boy was born after a 34-week pregnancy: birthweight 2·41 kg. He was a twin; the other twin

died of respiratory distress. The parents, who were of Polish, Austrian, and Spanish extraction and not related, were healthy and free of gastrointestinal symptoms. The infant was fed with a proprietory formula (Enfalac), but he vomited and did not gain weight. Diarrhoea began at 6 weeks of age and because of this the formula was changed to half-skimmed (2 % fat) milk.

He was first admitted to this hospital when 9 weeks old. He weighed 2·68 kg (Fig. 1) and had pallor, abdominal distension, and oedema of the face and legs. Hb 5·5 g/dl; reticulocytes 7·6%; erythrocyte morphology and white blood count normal; total serum proteins 2·7 g/100 ml. Because he fed poorly and vomited frequently the formula was changed to a soya bean preparation (Mull-Soy). He was given intravenous blood and albumin and discharged home. He was readmitted at 3 months of age because of continued vomiting, diarrhoea, and weight loss. He was now wasted, dehydrated, and had generalized oedema; weight 2·3 kg, Hb 8·8 g/dl; total serum proteins 3·2 g/100 ml, albumin 1·9, globulins 1·3 g/100 ml; sweat chloride concentration 28 mEq/l; x-rays of the chest and upper gastrointestinal tract normal. He was rehydrated and fed a protein hydrolysate (Nutramigen) which he vomited less, but diarrhoea continued. There was no
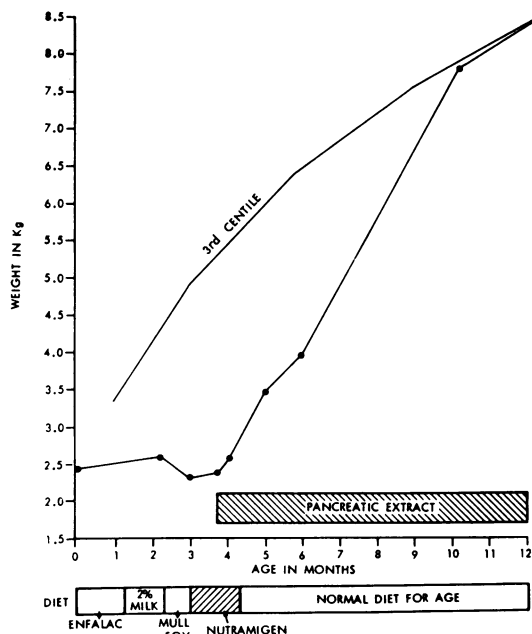
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weight gain and at 4 months he was still anaemic and hypoproteinaemic. Exocrine pancreatic deficiency was now suspected and pancreatic extract was added to the Nutramigen. He developed conjugate deviation of the eyes, nystagmus, a high pitched cry, and convulsions. During these episodes he had hypoglycaemia and hypocalcaemia; oedema increased and urinary volume fell to low levels. He was given albumin, blood, calcium gluconate, glucose, and mannitol intravenously. White blood count was now 21 000/mm³, platelets 8000/mm³. He was treated for sepsis with antibiotics, and intravenous protein hydrolysate was given to improve his nutrition.

After a month he was better and was investigated further. Sweat chloride concentration was again normal; a 3-day fat balance study showed a mean daily faecal fat excretion of 12 g (36% of dietary fat intake); after the intravenous injection of ^{51}Cr trichloride, only 0·2% of the label was excreted in the stool (normal <0·5%), indicating no increased loss of protein from the gut. Biopsy of the proximal duodenum showed normal histology (Fig. 2), and electron microscopy was also normal (Fig. 3), but disaccharidase activity was very low. Results of a pancreatic function test suggested that he had intestinal enterokinase deficiency (see below). He was now offered a regular diet with pancreatic extract, and gained weight rapidly (Fig. 1). He was discharged home after more than 2 months
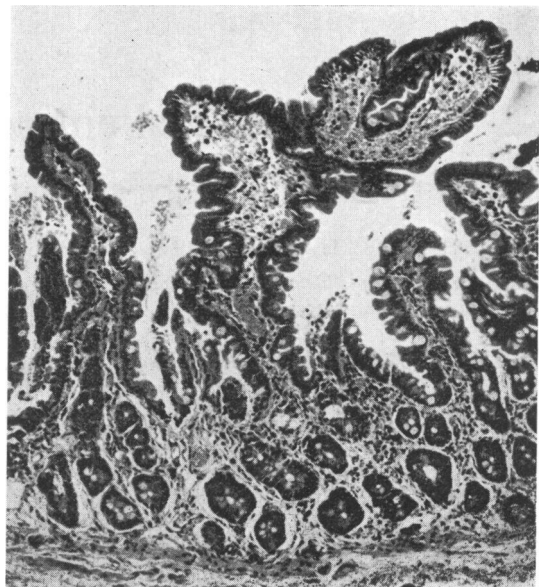
in hospital, weighing 3·95 kg; total serum protein 5·9 g/100 ml.

He continued to do well, and at 10 months he was reinvestigated. He looked well nourished and there were no physical abnormalities; weight 7·8 kg (3rd centile); height 68 cm (<3rd centile); total serum proteins 5·3 g/100 ml; mean daily faecal fat excretion 1·8 g (6% of dietary fat intake). Biopsy of the proximal duodenum was histologically normal but no lactase activity was detected. Results of the pancreatic function study are described below. At 16 months of age he was still well; weight 10·1 kg (10th centile); height 75 cm (<3rd centile); total serum proteins 5·3 g/100 ml, albumin 3·2, globulin 2·1 g/100 ml. Normal histology of the duodenal mucosa was again shown (Fig. 2); mucosal disaccharidase activity was now also normal. The defect in activation of the pancreatic proteolytic enzymes persisted.

His mother stopped the pancreatic extract when he was 22 months old because he disliked it and was refusing food. In spite of this he remained well and continued to grow; at 27 months his height and weight were about the 10th centile. He was last seen at the age of 44 months when he was eating a normal diet and having one formed stool a day; weight 16·4 kg, height 98·2 cm (both 25th to 50th centile), head circumference 52·5 cm (50th centile). There was, however, evidence of developmental delay; general performance was at the 30- to 36-month level and language development at about 24 months.

Case 2. The older sister of Case 1 was born after a 36-week gestation; weight 2·84 kg. Because of
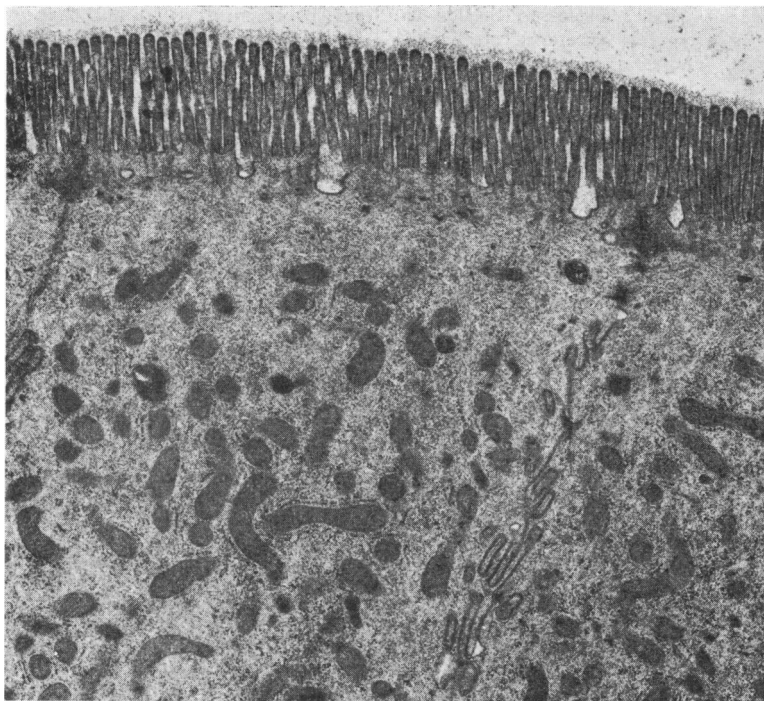
FIG. 3.—*Case 1. Electron photomicrograph of the luminal surface of two epithelial cells at the summit of a mucosal villus. The microvilli are normally orientated and covered with a thin layer of 'fluff'. The mitochondria are normal. (×12 672.)*

projectile vomiting she was admitted to this hospital at 2 weeks. There was no diarrhoea. Weight was 2·64 kg; she was mildly dehydrated but examination was otherwise normal. Hb 17 g/dl; white blood count 19 900/mm³ (normal differential); barium meal examination normal. She was rehydrated and seemed better.

She was fed cow's milk at home but soon developed diarrhoea; stools were large, loose, yellow, and foul-smelling. She was readmitted to hospital at one month of age. She now weighed only 2·92 kg and was mildly dehydrated and pale; Hb 6·6 g/dl; reticulocytes 15% and there was evidence of haemolysis. Bone marrow showed increased erythropoiesis; erythrocyte enzyme studies and fragility tests were normal; Coombs's test negative; hydrogen peroxide haemolysis test abnormal. Vitamin E deficiency was thought likely, and she was given a blood transfusion and vitamin E and folic acid by mouth. Total serum proteins after transfusion were 4·3 g/100 ml, albumin 3·1, globulin 1·2 g/100 ml. Diarrhoea persisted and intestinal malabsorption or milk allergy was suspected. Sweat chloride concentration was normal. She was then fed Nutramigen; the diarrhoea ceased and she began to gain weight. At 5 months she was fed a normal milk-containing diet and remained well. Because of the defect in her brother she was investigated when 3 years 11 months. She had been well and symptom-free in the interval. Her weight and

height were at the 50th centile; Hb 12·5 g/dl total serum proteins 7·1 g/100 ml. A duodenal biopsy showed normal mucosa and disaccharidase activity. The results of a pancreatic function study are given below.

She was last seen at 6½ years of age when she was completely well and symptom free; height and weight were at the 50th centile.

Methods

The technique of duodenal intubation and the methods used to measure the enzymes in the duodenal juice have been described (Haworth *et al.*, 1971). To investigate zymogen activation, 0·4 ml duodenal juice was mixed with 0·05 mol/l Na-citrate buffer at pH 5·6. This was incubated at 25 °C and trypsin, chymotrypsin, and carboxypeptidase activities were measured. Subsequently 50 μl enterokinase* solution (containing 100 EKU/ml†) were added to the juice. Proteolytic activities were again measured after 50 minutes' incubation at 25 °C. Preliminary studies showed that under these conditions at 50 minutes a plateau activity was

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*Porcine purified enterokinase obtained from Opochimie, BP 69, Monte Carlo, Monaco.
†1 EKU = enterokinase unit, corresponding to that enzymatic activity which liberates 1 μg trypsin/min.

reached which was not further increased by longer incubation. For measurements of enterokinase and disaccharidase activity, duodenal mucosa was homogenized with 50 parts of its volume of distilled water. Enterokinase activity was measured by incubating 20 μl mucosal homogenate with 100 μl bovine crystalline trypsinogen (2 mg/ml 0·001 mol/l HCl), 100 μl of 0·05 mol/l Na-citrate buffer (pH 5·6), 20 μl 100 mmol/l Natrium-glycodeoxycholate, and 0·51 ml distilled water. Glycodeoxycholate was added because it has a strong activating effect on the enzyme (Steiner et $al.$, 1972; Hadorn et $al.$, 1974). The amount of trypsin formed per unit time was measured by the spectrophotometric method of Hummel (1959). The disaccharidases were measured by the method of Dahlqvist (1964).

Results

Table I shows pH values and pancreatic enzyme activities in the duodenal contents of the 2 patients. For proteolytic enzymes, activities before and after addition of enterokinase are given. From Case 1 three samples were collected at different times. Sample CI and PI (5½ months) showed no trypsin or chymotrypsin activity. CI was collected without stimulation; PI was collected 10 minutes after stimulation of pancreatic function with pancreozymin (2 units/kg body weight I.V.).

In CI, lipase and amylase activity were present at a low level. Sample CII, collected at 10 months, showed very low trypsin and absent carboxypeptidase activity. Lipase and amylase were not measured. Sample CIII (16 months) showed low trypsin and chymotrypsin activities, carboxypeptidase was normal, lipase and amylase activities also normal. In samples CII and CIII a marked increase of

trypsin, chymotrypsin, and carboxypeptidase activity was observed after addition of enterokinase to the juice (zymogen activation test positive).

From Case 2 one unstimulated sample was collected at 3 years 11 months. It showed very low trpysin, absent chymotrypsin and carboxypeptidase activity, and normal lipase and amylase. Proteolytic enzymes increased markedly after the adddition of enterokinase (Table I). Table II shows enterokinase and disaccharidase activities in the duodenal biopsies of Case 1 at 16 months, Case 2, and their mother. Enterokinase activity was not measurable in the patients but disaccharidase activities were normal. The mother had enterokinase activity of 270 EKU/mg mucosa which, according to our limited experience in adults, is normal. The mother had an abnormally low lactase, but in view of normal sucrase and maltase activity, this was probably a case of isolated adult lactase deficiency.

Discussion

The clinical and biochemical findings in the 2 sibs were similar to those described in the earlier reported cases, but in the present cases vomiting was the first symptom and diarrhoea began later. Severe anaemia was also a prominent early feature; there was evidence of haemolysis, and vitamin E deficiency was suspected in the second case.

In this disorder, diarrhoea, failure to thrive, and hypoproteinaemia are readily explainable by the defect in protein digestion. During the early phase of the disease when patients suffer from hypoproteinaemia, they also show more generalized

TABLE I

pH values and pancreatic enzyme activities in duodenal contents of the two patients

Patients and nature of sample	Age at collection (m)	pH	Trypsin† (μg/ml)		Chymotrypsin† (μg/ml)		Carboxypeptidase† (IU × 10³/ml)		Lipase (IU/ml)	Amylase (IU/ml)
			Before E	After E	Before E	After E	Before E	After E		
Case 1										
CI		8·8	0·0	—	0·0	—	—	—	32	8
PI	5½		0·0	—	0·0	—	—	—	—	—
CII	10	7·0	6·0	1400	—	—	0·0	150	—	—
CIII	16	7·0	24·0	1700	14·0	550	40·0	200	1336	98
Case 2										
C	3y 11m	8·0	8·0	1100	0·0	720	0·0	500	550	58
Normal values*		>6·5	>45·0		>28·0		>20·0		>80	>35 (from 15 m)

*Lowest values observed in 50 children without pancreatic disease (unstimulated duodenal juice).
†Before E, activity before addition of enterokinase; after E, activity after addition of enterokinase.
C, without stimulation; P, after pancreozymin stimulation.

TABLE II

Enterokinase and disaccharidase activity in duodenal biopsies of Case 1 at 16 months, Case 2 at 3 years 11 months, and their mother

	Age	Enterokinase EKU*/g fresh weight	Disaccharidases IU†/g fresh weight		
			Lactase	Sucrase	Maltase
Case 1	16m	<0·01	4·2	6·4	30
Case 2	3y 11m	<0·01	4·7	8·4	27
Normal‡ values (children)		>135	>2·0	>1·0	>6·0
Mother		270	0·7	6·5	24
Normal§ values (adults)		>100	>1·1	>5·6	>21

*1 EKU = 1 μg trypsin formed/min.
†IU, international units = μmol substrate split/min.
‡Lowest values observed in 15 (enterokinase) and 19 (disaccharidases) children with normal mucosal histology.
§Lowest values observed in 6 adults with normal mucosal histology.

malabsorption. Most of them have steatorrhoea, levels of lipase and amylase are low in the duodenal juice, and disaccharidase activities of the duodenal mucosa are also low. It is likely that these changes are secondary to the protein malnutrition. Similar changes are found in kwashiorkor (Barbezat and Hansen, 1968; Bowie, Brinkman and Hansen, 1965; Prinsloo *et al.*, 1969). The possibility that entero-kinase deficiency in the present cases might also have been secondary to severe primary protein malnutri-tion was excluded by the demonstration that, with adequate therapy, disaccharidase, lipase, and amy-lase activities became normal, though enterokinase deficiency persisted (Tables I and II). Vitamin E deficiency could have been due to the secondary pancreatic insufficiency since vitamin E deficiency is known to occur in patients with pancreatic insufficiency due to cystic fibrosis (Harries and Muller, 1969).

In several duodenal samples from the present cases, and from 2 of the 4 previously published enterokinase-deficient patients, considerable activity of carboxypeptidase was found even before the addition of enterokinase to the juice. These samples also contained tryptic activity. Evidently very low activities of trypsin are sufficient for partial activation of procarboxypeptidase. If more tryp-sin was formed after the addition of enterokinase, however, carboxypeptidase activity also increased markedly (Hadorn and Haworth, 1973).

A striking clinical observation in Case 1 was that after the age of 22 months he no longer required pancreatic extract to control diarrhoea and to maintain adequate nutrition. In his sister (Case 2) substitution of Nutramigen for a cow's milk formula was sufficient to control symptoms between one and 5 months of age. Her case might easily have gone unrecognized if the tests had not been performed because of the disease in her brother. Evidently in some of these patients the defect in protein digestion is of clinical importance only during the first few months of life. The reason is not known, but there seem to be two possibilities. (1) Gastric peptic activity might compensate for the lack of pancreatic proteolytic enzymes. This mechanism was proposed by Crane for the signifi-cant (20%–50%) protein digestion and absorption shown in 2 patients after total pancreatectomy (Crane, 1969). Our experience with a child suffer-ing from total exocrine pancreatic insufficiency with no proteolytic activity in duodenal contents is similar; the nitrogen absorption coefficient was reduced to 40% of normal. Determinations of nitrogen absorption coefficients in enterokinase-deficient patients are in progress. (2) An important factor in the interpretation of the observed age dependency of clinical expression is growth velocity and protein requirement per unit body weight. Growth velocity decreases rapidly during the first 2 years of life: at 6 months it is 18 cm/year, at 12 months 13 cm/year, at 2 years 9 cm/year (50th centile growth velocity for boys, charts of J. M. Tanner and R. H. Whitehouse). Concomitant with this decrease of growth velocity is a decrease in the recommended protein intake of the infant from 2·4 g/kg per day during the first 3 months of life to 1·3 g/kg per day at one year and 1·1 g/kg per day at 3 years (Joint FAO/WHO Expert Committee, 1973). Thus, it is not surprising that a proteolytic defect which limits protein digestion to about 40% of normal may result in severe clinical symptoms and growth retardation only during periods of rapid growth. When the growth rate is slower, and in the adult, protein needs may just be met. In this con-

text it is relevant to mention an earlier patient (Haworth *et al.*, 1971) who was recently reinvestigated. This boy grew very poorly until the age of 8 when he was given pancreatic extract. He then showed catch-up growth and reached the 50th centile by the age of 12 years. When seen at 15 years of age he was again losing weight and had diarrhoea with fatty stools. The duodenal juice showed the same abnormalities as those found earlier. The dose of pancreatic extract was increased; the diarrhoea promptly ceased and he regained the lost weight. The decompensation and reappearance of symptoms in this patient may have been related to the increased protein needs during the pubertal growth spurt.

Intestinal enterokinase deficiency is a treatable disorder and should be looked for in infants presenting with unexplained vomiting, diarrhoea, anaemia, and hypoproteinaemia. The duodenal juice of such patients should be assayed for its enzyme activity; the diagnosis may be made if very low or absent proteolytic activity is found which can be activated after *in vitro* incubation with enterokinase. We performed this test in many other malabsorptive states, including coeliac disease, but in no instance did we see an increase of tryptic activity after *in vitro* addition of enterokinase (zymogen activation test positive). In all patients with positive zymogen activation tests we found deficiency of enterokinase activity by direct analysis of a duodenal mucosal specimen. During early infancy these patients require replacement therapy with pancreatic extract or enterokinase, but this may vary from one patient to another and may decrease with age. Though the finding of enterokinase deficiency in sibs strongly suggests that the condition is inherited, there is not yet enough information about the mechanism of inheritance. Our studies on the mother have not been helpful in this regard.

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