Efficiency in the Diagnosis of Acute Pancreatitis Increased by Improved Electrophoresis of Amylase Isoenzyme P₃ on Cellulose Acetate

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Serum from patients who have suffered acute pancreatitis contains P₃, an isoenzyme of pancreatic-derived amylase (EC 3.2.1.1). Heretofore, complete resolution of P₃ from the major salivary isoenzyme in serum, S₁, has not been possible, thus compromising the diagnostic potential of P₃ for pancreatitis. I describe an electrophoretic method for the essentially complete resolution of P₃ from S₁ by including CaCl₂, 1 mmol/L, in the Tris barbiturate electrophoresis buffer (25 mmol/L, pH 8.8). I evaluated the clinical utility of the method for 129 consecutive patients suspected of having pancreatitis, by using receiver operating characteristic curve analysis for results for total amylase, P₂, and P₃ activity. For a true-positive rate of 90% with a prevalence of pancreatitis of 7.8%, the diagnostic efficiency was increased from 82% (total amylase) to 91% (P₂) to 98% (P₃). Thus, including P₃ activity in the diagnostic criteria will eliminate most false-positive results for pancreatitis based on total amylase activity alone, and should decrease the need for expensive radiologic procedures currently required to confirm the presence of pancreatitis. I conclude that P₃ can be of significant value in the differential diagnosis of pancreatitis from other causes.

Additional Keyphrases: receiver operating characteristic curves, electrophoresis, cellulose acetate, hyperamylasemia associated with aspiration of saliva into the lungs, reference interval

Measurement of amylase (1,4-α-d-glucan glucanohydrolase; EC 3.2.1.1) isoenzymes in serum has been shown to improve the reliability of diagnoses of pancreatitis (1–9). In particular, estimates of the activity of the pancreatic isoenzyme, P₃, have a greater predictive value than total amylase activity (1, 2, 6, 10–12) in the diagnosis of acute and chronic pancreatitis. Yet the resolution of P₃ from the major salivary amylase, S₁, has been difficult at best. The two isoenzymes of amylase ordinarily present in serum, and the ability to estimate P₃ activities accurately can be impaired by the presence of a substantial amount of S₁.

I describe here a modification of the original electrophoresis method of Benjamin and Kenny (1) for essentially complete resolution of P₃ from S₁. I also report the results of a clinical study of 167 hospital patients that demonstrate the superior ability of P₃ activity to predict pancreatitis, as compared with either P₂ or total amylase activity.

Some cases will be discussed that suggest a new mechanism for hyperamylasemia by showing that increases in serum salivary-type amylase may follow aspiration of salivary secretions into the lung. Thus, analysis of results for amylase isoenzymes by the method I report can be of significant value in the differential diagnosis of pancreatitis, to distinguish it from other syndromes that can lead to hyperamylasemia.

Materials and Methods

Assay Procedure

Total amylase is measured in serum at 30 °C with "Statzyme Amylase" reagent (Worthington Diagnostics, Freehold, NJ 07728) with a routine method on the Micro Centrifugal Analyzer (Instrumentation Laboratory, Lexington, MA 02173). The Statzyme Amylase reagent contains a highly branched limit dextrin that is cleaved by amylase to yield fragments that are eventually measured by the extent of reduction of NAD⁺ (13). One unit (U) of amylase activity reduces 1 μmol of NAD⁺ per minute at 30 °C.

For electrophoretic separation of the amylase isoenzymes, I used 60 × 76 mm "Titan III" cellulose acetate plates (Helena Laboratories, Beaumont, TX 77704) with "HR" buffer (Tris sodium barbiturate, pH 8.6–9.0, 25 mmol/L) diluted to 2 L with de-ionized water and containing 1 mmol of CaCl₂ per liter. Three applications of serum or plasma samples (total volume applied, about 1 μL) were made about 1.5 cm from the cathode end of the plate, then subjected to electrophoresis for 1.5 h at 300 V (5 mA per plate) with ice in the central chamber and on the lid. To minimize contamination, I wore surgical gloves when placing the wicks in the chamber. After electrophoresis, I placed the Titan III plate on a 80 × 100 mm glass plate in a 140 mm-diameter Petri dish with a drop of water between the cellulose acetate and glass plates to ensure that the plate lay flat.

A slurry of one Phadebas Amylase test tablet (Pharmacia Diagnostics, Piscataway, NJ 08854) in 3 mL of CaCl₂ (8 mmol/L) was poured onto the electrophoresis plate, which was then quickly placed on a level, vibration-free surface in a 40 °C oven and incubated for 1 h. The Phadebas Amylase test tablet contains small beads of a water-insoluble cross-linked starch polymer to which a blue dye is bound (14). Hydrolysis by α-amylase releases soluble starch–dye fragments that diffuse into the electrophoresis plate at the sites of amylase activity.

After incubation, the plate was quickly washed under cold tap water and dropped into a bath of methanol for 5 min to fix the blue bands in the cellulose acetate matrix. The plate was then dried under warm, forced air and scanned densitometrically with a Helena FlurVis Scanner at 595 nm.

Analytical Variables

For quality control of the method I included a sample serum specimen (diluted 10-fold in human albumin, 50 g/L; final amylase concentration, 250 U/L) consisting of about 80% P₂ and 20% P₃, with a trace of S₁. The diluted control material was stored in aliquots at −20 °C. Freshly collected saliva, diluted 200-fold in isotonic saline (NaCl, 9 g/L), was always included in the run to locate salivary isoenzymes. Within-run precision was determined by spotting the diluted serum in all eight positions on a single cellulose acetate plate. Run-to-run precision was determined by measuring the amylase activity in the diluted serum included in one position during each run for each plate (n = 46).
percentage values for each fraction were determined densitometrically and used for calculating the mean and CV.

Linearity of the method (response vs concentration) was assessed by diluting with isotonic saline a urine specimen containing about 75% P2, 20% P3, and about 5% S1 (total amylase = 4500 U/L). This working stock solution contained 1125 U/L before further dilutions. Three replicate plates, to which various dilutions of working stock solution had been applied, were run simultaneously. The percentage for each fraction was determined densitometrically and used to compute the mean and SD activity concentration (U/L) for each fraction in each dilution.

Reference Intervals

Reference intervals for the amylase isoenzymes were determined with serum specimens from a normal population of hospital-personnel volunteers and selected outpatients: 87 women and 44 men, ages 17 to 79 years (95th percentiles), average 48 years. The normal reference interval was determined by a nonparametric method described by Butta and Lilje (15).

The values of activity for each amylase isoenzyme were arranged in rank order from lowest to highest; removing the high 2.5% and low 2.5% produced a 95% reference interval.

Clinical Studies

The first population of patients studied consisted of 129 consecutive subjects for whom total amylase had been requested by the attending physician; this included 22 cases of hyperamylasemia (total amylase greater than 190 U/L). For this population I determined amylase isoenzymes irrespective of the total activity. However, when it became apparent that P1 was never increased if the total amylase activity was within the normal reference interval (<190 U/L), I extended the initial patient population to include 34 more cases of hyperamylasemia, thus forming a population with a much wider scope of syndromes associated with hyperamylasemia.

Charts for all patients in the clinical study were examined to determine the final diagnosis made by the attending physician. A diagnosis of pancreatitis was usually based on a combination of several observations, including: (a) epigastric pain; (b) a clinical history that included alcohol abuse, cholecystitis and (or) cholestasis, and abdominal trauma; (c) increased total amylase activity in serum; (d) radiologic studies with computerized tomographic scans and ultrasound analysis; and (e) an improvement when the patient was placed on a diet of clear liquids. The results of the analysis for amylase isoenzymes were not available to the attending physician. I evaluated the clinical usefulness of the method on the basis of the highest values for total amylase, P2, and P3 activity during the course of hospitalization of each patient.

To evaluate the clinical performance of the method, I used receiver operating characteristic curves (ROC), as described by Robertson and Zweig (16), to discriminate between the ability of total amylase, P2, and P3 to separate the patients with pancreatitis from the remaining patients. These curves (e.g., see Figure 4, below) are plots of the true-positive rate, based on the subjects with pancreatitis (y-axis), vs the false-positive rate, based on the subjects for whom pancreatitis was ruled out (x-axis). ROC curves for total amylase, P2, and P3 activity were constructed for both the initial consecutive population (n = 129) and for the combined population (n = 167) at convenient decision levels of activity, as noted in frequency histograms of the data.

Predictive values were calculated according to the method of Galen and Gambino (17) for total amylase, P2, and P3 activity only for the initial consecutive population, this being a population that accurately reflects the prevalence of pancreatitis in the hospital population. The decision levels used in the predictive value calculations were those determined from the ROC curve at a true-positive rate of 90%.

Results

Analytical Considerations

The effect of CaCl2 on the migration of amylase isoenzymes is shown in Figure 1. The presence of CaCl2, 1 mmol/L, resolves the combined (P') peak by retarding the migration of P3, thereby allowing it to be clearly separated from the S1 band. All the other isoenzymes of amylase do not appear to be affected by the presence of CaCl2. In contrast, the presence of EDTA, 1 mmol/L in the buffer, completely breaks down the resolution of both the salivary and pancreatic isoenzymes. Including 8 mmol of CaCl2 per liter in the assay mixture overcame the effect of EDTA in the electrophoresis buffer and always provided an excess of this necessary co-factor for catalytic activity.

The densitometric resolution of P3 and the changes in concentrations of P3 activity during a mild attack of acute pancreatitis induced by exploratory laparotomy are shown in Figure 2. I used the same densitometer gain setting in all four scans, to allow a visual comparison of the relative changes of P2, P3, and S1 over a four-day period. The patterns on days 1 and 4 are typical of a normal amylase isoenzyme pattern, with only a trace of P3 present. On day 2, however, P3 increased by about 100-fold, whereas P3 activity only tripled.

The median activities and normal reference intervals for the amylase isoenzymes in serum are listed in Table 1. About half of the normal population in this study demonstrated no serum P3, which is reflected by a median value of 0.9 U/L for a reference interval that extends from 0 to 8 U/L.

Five individuals in the normal population (3.6%) and 11 in the combined patient population (6.5%) had serum P1, including four of the 33 patients diagnosed as having pancreatitis in the combined population (12.1%).

Figure 3 illustrates the range of linearity of the method for each isoenzyme. Upon dilution, the minor fractions, P3 and S1, apparently diminish in proportion to the major fraction, P2. Thus, this method appears to lose some sensitivity for isoenzymes present at less than roughly 10 U of activity per liter.

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Migration of amylase isoenzymes during electrophoresis in Tris-boracturate buffer with no additions, with added CaCl2 (1 mmol/L), or with added EDTA (1 mmol/L).

Positions: 1–4, pancreatitis; 5, labyrinthisis with vomiting; 6, saliva; 7, normal; 8, pancreatitis.
Fig. 2. Electrophoresis migration patterns for amylase isoenzymes in serum from a patient who suffered an attack of pancreatitis after exploratory laparatomy

Table 1. Median and 95% Reference Interval Activity Values for Total Amylase and Amylase Isoenzymes in a Normal Population (n = 134)

<table>
<thead>
<tr>
<th>Activity, U/L</th>
<th>Median</th>
<th>Reference Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total amylase</td>
<td>100</td>
<td>40-190</td>
</tr>
<tr>
<td>P2</td>
<td>50</td>
<td>5-115</td>
</tr>
<tr>
<td>P3</td>
<td>0.9</td>
<td>0-8</td>
</tr>
<tr>
<td>S1</td>
<td>40</td>
<td>0-105</td>
</tr>
<tr>
<td>S2</td>
<td>0.5</td>
<td>0-6</td>
</tr>
</tbody>
</table>

Table 2 summarizes the results of precision analysis of the method.

Comparison of Total Amylase, P2, and P3 for Diagnosing Pancreatitis

ROC curve analysis for the initial population of 129 consecutive patients for whom total amylase was requested is shown in Figure 4 (left) for various decision levels. Determining P3 allows for a greater true-positive rate with a smaller false-positive rate than does assaying for P2, which, in turn, is superior to determinations of total amylase. These results reflect the fact that total amylase exceeded the upper reference level (190 U/L in the normal population) in 22 cases but only 10 of these patients had pancreatitis.

At a true-positive rate of 90% in the 129 consecutive patients (Figure 4, left), predictive values were calculated for total amylase, P2, and P3 activity (Table 3). For the initial population (n = 129) at respective decision levels of 140, 90, and 12 U/L, the negative predictive values were 97-99% for all three tests [prevalence of pancreatitis = 7.7% (10/129)]. This reflects the fact that acute pancreatitis is almost always associated with hyperamylasemia, with very few false negatives.

However, the remarkable variation in positive predictive value (Table 3)—36% (total amylase), 45% (P2), and 82% (P3)—reflects the fact that many clinical conditions unrelat-ed to pancreatitis may result in hyperamylasemia, whereas pancreatitis appears to be the only disease in which P3 is increased.

When 34 additional cases of hyperamylasemia were added to the initial population to see whether the concentrations of P3 could still resolve pancreatitis patients from those with other syndromes, the superiority of P3 analysis was confirmed by the ROC curves (Figure 4, right).

Frequency histograms for the combined population (n = 167) are shown in Figure 5 for concentrations of total amylase, P2, and P3. Many different types of syndromes associated with hyperamylasemia but not with pancreatitis have been reported elsewhere (18-21) and are also included in Figure 5. One group of five patients (∆) with hyperamylasemia appeared to have a common syndrome heretofore unreported in the literature, involving suspected aspiration of salivary secretions into the lung and associated with vomiting. The respiratory therapists treating these patients confirmed probable aspiration in all five cases. To my knowledge, this is the first report of hyperamylasemia associated with aspiration of salivary secretions into the lung.

Other remarkable cases of hyperamylasemia are worthy
Fig. 4. ROC curve analysis for total amylase, P₂, and P₃ activity with a patient population of 129 consecutive patients for whom total amylase activity was requested by the attending physician (left) and for a combined patient population (n = 167) that included an additional 38 cases of hyperamylasemia (right).

Left, 10 cases of pancreatitis and 22 cases of hyperamylasemia; right, 33 cases of pancreatitis and 48 cases of hyperamylasemia. Upper limit of normal, 190 U/L.

Table 3. Predictive Values for Total Amylase, P₂, and P₃ for Pancreatitis* in 129 Consecutive Patients Tested for Total Amylase in Serum

<table>
<thead>
<tr>
<th>Total amylase</th>
<th>P₂</th>
<th>P₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decision level, U/L</td>
<td>140</td>
<td>90</td>
</tr>
<tr>
<td>Sensitivity, %</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>Specificity, %</td>
<td>81</td>
<td>91</td>
</tr>
<tr>
<td>Predictive value (+), %</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td>Predictive value (−), %</td>
<td>87</td>
<td>99</td>
</tr>
<tr>
<td>Efficiency, %</td>
<td>82</td>
<td>91</td>
</tr>
</tbody>
</table>

*aPrevalence = 7.7% (10/129).

The decision level was chosen from the ROC curves at a 90% true-positive rate (Figure 4).

of note. One case of macroamylasemia with symptoms of pancreatitis (hexagon-enclosed 1 in Figure 5) was clearly resolved by analysis of amylase isoenzymes. Macroamylasemia had been diagnosed on the basis of sustained increases of total amylase activity and a low amylase clearance/creatinine clearance ratio. The P₂ and P₃ fractions were easily seen on electrophoresis and were quantified by using baseline corrections to eliminate contributions from the smeared activity of the macroamylase, which migrates anodal to P₂ and P₃ (5, 7).

Two cases of hyperamylasemia were associated with upper gastrointestinal bleeding (diamond-enclosed points 1 and 2 in Figure 5). In both cases electrophoretic analysis revealed increased salivary amylase (S₁ and S₂), normal P₂, and no evidence of P₃. This suggests the possibility of upper gastrointestinal absorption of salivary amylase in cases of frank hemorrhage into the upper gastrointestinal tract.

One patient with hyperamylasemia (total amylase 888 U/L) and acute gangrenous cholecystitis (square-enclosed 8 in Figure 5) had increased activities of P₁ (504 U/L) and P₂ (337 U/L) but no P₃. Examination of the pancreas by the surgeon during exploratory laparotomy revealed a "thickened gallbladder with significant adhesions. The cystic artery was thrombosed." After removal of the gallbladder, "exploration of the retropancreatic region did not reveal any significant abnormalities nor any indurations in the area of the pancreas." The lack of any P₃ activity, even in the presence of above-normal pancreatic-type amylase, P₁ and P₂, correlates with the lack of gross evidence of pancreatitis.

Discussion

The measurement of P₃ as a diagnostic tool for pancreatitis has been recognized since the work of Benjamin and Kenny (1) and Legaz and Kenny (2), who used electrophoresis on cellulose acetate with a discontinuous buffer. They concluded that P₂ is increased in essentially all cases of pancreatitis.

Weaver et al. (8) and Clink et al. (9) used only slightly modified forms of the Benjamin and Kenny method for resolving the amylase isoenzymes, but were only concerned with resolving the pancreatic type from salivary type. Although they did not resolve P₃ from S₁, they concluded that the fractionation of amylase isoenzymes was of value in the differentiation of hyperamylasemia from nonpancreatic origin.

Using the Legaz and Kenny method for measuring P₃, Fung et al. (12) also concluded that the presence of significant amounts of P₃ in the serum of patients diagnosed for cholelithiasis was highly correlated with the finding of pancreatitis during surgery. Of 25 patients with cholelithiasis, 10 had pancreatitis—five with normal and five with above-normal values for total amylase. All 10 of the pancre-
Clinical Study for Pancreatitis and Total Amylase

- Negative
- Positive
- Vomiting
- GI Absorption, Parotitis
- Elevation of P and S Type
- Macroamylase

Fig. 5. Total amylase, \( P_2 \), and \( P_3 \) in 167 patients

Besides the six general categories of case histories indicated, exceptional cases are:

1. 18 cases of abdominal pain, etiology unclear, square-enclosed 2; seven cases of gastritis, gastroenteritis, square-enclosed 2; 15 cases of blunt injury to abdomen or chest, square-enclosed 2; five cases of cholecystitis and (or) cholelithiasis, square-enclosed 4; four cases of gastric ulcer, square-enclosed 5; four cases of acute appendicitis without peritonitis, square-enclosed 2; one case of hepatitis, square-enclosed 7; one case of acute gangrenous cholecystitis, square-enclosed 8; one case of pulmonary and saphenous vein embolism, square-enclosed 3; O, three cases of suspected pancreatitis, circle-enclosed 1; one case of ERCP exam, circle-enclosed 2; one case of trauma to abdomen, stomach, or duodenum.

Orders for amylase by various methods include a wide spectrum of clinical problems (Fig. 3). The S-type fraction, initially 46% (total amylase was 104 U/L at 30 °C with a defined substrate maltotetraose from BMC Canada, Dorval, HBP 1A9 Canada), decreased to 39% with a fourfold dilution; the P-type activity increased from 54 to 62% upon dilution. This deviation from linearity for minor fractions of amylase with sample dilution may be due to the heterogeneous nature of the substrate. A complex mechanism for staining activity bands requires upward diffusion of amylase proteins into the insoluble blue-dye/bead matrix of the substrate lying on the surface of the electrophoresis plate; subsequently, the blue-dye fragments must diffuse down into the matrix of the electrophoresis plate. This is in contrast to the simple diffusion or mixing of soluble reagents within the electrophoresis plate with situ generation of fluorescent or colored enzyme products to mark the site of enzyme activity bands in the plate (22). In any event, this lack of linearity for the Phadebas Amylase reagent for staining minor amylase fractions in electrophoresis plates does not appear to detract from the clinical utility of \( P_3 \) analysis.

Early in the clinical study I simply air-dried the plates. However, with some lots of cellulose acetate plates, the blue bands were distorted. In seeking a better method of drying the plates while fixing them, I found that the soluble blue-dye fragments are very insoluble in methanol. Midway in the clinical study, therefore, the procedure was altered to include the methanol-fixative and drying technique. Correlation of all amylase isoenzyme values (percentage of total amylase) in 24 different serum specimens for the methanol-soaked (x) vs air-dried (y) plates yielded: \( y = 0.959 x + 2.0 \) (r = 0.998).

Efforts to quantify \( P_3 \) by other electrophoresis methods that do not resolve \( P_3 \) from \( S_1 \) have resulted in the calculation of the \( P_3 \) index as suggested by Frost (10) and Collins et al. (11). Both reports recognized that \( P_3 \) could not be clearly resolved from the slightly-faster-migrating \( S_1 \) and named the smeared band, which appears in the area of \( S_1 \) and \( P_3 \), as \( P' \) (as shown in Figure 1 when CaCl\(_2\) was not added to the buffer). Quantification of the \( P_3 \) component of \( P' \) required

- circle-enclosed 3; four cases of cholelithiasis and (or) cholecystitis, circle-enclosed 4; one case of gastric ulcer, circle-enclosed 5; three cases of metastatic cancer, circle-enclosed 6; one case of histocytic lymphoma circle-enclosed 7; one case of acute appendicitis with peritonitis, circle-enclosed 8; one case of intestinal adhesion, circle-enclosed 9; two cases of pancreatic pseudocyst, circle-enclosed 10; one case of calcification of pancreas, circle-enclosed 11; \( \gamma \), one case of abdominal aortic aneurysm with tear in the duodenum, inverted triangle-enclosed 1; one case of acute necrotizing pancreatitis inverted triangle-enclosed 2; \( \leftarrow \), one case of macroamylase with pancreatitis, hexagon-enclosed 1; O, two cases of parotiditis, diamond-enclosed 1; one case of gastric with upper GI bleeding, diamond-enclosed 2; one case of duodenal ulcer, acute hemorrhage, diamond-enclosed 3; \( \sqcap \), six cases of vomiting with epigastric pain, triangle-enclosed 1; one case of brain-stem aneurysm with vomiting, triangle-enclosed 2; one case of pulmonary disease with vomiting, triangle-enclosed 3; one case of chronic obstructive pulmonary disease, triangle-enclosed 4.

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careful measurement of migration distances of P' and P2, thereby to derive a P3 index.

The data I report here clearly show that P' can be essentially completely resolved into separate bands of P3 and S1 by including CaCl2 in the electrophoresis buffer. The fact that EDTA appears to cause gross smearing of all the amylase bands during migration suggests that Ca2+ is probably tightly bound to all of the isoenzymes. That P3 moves slightly faster in the absence of calcium ion in the buffer suggests a smaller binding constant for the P3-Ca2+ complex than for complexes with the other amylase isoenzymes. Removal of the positively charged calcium ion from the amylase isoenzyme would explain the faster migration toward the anode.

ROC curve analysis for total amylase, P2, and P3 activity in the clinical study presented in this report clearly demonstrates the superior performance of P2 activity as a useful diagnostic indicator of pancreatitis, confirming earlier reports of its value (2, 6). For a 90% true-positive rate in the 129 consecutive patients for whom total amylase was requested, the efficiency for diagnosing pancreatitis in this report improved from 82% when based on total amylase activity, to 91% for P2 activity, to 98% for P3 activity. Thus this method should be useful in confirming the presence of pancreatitis without requiring such time-consuming and expensive procedures as amylase clearance/creatinine clearance ratios, ultrasonography, and computerized tomographic scanning, as suggested by Weaver et al. (6).

Increases in S1 and S2 activities in serum of patients who have vomited suggest the possibility of aspiration of salivary secretions into the lung, with absorption of amylase into the circulation, a mechanism for hyperamylasemia that has not been previously seen (21). The concentration of amylase activity in saliva may be on the order of several hundred thousand units per liter. If 5% of the weight of a 70-kg individual is assumed to be blood with a 50% hematocrit, aspiration of only about 3 or 4 mL of saliva (e.g., at 200 000 U/L) would increase the serum concentration of total amylase by at least 50 U/L. Therefore, a patient who presents to the emergency room with epigastric pain and vomiting may have an above-normal amylase due to salivary aspiration into the lung. Analysis of amylase isoenzymes would clarify the diagnosis. Alternatively, analysis for these isoenzymes may help to clarify the causes of hyperamylasemia associated with epigastric pain and upper gastrointestinal tract bleeding. However, further research is required to verify this possibility.

In conclusion, the method of analyzing for P3 and other amylase isoenzyme activity presented here is faster than other methods (I, 5, 7), requiring only about 3 h between applying samples to the cellulose acetate plate and obtaining a stained and dried plate ready for scanning. The method is relatively easy, requiring no overlay of agarose mixtures on the electrophoresis plate (I). Fixing the blue-dye fragments released from the Phadebas substrate in the cellulose acetate plate in methanol provides distortion-free activity bands and allows easy and fast drying (1 min), whereas drying agarose plates takes longer. The method is relatively inexpensive, requiring a single cellulose acetate plate and one Phadebas Amylase test tablet for eight serum samples. And finally, the P3 band is clearly resolved from the S1 band, allowing a simple and precise computation of P3 activity. On the basis of the clinical study reported here, I conclude that P3 analysis by this method is clearly superior to determinations of total amylase or P2 activity and should be used for the differential diagnosis of pancreatitis in cases of hyperamylasemia.

I gratefully acknowledge William Butts, Jack Laseroth, Steve Sarwitcz, Jim Hushnergarth, Selig Leyser, Duane Carlson, Jerry Luetteke, and Leila McCleary for their assistance in the evaluation of difficult case histories, and I thank Rita Ellerbrook of Helena Laboratories for supplying the Titan III electrophoresis plates used in much of this research. I also thank the medical technologists in the clinical laboratory for their patience and help in measuring total amylase activity in many of the blood specimens analyzed in this research.

References