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Shwackman's syndrome
trypsin

Plasma Immunoreactive Pancreatic Cationic Trypsinogen in Cystic Fibrosis: a Sensitive Indicator of Exocrine Pancreatic Dysfunction

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Summary

Plasma immunoreactive cationic trypsin(ogen) levels were determined in 32 control subjects and 43 patients with varying degrees of pancreatic insufficiency including 35 with cystic fibrosis (CF) and eight with Shwachman's syndrome. In six CF infants less than 2 years of age, plasma trypsin(ogen) levels were significantly elevated (97.3 ± 62.2 ng/ml) above the normal range for nine controls (7.0 ± 5.9 ng/ml; $P < 0.025$). Four of these infants had steatorrhea, three of whom had undetectable duodenal trypsin activity after stimulation with secretin-cholecystokinin. In two CF infants, molecular size fractionation by gel filtration of plasma followed by radioimmunoassay of the column fractions demonstrated that trypsinogen was the only immunoreactive species in the circulation.

In contrast, in older CF patients with steatorrhea (mean age, 15.3 ± 4.6 years), plasma cationic trypsin(ogen) levels were undetectable or low (1.1 ± 1.7 ng/ml). This finding clearly distinguished them from older CF patients without steatorrhea (mean age, 14.3 ± 3.9 years) in whom cationic trypsin(ogen) levels were significantly higher (23.3 ± 17.6 ng/ml; $P < 0.01$). The mean trypsin(ogen) concentration in the older CF patients without steatorrhea did not differ from the mean value for 23 normal subjects of similar age. Plasma cationic trypsin(ogen) levels in two Shwachman's patients with steatorrhea (0.19 and 0.86 ng/ml) were significantly lower than the values found in six Shwachman's patients without steatorrhea (5.9 ± 2.3 ng/ml; $P < 0.025$). Furthermore, in nine older CF patients and eight Shwachman's patients, circulating trypsin(ogen) levels were highly correlated with duodenal trypsin output after secretin-cholecystokinin stimulation ($r = 0.946$, $P < 0.01$; $r = 0.899$, $P < 0.01$, respectively). These results suggest that in CF infants high levels of circulating trypsin(ogen) persist even in those with complete pancreatic insufficiency. In older CF patients and those with Shwachman's syndrome, however, circulating trypsin(ogen) accurately reflects residual pancreatic function.

Speculation

Cystic fibrosis (CF) infants often possess viable but ductally obstructed pancreatic tissue, which may be destroyed with disease progression. The correlation between pancreatic exocrine function and circulating trypsin(ogen) in older CF patients and Shwachman's patients, however, indicates that ductal obstruction is not a prominent feature in these patients.

Patients with cystic fibrosis (CF) have been found to have elevated immunoreactive trypsin in serum or dried blood spots obtained in the newborn period and during early infancy (2, 8, 19). In CF children 2 years of age or older, however, Crossley *et al.* (8) found serum immunoreactive trypsin levels to be similar to or slightly below those in other children without CF. Dandona *et al.* (9) studied 29 CF patients between 2 and 25 years of age and found elevated trypsin levels in only three, with low or undetectable values in the remaining 26 patients (82%). They suggested that this test may be a useful index of pancreatic function in CF but did not provide direct evidence for this suggestion.

To explore the possibility that the measurement of circulating immunoreactive trypsin could be a useful indicator of pancreatic function, we have determined immunoreactive cationic trypsin(ogen) in plasma of children with varying degrees of pancreatic insufficiency, including CF infants and older CF patients as well as children with exocrine pancreatic hypoplasia, or Shwachman's syndrome (23). We have used a radioimmunoassay technique developed in this laboratory which can detect circulating cationic trypsinogen as well as trypsin bound to α_1-protease inhibitor (α_1-antitrypsin) (12). In contrast to methods described in other reports (2, 8, 19), this procedure involves chemical blocking of the active site of the radioiodinated trypsin tracer used in the assay to prevent it from binding to plasma protease inhibitors while maintaining full immunoreactivity.

MATERIALS AND METHODS

PATIENTS

We studied 43 patients with varying degrees of pancreatic insufficiency including 35 with CF and eight with Shwachman's syndrome. The diagnosis of CF had been established by typical clinical manifestations of this disease as well as by at least two abnormal sweat chloride determinations (>60mEq/liter). All the CF patients were being followed in the CF clinic at the Hospital for Sick Children, Toronto. The ages of the CF patients were between 0.08 and 27.59 years (mean, 13.32 years). Nineteen patients were female, and 16 were male. Eight patients (six females and two males) were below the third percentile for weight, and four of these patients (three females and one male) are also below the third percentile for height. All CF patients with evidence of growth failure had pancreatic insufficiency. In 28 patients old enough to perform pulmonary function studies, the most recent assessment revealed a wide range of pulmonary disease. Nine patients had mild pulmonary disease, 11 had moderate disease, and nine were severely affected. Three patients have since died of pulmonary complications. A 12-year-old female with pancreatic insufficiency developed massive hepatosplenomegaly at 11 years of age. Liver biopsy revealed advanced cirrhosis with histologic features compatible with chronic liver disease associated with CF.

Six patients with Shwachman's syndrome had intermittent neutropenia, and two had persistent neutropenia. The ages of the Shwachman's patients were between 2.25 and 18.0 years (mean, 8.1 years). Four patients were male, and two were female. Height and weight measurements were persistently below the third per-

centile in seven patients. Four Shwachman's patients had metaphyseal dysostosis involving long bones. Sweat chloride determinations were performed in seven Swachman's patients; all values were within normal limits.

The patients are reported in two separate groups: six CF infants who were less than two years of age comprise one group (mean age, 0.80 ± 0.75 years); a larger group consists of eight patients with Shwachman's syndrome (mean age, 8.1 ± 5.5 years) and 29 CF patients (mean age, 14.6 ± 3.9 years) who were 3 years of age or older. Thirty-two subjects with no apparent history of pancreatic or renal disease served as controls (mean age, 13.2 ± 5.7 years).

ASSESSMENT OF PANCREATIC FUNCTION

Fat balance studies were performed on all patients. Accurate records of daily fat intake were maintained throughout each balance period. Feces were collected over 5 days, stored at 4°C, weighed, and then analyzed for fecal fat content (27). Mean daily fat losses were expressed as a percentage of mean daily fat intake. In 17 patients including two CF infants, nine CF patients over 3 years of age, and six with Shwachman's syndrome, 5-day fecal fat excretion was 9% of intake or less (mean, 4.44 ± 2.68%). None of these patients required pancreatic enzyme replacement therapy, and pancreatic function was therefore considered to be adequately preserved for digestive purposes. Fecal fat excretion was 16% of intake or greater in the remaining 26 patients, including four CF infants, 20 older CF patients, and two patients with Shwachman's syndrome. These patients were considered to have steatorrhea and were given exogenous pancreatic enzyme supplements with meals.

Total duodenal trypsin output during constant intravenous infusion of secretin-cholecystokinin (Boots Drugs, UK; 0.125 units/kg/min over 1 hr) was determined in 22 patients (14). This group included all eight patients with Shwachman's syndrome, five CF infants less than 2 years of age, and nine older patients with CF. After an overnight fast, a double lumen tube was positioned in the duodenum under fluoroscopic control with the tip (aspiration port) at the ligament of Treitz and the proximal tube (infusion port) within the first part of the duodenum. A nasogastric tube was positioned in the body of the stomach, and gastric contents were aspirated using intermittent suction throughout the study. During the procedure, the duodenum was perfused at a constant rate with a marker solution of Bromosulfo-phthalein (0.01% in 5% mannitol) at 1.67 ml/min. After a baseline collection over a 20-min period, duodenal contents were aspirated at 20-min intervals during secretin-cholecystokinin infusion for 1 hr, and the volume of each collection period was recorded. Trypsin activity and Bromosulfo-phthalein concentration for each collection period were determined (17, 22), and total duodenal trypsin output was calculated in units per kilogram body weight per hour (14) from the three collections during stimulation.

BLOOD SAMPLES

Random blood samples from patients and controls were collected using heparin or EDTA as anticoagulent. In patients with malabsorption, blood samples were obtained without discontinuing enzyme replacement therapy. In addition, serial blood samples were obtained before and during pancreatic stimulation (0, 20, 40, and 60 min) in one patient with Shwachman's syndrome and in six older CF patients. The plasma was separated by centrifugation and stored at −70°C until assayed.

RADIOIMMUNOASSAY FOR CATIONIC TRYPSIN(OGEN)

The radioimmunoassay procedure for cationic trypsin(ogen) has been previously described (12). Human cationic trypsin was purified from acetone powders of pancreatic tissue following the procedure of Feinstein *et al.* (11). The trypsin used as tracer in the assay was inactivated with tosyl-L-lysine chloromethyl ketone (TLCK) to prevent binding of the tracer to plasma protease inhibitors (25). TLCK-cationic trypsin was radioiodinated by a modification (13) of the chloramine-T procedure of Hunter and Greenwood (18). All samples were diluted with buffer containing 50 mM Tris-HCl (pH 7.6), 0.14 M NaCl, 0.4% bovine serum albumin, and 0.12% normal rabbit IgG. After adding sufficient buffer to make a final incubation volume of 1 ml, 0 to 150 μl of standard cationic trypsin (20 ng/ml) or sample, 0.1 ml of cationic antiserum (diluted to 1:175,000 with standard buffer), and 0.1 ml of ^{125}I-labeled TLCK-cationic trypsin (10,000 cpm) were added in the order given. After incubation at 4°C for 4 days, bound and free labeled antigen were separated by the double antibody procedure described by Odell *et al.* (21).

The molecular size distribution of immunoreactive human pancreatic cationic trypsin(ogen) in CF plasma was determined by gel filtration of 200 μl aliquots of plasma according to the procedure previously described in this laboratory (12). To detect α_2-macroglobulin-cationic trypsin complexes, aliquots of each of the fractions that contained α_2-macroglobulin were treated with 15 μl of 0.32 M formic acid and incubated for 1 hr at 37°C followed by radioimmunoassay in duplicate (7).

STATISTICAL ANALYSIS

Results are presented as mean ± standard deviation. The significance of the difference of the means was assessed by unpaired Student's *t* test. Correlation coefficients were evaluated by linear regression, and the corresponding significance levels were determined by standard methods involving the z′ transformation of r.

RESULTS

CF INFANTS

In six CF infants less than 2 years of age (Table 1), plasma cationic trypsin(ogen) levels were significantly elevated (97.3 ± 62.2 ng/ml) above the normal range for nine controls of similar age (7.0 ± 5.9 ng/ml; $P < 0.025$). Four of the CF infants had steatorrhea, three of whom had undetectable duodenal trypsin output after stimulation with secretin-cholecystokinin, and circulating cationic trypsin(ogen) levels in these patients were dramatically elevated. In one of these patients (patient 1) plasma cationic trypsin(ogen) tended to decrease with advancing age, and levels of 155, 80, 27 ng/ml were obtained at 0.08, 0.33 and 1.33 years, respectively. In contrast, two CF infants (patients 5 and 6) did not have steatorrhea, and in both patients, duodenal trypsin was detectable after hormonal stimulation of the pancreas. However, the plasma cationic trypsin(ogen) level in one of these patients (patient 5) with the highest duodenal trypsin output was similar to values obtained for the control group.

Gel filtration of plasma samples from two CF infants with

Table 1. *Plasma cationic trypsin(ogen) levels in six CF infants under 2 years of age*[1]

Patient	Sex (M or F)	Age (yr)	Fecal fat (% intake)	Mean duodenal trypsin output (unit kg/hr)	Plasma Cationic trypsin(OGEN) (ng/ml)
1	M	0.08	55	0	155.1
		0.33			80.5
		1.33			26.9
2	F	0.16	37	N.D.[2]	149.7
3	F	0.67	27	0	145.6
4	M	1.4	42	0	126.5
5	M	0.5	9	333	8.7
6	F	2.0	8	21.7	44.5

[1] Nine controls (six males and three females), mean age, 1.2 years (0.3 to 2.0 years); mean plasma cationic trypsin(ogen), 7.0 ± 5.9 ng/ml.

[2] N.D., not done.

steatorrhea and high levels of circulating trypsin(ogen) followed by radioimmunoassay of the column fractions demonstrated the presence of only the zymogen form (results not shown). No active trypsin could be detected complexed with α_1-protease inhibitor. Furthermore, no cationic trypsin could be detected after acid treatment of the fractions containing α_2-macroglobulin.

OLDER CF PATIENTS AND SHWACHMAN'S PATIENTS

Figure 1 compares plasma cationic trypsin(ogen) levels between the various groups studied. It is apparent that in older CF patients with steatorrhea (mean age, 15.3 ± 4.6 years) plasma cationic trypsinogen levels were undetectable or extremely low (1.1 ± 1.7 ng/ml) which clearly distinguished them from older CF patients without steatorrhea (mean age, 14.3 ± 3.9 years) in whom cationic trypsin(ogen) levels were significantly higher (23.3 ± 17.6 ng/ml; $P < 0.01$). The mean cationic trypsin(ogen) concentration in the CF patients without steatorrhea was not significantly higher than the mean value for the control group (13.04 ± 3.65 ng/ml), but individual trypsin(ogen) values among these CF patients extended over a much wider range.

In the patients with Shwachman's syndrome (mean age, 8.1 ± 5.5 years), plasma cationic trypsin(ogen) levels in two patients with steatorrhea (0.19 and 0.86 ng/ml) were significantly lower than values found in six Shwachman's patients without steatorrhea (5.9 ± 2.3 ng/ml; $P < 0.025$). Cationic trypsin(ogen) levels were significantly lower than the control group in both the CF group with steatorrhea ($P < 0.0005$) and the Shwachman's patients with steatorrhea ($P < 0.001$).

PLASMA CATIONIC TRYPSIN(OGEN) AND DUODENAL TRYPSIN OUTPUT

An average value of 2078 ± 822 units/kg/hr was obtained for duodenal trypsin output in 52 children previously investigated at the Hospital for Sick Children, Toronto, and without obvious pancreatic insufficiency or CF. Six of eight Shwachman's patients and six of nine older CF patients studied had either undetectable or reduced (at least 2 S.D. below the mean) duodenal trypsin output during secretin-cholecystokinin stimulation when compared to the normal values. Figure 2 shows a plot of random plasma cationic trypsin(ogen) values in these patients *versus* total duodenal trypsin output during pancreatic stimulation. It can be seen that plasma cationic trypsin(ogen) values are highly correlated with duodenal trypsin output in both the Shwachman's group ($r = 0.899$; $P < 0.01$) and the group of older CF patients ($r = 0.946$; $P < 0.01$). Five of the nine older CF patients studied had steatorrhea. In four of these patients, duodenal trypsin outputs were undetectable, and plasma cationic trypsin(ogen) levels were 0.19, 0.40, 0.51, and 0.55 ng/ml. Another CF patient had mild steatorrhea (16% of intake), but duodenal trypsin activity was detectable (22 μ/kg/hr). This was reflected by an intermediate plasma cationic trypsin(ogen) value of 5.75 ng/ml. It is possible, therefore, that this patient had adequate proteolytic activity for protein digestion but inadequate lipolytic capacity to prevent fat malabsorption. In four CF patients without steatorrhea, plasma cationic trypsin(ogen) levels closely paralleled duodenal trypsin output. Two Shwachman's patients with steatorrhea had duodenal trypsin outputs of 0 and 33.9 μ/kg/hr and correspondingly low plasma cationic trypsinogen levels of 0.19 and 0.86 ng/ml, respectively. Six Shwachman's patients without steatorrhea had duodenal trypsin outputs which were closely reflected by plasma cationic trypsin(ogen) levels.

Plasma samples were drawn before and during stimulation (0, 20, 40, and 60, min) with secretin-cholecystokinin in six older CF patients and one patient with Shwachman's syndrome. No significant changes in circulating cationic trypsin(ogen) could be detected after hormonal stimulation, even in three CF patients and one Shwachman's patient without malabsorption. In these four patients, hormonal stimulation of the pancreas produced at least three-fold increases in duodenal trypsin output.

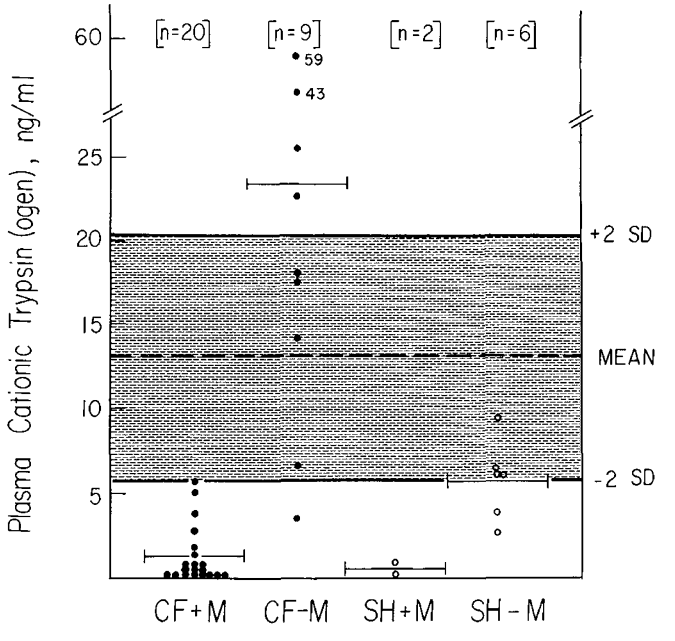

Fig. 1. Immunoreactive cationic trypsin(ogen) and fat malabsorption in older CF patients and patients with Shwachman's syndrome. Older CF patients with (CF + M) and without (CF − M) malabsorption and Shwachman's patients with (SH + M) and without (SH − M) malabsorption are compared. *Shaded area* shows mean cationic trypsin(ogen) (±2 S.D.) for 23 control subjects of similar age (13.2 ± 5.7 years).

Significance by unpaired *t* test: CF + M *versus* CF − M, $P < 0.01$; SH + M *versus* SH − M, $P < 0.025$; CF + M *versus* controls, $P < 0.0005$; CF − M *versus* controls, not significant; SH + M *versus* controls, $P < 0.001$; SH − M *versus* controls, $P < 0.025$.

DISCUSSION

In recent years, the radioimmunoassay technique has been used to detect and characterize the molecular forms of immunoreactive

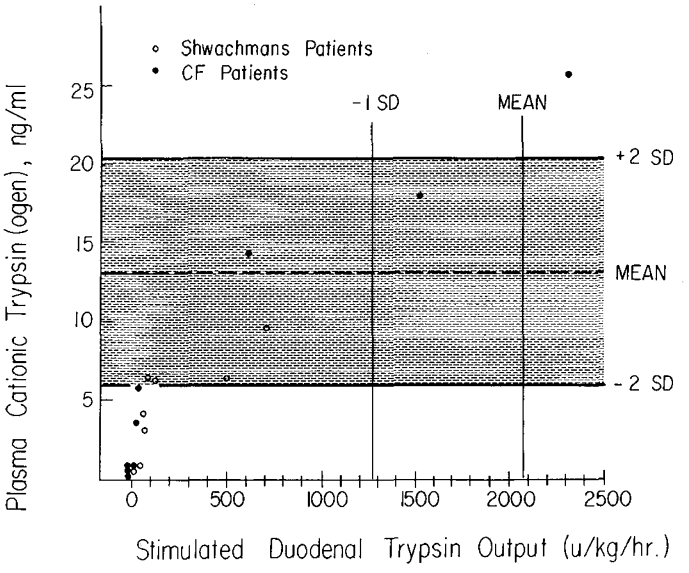

Fig. 2. Plasma immunoreactive cationic trypsin(ogen) levels plotted against stimulated duodenal trypsin outputs in nine older CF patients and eight patients with Shwachman's syndrome. The *shaded area* shows mean plasma cationic trypsin(ogen) (±2 S.D.) for 23 control subjects of similar age (13.2 ± 5.7 years). The *vertical lines* represent the normal range (mean − 1 S.D.) for stimulated duodenal trypsin output in 52 patients without pancreatic disease who were previously studied at the Hospital for Sick Children, Toronto.

pancreatic cationic and anionic trypsin in human plasma and serum. These studies have demonstrated that the circulating immunoreactive trypsin exists solely as trypsinogen in normal individuals (4, 12, 20, 26). Abnormal immunoreactive trypsin(ogen) levels have been found in patients with renal failure (10), common bile duct stones (10), carcinoma of the pancreas (1, 10), chronic pancreatitis (1, 10), and acute pancreatic inflammation (5, 7, 10). Recently, Crossley *et al.* (8) reported abnormally high levels of immunoreactive trypsin among CF infants during the first year of life. Our results confirm that cationic trypsin(ogen) levels are indeed elevated in the plasma of CF infants. Furthermore, we have demonstrated that in two CF infants with elevated cationic trypsin(ogen), all the immunoreactive material eluted from gel filtration columns as a single peak consistent with free trypsinogen. In normal individuals, the zymogen nature of this 23,000 molecular weight peak has previously been confirmed by activation with enteropeptidase (12).

The highest levels of immunoreactive trypsin(ogen) were found in the youngest CF infants with undetectable duodenal trypsin activity. This observation suggests that pancreatic zymogens are entering the circulation at an enhanced rate from viable but ductally obstructed pancreatic acinar tissue. A progressive decline in plasma levels of circulating cationic trypsin(ogen) with age was observed in one infant. This finding, especially if confirmed by additional studies, suggests that the viable pancreatic tissue is progressively destroyed.

Plasma levels of immunoreactive trypsin(ogen) in older CF children were much lower than those of CF infants. In older CF patients, circulating trypsin(ogen) appears to closely reflect the residual amount of functioning pancreatic tissue. Low or undetectable levels of trypsin(ogen) were found only in the group with steatorrhea, whereas significantly higher levels of this proenzyme were detected in the patients without malabsorption. Furthermore, as shown in Figure 2, random plasma cationic trypsin(ogen) levels were highly correlated with duodenal trypsin output after secretin-cholecystokinin stimulation. These results, based on a small number of patients, suggest that plasma cationic trypsin(ogen) levels may be as effective as duodenal trypsin output in determining subtle changes in pancreatic function among these patients. Thus, unlike most CF infants, this older group of CF patients probably have relatively unobstructed pancreatic ducts. The fact that none of the older CF patients studied exhibited a rise in circulating cationic trypsin(ogen) after hormonal stimulation further supports this conclusion.

A small number of CF patients without steatorrhea appear to be susceptible to recurrent attacks of acute pancreatitis during adolescence and adulthood (24). However, none of the older CF patients without steatorrhea included in this study showed clinical or laboratory evidence of acute pancreatitis. It is possible, however, that the high serum cationic trypsin(ogen) seen in the CF infants reflects some degree of persisting pancreatic inflammation.

The histopathology of the pancreas of patients with Shwachman's syndrome is different from that found in CF. Pancreatic acinar tissue is scarce or even absent, but the pancreatic ducts and ductular epithelium appear normal (3, 15). Although enzyme output is usually reduced or absent, normal secretory volumes with normal or reduced bicarbonate content are usually obtained during secretin-cholecystokinin stimulation (16). The high degree of correlation between plasma cationic trypsin(ogen) levels and duodenal trypsin output in these patients is consistent with the presence of relatively unobstructed pancreatic ducts. The levels of pancreatic trypsin(ogen) in the plasma of Shwachman's patients appear to reflect the residual secretory capacity of acinar tissue in each patient.

We have previously stressed that a catalytically inactive but fully immunoreactive form of trypsin must be used as tracer for radioimmunoassay of trypsin(ogen) in plasma or serum to prevent its binding plasma protease inhibitors. Recently, Borgstrom and Ohlsson (4) using a similar approach, reported a radioimmunoassay technique for measuring cationic trypsin(ogen) in human plasma using trypsin inactivated with diisopropylfluorophosphate as radioiodinated tracer. These authors found plasma cationic trypsin(ogen) levels of approximately 25 ng/ml in normal adults, in agreement with values reported from this laboratory (12). In our infants and children used as controls, however, levels were somewhat lower. Crossley *et al.* (8) and others (2, 9, 10) have used a radioimmunoassay kit (commercially available from Hoechst Pharmaceutical Laboratories, United Kingdom) and have reported mean serum trypsin concentrations of 104 ± 52 ng/ml in non-CF, nondiabetic children (8), whereas higher values (272 ± 67 ng/ml) have been demonstrated in normal adults (10). Although few specific details of this commercially prepared kit are available, Elias *et al.* (10) have stated that a semipurified trypsin preparation (designated Ag5) was used as standard. Wood *et al.* (28) later reported that the Ag5 standard has only 45% of the immunoreactivity of highly purified trypsin and have described a method for the conversion of trypsin values obtained with Ag5 trypsin to values for pure trypsin. However, the trypsin values obtained by these authors after conversion remain much higher than the levels reported by Borgstrom and Ohlsson (4) and by this laboratory (12). It is possible that the higher levels of immunoreactive trypsin reported by workers using the Hoechst kit result from the use of active or partially active trypsin as tracer and that binding of tracer to serum protease inhibitors could be responsible for artificially higher values.

Although additional studies are needed, our results suggest that plasma cationic trypsin(ogen) levels may be a sensitive, noninvasive indicator of subtle changes in pancreatic function among pediatric patients. Inasmuch as there is no apparent postprandial rise in circulating cationic trypsin(ogen) (6), random blood samples seem suitable for this test. We intend to evaluate the usefulness of this immunoassay in a prospective manner, using pediatric patients with various intestinal, pancreatic, and other disorders to provide comparative data with other recognized clinical techniques for measuring exocrine pancreatic function.

REFERENCES AND NOTES

1. Adrian, T. E., Besterman, H. S., Mallinson, C. N., Pera, A., Redshaw, M. R., Wood, T. P., and Bloom, S. R.: Plasma trypsin in chronic pancreatitis and pancreatic adenocarcinoma. Clin. Chim. Acta, *97:* 205 (1979).
2. Applegarth, D. A., Davidson, A. G. F., Kirby, L. T., Bridges, M., Sorenson, P., Wong, L. T. K., and Hardwick, D. F.: Dried blood spot screening for cystic fibrosis. Lancet, *2:* 1236 (1979).
3. Bodian, M., Sheldon, W., and Lightwood, R.: Congenital hypoplasia of the exocrine pancreas. Acta Paediatr., *53:* 282 (1964).
4. Borgstrom, A., and Ohlsson, K.: Radioimmunological determination and characterization of cathodal trypsin-like immunoreactivity in normal human plasma. Scand. J. Clin. Lab. Invest., *36:* 809 (1976).
5. Borgstrom, A., and Ohlsson, K.: Immunoreactive trypsin in serum and peritoneal fluid in acute pancreatitis. Hoppe-Seylers Z. Physiol. Chem., *359:* 667 (1978).
6. Borgstrom, A., and Ohlsson, K.: Studies in the turnover of endogenous cathodal trypsinogen in man. Eur. J. Clin. Invest., *8:* 379 (1978).
7. Brodrick, J. W., Geokas, M. C., Largman, C., Fassett, M., and Johnson, J. H.: Molecular forms of immunoreactive cationic trypsin in pancreatitis patients sera. Am. J. Physiol., *237:* E474 (1979).
8. Crossley, J. R., Elliott, R. B., and Smith, P. A.: Dried-blood spot screening for cystic fibrosis in the newborn. Lancet, *1:* 472 (1979).
9. Dandona, P., Hodson, M., Bell, J., Ramdial, L., and Batten, J. C.: Serum immunoreactive-trypsin concentrations in cystic fibrosis. Lancet, *1:* 1032 (1979).
10. Elias, E., Redshaw, M., and Wood, T.: Diagnostic importance of changes in circulating concentrations of immunoreactive trypsin. Lancet, *2:* 66 (1979).
11. Feinstein, G., Hofstein, R., Koifman, J., and Sokolovsky, M.: Human pancreatic proteolytic enzymes and protein inhibitors. Eur. J. Biochem., *43:* 569 (1974).
12. Geokas, M. C., Largman, C., Brodrick, J. W., and Johnson, J. H.: Determination of human pancreatic cationic trypsinogen in serum by radioimmunoassay. Am. J. Physiol., *236:* E77 (1979).
13. Geokas, M. C., Wollesen, H., and Rinderknecht, H.: Radioimmunoassay for pancreatic carboxypeptidase B in human serum. J. Lab. Clin. Med., *84:* 574 (1974).
14. Go, V. L. W., Hofmann, A. F. and Summerskill, W. H. J.: Pancreozymin bioassay in man based on pancreatic enzyme secretion. J. Clin. Invest., *49:* 1558 (1970).
15. Hadorn, B.: The exocrine pancreas. *In*: C. M. Anderson, V. Burke: Pediatric Gastroenterology. pp. 289–327 (Blackwell Sci., Oxford, 1975).
16. Hadorn, B., Zoppi, G., Shmerling, D. H., Prader, A., McIntyre, I., and Anderson, C. M.: Quantitative assessment of exocrine pancreatic function in infants and

children. J. Pediatr., *73:* 39 (1968).
17. Hummell, B. C. W.: A modified spectrophotometric determination of chymotrypsin, trypsin and thrombin. Can. J. Biochem. Physiol., *37:* 1393 (1959).
18. Hunter, W. M., and Greenwood, F. C.: Preparation of iodine-131 labeled human growth hormone of high specific activity. Nature (Lond.), *194:* 495 (1962).
19. King, D. N., Heeley, A. F., Walsh, M. P., and Kuzemko, J. A.: Sensitive trypsin assay for dried-blood specimens as a screening procedure for early detection of cystic fibrosis. Lancet., *2:* 1217 (1979).
20. Largman, C., Brodrick, J. W., Geokas, M. C., and Johnson, J. H.: Demonstration of human pancreatic anionic trypsinogen in normal serum by radioimmunoassay. Biochim. Biophys. Acta, *543:* 450 (1978).
21. Odell, W. D., Parlow, A. F., Cargille, C. M., and Ross, G. T.: Radioimmunoassay for human follicle-stimulating hormone: physiological studies. Clin. Invest., *47:* 2551 (1968).
22. Seligson, D., Marino, J., and Dodson, E.: Determination of sulfobromophthalein in serum. Clin. Chem., *3:* 638 (1957).
23. Shwachman, H., Diamond, L. K., Oski, F. A., and Khaw, K-T.: The syndrome of pancreatic insufficiency and bone marrow dysfunction. J. Pediatr., *65:* 645 (1964).
24. Shwachman, H., Lebenthal, E., and Khaw, K-T.: Recurrent acute pancreatitis in patients with cystic fibrosis with normal pancreatic enzymes. Pediatrics, *55:* 86 (1975).
25. Shaw, E.: Site specific reagents for chymotrypsin, trypsin and other serine proteases. Methods Enzymol., *25:* 655 (1972).
26. Temler, R. S., and Felber, J. P.: Radioimmunoassay of human plasma trypsin. Biochim. Biophys. Acta, *445:* 720 (1976).
27. Van der Kamer, J.: *In*: D. Seligson: Standard Methods of Clinical Chemistry. Vol. 2, p. 34 (Academic Press, Inc., New York, 1958).
28. Wood, T. P., Redshaw, M. R., and Rousell, R. H.: Units for immunoreactive trypsin. Lancet, *1:* 781 (1979).
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