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# Protein metabolism. Determination of transaminases

Dietary protein is a vital source of amino acids. Proteins ingested in the diet are digested into amino acids or small peptides that can be absorbed by the intestine and transported in the blood. Another source of amino acids is the degradation of defective or unneeded cellular proteins.

Protein digestion begins in the stomach, where the acidic environment favors protein denaturation. Denatured proteins are more accessible as substrates for proteolysis than are native proteins. The primary proteolytic enzyme of the stomach is ***pepsin***, a nonspecific protease that, remarkably, is maximally active at pH 2. This is the reason that pepsin can be active in the highly acidic environment of the stomach, even though other proteins undergo denaturation there.

Protein degradation continues in the lumen of the intestine owing to the activity of proteolytic enzymes secreted by the pancreas (***trypsin***, ***chymotrypsin***, ***elastase***, *and the* ***carboxypeptidases***). These proteins are secreted as inactive zymogens and then converted into active enzymes. The battery of enzymes displays a wide array of specificity, and so the substrates are degraded into free amino acids as well as di- and tripeptides. Digestion is further enhanced by proteases, such as aminopeptidase N, that are located in the plasma membrane of the intestinal cells. Aminopeptidases digest proteins from the amino-terminal end. Single amino acids, as well as di- and tripeptides, are transported into the intestinal cells from the lumen and subsequently released into the blood for absorption by other tissues.

## Transamination Reactions

Transamination is the major process for removing nitrogen from amino acids. In most instances, the nitrogen is transferred as an amino group from the original amino acid to α-ketoglutarate, forming glutamate, whereas the original amino acid is converted to its corresponding α-keto acid. For example, the amino acid aspartate can be transaminated to form its corresponding α-keto acid, oxaloacetate. In the process, the amino group is transferred to α-ketoglutarate, which is converted to its corresponding amino acid, glutamate.

All amino acids except lysine and threonine undergo transamination reactions. The enzymes catalyzing these reactions are known as **transaminases** or **aminotransferases**. For most of these reactions, α-ketoglutarate and glutamate serve as one of the α-keto acid–amino acid pairs. Pyridoxal phosphate is the cofactor.

Overall, in a transamination reaction, an amino group from one amino acid becomes the amino group of a second amino acid. Because these reactions are readily reversible, they can be used to remove nitrogen from amino acids or to transfer nitrogen to α-keto acids to form amino acids. Thus, they are involved both in amino acid degradation and in amino acid synthesis.

The transaminase enzymes are important in the production of various amino acids, and measuring the concentrations of various transaminases in the blood is important in the diagnosing and tracking many diseases. For example, the presence of elevated transaminases can be an indicator of liver and cardiac damage. Two important transaminase enzymes are **aspartate transaminase (AST)**, also known as **glutamic oxaloacetic transaminase (GOT)**; and **alanine transaminase (ALT)**, also called **alanine aminotransferase (ALAT)** or **glutamate-pyruvate transaminase (GPT)**.

ALT catalyzes the transfer of an amino group from L-alanine to α-ketoglutarate, the products of this reversible transamination reaction being pyruvate and L-glutamate.

ALT is commonly measured clinically as part of liver function tests. When used in diagnostics, it is almost always measured in international units/liter (IU/L) or μkat.

Significantly elevated levels of ALT (SGPT) often suggest the existence of other medical problems such as viral hepatitis, diabetes, congestive heart failure, liver damage, bile duct problems, infectious mononucleosis, or myopathy, so ALT is commonly used as a way of screening for liver problems. Elevated ALT may also be caused by dietary choline deficiency. However, elevated levels of ALT do not automatically mean that medical problems exist. Fluctuation of ALT levels is normal over the course of the day.

Aspartate transaminase catalyzes the interconversion of aspartate and α-ketoglutarate to oxaloacetate and glutamate.

AST is similar to alanine transaminase (ALT) in that both enzymes are associated with liver parenchymal cells. The difference is that ALT is found predominantly in the liver, with clinically negligible quantities found in the kidneys, heart, and skeletal muscle, while AST is found in the liver, heart (cardiac muscle), skeletal muscle, kidneys, brain, and red blood cells. As a result, ALT is a more specific indicator of liver inflammation than AST, as AST may be elevated also in diseases affecting other organs, such as myocardial infarction, acute pancreatitis, acute hemolytic anemia, severe burns, acute renal disease, musculoskeletal diseases, and trauma.

AST is commonly measured clinically as a part of diagnostic liver function tests, to determine liver health. However, it is important to keep in mind that the source of AST (and, to a lesser extent, ALT) in blood tests may reflect pathology in organs other than the liver.

**Optical test**

**Principle**

It is not possible to monitor transaminase reactions directly using the optical test, transaminases having coenzyme pyridoxal phosphate, but the advantages of continuous-monitoring assays can be obtained by coupling the transaminase reactions to specific dehydrogenase reactions. The ketoacid formed in the transaminase reaction are measured indirectly by enzymatic reduction to the corresponding hydroxyacids, and the accompanying change in NADH concentration is monitored spectrophotometrically at 340nm.

The coupled reactions for the two enzymes are:

**<u>For GOT</u>:**

Oxaloacetate, formed in the GOT reaction, is reduced to malate in the presence of malate dehydrogenase (MDH):

$$\text{L-aspartate} + \alpha\text{-ketoglutarate} \xrightleftharpoons{\text{GOT}} \text{oxaloacetate} + \text{glutamate}$$

$$\text{oxaloacetate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{MDH}} \text{malate} + \text{NAD}^+$$

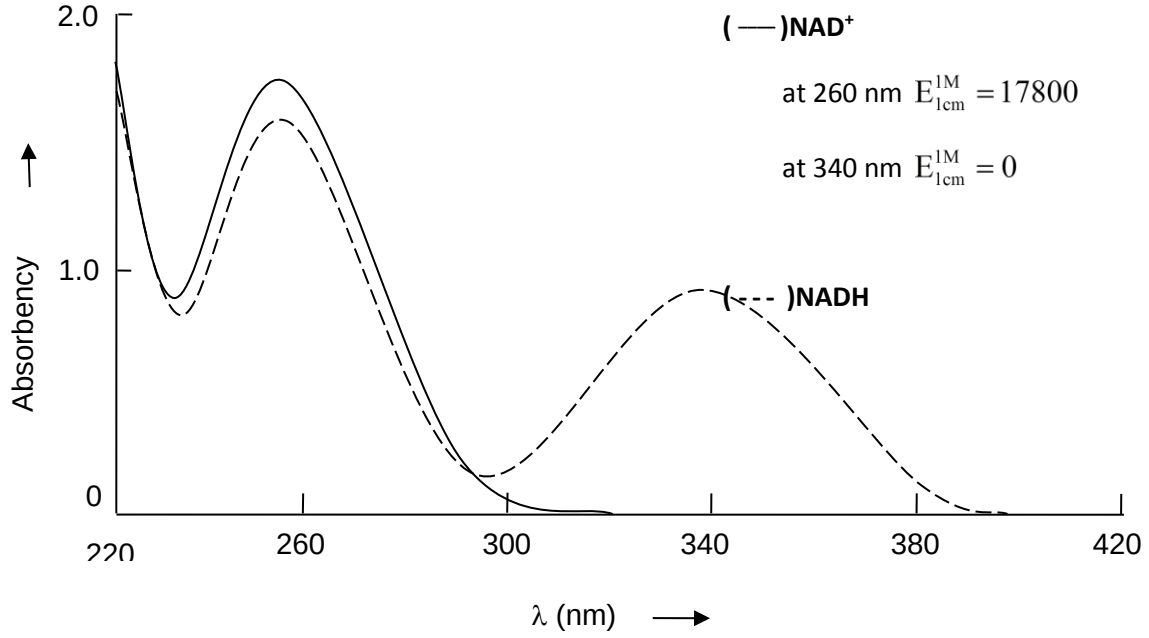
**<u>For GPT:</u>**

Pyruvate, formed in the GPT reaction, is reduced to lactate be lactate dehydrogenase (LDH):

$$\text{L-alanine} + \alpha\text{-ketoglutarate} \xrightleftharpoons{\text{GPT}} \text{pyruvate} + \text{glutamate}$$

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{\text{LDH}} \text{lactate} + NAD^+$$

The substrate, NADH, and the auxiliary enzymes, MDH and LDH must be present in sufficient quantity so that the reaction rate is limited only by the amounts of GOT and GPT respectively. As the reactions proceed, NADH is oxidized to $NAD^+$. The disappearance of NADH is followed by the decrease in absorbance at 340 nm, which is measured for several minutes either continuously or at frequent intervals. The change in the absorbance per minute ($\Delta A/min$) is related directly to micromoles of NADH oxidized and, in turn, to micromoles of substrate transformed per minutes (International Units).




The absorption spectra of $NAD^+$ and NADH

**Working procedure for GOT determination:**

In a cuvette, pipet1000 µl of working reagent and 100 µl of serum. Homogenize by pipetting up and down, incubate 1 minute at room temperature, and then read the absorbance against air at la 340 nm, after 1, 2 and 3 minutes. Calculate ΔE/minute.

**Calculation of enzymatic activity for ASAT (U/L) = ΔE/minute x 1750**

**Normal values:** **- men: <55U/l**
**- women: <35 U/l**.

**Clinical significance**

Transaminases are widely distributed in human tissues. Both GOT and GPT are normally present in human plasma, bile cerebrospinal fluid, and saliva, but none is found in urine unless a kidney lesion is present.

In *viral hepatitis* and other forms of liver disease associated with hepatic necrosis, levels of serum GPT and GOT are elevated even before the clinical signs and symptoms of disease, such jaundice, appear. Activities of both enzymes may reach values as 100 times the upper reference limit, although 20 - 50 fold elevation are most frequently encountered.

Moderately increased levels of GOT and GPT activity may also be observed in *extrahepatic cholestasis.* Levels observed in *cirrhosis* vary with the status of the cirrhotic process: they range from upper normal to some four to five times normal, with the level of GOT activity higher than that of GPT activity. Five- to tenfold elevations of the two enzymes may occurs in patients with primary or metastatic *carcinoma of the liver* with GOT usually being higher than GPT, but levels are often normal in the early stages of malignant infiltration of the liver.

Slight or moderate elevations of both GOT and GPT activities may be observed after intake of alcohol, during delirium tremens, and after administration of drugs such as opiates, salycilates, or ampicillin.

Although serum levels of both GOT and GPT become elevated whenever disease process affects liver cell integrity, GPT is the more liver-specific enzyme. Serum elevations of GPT activity are rarely observed in conditions other than parenchymal liver disease. Moreover, elevation of GPT activity persists longer than do those of GOT activity.

After *myocardial infarction* an increased level of GOT activity appears in serum. GPT levels are within normal limits, or are only marginally increased, in uncomplicated myocardial infarction, since the concentration of GPT in heart muscle is only a fraction of that of GOT. However, GOT is increased in liver damage secondary to heart failure.

GOT and occasionally GPT activity levels are increased in *progressive muscular distrophy and dermatomyositis*, reaching levels up to 8 times normal; they are usually normal in muscle diseases of neurogenic origin. *Pulmonary emboli* can rise GOT levels to two to three times normal, and slight to moderate elevations (2-5 times normal) are seen in *acute pancreatitis, crushed muscle injuries, gangrene, and hemolytic disease.*