Amylase (AMS)

AMS is a digestive enzyme that functions to hydrolyze glycosidic bonds in polymers of glucose. Two isoenzymes of AMS have been identified: a pancreatic isoenzyme (P-type) and a salivary isoenzyme (S-type). Most general enzymatic assays for AMS measure total activity.

AMS was one of the earliest biochemical markers to be used to provide laboratory evidence of a particular disease - acute pancreatitis. Acute pancreatitis is caused by a blockage of the pancreatic duct or by direct injury to pancreatic tissue (e.g., toxins, inflammation, trauma, etc.). As a result, enzymes, such as AMS, that should be released into the intestine for digestion, back up into the blood stream. AMS activity in the blood stream increases within the first 12 - 24 hours following the onset of acute pancreatitis and reaches its peak level typically by 72 hours. AMS levels return back to normal within 3 - 5 days. Appropriate use of the pancreatic enzymes to diagnose pancreatitis includes repeat studies during the first 24 - 48 hours after admission.

AMS is a relatively small enzyme, small enough to be filtered by the glomerulus and enter into the urine. Thus, during acute pancreatitis, urine levels of AMS will be elevated. Urine levels of AMS have been found to be elevated as long as 7 - 10 days following the onset of acute pancreatitis.

The increased renal clearance of AMS has been found to be useful diagnostically. This is especially true when the urinary excretion of AMS is compared to the relatively constant urinary excretion of creatinine. Thus, in the evaluation of acute pancreatitis, a useful calculation is the amylase:creatinine clearance ratio (ACCR).

The normal reference range for ACCR is 3.0 - 5.0%. In cases of acute pancreatitis, the ACCR is most likely in a range of 6.3 - 13.3%. Regarding the ACCR, Tietz notes: "*In acute pancreatitis, tubular reabsorption of amylase and other proteins is reduced (probably secondary to competition from other low molecular weight proteins) and ACCR is increased; values exceeding 8% are not uncommon. Caution must be exercised when interpreting increased ACCR values because elevations have also been observed in burns, ketoacidosis, renal insufficiency, myeloma, light-chain proteinuria, and march hemoglobinuria....*"

Returning to AMS isoenzymes, both P-type and S-type isoenzymes are present in the blood stream. AMS levels are not specific for just pancreatitis. Lipase, the next enzyme in this unit, is considered the pancreatic enzyme of choice in detecting laboratory evidence of acute pancreatitis. However, AMS was the first marker used for this purpose and remains firmly entrenched in the diagnostic protocols for acute pancreatitis.

Elevated AMS activity may also be due to **macroamylasemia**. In this condition, AMS is bound to immunoglobulin (typically IgG) in serum. The immunoglobulin-AMS complex is too large to be excreted into the glomerular filtrate, thus it remains in the circulation longer and results in increased serum AMS activities, but without signs or symptoms of acute pancreatitis. Although the condition of macroamylasemia is benign, the resulting rise in serum amylase activity may

lead to unnecessary investigation of pancreatic function. Patients with macroamylasemia evaluated with the ACCR will show decreased urine AMS activities with low ACCR. Recall the ACCR is typically increased in acute pancreatitis.

There are two additional general notes regarding AMS.

1. Although there should be absolutely no pipetting by mouth in the clinical laboratory, this is especially true when working with AMS because of the possibility of saliva containing AMS contaminating the measurement system for AMS.
2. Although typically not performed, we would find elevated AMS (S-type) associated with the MUMPS. ↑mumps

Even though we have not focused of the actual assay/substrate used to measure each enzyme, I would like to you to be familiar with the three main starch-based methods that may be used in the measurement of AMS. These methods include: amyloclastic assays, saccharogenic assays, and chromogenic assays. These methods are discussed on page 618 in Tietz.

The following are clinically important characteristics of AMS that affects the laboratory assay and the results obtained by that assay.

- AMS is inhibited by EDTA, oxalate, and citrate.
- AMS has typical stability.
- AMS has no significant interferences.

Lipase (LPS)

The pancreas is the major source of LPS. In contrast to AMS, LPS is not present in the salivary gland. The presence of colipase and bile salt is required for full catalytic activity of pancreatic LPS. Both LPS and colipase are secreted by the pancreas and are present in the blood stream. Colipase is present in the blood of patients with pancreatitis, but in variable concentrations and usually below normal and below the amount needed activate pancreatic LPS fully. To determine accurately and fully the pancreatic LPS activity in patients with pancreatitis, it is essential to add colipase to the reagent.

Following an attack of acute pancreatitis, LPS levels elevate earlier and to a greater extent than do AMS levels. Typically, LPS activity can be found elevated in serum 4 - 8 hours after the onset of pancreatitis, peaks at about 24 hours after onset, and returns back to normal levels within 8 - 14 days. 4-8° onset Peak 24°

LPS, and AMS, are utilized in the diagnosis of pancreatitis. In the past, AMS was the test of choice for the diagnosis of pancreatic disease because, until recently, the precision and accuracy of LPS assays were poor. Measurements of LPS activity have been improved by (1) incorporating colipase in the assay to ensure a sufficient supply of coenzyme and (2) developing defined substrates for the lipase reaction. LPS is now considered to be more sensitive and specific than AMS as a marker of pancreatitis.

The following are clinically important characteristics of LPS that affects the laboratory assay and the results obtained by that assay.

- LPS has no significant inhibitors.
- LPS has typical stability.
- LPS has no significant interferences.

One final note regarding LPS and AMS. Elevation of serum AMS has been associated with diabetic ketoacidosis (DKA). The origin of the elevated AMS level is disputed, but the elevated AMS appears to be of the S-type. In addition, LPS levels are also elevated in DKA. Both AMS and LPS are elevated in DKA without clinical symptoms of pancreatitis – such as abdominal pain. CT exams of the pancreas show a normal organ with no evidence of pancreatitis in these patients. Also, diabetic patients who are under control (i.e., with insulin, diet etc.) and without DKA have normal AMS and LPS levels. Thus, in patients with DKA, elevated AMS and LPS may be present in the absence of pancreatitis.

Miscellaneous Enzymes

Cholinesterases

There are two different cholinesterase isoenzymes. **True cholinesterase** (AChE) is found in high concentration in the CNS. It is also found in erythrocytes, lung, and spleen. AChE functions to metabolize the neurotransmitter acetylcholine. **Pseudocholinesterase** (SChE) is produced by the liver and is found in serum. The normal function of SChE is not known, but it is important in the metabolism of succinylcholine, a muscle relaxant used during surgery. SChE is the isoenzyme we focus on in the clinical laboratory.

SChE is characteristically **decreased** in patients with various types of liver disease including:

- viral hepatitis
- cirrhosis
- metastatic carcinoma
- abscess of the liver
- hepatic congestion of heart failure

In acute hepatitis, SChE is at its lowest level at the peak of the disease. With recovery, it returns to normal. It has been suggested that this enzyme may serve as an index to recovery. SChE, however, is not frequently used in diagnostic enzymology to evaluate liver function.

SChE finds its greatest clinical utility in cases of poisoning with organophosphate insecticides such as *Malathion* and *Parathion*. Organophosphate insecticides are potent inhibitors of the cholinesterases. Both cholinesterases are inhibited by these insecticides, but SChE activity drops more rapidly than AChE activity following exposure. Thus, following insecticide poisoning, we monitor SChE levels. A drop of 40% in serum enzyme activity is typically seen before symptoms of poisoning develop. Typically a drop of ~80% is required before serious neuromuscular affects become apparent. In the clinical laboratory, multiple samples are tested

over a period of time looking for evidence that the activity of SChE is beginning to increase. This finding shows that treatment efforts of the medical staff are beginning to work and the patient is beginning to recover.

The following are clinically important characteristics of SChE that affects the laboratory assay and the results obtained by that assay.

- SChE is inhibited by fluoride, citrate, and oxalate.
- SChE has typical stability.
- Hemolysis should be avoided because of AChE presence in the erythrocyte.

Ceruloplasmin (Cp)

Cp is an α_2-globulin which has copper transport as its primary function. Cp can bind a total of six copper atoms for transport; four are tightly bound and two are loosely bound.

Copper atoms impart a blue color to the protein. Normally this is not a high enough concentration to see, but when a female is on birth control pills, or during pregnancy, the level of Cp doubles. At these Cp levels, the blue color becomes more evident. When this blue color is added to the yellow color of the serum, a green color may develop for the serum.

Cp also has enzymatic activity. This enzymatic activity only develops after Cp has its full complement of copper. When it becomes active, Cp begins to oxidize substrates such as ascorbic acid, epinephrine, and serotonin in the blood stream. The purpose for this is not fully understood.

The most important enzymatic action of Cp is associated with iron metabolism. Here Cp can function as either an oxidant or an antioxidant.

Cp is vitally important in regulating the ionic state of iron, in particular oxidizing Fe^{+2} to Fe^{+3}, which allows iron to be incorporated into transferrin for transport. Thus Cp helps prevent the toxic effects of iron.

The following are clinically important characteristics of Cp that affects the laboratory assay and the results obtained by that assay.

- Cp is inhibited by EDTA. The way that EDTA inhibits the enzymatic activity of Cp is by displacing the 2 loosely bound copper atoms.
- Cp has typical stability.
- Cp has no significant interferences.