In this immunocatalytic assay for alpha-amylase (EC 3.2.1.1) of pancreatic origin, a highly specific monoclonal antibody coupled to plastic beads is used to extract pancreatic amylase from samples, leaving salivary amylase in solution. The catalytic activity of the bound pancreatic amylase is then determined with blocked p-nitrophenyl maltoheptaoside as substrate. The method shows no cross-reactivity with salivary amylase, analytical recovery is 89–109% for pancreatic amylase, and interassay imprecision is 7.1–7.7%. We used the method to determine pancreatic amylase in serum and urine from healthy controls and different patient groups. The reference intervals for 34 supposedly healthy controls were: serum, 10–48 U/L (mean 27 U/L); urine, <20–435 U/L (mean 104 U/L). Results by the present assay correlated well with a salivary amylase inhibition assay (Boehringer Mannheim). We conclude that the described immunocatalytic assay is clinically useful for detecting increased activities of pancreatic amylase in serum and urine.

Human alpha-amylase (EC 3.2.1.1) consists of two major isoenzymes, pancreatic and salivary. Although these are closely related, they are encoded by two different genes (1, 2). Pancreatic amylase is synthesized only in pancreatic tissues, whereas the salivary-type amylase has been found in salivary glands and in pulmonary, female genital, hepatic, and various malignant tissues (3). Amylase determinations are widely used for the diagnosis and evaluation of pancreatic disease. In many cases, however, determinations of total amylase give misleading information, because a high concentration of salivary amylase is present (4–7).

Attempts to produce polyclonal antibodies exclusively specific for pancreatic amylase have been unsuccessful, mostly owing to the extensive structural similarities between pancreatic and salivary isoenzymes, although some groups have succeeded in obtaining partly or highly specific polyclonal antisera (8–10). Monoclonal antibody techniques have recently yielded specific isoenzyme antibodies, most of which have been directed against salivary amylase (11–18), and we have prepared several monoclonal antibodies that are specific for pancreatic amylase (19).

Instead of inhibiting or binding salivary amylase, an approach subject to limitations in cases with salivary amylase in large excess, we have developed a direct immunocatalytic assay in which the pancreatic amylase is bound by a specific immobilized monoclonal antibody and subsequently analyzed for catalytic activity. This assay is theoretically superior to the aforementioned assays, because the pancreatic amylase is specifically extracted and determined with no interference from the salivary isoenzyme in the original sample. We also evaluated the assay by analyzing samples from different groups of patients.

**Materials and Methods**

**Patient groups.** These consisted of (a) 34 supposedly healthy controls, (b) 18 patients with acute pancreatitis, (c) 40 patients with gastrointestinal pain of unknown etiology, and (d) 18 patients with renal insufficiency.

**Samples.** Serum and urine samples were collected and stored frozen at −20 °C until analyzed. Instead of serum, heparin- or EDTA-treated plasma can be used.

**Antibodies.** Monoclonal antibody, specific for human pancreatic amylase, was developed by immunizing Balb/c mice with pancreatic amylase purified from human pancreatic tissue (19, 20). Salivary amylase was obtained from Sigma Chemical Co., St. Louis, MO, or we purified it from human mixed saliva (20).

We coated anti-pancreatic amylase monoclonal antibodies onto styrene-maleic anhydride beads (Dylark, diameter 6.35 mm, Sekisui Chemical Co., Osaka, Japan) by incubating them overnight in antibody solution containing 5 μg of antibody per bead in phosphate-buffered saline, which contained 138 mmol of NaCl, 2.6 mmol of KCl, 1.5 mmol of KH₂PO₄, and 8.1 mmol of Na₂HPO₄ per liter. After antibody-coating, the residual binding sites were blocked with bovine serum albumin, 1 mg/L, in 1,4-piperazinediethanesulfonic acid (pH 7.1).

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5 Nonstandard abbreviations: BpNPG, p-nitrophenyl maltoheptaoside; p-npzs, 1,4-piperazinediethanesulfonic acid; and cmz, 2-(N-cyclohexylamino)ethanesulfonic acid.
Substrate. Blocked p-nitrophenyl maltoheptaoside (BpNPG7), alpha-glucosidase (EC 3.2.1.20) from yeast, and glucoamylase (EC 3.2.1.3) from Aspergillus niger were purchased from Genzyme Biochemicals Ltd., Maidstone, U.K. The terminal glucose unit of the substrate is blocked by bonding to a substituent, which inhibits cleavage by the aforementioned exoenzymes. 4-Nitrophenol, Standard Reference Material no. 936, was obtained from the U.S. National Bureau of Standards, Gaithersburg, MD.

Other reagents. PIPES and 2-(N-cyclohexylamino)ethanesulfonic acid, ches, were purchased from Sigma Chemical Co.

All reagents used were of analytical grade.

Commercial kits. "Alpha-Amylase PNP" and "Pancrease alpha-Amylase PNP" test kits from Boehringer Mannheim GmbH, Mannheim, F.R.G., were used for determination of total amylase and pancreatic amylase, respectively.

The amylase assay reagent was PIPES buffer (50 mmol/L, pH 7.1) containing, per liter, 1.6 mmol of BpNPG7, >20 kU of alpha-glucosidase, >5 kU of glucoamylase, 50 mmol of sodium chloride, and 1 mmol of calcium chloride. Stored lyophilized the reagent is stable for at least a week at 4°C after reconstitution.

The assay buffer consisted of PIPES buffer (100 mmol/L, pH 7.1) containing 50 mmol of sodium chloride and 1 mmol of calcium chloride per liter.

The washing solution was PIPES buffer (10 mmol/L, pH 7.1) containing 0.5 mL of Tween 20 (polyoxyethylene (20) sorbitan monolaurate) per liter.

The stopping solution was ches buffer (100 mmol/L, pH 10.3).

Standards. The standards were made from purified human pancreatic amylase in bovine serum, adjusted to pH 6.0 with solid PIPES. Enzyme activity of the standards was determined kinetically at 37°C, with the assay reagent described above. One milliliter of sample was mixed with 40 mL of amylase reagent. The molar absorbptivity of 4-nitrophenol at 405 nm was determined to be 9600 L mol⁻¹ cm⁻¹. One unit (1 U) of enzyme activity is defined as the amount of pancreatic amylase catalyzing the hydrolysis of 1 mol of BpNPG7 per minute per liter under the described assay conditions; 1 mol of 4-nitrophenol is released per mole of BpNPG7 (27).

Assay procedure. Pipet 200 mL of assay buffer and 100 mL of standards or samples into 12-mm (diameter) test tubes. Before assay, dilute urine samples with an equal volume of zero standards. Place one antibody-coated bead in each tube. Incubate on a horizontal shaker at room temperature for 30 min. Aspirate the liquid, then wash the beads twice with 3-mL portions of washing solution. Drain and discard all liquid, then add 300 mL of amylase reagent to each bead and incubate for 10 min at 37°C. Pipet 700 mL of stopping solution into all tubes. Measure the absorbance of each test solution at 405 nm and evaluate the pancreatic amylase activity of unknown samples by comparison with a standard curve. For urine samples, correct for the dilution factor.

Results and Discussion

Table 1 shows data on precision of this assay. There was no detectable cross-reaction with salivary amylase (Figure 1). The detection limit is 4 U/L, which with a confidence of 99.7% can be distinguished from the zero calibrator. Analytical recovery ranged from 89 to 109% (mean 99.3%, n = 6), as assessed by adding known amounts of purified pancreatic amylase to patients' samples and assaying them. The analytical range of the assay is 4 to 1000 U/L.

Results of this assay correlated well with total amylase (r = 0.996) for patients with acute pancreatitis, but for 23% of patients with various gastrointestinal disease total amylase gave misleading information, and also for 27% of patients with renal insufficiency. These numbers agree well with earlier reports (5, 6). For the above-mentioned groups of patients, results of the immunocatalytic assay correlated

![Table 1. Precision of the Immunocatalytic Assay of Pancreatic Amylase](attachment:table1.png)

![Fig. 1. Standard curves for the immunocatalytic assay, based on assays of purified preparations of pancreatic and salivary amylases Salivary amylase up to 5000 U/L gave no detectable signal in the assay](attachment:fig1.png)

![Fig. 2. Correlation of immunocatalytic pancreatic amylase assay with total amylase and pancreatic amylase determined by an immunoinhibition assay in patients with gastrointestinal and renal disease The upper reference limits of the assays are indicated by the interrupted lines](attachment:fig2.png)
well with those by a commercial assay involving immunoinhibition of salivary amylase: $r = 0.957$ and 0.946 (Figure 2).

The described immunocatalytic assay of pancreatic amylase is simple to perform, gives final results within 1 h, shows good performance characteristics, and is highly specific for pancreatic amylase in samples from different types of patients.

References


Hypomagnesemia and Low Alkaline Phosphatase Activity in Patients’ Serum after Cardiac Surgery

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Significant decreases in magnesium (Mg) concentration and alkaline phosphatase (ALP; EC 3.1.3.1) activity in serum were seen in patients after cardiac surgery with cardiopulmonary bypass (Group 1), as compared with non-cardiac-surgery patients after general anesthesia (Group 2) or only spinal anesthesia (Group 3). Mean changes for Mg and ALP by the first postoperative day, compared with pre-operative baseline values, were as follows: Group 1: Mg -7.5 mg/L (-38.3%), ALP -46 U/L (-48.4%); Group 2: Mg -3.3 mg/L (-17.4%), ALP -17 U/L (-16.5%); and Group 3: Mg -1.9 mg/L (-10.0%), ALP -15 U/L (-14.0%). The decreases in Mg and ALP observed in post-cardiac-surgery patients appear to be a consequence of the cardiac surgery and the cardiopulmonary bypass pump. Measurement of Mg and ALP in a subgroup of 10 cardiac-surgery patients for 10 days postoperatively showed initial decreases, with gradual recovery to near-normal values by the 10th day. That the changes in Mg and ALP seen postoperatively were not attributable to hemodilution alone was confirmed by measuring total-protein concentrations before and after operation. ALP requires Mg ion in vitro for optimal activity, but addition of Mg in the appropriate amounts to sera with low ALP activity did not restore ALP activity. The low ALP activity seen in post-cardiac surgery patients in vivo may perhaps be related to factors other than Mg that were removed by the cardiopulmonary bypass pump.

Additional Keyphrases: magnesium · alkaline phosphatase · total protein · cardiopulmonary bypass · general and spinal anesthesia

Several investigators (1–4) have noted hypomagnesemia in patients after cardiac surgery—as have we. We have also seen significant decreases in serum alkaline phosphatase.

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