Intestinal Enterokinase Deficiency

A Newly-recognized Disorder of Protein Digestion

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Tarlow, M. J., Hadorn, B., Artherton, M. W., and Lloyd, J. K. (1970). *Archives of Disease in Childhood*, 45, 651. **Intestinal enterokinase deficiency. A newly-recognized disorder of protein digestion.** Enterokinase is an intestinal enzyme necessary for the activation of pancreatic proteolytic zymogens. Two children with absence of enterokinase activity in the duodenal juice and intestinal mucosa are described. Both had diarrhoea from birth and failed to thrive; one developed hypoproteinaemic oedema. Oral administration of pancreatic extract resulted in prompt control of symptoms and acceleration of growth.

A simple screening test for enterokinase deficiency is described.

Enterokinase is an enzyme secreted by the mucosa of the small intestine and is responsible for the conversion of trypsinogen to its active product, trypsin. Its key role in initiating the formation of proteolytic enzymes from their inactive precursors is illustrated in Fig. 1. When the pancreatic zymogens reach the duodenum, trypsin is formed by the action of enterokinase on trypsinogen and this trypsin then activates the other pancreatic proteolytic zymogens (chymotrypsinogens and pro-carboxypeptidases).

Enterokinase was first discovered in 1899 by Schepownalnikov working in Pavlov's laboratory, and because of its enzymic action on another enzyme, Pavlov (1967) aptly described it as a 'ferment of ferments'. Despite considerable interest in the physiology of enterokinase during the earlier part of this century, and the fact that its absence should result in marked impairment of protein digestion, it has not excited much medical interest.

We describe 2 children who presented with diarrhoea and failure to thrive, in whom the underlying abnormality was found to be enterokinase deficiency. A brief report of Case 1 has previously appeared as a preliminary communication (Hadorn *et al.*, 1969) and Case 2 has been recorded in abstract form (Tarlow and Hadorn, 1970).

Case Reports

Case 1. This girl, the elder child of healthy unrelated parents, was born at term on 23 June 1967 and

weighed 3.4 kg. Diarrhoea was present from birth, she failed to gain weight, and was admitted to hospital at the age of 3 weeks. Changes in the feeding regimen failed to control the symptoms, and at the age of 3 months her

FIG. 1.—Activation of pancreatic proteolytic enzymes.

weight was only 3.05 kg. At 4 months she became severely ill and oedematous; total serum proteins 3.2 g./100 ml. and Hb 6.4 g./100 ml. Total and differential white blood cell count normal. Duodenal juice contained very low levels of amylase, lipase, and proteolytic activity. Faecal fat 14 g. in a single 24-hour specimen. Cystic fibrosis excluded by finding a normal sweat sodium concentration. Barium meal and follow-through normal. Intravenous albumin and blood were given, pancreatic extract (250 mg. Pancrex V forte*) was added to the feeds, and her general condition improved considerably. Two further samples of duodenal juice were obtained at 7 and 12 months of age; amylase and lipase levels were then normal, but virtually no proteolytic activity was detected in either sample (3 units and 1 unit, respectively, by the technique of McGowan and Wills, 1962). Tryptic activity in stools, however, was normal, both when measured by a non-

specific technique for proteolytic activity (McGowan and Wills, 1962) and by a specific trypsin assay (Hummel, 1959). Withdrawal of oral pancreatic extract at the age of 12 months resulted in recurrence of diarrhoea and clinical deterioration within a few days, and substitution therapy was therefore restarted and continued in a dose of 250 mg. Pancrex V forte with each meal.

The patient was reinvestigated at the age of 17 months when her height was 72.4 cm. and weight 8.6 kg. (both just below the 3rd centile). Oral pancreatic extract was stopped during the period of this investigation. Total serum proteins were 6.0 g./100 ml. and albumin was normal on electrophoresis; Hb 11.2 g./100 ml. and white blood count normal; faecal fat 3.0 g./day (mean of 4 days). Jejunal biopsy showed a normal leaf-like villous pattern and was normal to light and electron microscopy; lactase activity 37 μ moles/min. per g. protein (normal). Investigations of the duodenal juice for enterokinase activity are described later.

When last seen at the age of 2½ years the patient was well and her growth rate and bone age were normal (Fig. 2). For a six-month period the dose of Pancrex V forte had been doubled (500 mg. with each meal), but as this had made no difference to her growth rate or general well-being, she is now being maintained on the smaller dose of 250 mg. with each meal.

Case 2. This girl is a non-identical twin child of healthy Pakistani parents who are second cousins. Neither her twin nor an elder sib have any clinical evidence of gastro-intestinal disease. She was born on 8 January 1965 and weighed 2.0 kg. Diarrhoea with loose offensive stools was noticed from birth, and she failed to gain weight normally. At the age of 2 months she was found to be anaemic (Hb 5.8 g./100 ml.) and to have steatorrhoea with faecal fat of 7.6 g./day (mean of 5 days). Faecal proteolytic activity (Shwachman, Farber, and Maddock, 1943) was undetectable. Cystic fibrosis was excluded by the repeated finding of normal sweat sodium concentrations. Oedema was not present and serum proteins were not estimated. Despite various changes of milk formula, she had persistent diarrhoea and failed to gain weight; she was therefore reinvestigated at 9 months of age, when Hb 10.8 g./100 ml.; total and differential white blood count normal; serum proteins normal (total 6.1 g./100 ml. albumin 3.7 g./100 ml., globulin 2.4 g./100 ml.). Urine xylose excretion over a 5-hour period normal. Proteolytic activity in stools (Shwachman *et al.*, 1943) detectable to a dilution of 1:1024, but 4 duodenal intubations between the ages of 11 months and 4 years showed an identical pattern with absent proteolytic activity in the duodenal juice (even after stimulation with pancreozymin) but normal levels of amylase and lipase.

A diagnosis of trypsin deficiency was made, and oral pancreatic extract (Cotazym* 1 capsule with each meal) was started at the age of 10 months. There was immediate improvement in her clinical condition, her stools became normal, and she gained weight. When

the pancreatic extract was stopped after 6 weeks there was recurrence of diarrhoea and she again lost weight. She was therefore maintained on the previous dose of pancreatic extract.

FIG. 2.—Effect of oral pancreatic extract on weight gain in Cases 1 and 2.

At the age of 4 years her general health was good, her growth rate and bone age were normal (Fig. 2). Faecal fat 0.9 g./day (mean of 3 days), Hb 12.2 g./100 ml., and serum proteins 6.5 g./100 ml. Jejunal biopsy showed normal mucosa by light and electron microscopy, and the presence of alkaline and acid phosphatase, leucine naphthylamidase, a γ -L-glutamyl transpeptidase-like enzyme, and an α -glucosidase were demonstrated by histochemical techniques (Dr. B. D. Lake). The results of duodenal intubation and studies of enterokinase activity are described with those of Case 1.

Investigations of Intestinal Proteolytic Activity

Duodenal juice was collected by intubation after stimulation by intravenous pancreozymin (2 units/kg. body weight). Trypsin activity was assayed either by the non-specific proteolytic assay of McGowan and Wills

*Organon.

TABLE I
Enterokinase, Trypsin, and Chymotrypsin Concentrations in Duodenal Juice

	Case 1		Case 2		Normal Values
	Initial Value	After Addition of Juice Containing Enterokinase	Initial Value	After Addition of Juice Containing Enterokinase	Mean Range
Trypsin ($\mu\text{g./ml.}$)	0	488	0	210	190* 45-800
Chymotrypsin ($\mu\text{g./ml.}$)	0	225	0	480	220* 39-410
Enterokinase (EKU/ml.)	< 0.01		< 0.01		2.7† 0.4-7

*Data from 25 children without pancreatic disease. †Data from 4 children with normal protein digestion and 1 with cystic fibrosis.

(1962), or by a specific esterase technique using Lundh's modification (1957) of the method of Schwert and Takenaka (1955). Chymotrypsin was measured by Lundh's modification of the method of Schwert and Takenaka. Activation of trypsinogen by exogenous trypsin was attempted using the method of Desnuelle, Reboud, and Abdeljlil (1962) with a concentration of bovine trypsin of 20 $\mu\text{g./ml.}$ and added calcium of 0.02M in the incubation mixture, and incubating for up to 24 hours at pH 7.6.

A modification (Hadorn, 1969) of the technique described by Kunitz (1939) was used for the assay of enterokinase. 0.1 ml. duodenal juice was precipitated with about 10 ml. cold ethanol and redissolved in 1.0 ml. 0.9% saline, as described by Lundh; 0.8 ml. of this solution (equivalent to 0.8 ml. of the original juice) was chromatographed on a G-100 Sephadex column (Pharmacia 15/30) using tris buffer (0.05 M tris, pH 7.5, containing 0.1M KCl). 2 ml. samples of the eluate were collected and 0.1-1.0 ml. aliquots incubated with 0.1 ml. trypsinogen solution (bovine trypsinogen (Sigma) 1 mg./1 ml. in 0.005M HCl) and 0.1 ml. trismaleate buffer (pH 6.0, 0.1M) the volume being made to 1.5 ml. with distilled water. Calcium chloride was added to the incubation mixture to give a final concentration $5 \times 10^{-4}\text{M}$. This solution was incubated at 25 °C. and aliquots were assayed for trypsin at intervals up to 120 minutes. The initial rate of formation of trypsin under these conditions is linear (Hadorn, 1969) and is proportional to the enterokinase concentration. One enterokinase unit (EKU) is the amount of enzyme which converts 1 $\mu\text{g.}$ trypsinogen to trypsin per minute under the defined conditions. This does not correspond to the EKU defined by Kunitz (1939) who used different assay conditions.

Enterokinase activity of duodenal mucosa was measured in the same way after the biopsy had been homogenized in a Potter homogenizer using about 20 times its volume of the tris buffer, and the supernatant separated by centrifugation at 800 g. for 20 minutes at 4 °C.

Screening test for enterokinase deficiency. The proteolytic enzymes in the duodenal juice of the patients with enterokinase deficiency were activated by adding exogenous enterokinase in the form of juice from a

patient with exocrine pancreatic insufficiency (cystic fibrosis); this juice contained enterokinase but no trypsin. 0.1 ml. each juice was mixed and incubated for 15 minutes at 37 °C. and the mixture assayed for trypsin in the standard way (Lundh, 1957).

Results

In neither patient was trypsin or chymotrypsin detectable in the duodenal juice, and enterokinase activity was barely detectable (< 0.01 EKU ml.). After the addition of juice containing enterokinase (but no trypsin), activation of trypsinogen occurred and normal levels of trypsin and chymotrypsin were found in both cases (Table I). After incubation with exogenous trypsin there was no activation of trypsinogen in the juice of either patient, though normal levels of chymotrypsin appeared.

No enterokinase activity could be detected in the mucosal biopsies of either patient.

Discussion

The evidence that deficiency of enterokinase is responsible for the defect of protein digestion in our patients is provided by the absence of enterokinase activity in both the duodenal juice and the mucosal homogenate, and the correction of the proteolytic defect by the addition of duodenal juice which contained enterokinase but no trypsin. Addition of purified human enterokinase was also shown to have the same action in the first patient (Hadorn *et al.*, 1969). The appearance of trypsin and chymotrypsin in the juice after the addition of enterokinase clearly showed the presence of the pancreatic zymogens trypsinogen and chymotrypsinogen, and the demonstration of normal lipase and amylase activities (after correction of general malnutrition) suggested normal pancreatic function. The finding of steatorrhoea in both patients in the early months of life may be attributed to enzyme deficiencies secondary to general protein malnutrition. In the first patient low levels of amylase

TABLE II

Comparison of Clinical and Laboratory Features in Trypsinogen Deficiency Disease and Enterokinase Deficiency

	Townes (1965)	Townes <i>et al.</i> (1967)	Morris and Fisher (1967)	Present Cases	
				1	2
Sex	M	M	F	F	F
Failure to thrive	+	+	+	+	+
Hypoproteinaemic oedema	+	+	+	+	-
Initial steatorrhoea	+	Not known	+	+	+
Pancreatic enzymes after treatment	<div> <div>T</div> <div>L</div> <div>A</div> </div> Nil	<div> <div>T</div> <div>L</div> <div>A</div> </div> Nil	<div> <div>T</div> <div>L</div> <div>A</div> </div> Nil	<div> <div>T</div> <div>L</div> <div>A</div> </div> Nil	<div> <div>T</div> <div>L</div> <div>A</div> </div> Nil
Stimulation with exogenous trypsin	CT Normal	CT Normal	CT Normal	CT Normal	CT Normal
Enterokinase	Not known	Not known	Not known	Absent	Absent

T = Trypsin. CT = Chymotrypsin. L = Lipase. A = Amylase.

and lipase as well as low proteolytic activity were found at this stage. Similar depression of pancreatic enzyme secretion has been described in children with kwashiorkor (Thompson and Trowell, 1952). In these children, as in our patients, fat absorption returned to normal as the general nutritional state improved.

Evidence that the deficiency of enterokinase is a primary abnormality in our patients is provided by the finding of otherwise normal intestinal mucosa when examined by light microscopy, electron-microscopy, and histochemical studies, and by the failure to demonstrate any other abnormality of intestinal function. It is not yet known whether secondary deficiency of enterokinase can occur in association with other intestinal disease. Hadorn (1969) showed no correlation between enterokinase activity and disaccharidase activities in the mucosa in patients with coeliac disease, and concluded that the mucosal atrophy in this condition did not result in reduced enterokinase activity.

Normal human pancreatic juice contains a small amount of trypsin, which is probably rendered inactive by association with a trypsin-inhibitor in order to prevent digestion of the pancreas. The presence of trypsin inhibitor has been shown in human pancreatic juice by Keller and Allan (1967). In our first patient, thirtyfold dilution of the duodenal juice resulted in spontaneous activation of the trypsinogen (B. Hadorn, unpublished observations), possibly because of dissociation of the trypsin-inhibitor complex. A similar mechanism may lead to the activation of trypsinogen during its passage down the intestine and account for the presence of trypsin in the stools. Faecal trypsin in our patients may also be due to bacterial activation of trypsinogen.

Despite the good clinical response to treatment

with relatively small amounts of pancreatic extract (which does not contain enterokinase) (M. J. Tarlow and O. Tarlow, unpublished observations), the growth of both our patients has remained at or just below the 3rd centile. Short stature has not been a feature in either family and the twin brother of Case 2 is both taller and heavier than his sister despite being of smaller birthweight. Possibly the protein deficiency which was present from the earliest days of life has produced permanent limitation of growth; suboptimal growth has also been observed in children with severe malnutrition who have been followed for several years after clinical recovery and the introduction of adequate feeding (Krueger, 1969; Garrow and Pike, 1967).

In addition to the two patients described here, a third case of enterokinase deficiency has recently been reported (Polonovski and Bier, 1970), and all these patients have a similarity to patients with trypsinogen deficiency disease (Townes, 1965; Townes, Bryson, and Miller, 1967; Morris and Fisher, 1967). Indeed this diagnosis was originally considered in our patients and in the patient of Polonovski and Bier. Townes (1965) based his diagnosis of trypsinogen deficiency (rather than enterokinase deficiency) on the failure of added trypsin to activate trypsinogen. However, in both our patients, and in the patient described by Polonovski and Bier, incubation of the duodenal juice with trypsin under the conditions used by Townes also failed to result in trypsinogen activation. Polonovski and Bier (1970) suggest that higher concentrations of exogenous trypsin than that used by Townes may be necessary for activation of human trypsinogen, though the lower concentration is sufficient to activate chymotrypsinogen. As can be seen from Table II there is no single clinical or

laboratory feature which differentiates between the two conditions, and as enterokinase activity was not investigated in the children with trypsinogen deficiency disease it is possible that these children also have enterokinase deficiency.

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