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cathodal elastase
celiac disease
cow's milk protein intolerance
duodenum
pancreatic insufficiency

Cathodal Elastase in Duodenal Juice from Children with Gastrointestinal Disorders

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Summary

Immunoreactive cathodal elastase, elastinolytic activity, and activity on the low-molecular elastase substrate succinyl-trialanine were assayed in duodenal juice from 89 fasting children with different malabsorption problems. Cathodal elastase immunoactivity (mean value, 0.06 g/liter) averaged 1% of the total protein content in duodenal juice and 1/16 of the succinyl-trialanine-splitting activity. A strong influence of age was found for immunoactivity and elastinolytic activity, indicating continuing development of the cathodal elastase during the first 24 months of life.

In 81 children with normal pancreatic function, significantly lower levels for all parameters including total protein were found for 14 with coeliac disease than for 34 children with unclassified gastrointestinal disorders and 33 with cow's milk protein intolerance. In eight children with pancreatic insufficiency, seven lacked detectable immunoactive cathodal elastase; low levels of succinyl-trialanine-splitting activity were found in six, and remnants of elastinolytic activity in three.

Speculation

Cathodal elastase, which accounts for the major part of the elastinolytic activity in duodenal juice, is present in low amounts in infancy and is fully developed first at about 2 years of age. Low amounts of this enzyme as in delayed maturation may play a role in protein malabsorption in the first years of life.

Among the enzymes with proteolytic properties excreted from the pancreas, a unique functional position is held by the elastases with their ability to digest elastin besides other substrates. From studies on human pancreatic extracts (14), the existence of two different enzymes has been postulated, one anionic and one cationic, with activity against succinyl-trialanine, a substrate reported to be highly specific for porcine pancreatic elastase (2). The cationic variant, named elastase 2 by Largman *et al.* (14), has unanimously been reported to have elastinolytic properties, whereas conflicting data in this respect have been published about the anionic form (elastase 1) (10, 14, 17).

Knowledge about these enzymes or enzyme forms in human duodenal content in various physiologic and pathologic conditions is scanty and incomplete. In the duodenal juice, the elastin-digesting capacity has hitherto been reported to be confined to an enzyme with cathodal mobility in agarose gel electrophoresis at pH 8.6 (5, 6, 11). The succinyl-trialanine-splitting activity appears under the same circumstances also in an anodal region (6, 11).

The lack of cross-reaction of the anodal trialanine-splitting portion with rabbit antibodies against human cathodal elastase (6, 14) provides a possibility for separate determination of the cathodal elastase by immunologic methods (6). This study made immunochemical determinations of cathodal elastase parallel to estimates of elastinolysis, succinyl-trialanine-splitting activity, and the total protein content in the duodenal juice from 89 children of various ages and various gastrointestinal disorders.

PATIENTS

The study group, 48 boys and 41 girls, aged 6 wk to 8 years, were submitted in 1974–1978 to gastroenterological examination at the Department of Pediatrics for suspected malabsorption with signs of poor weight gain and/or stool problems. None were acutely ill, and none received parenteral nutrition when investigated. Further details are given in Table 1.

The examination program (1) included, besides routine clinical and laboratory examination, intestinal mucosal biopsy, duodenal juice aspiration, and fecal microscopy. Suspicions of food intolerance were followed up with proper eliminations and challenges. Pancreatic function was assessed from duodenal juice analyses including determination of amylase and trypsin activities together with agarose gel electrozymogram (5).

The study was approved by the Ethical Committée of the University of Lund.

COLLECTION OF DUODENAL JUICE

All patients received alimemazine 2 mg/kg body weight 2 hr before intubation. Duodenal juice was collected into tubes on crushed ice via a double lumen tube (Salem Sump Fr Size 10 Ch) from a position immediately proximal to the duodeno-jejunal flexure (fluoroscopic control). All samples were collected in fasting state (infants, 6 hr; children, overnight) and kept frozen at −20°C until analysed, control studies (6) having shown no significant losses of activity—immunological or enzymatic—under these conditions. pH's of all samples were 6 or more.

MATERIALS

Agarose was from Marine Colloids Inc; Miles Lab. Ltd., Stoke Poges, United Kingdom; succinyl-trialanine-*p*-nitroanilide [Suc(Ala)$_3$NA] was from Peptide Institute Inc., Protein Research Foundation, Osaka, Japan; bovine elastine was from Worthington Biochemical Corp., Freehold NJ; bovine albumin, *n*-benzoyl-DL-arginine-*p*-nitroanilide was from Sigma Chemicals, St Louis, MO, and Phadebas Amylase Test was from Pharmacia, Uppsala, Sweden.

The purified human cathodal elastase and rabbit antisera against this purified enzyme were produced at our laboratory (6).

METHODS

ENZYME ACTIVITY

Elastase esterolytic activity was measured according to Fric et al. (11) with Suc(Ala)$_3$NA as substrate and with the purified human cathodal elastase as standard. Elastase elastinolytic activity was assayed with radial diffusion in bovine elastin-agarose gel (19) and with spectrophotometry according to Ardelt as modified by Gertler (12). Trypsin activity was measured with *n*-benzoyl-DL-arginine-*p*-nitroanilide (8), and amylase was assayed with Phadebas Amylase Test (7).

Table 1. *Clinical material*

Diagnostic groups	*n*	Age (mos.) Mean Range	Small intestinal mucosa Flat	Convoluted	Ridged	Villous
Pancreatic insufficiency						
Shwachman's syndrome	2	20 1–84				
Cystic fibrosis	6					
Normal pancreatic function						
Celiac disease[1]	14	11 6–22	14	0	0	0
Cow's milk protein intolerance[2]	33	12 1–36	0	6	16	11
Unclassified gastrointestinal disorders[3]	34	25 5–96	0	3	18	13

[1] Diagnostic criteria: flat mucosa on a gluten-containing diet, clinical and histologic improvement on a gluten-free diet, and a histologic relapse when gluten was reintroduced in the diet (9).

[2] Diagnostic criteria: free of symptoms on a cow's milk-free diet and return of identical symptoms at two challenges with cow's milk.

[3] Failure to thrive, vomiting, and/or diarrhoea for more than 3 wk, with normal laboratory tests. Symptoms disappearing within 3 months.

IMMUNOACTIVITY

Cathodal elastase immunoactivity was determined with electroimmunoassay (15) at pH 8.6 in agarose gels containing 2% rabbit antibodies against human cathodal elastase with the purified human elastase as standard (6).

AGAROSE GEL ELECTROPHORESIS

Agarose gel electrophoresis was run in pH 8.6 (5).

PROTEIN CONCENTRATION

Protein concentration was measured according to Lowry *et al.* (16), with bovine albumin as standard.

STATISTICAL EVALUATION

For comparisons of groups Student's *t* test as well as the nonparametric Mann-Whitney *U* test were used.

RESULTS

Figure 1 shows the age distribution for the levels of immunoactive cathodal elastase in children less than 2 years old and with normal pancreatic function. In the age interval 0 to 7 months, no estimate exceeded 0.05 g/liter. The correlation coefficient was 0.61 (0 to 18 months = 0 to 24 months), indicating significant age influence ($P < 0.001$). In the interval 9 to 24 months the coefficient was 0.51 ($P < 0.001$). In the ages over 2 years, no influence of age was demonstrated.

Figure 2 gives the corresponding distribution for elastinolysis determined according to Ardelt *et al.* The correlation coefficient was 0.43 ($P < 0.01$).

No such influence of age was found for the estimates of radial gel elastinolysis and for Suc(Ala)$_3$NA activity (correlation coefficients, 0.08 and 0.04, respectively).

Figure 3 gives the ratio Suc(Ala)$_3$NA activity:cathodal elastase immunoactivity related to the age of the children.

The correlation between estimates of elastinolysis by the two different methods was poor ($r = 0.21$), as was also the correlation between both these elastinolysis measurements and Suc(Ala)$_3$NA activities ($r < 0.3$ for both).

As can be expected, a good covariance existed between estimates of immunoactive cathodal elastase and elastinolysis (Ardelt *et al.*) ($r = 0.54$), but analysis of paired data indicated two significant counteracting factors (paired *t* test = 3.66, $P < 0.01$). In 45 of 81 children, the elastinolysis estimate exceeded the immunoactivity estimate by more than 50%; the reverse was valid for 15 of 81.

Table 2 gives the results of elastase assays for the three groups of children with apparently normal pancreatic function. For protein and Suc(Ala)$_3$NA activity, the mean values for the children with coeliac disease were significantly lower than the means for both other groups ($P < 0.01$).

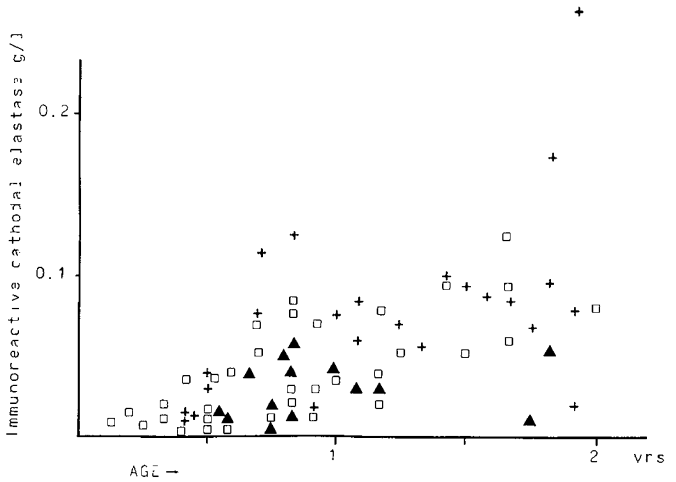

Fig. 1. Age distribution for immunoactive cathodal elastase in duodenal juice from children with normal pancreatic function. ▲, coeliac disease; +, unclassified malabsorption; □, cow's milk protein intolerance.

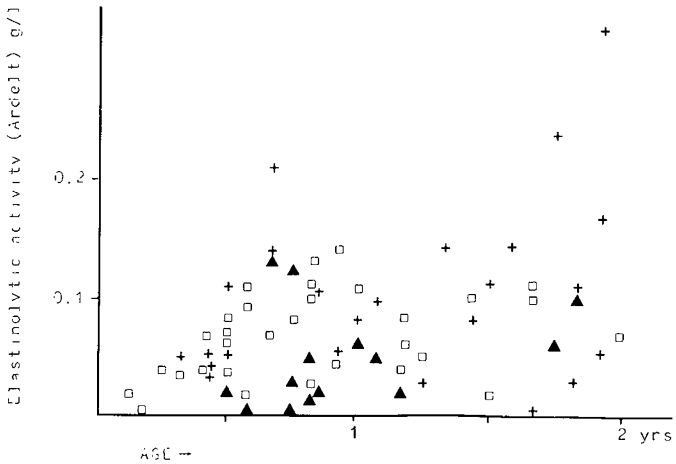

Fig. 2. Age distribution for elastinolytic activity determined according to Ardelt in duodenal juice from children with normal pancreatic function. ▲, coeliac disease; +, unclassified gastrointestinal disorders; □, cow's milk protein intolerance.

For the immunoactivity and elastinolysis (Ardelt *et al.*) shown in Figures 1 and 2 to be age-dependent, the difference was significant ($P < 0.01$) only between the groups with coeliac disease and unclassified gastrointestinal disorder. The age distribution of the groups was different, as shown in Table 2.

Comparison of age-matched samples from the groups, however, displays significantly ($P < 0.01$) lower means for the coeliac group than both other groups for immunoactivity and elastinolysis.

Table 3 lists the results for the patients with pancreatic insufficiency. In 7 of 8 an absence of detectable cathodal elastase immunoactivity is seen. Suc(Ala)$_3$NA activity, however, is present in six patients, and remnants of elastinolysis can be detected in three of them. Radial gel elastinolysis obtained zero values for all in this group.

DISCUSSION

The estimates of immunoreactive cathodal elastase, elastinolysis, and Suc(Ala)$_3$NA activity were made in duodenal juice samples from fasting subjects. The aim of this study was to provide data suitable for comparative purposes rather than for absolute determination of maximum secretory capacities. These presumptions, together with accessibility and reproducibility in clinical routine, made the fasting condition preferable.

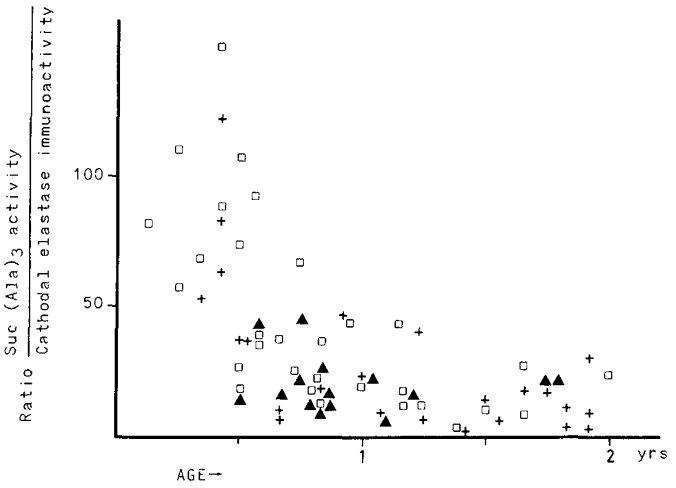

Fig. 3. Age distribution of the succinyl-trialanin activity:cathodal elastase immunoactivity ratio in duodenal juice from children with normal pancreatic function. ▲, coeliac disease; +, unclassified gastrointestinal disorders; □, cow's milk protein intolerance.

No studies of elastase and elastase-like enzymes in the duodenal juice from children providing comparable data have been found in literature.

In adults with pancreatic insufficiency, the Suc(Ala)$_3$NA activity in the duodenal juice has been reported decreased, parallel (21) or otherwise to (11) the reduction of amylase levels. As pointed out in the introduction, the Suc(Ala)$_3$NA activity is accounted for by at least two enzymes or enzyme variants: the cathodal elastase 2 with well-established elastinolytic activity and the anodal elastase 1 with doubtful elastinolytic activity. Our study illustrates this by the lack of correlation between age and Suc(Ala)$_3$NA activity contrasting with the age correlations in infancy found for cathodal elastase immunoactivity and for elastinolysis. As shown in Figure 3, the Suc(Ala)$_3$NA activity in infants below 7 months of age seems to be derived mostly from an enzyme not reacting with cathodal elastase antibodies.

Our study indicates that the cathodal elastase accounts for a major part of the total elastinolytic activity detected by the method of Ardelt *et al.* However, the finding that in 45 of 81 subjects the elastinolysis levels exceeds the immunoactivity levels suggests the presence of other sources of elastinolytic activity in the duodenal juice, one possibly being the anionic elastase, as claimed by Largman *et al.* (14). Attempts to demonstrate elastinolysis in anodal regions of the electrozymogram of duodenal juice with the radial gel technique were unsuccessful (6) possibly due to that method's low sensitivity. This is supported by our experience in this study with the poor correlation between this method and the other assays.

Compared with the findings for trypsin (3, 4), the proportion of the total protein content made up by immunoreactive cathodal elastase is strikingly low, averaging 1%. This contrasts to the proportion of 10% in the pancreatic juice from adults reported by

Table 2. *Elastase estimations and total protein, mean, and range in duodenal juice from patients with normal pancreatic function*

	Suc (Ala)$_3$ NA activity (g/liter)	Cathodal elastase immunoactivity (g/liter)	Elastinolysis (Ardelt *et al.*) (g/liter)	Total protein (g/liter)
Coelic disease (n = 14; mean age, 11 mos.)	0.54 ± 0.29[1] (0.22–1.28)[2]	0.026 ± 0.015 (0.008–0.05)	0.048 ± 0.04 (0–0.138)	4.37 ± 1.69 (2.1–9.1)
Cow's milk protein intolerance (n = 33; mean age, 12 mos.)	1.11 ± 0.79 (0.11–3.09)	0.037 ± 0.015 (0.002–0.114)	0.076 ± 0.045 (0–0.179)	6.05 ± 1.70 (2.0–9.4)
Unclassified gastrointestinal disorders (n = 34; mean age, 25 mos.)	1.09 ± 0.75 (0.25–3.14)	0.058 ± 0.056 (0.001–0.27)	0.102 ± 0.068 (0–0.326)	6.97 ± 1.80 (4.0–10.8)

[1] Mean ± S.D.
[2] Numbers in parentheses, range.

Table 3. *Elastase and total protein in duodenal juice from patients with pancreatic insufficiency*

	Age			Elastinolysis			
Patient[1]	yr	mos.	Suc(Ala)$_3$NA activity (g/liter)	Ardelt *et al.* (g/liter)	Radial gel (g/liter)	Cathodal elastase immunoact (g/liter)	Total protein (g/liter)
1	7	0	0.00	0	0	0.004	2.2
2	3	1	0.06	0.04	0	0	5.0
3	1	0	0.05	0	0	0	0.9
4	0	1	0.03	0	0	0	2.4
5	1	5	0.02	0.01	0	0	11.8
6	0	7	0.07	0.02	0	0	1.9
7	0	1	0.05	0	0	0	2.7
8	0	1	0.00	0	0	0	10.8

[1] Diagnoses: patients 1 and 2, Shwachman's syndrome; patients 3 to 8, cystic fibrosis.

Ohlsson and Ohlsson (18). The age of our patients and the source of enzyme can only partly explain this discrepancy. The proportion that can be roughly calculated from the purification of the enzyme (6) points at the same level, around 1%. Conclusions in this respect must await the separate determinations of the anionic elastase and its relative activity on elastin and Suc(Ala)$_3$NA.

We found an age correlation, suggesting that the immunoreactive cathodal elastase is not fully developed at birth, but similar to amylase (13) develops at least during the first 12 or 24 months. Low amylase levels in our material coincided with low elastase immunoactivity, but high amylase activity was also found in some patients with low elastase immunoactivity. This suggests a nonsimultaneous development of the two enzymes. In contrast, trypsin activities analysed in the same samples showed no age correlation (3).

The significance of these relatively low amounts of cathodal elastase in infancy for the intraluminal digestion of proteins is still uncertain. We found no evidence that pointed to the low cathodal elastase content as a contributing factor to cow's milk protein intolerance and to celiac disease.

The lower levels found in children with coeliac disease are probably, as has been suggested for the parallel phenomena for trypsins (3), due to increased water content, proposed by Polak *et al.* (20) in turn to be caused by hyperactivity in the secretin-producing cells in the intestinal mucosa in coeliac disease.

No subject with normal pancreatic function showed total absence of immunoactive cathodal elastase. On the other hand, our assay failed to detect any such elastase in seven of eight children with pancreatic insufficiency, despite demonstrable Suc(Ala)$_3$NA activity in six of eight subjects (and elastinolytic activity in three). The remnants of these activities might be derived from the anionic enzyme; if so, this is a phenomenon analogous to our findings for trypsin, whose anionic variant seems to be the last to vanish from the diseased pancreas (3).

Separate determination of the anionic elastase with immunochemical method might elucidate this.

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