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BIOCHEMICAL DIAGNOSIS OF LIVER DISEASE.

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ABSTRACT

It is important that clinicians and laboratorians, including clinical chemists and pathologists, recognize and understand the clinical significance of abnormal liver function tests. The liver regulates many important metabolic functions. Hepatic injury is associated with distortion of these metabolic functions. Hepatic disease can be evaluated and diagnosed by determining serum concentrations of a number of serum analytes. Many serum analytes exist to assist in the biochemical diagnosis of liver disease. The focus of this paper is on the analytes which are associated with hepatic necrosis, cholestasis, defects in excretion and end stage hepatic disease which results in decreased synthetic function. The abnormalities of these serum analytes will be correlated with the important types of liver disease.

KEY WORDS : Liver function tests, albumin, bilirubin, alkaline phosphatase and transaminases.

1. INTRODUCTION

The liver regulates many important metabolic functions (Table 1). Diffuse severe hepatic injury is associated with marked distortion of these metabolic functions and can be evaluated by determining serum concentrations of a relatively small number of analytes (Table 2).

In general, only four serum biochemical tests are necessary to assess hepatic abnormalities (1). These analytes are (1) alanine aminotransferase (ALT) (EC 2.6.1.2) and aspartate aminotransferase (AST), (EC 2.6.1.1), (2) alkaline phosphatase (ALP) (EC 3.1.3.1), prealbumin or prothrombin to measure hepatic synthetic function (Table 3).

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Table 1. Metabolic Functions of the Liver

Metabolism and regulation

- Carbohydrates
- Amino acids and ammonia
- Proteins
- Cholesterol and other lipids

Synthesis

- Urea
- Serum proteins (e.g., albumin, coagulation proteins, carrier proteins, and acute phase proteins)
- Bile acids

Metabolism and degradation

- Proteins
- Drugs
- Hormones
- Endogenous substances (e.g., bilirubin)

The aminotransferases are elevated with acute hepatic cell injury, in contrast to ALP, which is elevated with cholestasis. Serum bilirubin is important in assessing the ability

Table 2. Important Analytes to Evaluate Liver Function

Alanine aminotransferase (ALT) (serum glutamate
pyruvate transaminase [SGPT]}
Alkaline phosphatatase (ALP)
Ammonia
Aspartate aminotransferase (AST) (serum glutamate oxaloacetate transaminase [SGOT]}
Total bilirubin
Unconjugated (indirect)
Conjugated (direct)
γ-Glutamyl transferase (GGT)
Lactate dehydrogenase (LD)
5' Nucleotidase (5' NT)
Pseudocholinesterase
Ceruloplasmin
Alpha feto protein
Bile acids
Ornithine carbamoyltransferase
Carbohydrate deficient transferrin
Fatty acid ethyl esters

Table 3. Information Obtained From Liver Function Analytes

Analyte	Indicator
Aspartate aminotransferase, alanine aminotransferase	Necrosis of liver
Alkaline phosphatase	Cholestasis
Bilirubin	Excretion defects
Pseudocholinesterase or albumin	Synthesis defects

of the hepatic cell to conjugate and excrete an organic anion. Decreased serum albumin signifies decreased hepatic synthetic ability (2).

The pattern of results from the above four tests allows classification of liver disease into the broad categories of (1) acute hepatitis, (2) chronic hepatitis, (3) cirrhosis, (4) cholestasis, and (5) neoplasms, (Table 4). In conjunction with orther clinical parameters, the pattern of liver function test results is useful in classfying the type of liver disease present, following the course of hepatic disease, and predicting prognosis. Each of the four analytes will be correlated to classify each type of liver disease. A diagnostic pattern of abnormalities in these four analytes is extremely useful to establish the diagnostic pattern of abnormalities in these four analytes is extremely useful to establish the diagnosis in patients afflictfed with hepatic disease (3,4).

II. BASIC LIVER FUNCTION TESTS TO IDENTIFY ABNORMALITIES IN HEPATIC SYNTHESIS

Albumin

Albumin is synthesized in the liver. Its half life is approximately twenty days The rate of synthesis is variable depending on nutritional status and age. Albumin is not stored in the liver. The physiologic functions of albumin are to maintain vascular osmotic pressure, transport endogenous and exogenous compounds, and act as a protein reserve. Albumin is a source of amino acids for body tissues. Albumin production correlates with changes in the colloid content of the extracellular space.

Albumin also plays a major role in binding bilirubin, fatty acids, hormones such as thyroxine, triiodothyronine, cortisol and aldosterone. It also binds calcium and magnesium and various drugs such as warfarin and salicylate.

Prealbumin is also synthesized by the liver. It has a half life of two days and is another serum analyte to identify decreased hepatic synthetic function. Prealbumin is also a transport protein. It binds and transports tetraiodothyronine and triiodothyronine.

Table 4. Classification of Liver Diseases

- Hepatitis
  - Acute
    - Acute viral hepatitis, type A
    - Acute viral hepatitis, type B
    - Acute viral hepatitis, type C
    - Hepatitis associated with systemic viral infection
      - Infectious mononucleosis hepatitis
      - Cytomegalic virus hepatitis
    - Alcoholic hepatitis
    - Drug-induced hepatitis
    - Chemical hepatitis
  - Chronic
    - Chronic active hepatitis
    - Chronic persistent hepatitis
- Cirrhosis
  - Alcoholic
  - Following viral hepatitis
  - Following biliary obstruction; secondary biliary cirrhosis
  - Secondary to chronic heart failure
- Cholestasis
  - Intrahepatic
    - Acute
    - Drug-induced or chemical induced
    - Virus-induced
    - Alcohol-induced
    - Associated with neoplastic and other infiltration
    - Recurrent cholestasis of pregnancy
    - Primary biliary cirrhosis
  - Extrahepatic
    - Biliary atresia
    - Choledocholithiasis
    - Stricture
    - Neoplasm
    - Suppurative cholangitis
- Neoplasms
  - Primary
    - Adenoma
    - Hepatoma
  - Secondary metastases from lung, breast, stomach, colon or pancreas

Albumin has more than twenty genetic variants. When genetic variants are present,they can be detected by performing serum protein electrophoresis. This abnormality is termed bisalbuminemia and is characterized by two bands in the albumin area on the electrophoretic plate. An acquired form of bisalbuminemia is caused by the inhibition of migration of albumin molecules on electrophoretic plates by drugs in serum such as penicilin bound to albumin molecules. Analbuminemia is a genetic condition caused by absence of albumin. This condition will result in decreased vascular oncotic pressure with resultant edema.

Decreased serum albumin is caused by many diseases. Hypoalbuminemia is frequently found in end stage liver disease (Table 5). Cirrhosis due to many diseases is associated with diminished numbers of hepatocytes and thus decreased hepatic capacity to synthesize albumin (5). Impaired synthesis also occurs due to diminished protein intake. Portal hypertension in cirrhotic patients will result in sequestering albumin into ascitic fluid in the extravascular space causing further decrease in serum albumin. Various investigations have demonstrated that low serum albumin is an indicator of increased morbidity and mortality in liver disease and other conditions associated with hypoalbuminemia (6).

Table 5. Factors Leading to Hypoalbuminemia in Liver Disease

- Pathogenic Factors
  - Decreased albumin synthesis
  - Malnutrition
  - Abnormalities of albumin distribution (ascites)

Decreased serum albumin is an important indicator of end stage liver disease. Other serum proteins are also decreased in cirrhosis. These include antitrypsin, fibrinogen, ceruloplasmin, transferrin and pseudocholinesterase.

Albumin is decreased in many other conditions other than cirrhosis. Protein losing enteropathy and malabsorption conditions such as chronic pancreatitis or small intestinal malabsorptive diseases such as sprue result in hypoalbuminemia. Nephrotic syndrome caused by diseases such as diabetic nephropathy results in marked proteinuria causing hypoalbuminemia. Severe generalized dermatitis or severe burns will also result in depletion of serum albumin due to denudation of skin. Catabolism of albumin is increased in hyperthyroidism and diseases associated with excessive production or utilization of cortisol.

Many coagulation proteins are synthesized by the liver. A few of the more important ones are II, VII, IX and X. The prothrombin time coagulation test is abnormally prolonged in end stage liver disease because various coagulation proteins produced by the hepatocyte are deficient due to decreased synthesis.

In conclusion, the two analytes which are useful to evaluate decreased synthetic function are serum albumin and the plasma prothrombin time.

Determination of albumin in serum or plasma is usually based on the binding behaviour of the protein with the anionic dyes bromcresol green (BCG) or bromcresol purple (BCP) in a manual or automated procedure. Immunochemical methods such as nephelometry are highly specific and more sensitive. Albumin assay by an immunochemical method does not pose the same problem as immunochemical methods for other specific proteins. Reliable, consistent antisera can be generated because highly purified protein is available, and the pure protein can also be used (7).

III. TESTS OF SUBSTANCES METABOLIZED AND EXCRETED BY THE LIVER

Bilirubin is an orange-yellow pigment produced from protoporphyrin. Biliverdin is a green pigment. The total daily bilirubin production is approximately 300 mg and is derived from the heme moiety of hemoglobin. Bilirubin is useful as an indicator of the excretory ability of the liver. Most of the bilirubin is derived from heme from senescent RBC's by the reticuloendothelial system, while a minor portion is derived from other substances containing heme such as myoglobin. The bilirubin is then transported from the peripheral tissues to the liver.

Two proteins in the hepatocyte, ligandin and Z protein bind the bilirubin. The bilirubin is subsequently conjugated with glucuronic acid to form biliurbin glucuronide, primarily diglucuronide and a minor monoglucuronide, which is excreted by the hepatocyte into the bile ductules and eventually by the extrahepatic bile ducts into the duodenum (8). The bilirubin glucuronides are converted back to unconjugated bilirubin by beta glucuronidase in the small intestine. Subsequently, the unconjugated bilirubin is converted to urobilinogen by anaerobic intestinal bacteria. Twenty percent of intestinal urobilinogen recirculates back to the liver from the intestine through the enterohepatic circulatory system. Approximately five percent then appears in the peripheral blood and is secreted by the kidneys into urine. Approximately eighty percent of the intestinal

urobilinogen is oxidized to stercobilin which is orange-brown and imparts the yellow, orange-brown color to feces. With marked extrahepatic cholestasis the stool color is clay colored (silvery appearance). The normal color of urine is related to the color of urobilin. With marked extrahepatic cholestasis the urine color becomes a darker yellow-orange related to excessive bilirubin glucuronide.

The normal serum total bilirubin is less than 1.2 mg/dl In healthy individuals, almost all bilirubin is unconjugated : conjugated bilirubin is up to about 0.2 mg/dl (Table 6). If the hematocrit is slightly decreased due to anemia other than hemolytic anemia, the total bilirubin may be lower than 1.2 mg/dl due to decreased unconjugated bilirubin. Women who are still menstruating may also have a slight decrease in their total bilirubin due to decreased unconjugated bilirubin. A decreased serum albumin may also result in a slight decrease in total bilirubin due to decreased intravascular transport of unconjugated bilirubin.

Table 6. Differential Diagnosis of Jaundice

Elevated unconjugated bilirubin
- Hemolytic anemia
- Transfusions (especially of stored blood)
- Resorption of hematomas, blood in extravascular spaces

Hepatic dysfunction elevated unconjugated and conjugated bilirubin
- Hepatitis-like picture
  - Halothane anesthesia
  - Drugs, chemicals
  - Shock or heart failure
  - Infection with hepatitis viruses
- Cholestatic picture
  - Hypotension
  - Drugs, chemicals
  - Sepsis in liver

Extrahepatic obstruction of elevated conjugated bilirubin
- Bile duct injury
- Choledocholithiasis
- Acute pancreatitis

IV. ETIOLOGY OF INCREASED UNCONJUGATED BILIRUBIN

The major cause of increased serum unconjugated bilirubin is hemolytic anemia (Table 7). Hemolysis results in increased serum unconjugated bilirubin in the absence of liver disease. The hemolysis of sickle cell disease can also be associated with a slight increase in conjugated bilirubin due to infarction of the liver. The neonate has an increased serum unconjugated bilirubin. This elevation is known as physiologic jaundice. It results from increased hemolysis of erythrocytes and immaturity of the liver in premature neonates causing decreased ability to conjugate bilirubin.

Table 7. Differential Diagnosis of Elevated Unconjugated Bilirubin

- Gilbert syndrome
- Crigler-Najjar syndrome
- Hemolytic anemia
- Resolving hematomas
- Transfusion of blood that is old and has a short survival
- Hemorrhagic pulmonary infarcts

Two genetic diseases are associated with increased serum unconjugated bilirubin. Gilbert's syndrome is associated with decreased hepatic UDP glucuronyltransferase or with defects in hepatic membrane transport of unconjugated bilirubin. A clinical test for Gilbert's syndrome is to request the Gilbert's individuals to reduce their caloric intake for 48 hours. The serum unconjugated bilirubin increases. This occurs because during fasting metabolic products of

fatty acids being utilized for calories are preferentially conjugated by the liver cell UDP glucuronyltransferase.

The Crigler-Najjar syndromes are classified as Type I and Type II. The Type I is associated with complete absence of hepatic UDP glucuronyltransferase while in the Type II the enzyme is present but reduced in amount. Both of these syndromes are associated with increased serum unconjugated bilirubin. The infant afflicted with the Type I syndrome may die due to destruction of the basal ganglia by unconjugated bilirubin which has an affinity for the basal ganglia lipid moieties.

V. ETIOLOGY OF INCREASED CONJUGATED BILIRUBIN

Cholestasis connotes a static or slowing of the excretion of bile. Any complete or partial obstruction of the biliary system will result in decreased ability to excrete conjugated bilirubin (Table 8). The biliary obstruction may be intrahepatic involving the bile canaliculi or bile ductules or extrahepatic due to obstruction of bile duct such as the common bile duct or the ampulla of Vater.

Table 8. Differential Diagnosis of Elevated Conjugated Bilirubin.

Cholestasis, intrahepatic and extrahepatic
Postoperative intrahepatic and extrahepatic cholestasis
Recurrent intrahepatic cholestasis during pregnancy
Dubin-Johnson syndrome
Rotor syndrome

Intrahepatic cholestasis may be caused by drugs, viruses, alcohol and chemical agents. Drugs such as the phenothiazines and oral contraceptives have the propensity to induce intrahepatic cholestasis. Alcohol and viral hepatitis damage the bile canaliculi and bile ductules and result in intrahepatic cholestasis with an increase in conjugated bilirubin. Another cause for intrahepatic cholestasis is inflammation of the interlobular bile ductules in the portal triads. Fibrosis may then ensue and result in sclerosing cholangitis. Autoimmune primary biliary cirrhosis or non-Hodgkin's lymphomas may also obstruct bile ductules in the portal triads and cause an increase in the serum conjugated bilirubin. Primary hepatomas and metastatic carcinomas involving the liver may obstruct larger intrahepatic bile ducts with subsequent increase in the serum conjugated bilirubin.

Extrahepatic obstruction of the bile duct system with a greater increase in serum conjugated bilirubin than is found in intrahepatic cholestasis occurs in carcinomas of the large bile ducts such as the Klatskin bile duct carcinoma, carcinoma of the head of the pancreas or ampulla of Vater, choledocholithiasis or strictures of the common bile duct secondary to inflammatory lesions or injury from surgical procedures.

Recurrent cholestasis of pregnancy occurs during pregnancy resulting from intrahepatic cholestasis caused by the steroid hormones of pregnancy on the biliary canaliculi. In addition, in the Dubin-Johnson syndrome, conjugated bilirubin excretion is impaired. Alkaline phosphatase is not increased in the serum. The increase in conjugated bilirubin is triggered by pregnancy or utilization of oral contraceptives.

Whenever acute hepatitis is present caused by alcohol, drugs or viruses, both unconjugated bilirubin and conjugated bilirubin are increased in serum. The elevated unconjugated bilirubin is caused by hepatic cell injury and impaired conjugation of bilirubin. Unconjugated bilirubin may also eventually increase due to chronic cholestasis resulting in hepatocellular injury. The cholestasis results in the elevation of conjugated bilirubin. These agents cause prominent swelling of hepatocytes compressing bile canaliculi and bile ductules.

Marked increases in conjugated bilirubin occurs in complete obstruction of the common bile duct. Conjugated bilirubin can be excreted by the kidney into urine. However, if renal failure ensues, for example in hepatorenal syndrome, there will be a pronounced elevation in serum bilirubin due to decreased renal clearance of conjugated bilirubin.

VI. PROCEDURES TO DETERMINE TOTAL AND CONJUGATED BILIRUBIN

The total bilirubin clinical chemistry method utilizes a detergent to prevent protein precipitation. The diazo reagent 2,5-dichlorophenyldiazonium tetrafluoroborate (DPD) reacts very rapidly coupling with bilirubin under acidic conditions. The intensity of the color of the azobilirubin produced is proportional to the total bilirubin concentration and can be measured photometrically.

Total bilirubin + DPD (acidic condition) = Azobilirubin.

The method for the determination of conjugated bilirubin utilizes the following assay : Acidified sodium nitrite produces nitrous acid, which reacts with sulfanilic acid (in acidic solution) to form a diazonium salt. The diazotized sulfanilic acid then reacts with bilirubin to form isomers of azobilirubin. In the conjugated bilirubin assay, only conjugated bilirubin is converted by the diazotized sulfanilic acid. The intensity of the red color of azobilirubin is measured photometrically and is proportional to the conjugated bilirubin concentration (9).

VII. LIVER FUNÇTION TESTS ASSESSING HEPATIC CELL INJURY UTILIZING ENZYMES. ASPARTATE AMINOTRANSFERASE (AST) AND ALANINE AMINOTRANSFERASE (ALT)

AST and ALT are aminotransferases that catalyze the interconversion of amino acids and alpha-oxo-acids by transfer of amino groups. AST (EC 2.6.1.1) was previously known as SGOT and ALT (EC 2.6.1.2) was known as SGPT. AST is present in the cytoplasm of the hepatic cell and is known as C-AST and is also present in the mitochondria and is known as M-AST. In contrast, ALT is only located in the hepatic cell cytoplasm. In all amino transfer reactions the alpha-oxo-glutarate and L-glutamate couple serves as one amino group acceptor and donor pair (Table 9).

Table 9. Locations of Enzymes in Liver

Cell membrane	: ALP, GGT, NTP
Mitochondria	: M-AST
Cytoplasm	: LD, ALT, AST
Canaliculi	: ALP, GGT, NTP

Pyrodoxal-5'-phosphate and its amino analogue, pyridoxamine phosphate function as coenzymes in the amino transfer reactions. The

pyridoxal 5'-phosphate is bound to the enzyme and participates as a true prosthetic group. The IFCC expert panel specifies that pyridoxal-5'-phosphate be added in the clinical chemistry laboratory to function as the coenzyme. Transamination plays an important role in intermediary metabolism because it functions in the synthesis and degradation of amino acids in the endoplasmic reticulum of the cell. The three amino acids, glutamic acid, aspartic acid and alanine can be transaminated to their ketoacids which then enter the citric acid cycle and become a source of energy.

ALT and AST are present in liver, heart, skeletal muscle, kidney, in high activity with lesser amounts in many other organs (Table 10). AST is a dimer of two identical subunits. The molecular mass of the holoenzyme from human liver is 93,000 daltons. M-AST is also a dimer with a molecular mass of 90,400 daltons (Table 11).

Table 10. Tissue Activities of Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) Relative to Serum

Tissue	ALT	AST
Liver	2750	7100
Kidney	1188	4550
Heart	444	7800
Skeletal muscle	300	4950
Pancreas	125	1400
Spleen	75	700
Lung	44	500
Erythrocytes	4	8
Normal serum	1	1

Table 11. Biochemistry of Cytoplasmic and Mitochondrial Asparate Aminotransferase

	Cytoplasmic	Mitochondrial
Heat stability	Poor	Good
Inhibition by phosphate	No	Yes
Serum half-life of enzyme	Long	Short
DEAE chromatography with 8 mmol/L phosphate buffer, pH 7	Retained	Eluted
Present in normal serum	Yes	<12% of total
Electrophoretic mobility	Anodal	Cathodal

Reference ranges have been determined for serum ALT and AST on individuals between the ages of six and 99 years. A nadir occurs at ages 15 to 20 for both enzymes. ALT and AST peak activities occur at ages 30 to 40 for men and ages 50 to 60 for women. Obese men and women have higher values due to fatty livers. AST and ALT are eliminated without degradation. Both are found in bile but there may be other routes of elimination. The half life of ALT in serum is 50 hours while AST has a half life of 12 hours.

The transminases serve as liver function tests which reflect necrosis of the hepatic cell. If the serum transaminases are abnormal, liver disease must be part of the differential diagnoses.

ALT and AST are present in hepatocytes in large amounts relative to serum. The AST activity in hepatocytes is about three times that of ALT. AST is present in both the cytoplasm and the mitochondria in contrast to ALT which is only present in the cytoplasm. Both ALT and AST are useful in establishing the presence of liver cell

injury of any cause. In general, ALT is the more sensitive test in acute and obstructive liver diseases, whereas AST is more sensitive in chronic and infiltrative lesions (10).

Drugs, viruses, alcohol and chemicals are able to cause necrosis of the hepatocyte (11). The permeability of the hepatic cell membranes increase and degeneration of the cell ensues. In acute viral hepatitis, ALT levels are higher than AST levels. In acute hepatocellular injury, AST and ALT activity in excess of forty times the upper reference limit is not unusual. An activity higher than twenty times the upper reference limit, approximately 1000 u/L is indicative of acute viral hepatitis, drug induced liver injury (e g., acetaminophen) or ischemic hepatic injury. The serum aminotransferase levels gradually rise for one to two weeks before the patient becomes jaundiced. Within a few weeks, the aminotransferase activity returns to normal accompanied by normalization of bilirubin. Persistent elevation longer than two weeks in viral hepatitis is a sensitive indicator of the development of chronic viral hepatitis. Hepatocellular damage with necrosis is decreased in chronic viral hepatitis and the changes in aminotransferase activities are usually moderate.

Permanently elevated levels of the aminotransferase indicate chronic active or chronic persistent viral hepatitis. Elevation of AST significantly above the upper reference limit is a very poor prognostic sign in chronic active hepatitis. This should be confirmed by a liver biopsy. Cirrhosis without active viral hepatitis, alcohol abuse, drugs or chemical hepatitis is not associated with aminotransferase elevation in serum. Elevations of the aminotransferases in patients suffering from cirrhosis only occur if viral activity is persistent or active or with active alcohol abuse, or if there is persistent chemical or drug hepatocellular injury.

The De Ritis ratio AST to ALT is important in identifying certain etiologic factors causing hepatitis (12). An AST/ALT ratio of two is characteristic of alcoholic hepatitis. This pattern is also seen in severe ischemic hepatic injury or severe hepatotoxic drug injury. Acute or chronic viral hepatitis does not give this pattern. ALT is greater than AST in general in viral hepatitis.

Moderately increased aminotransferase serum elevations are occasionally found in cholestasis. The activity of AST and ALT rarely exceeds a two fold increase above the upper reference limit. This may result from regurgitation of AST and ALT from bile in cholestasis since ALT and AST are normally present in bile or increased synthesis of the enzymes.

In summary, hepatic necrosis due to viral hepatitis and toxic drugs may cause a twenty to fifty fold elevation in the serum transaminases. Peak values occur between the seventh and twelfth days with values returning to normal by the third to fifth week if recovery is uneventful.

Alcoholic hepatitis causes transaminases to increase up to 300 IU with AST greater than ALT (Table 12) due to release of M-AST (13). Levels greater than 300 IU suggest the presence of alcoholic rhabdomyolysis and/or alcoholic cardiomyopathy. In viral hepatitis ALT is higher than AST. AST and ALT are usually normal in patients with cirrhosis. Elevations of

AST and ALT in cirrhosis indicate continuing hepatocellular necrosis (14).

Table 12. Changes Specifically Associated with mAST

Any disease effecting or causing abnormal mitochondrial function, which initiates release of mAST
- Acute alocholic hepatitis
- Reye's syndrome
- Acute diminished hepatic perfusion
- Shock liver or congestive heart failure

Chemicals or drugs-methotrexate, acetaminophen and CCL_4 increase both total and mAST

Hepatomas and metastatic carcinomas of the liver cause minimal increase in AST and ALT in the early stages of malignant liver infiltration. If the malignancy causes extensive necrosis, five to ten fold elevations of both enzymes may occur.

Serum elevations of AST and ALT may be observed in conditions other than hepatic necrosis. Serum AST is increased in most patients with acute myocardial infarction, particularly transmural infarcts. AST was the first enzyme used in the diagnosis of acute myocardial infarction; it is now obsolete and cost ineffective. Myocarditis usually increases the serum AST. Patients with congestive heart failure have increased AST, which is attributed to hepatic congestion and necrosis.

AST is present in high activity in skeletal muscle, and the ALT activity is about one-fourth of the AST activity. In disorders of skeletal muscle, the enzymes of clinical importance are creatine kinase, pyruvate kinase, aldolase, AST and ALT. ALT and AST are of somewhat lesser clinical importance due to their lower clinical sensitivities. In Duchenne oystrophy, the transaminases are increased from about six to ten times normal; in the more benign dystrophies like Becker dystrophy, limb-girdle, fascioscapulohumeral and ocular dystrophies, the increases in ALT and AST are substantially less, and the values may be normal (15). Accidental trauma and major surgery increase serum enzymes in most patients. CK is the most sensitive test, AST less so, and ALT is the least sensitive. Rhabdomyolysis, or destruction of skeletal, muscle tissue, is nearly always accompanied by increases in CK, AST and myoglobin. Important causes are trauma, alcohol, seizure disorders and physical exertion (16, 17).

Obese individuals have an increased AST if there is coincident fatty change in the liver. In the absence of alcoholism and diabetes mellitus, the likelihood of severe liver damage in obesity is slight. Since the transaminases are widely distributed in the body, increases in acute illnesses that affect the liver, heart, skeletal muscle or kidney are not surprising.

The almost universally used clinical chemistry reaction scheme to determine ALT is as follows:

L-alanine + P5P enzyme complex $\rightarrow$
P5P enzyme complex+ pyruvate
α-ketoglutarate + P5P enzyme complex $\rightarrow$
P5P enzyme complex + L-glutamic acid
pyruvate + NADH + $H^+ \rightarrow$ L-lactate + NAD^+

The decrease in absorbance with time is followed at 340 nm.

ALT is a very stable enzyme; its values do not change in 24 hours, even when left in contact

with the clot at room temperature. ALT is stable for at least one week at 4° C.

For AST, the very widely used reaction scheme is as follows :

L-aspartate + P5P enzyme complex →
P5P enzyme complex + oxaloacetate

α-ketoglutarate + P5P enzyme complex →
P5P enzyme complex + L-glutamic acid

oxaloacetate + NADH + H^+ + MDH →
L-malate + NAD^+

As for ALT, the reaction rate is monitored at 340 nm. AST is stable for at least 24 hours at 4^0 C and AST for 48 hours at 25^0 C even in contact with the clot.

VIII LIVER FUNCTION ENZYME TESTS DEMONSTRATING CHOLESTASIS

Enzymes released from biliary and hepatocyte plasma membranes, canalicular membrane, endothelium of hepatic veins or enzymes synthesized at an increased rate in cholestasis.

IX ALKALINE PHOSPHATASE (ALP)

Four separate gene loci are responsible for encoding for human alkaline phosphate isoenzymes. The four human ALP isoenzymes are (1) tissue-nonspecific found in liver, bone, and kidney; (2) placental; (3) intestinal, and (4) placental-like or germ cell. The tissue nonspecific gene is located at the tip of the short arm of chromosome 1, while the placental, intestinal and placental-like or germ cell ALP genes are found in a cluster on the tip of the long arm of chromosome 2. Routinely, the usual alkaline phosphatase isoenzymes identified by serum electrophoresis in the clinical laboratory are liver, bone, placenta, and intestine. However, multiple ALP isoforms may be derived from the liver when cholestasis is present. These isoforms are the high-molecular-mass ALP, and ALP lipoprotein-X complex (18, 19). Alkaline phosphatase (ALP) designates a group of phosphate esterases having low substrate specificity. Many phosphate esters can serve as substrates including nonphysiological substances such as p-nitrophenyl phosphate (20). Liver ALP has a molecular mass of 92,400 daltons. The bone ALP molecular mass is 94,800 daltons. ALP from all body sources is a zinc containing glycoprotein with a serine residue at the active center.

ALP is an ubiquitous enzyme and is present in the cell membranes of organs that exhibit high absorptive or excretory capacity. These are the epithelial cells of renal tubules, the brush border cells of the small intestine, the cells of the placental villi, membranes lining bile canaliculi, cells lining liver sinusoids, salivary epithelial cells, breast duct epithelial cells and osteoblasts. The organs with the highest ALP activities are placenta, small intestine, bone, kidney, and liver. ALP is invariably bound to cell membranes or microsomes (21). After disruption, ALP is present in the sediment containing membrane fragments. The function of ALP is to catalyze the transfer of a phosphate moiety between a donor and acceptor molecule. ALP facilitates the transfer of metabolites such as lipids, proteins, and carbohydrates across cell membranes. ALP hydrolyzes phosphate esters, synthesizes

phosphate esters and is essential in calcification of bone. The half life of hepatic ALP in serum is approximately seven days as is placental ALP. Bone ALP has a half life of two days while intestinal ALP has a half life in serum of minutes.

Day to day changes of 4% on serum ALP activity in normal persons occurs. Confinement to bed, prolonged fasting and minimal physical activity do not affect serum ALP. The postprandial increase of ALP in blood type O and B secretor individuals is intestinal ALP. In normal pregnancy ALP increases in the third month and doubles by the ninth month. ALP remains elevated for up to one month after delivery (22). Diseases associated with increased serum ALP are cholestatic hepatic conditions, benign and malignant osteoblastic lesions of the bone, pulmonary or renal infarction acute pancreatitis, and neoplastic ectopic production by malignant tissues (carcinoplacental ALP, Regan and Nagao enzymes) (Table 13).

When the serum ALP is significantly decreased below the lower limit of the reference range, the differential diagnoses include (1) congenital hypophosphatasemia (the converse of familial hyperphosphatasemia), in which infants and children are severely affected by arrested bone growth and have a propensity for fracture of multiple bones, (2) zinc deficiency; (3) magnesium deficiency; (4) hypothyroidism; and (5) vitamin B_{12} deficiency (Table 14) (23, 24).

The condition familial hyperphosphatasemia of childhood is associated with an elevated serum ALP in healthy children who do not have hepatic or bone diease. They have a genetic familial propensity for excessive tissue production of ALP.

Table 13. Pathologic Lesions of Various Organs Associated with Elevated Serum Alkaline Phosphatase Level

Liver
- Cholestatic lesions

Bone
- Osteoblastic lesions

Heart
- Organization of infarct
- Cardiac failure

Lung
- Organization of infarct

Pancreas
- Acute pancreatitis

Gastrointestinal
- Giant peptic ulcer
- Erosive or ulcerative lesion of small intestine in malabsorption
- Acute infarction of small intestine
- Erosive ulcerative lesions of colon

Kidney
- Organization of acute infarction

Neoplastic ectopic production
- Regan isoenzyme
- Nagao isoenzyme
- Hepatoma isoenzyme
- Kasahara isoenzyme

Table 14. Decreased Serum Alkaline Phosphatase

Congenital hypophosphatasia
Pernicious anemia-decreased vitamin B12
Hypothyroidism
Zinc deficiency
Malnutrition
Magnesium deficiency

The ALP serum elevation is exhibited by other family members. The intestinal ALP isoenzyme accounts for a large part of the elevated serum ALP in this genetic condition. Liver, bone and kidney isoenzymes also are found in the serum of these individuals. Transient hyperphosphatasemia occurs in children and rarely in adults and causes marked elevation of ALP for up to three months. The ALP elevation consists

of liver and bone isoenzymes. The etiology may be due to a viral infection (24).

An increase in serum ALP activity is usually associated with either physiologic causes or pathologic lesions of liver and/or bone. Physiologic causes include pregnancy (ALP derived from the placenta), normal bone growth in children (ALP derived from bone osteoblasts), and the intestinal ALP isoenzyme present in serum in healthy individuals with blood type O and B who are secretors of H-blood group substance and who are postprandial (25-28).

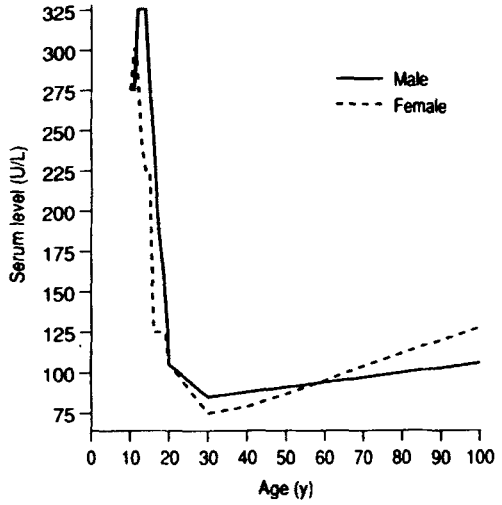

Fig. 1. Age-and sex-related upper limits for serum alkaline phosphatase.

Increased serum ALP associated with liver disease is caused by intra- or extrahepatic cholestasis and some destruction of hepatic cell membranes.

Biliary ALP is associated with hepatic cell membrane fragments; an increase of biliary ALP is usually accompanied by an increase in gamma glutamyltransferase and 5'-nucleotidase. These enzymes are also bound to the hepatocyte membrane Biliary ALP is increased in serum in cholestasis. Elevation of serum ALP is observed in patients who have some form of extrahepatic or intrahepatic bile duct obstruction. Any mechanism that impedes the excretion of ALP in bile will result in regurgitation of the enzyme into the circulation via the hepatic sinusoids. The increased serum ALP present in patients with liver diseases closely resembles the ALP that can be extracted from liver. Cholestasis stimulates the synthesis of ALP by the bile ductule cells, providing more ALP, which ultimately enters the blood. The amphiphilic nature of bile salts facilitates the release of ALP from its membrane bound sites and entry into blood

The magnitude of the ALP elevation is of some help in the differential diagnosis of hepatobiliary disorders. The highest ALP activities are found in patients with primary or secondary liver malignancy and in patients with extrahepatic cholestasis. Intraphepatic cholestasis caused by alcoholic hepatitis, viral hepatitis, chemical hepatitis or drug hepatitis may cause an elevation of ALP two times or three times above the upper limit of the reference range in contrast to ALP serum elevations of five to ten times above the upper limit of the reference range in complete extrahepatic obstruction as is found in cancer of the extrahepatic bile ducts or choledocholithiasis or carcinoma of the head of the pancreas.

Routinely, the usual ALP isoenzymes identified by serum electrophoresis in the clinical laboratory are derived from the liver, bone, placenta and intestine. However, multiple ALP isoforms may be derived from the liver when cholestasis is present. These isoforms are high-molecular-mass ALP and ALP lipoprotein-X complex (Fig. 2).

Table 15. Types of Hepatic Diseases Associated with Elevated Serum Alkaline Phosphatase Level

Two fold increase
- Acute viral hepatitis
- Acute drug or chemical hepatitis
- Acute alcoholic hepatitis
- Acute fatty liver
- Shock liver or congestive heart failure

Five fold increase
- Drug cholestatic hepatitis
- Carcinoma of head of pancreas
- Choledocholithiasis
- Primary biliary cirrhosis
- Hepatoma or metastatic carcinoma

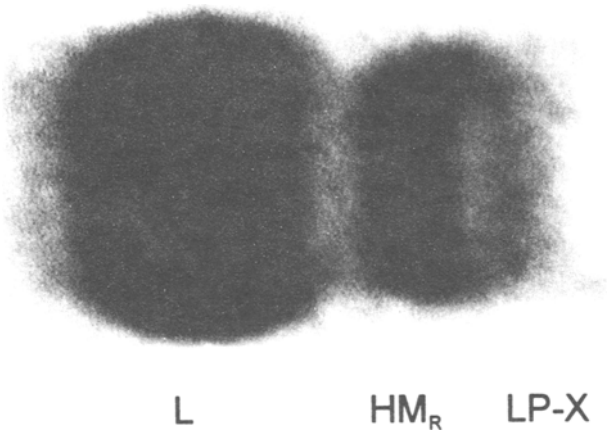

Fig. 2 Electrophoresis of serum in a patient with colon cancer metastatic to the liver, demonstrating 3 ALP hepatic isoforms liver ALP (L), high-molecular-mass (HMr) ALP and ALP LP-X complex (LP-X). Agarose gel and P-toluidinium 5 - bromo - 4 - chloro - 3 - indolyl phosphate substrate.

The high-molecular-mass ALP or fast liver is derived from the liver cell plasma membrane. The enzyme is anchored in the plasma membrane by means of a covalent linkage to membrane phospholipid moieties called glycosyl-phosphatidylinositols, the ultrafast ALP isoform or ALP lipoprotein-X is a complex of ALP with lipoprotein receptors on the hepatic cell plasma membranes. When high-molecular-mass ALP and ALP lipoprotein-X are found in serun the patient's condition is cholestasis due to malignancy affecting the liver or alcoholic, drug, heart failure ischemic or viral hepatitis. An abnormally increased ALP intestinal isoenzyme may be an indicator of end stage chronic liver disease, particularly cirrhosis or primary liver cancer.

The sensitivity of ALP in hepatobiliary disorders makes it an excellent screening test, but its nonspecificity requires more definite procedures, for example, sonography, computerized tomography, isotope uptake studies, biopsy, and so on, to make specific diagnosis.

Serum bone ALP mirrors the activity of osteoblasts (29). Thus in bone growth, healing of a fracture, rickets or Paget's disease, serum ALP is increased. In patients with increased osteoblastic activity, an increased bone ALP isoenzyme can usually be demonstrated, and the isoenzyme is especially prominent during periods of osteogenesis. Increases in bone ALP occur in patients with osteomalacia produced by vitamin D deficiency, in hyperparathyroidism, in acromegaly, in various metabolic diseases involving bone, and in post menopausal osteoporosis. ALP activity provides phosphate for calcification in bone production. If only bone lytic activity occurs without bone production, serum ALP will not be elevated such as in bone lysis by osteoclasts in multiple myeloma (30). Since ALP is present in the pancreas, acute pancreatitis may cause an increase in serum ALP. ALP is normally present in vascular endothelial cells. Thus, granulation tissue

associated with lung infarcts may cause an increase in serum ALP. Renal tubules contain ALP wich may be released into serum as a result of renal infarction. ALP is present in the mucosa of the gastrointestinal tract. Large necrotic lesions on the gastrointestinal mucosa may result in minimal increaee in serum ALP (Table 16).

Table 16. Chemical Properties of Normal Alkaline Phosphatase Isoenzymes

	ALP Isoenzymes				
Property	Biliary	Hepatic	Bone	Placenta	Intestine
Heat stability inhibition	Unstable	Unstable	Very unstable	Stable	Unstable
L-Phenylalanine inhibition	Weak	Weak	Weak	Strong	Strong
L-Homoarginine inhibition	Moderate	Strong	Strong	Weak	Weak
L-Leucine inhibition	Weak	Moderate	Moderate	Weak	Weak
Urea inactivation	Strong	Strong	Very strong	Moderate	Moderate

Neoplastic ectopic production by malignant cells may cause increases in serum ALP as is found in carcinoplacental ALP, e.g., Regan or Nagao isoenzymes (31). Fishman was the first to describe an unusual ALP isoenzyme ("Regan") found in a patient with bronchogenic carcinoma; it is an isoenzyme that has proven to be chemically and functionally similar to placental ALP (32,33). One difference is the greater sensitivity of the placental isoenzyme to urea inhibition, which suggests differences in the tertiary structure. The Regan isoenzyme is more sensitive to urea inhibition than the pregnancy placental form thus suggesting differences in their tertiary structure.

Two other cancer - associated ALP isoenzymes are the Nagao and Kasahara forms. The Nagao isoenzyme was found in a patient with pleural carcinoma; it resembled the Regan isoenzyme in its heat stablity, electrophoretic migration, and precipitation with antiplacental ALP antibody. It differed from the Regan form by being more sensitive to inhibition by L-phenylalanine or leucine. The Kasahara ALP, first found in a patient with hepatoma, migrated

Table 17. Chemical Properties of Tumor-Associated Alkaline Phosphatase Isoenzymes

	ALP Isoenzymes			
Property	Hepatoma	Kasahara	Nagao	Regan
Heat stability	Unstable	Stable	Very stable	Very stable
L-Phenylalanine inhibition	Moderate	Moderate	Strong	Strong
L-Homoarginine inhibition	Weak	Weak	Weak	Weak
L-Leucine inhibition	Weak	Moderate	Moderate	Weak
Urea inhibition	Moderate	—	—	Strong
EDTA inhibition	Strong	—	Strong	Weak

much further toward the anode than the Regan or Nagao forms (Table 17) (34, 35).

Patients with hepatoma generally have a heat-sensitive ALP in serum that is different than the three cancer-associated ALP isoenzymes described above, and is different from normal liver ALP. This ALP is heat sensitive, but resistant to inactivation by L-homoarginine, which inhibits normal liver ALP.

A reference method for ALP has been described, which was the culmination of elaborate investigation. The reaction conditions were devised to provide a method that was suitable for routine use. P-Nitrophenyl-phosphate in an alkaline transphosphorylating buffer such as diethylamino-ethanol or 2-amino-2-methyl-1-propanol is the recommended substrate. Agreement on temperature is not complete; some prefer 30° C and others 37° C (36).

A popular substrate is p-nitro-phenylphosphate (PNPP). This rate of ALP activity is enhanced if amino alcohols are used as buffers. An excellent one is 2-methyl-2-aminopropanol-1 (MAP), as is diethanolamine (DEA), which is popular in Europe. The amino alcohol enhances sensitivity and decreases reaction times.

A continuous-monitoring procedure uses PNPP as the substrate and is colorless. Alkaline phosphatase releases the phosphate, and p-nitro-phenol (PNP) forms; PNP is colorless in the acid form. However, with an alkaline pH, it is changed to p-nitrophenoxide and assumes a quinoid structure that is very yellow. The rate of yellow color formation is a measure of the reaction and is monitored spectrophotometrically.

ALP electrophoresis offers a sensitive, easy and reproducible method to identify ALP liver isoforms (Table 18). In the future, specific monoclonal antibodies to these isoforms may be useful to clinically evaluate these serum ALP isoforms, without the use of electrophoresis. Before electrophoresis, agarose agar gels are equilibrated for approximately 30 minutes in a buffer containing a non-ionic detergent (Triton X 100), which allows the serum ALP to penetrate the agarose gel. In addition the gel is also equilibrated with an anionic detergent (sodium salt 1-heptane sulfonic acid) which imparts a similar charge to the ALP isoenzymes permitting them to be separated according to their individual molecular weights. Ten microliters of serum are applied to the gels. Electrophoresis is performed at 150V for 30 minutes, followed by incubation with the substrate for 30 minutes. The gel is next rinsed in distilled water and scanned on a densitometer.

To improve the electrophoretic separation of the ALP isoenzymes and to allow better quantitative estimation of the densitometric scans, the following procedures are needed to improve resolution of the isoenzymes (37-43).

Table 18. Methods for Alkaline Phosphatase Isoenzymes

Physical	Heat
Chemical	L-phenylalanine, other amino acids, 3M urea
Column Chromatography	
Specific Antibody	Placental : Intestinal bone
Electrophoresis	Acetate Agarose Polyacrylamide
Bone separation is accelerated by adding wheat germ lectin	
Electrofocusing	

1 Add wheat germ lectin to the buffer prior to electrophoresis. Final concentration lectin 50mg/L prepared in distilled water a 5 g/L (139 mol/L aqueous solution of wheat germ lectin available as a lyophilized powder). The wheat germ lectin buffer mixture is used to soak the support media membrane. This procedure exploits the difference in carbohydrate moieties of bone and liver ALP. Wheat germ lectin retards bone ALP much more severely than liver ALP and allows better separation of these isoenzymes.

2. Add 5 μl of Cetavlon-diethyl ether to 20 μl of serum sample to extract the ALP - lipoprotein-X complex. The ether extract is discarded and the aqueous phase is used for electrophoretic investigation.

3. Heat stability test to differentiate liver ALP, bone ALP, intestinal ALP and placental ALP. Heat 100 μl of serum sample at 56° C for 10 minutes. The placental isoenzyme is over 90% stable, the liver and intestinal isoenzymes are 60-70% stable and the bone isoenzyme is less than 10% stable. The heat test is especially important in electrophoresis to inactivate the bone isoenzyme.

4. Add 10 microliters of neuraminidase 2u/l to 50 microliters of serum sample, and incubate for 12 hours at 37° C to cause desialylation and improve electrophoretic resolution of the bone and liver ALP and the intestinal variant isoenzymes.

5. Add L-phenylalanine (10mmole/L) to the gel, to inhibit intestinal ALP and intestinal variant ALP, with no inhibition of liver and bone ALP isoenzymes.

X. 5 PRIME NUCLEOTIDASE

5 Prime nucleotidase (EC 3.1.3.5) NTP is an enzyme that is ubiquitous in many tissues but is especially useful in the assessment of cholestatic disease (44, 45). The enzyme is present on the plasma membrane of the hepatic cell similar to ALP. The enzyme releases inorganic phosphate from nucleoside-5-prime phosphates such as adenosine-5-prime phosphate. Its pH optimum is at 7. The upper normal level is 12 U/L. Minimal elevations occur in conditions causing intrahepatic cholestasis. Extrahepatic lesions may cause serum elevations five times normal such as choledocholithiasis or carcinomas of the extrahepatic bile ducts. The serum elevation of 5 prime nucleotidase resembles that of ALP. 5 Prime nucleotidase elevations persist longer than ALP. Osteoblastic bone disease does not cause elevated levels of 5 prime nucleotidase. Thus, when serum ALP is increased the usual interpretation is that either cholestatic liver disease or osteoblastic bone disease is present. The determination of 5 prime nucleotidase is useful to differentiate cholestatic liver disease from osteoblastic bone disease.

5 Prime nucleotidase has a hepatobiliary isoform and primarily a high molecular mass isoform. Nickel ions are used in the clinical chemistry procedure to precipitate the high molecular isoform. The substrate used in the clinical chemistry laboratory is adenosine-5-prime-phosphate. This substrate may also be hydrolyzed by nonspecific alkaline phosphatases. 5-Prime-nucleotidase is inhibited by nickel ions and ALP is not inhibited. In the clinical chemistry procedure, serum is incubated with adenosine-5 prime-phosphate with and without nickel ions.

After 30 minutes, the amount of inorganic phosphate liberated is determined. Phosphate which is produced in the absence of nickel equals the combined activities of ALP and 5 prime nucleotidase while that produced in the presence of nickel represents the activity of ALP. The difference between the two values of phosphate is equal to the activity of the 5 prime nucleotidase in the serum. Manganese is a useful activator of 5 prime nucleotidase.

XI GAMMA GLUTAMYLTRANSFERASE

Gamma glutamyltransferase (GGT) (EC 2.3.2.2) transfers the gamma glutamyl group from peptides and compounds that contain it to an acceptor (46-50). GGT functions as an amino acid transferase and is an ubiquitous enzyme. It is primarily found in hepatobiliary tissues. It also is present in high amounts in renal tubules and in lesser amounts in the mucosa of the small intestine, pancreas and prostate. Most of GGT is located in cell membranes where its function is to transport amino acids and peptides into the cell across cell membranes.

Since GGT is present in the cell membranes of the hepatobiliary system, it is an extremely sensitive enzyme to identify cholestatic disease both intra and extrahepatic cholestasis. GGT is more sensitive than ALP in detecting cholestatic hepatic disease. Twice to five times normal values may occur in viral hepatitis. Fatty livers and drug induced hepatic cholestasis also cause twice to five times normal levels. Higher increases of GGT occur in extrahepatic cholestasis caused by carcinomas of bile ducts or choledocholithiasis. Since the enzyme is present in the pancreas, pancreatitis and pancreatic carcinoma also cause increases in serum GGT. Likewise renal tubular injury causes serum GGT elevations (Table 19).

Table 19. Nonhepatobiliary Causes of Increased Serum γ-Glutamyl Transferase

Drugs	Magnitude of Increase*
Anticoagulants (e.g., coumarin)	Slight
Antihyperlipidemics (clofibrate)	Slight
Oral contraceptives (estrogens)	Slight
Analgesics (e.g., acetamenophen)	Moderate
Anticonvulsants (e.g., phenytoin)	Moderate
Antidepressants (tricyclics)	Moderate
Barbiturates	Moderate
Alcohol	Moderate to marked
Disorders	
Diabetes mellitus	Slight
Hyperthyroidism	Slight
Kidney diseases	Slight
Neurologic disorders	Slight
Obesity	Slight
Pulmonary diseases	Slight
Rheumatoid arthritis	Slight
Hyperlipidemia	Slight to moderate
Myocardial injury	Slight to moderate
Exocrine pancreatic disease	Moderate to marked
Malignancy	Slight to marked

*Slight increase, serum GGT < 2 x upper reference limit (URL); moderate, > 2 and < 5 x URL; marked, > 5 x URL.

One of the main advantages of determining GGT is that it is not present in bone. Thus, if ALP is elevated, the determination of GGT is useful to differentiate the etiology of ALP elevation, since serum GGT is not increased in bone disease. GGT is present in vascular endothelium. Minimal elevations occur in patients who are resolving acute myocardial infarcts. Infarcts hepatic ischemia following heart failure resulting from an accute myocardial infarct may also account for increased serum GGT in patients suffering from acute myocardial infarction due to

heart failiure.

GGT is present in seminal fluid and in the prostate This accounts for higher normal serum levels in males. Serum GGT is increased in patients suffering from carcinoma of the prostate. An important reason why physicians who treat alcoholics request serum GGT is that alcohol is a hepatic enzyme inducer, alcohol is toxic to hepatic microsomes and induces enzyme production. It remains elevated with the duration of persistent alcohol abuse. Barbiturates and anticonvulsants such as pheytoin, acetaminophen and tricyclic antidepressants also stimulate hepatic enzyme induction (51-53). Diseases other than hepatobiliary conditions, pancreatic, prostatic and renal conditions may also cause serum GGT increases. These include diabetes mellitus, hyperthyroidism and obesity (54).

The method for determination of GGT utilises L gamma-glutamyl-p-nitroaniline as the substrate with glycylglycine serving as the gamma glutamyl residue acceptor. The buffer is tris and glycylglycine. The increase in absorbency is monitored at 405nm with p-nitroaniline formation at a temperature of 37°C; the IFCC reference method employs L-gamma-glutamy1-2 carboxy-4-nitroaniline as the substrate and glycylglycine as the acceptor. The temperature is 30°C, pH 7.90. The formation of 5-amino-2 nitrobenzoate is monitored at 410nm.

XII OTHER LIVER FUNCTION ANALYTES

Ammonia

Plasma ammonia is useful for diagnosis, screening and follow up of patients with hepatic encephalopthy (Table 20) (55,56). The most common cause for an increase in plasma ammonia is end-stage liver disease, resulting in a defective hepatic urea cycle and inability of the liver to transform ammonia to urea. Plasma ammonia is derived from several sources. Although some ammonia is derived from skeletal muscle metabolism, most of the ammonia is produced from bacterial metabolic action on protein in the intestine. The ammonia is transported to the liver via the portal vein where it is converted to urea.

Table 20. Etiology of Elevated Plasma Ammonia

Cirrhosis
Reye syndrome
Genetic urea cycle disease
Genetic urea cycle disease
- Ornithine transcarbamylase deficiency
- Carbamyl phosphate synthetase deficiency
- Arginosuccinate synthetase deficiency

Organic acidemias
- Methylmalonic acidemia
- Propionic acidemia

Elevated plasma ammonia levels in neonates and children can be caused by a number of genetic disorders including urea cycle enzyme deficiencies such as carbamoyl-phosphate synthetase or ornithine transcarbamylase deficiency. Plasma ammonia also may become elevated in patients with certain congenital organic acidemias, notably methylmalonic and propionic acidemia. Increased plasma ammonia is associated with development of hepatic encephalopathy in which there is decreased mental capacity and eventually stupor, coma, and death. Patients with hepatic disease have a higher risk for developing hepatic coma when various complications occur, such as gastrointestinal hemorrage, metabolic alkalosis,

and hepatorenal syndrome. Plasma ammonia increases in patients with gastrointestinal hemorrage because of the production of ammonia by bacterial action on the bllod protein and absorption of the ammonia from the gastrointestinal tract. Hypokalemia, which leads to metabolic alkalosis, causes increases in production and absorption of ammonia. Renal failure caused by acute tubular necrosis or hepatorenal syndrome leads to excessive urea in the gastrointestinal tract. Bacterial action converts the urea to ammonia which then is absorbed, leading to elevated plasma ammonia.

The clinical chemistry procedure to determine serum ammonia is in the reaction catalyzed by glutamate dehydrogenase (GLDH), ammonia reacts with the alpha-ketoglutarate and NADPH to form glutamate and NADP (57).

Alpha-ketoglutarate + NH_4 + NADPH + GLDH -> L-Glutamate + $NADP^+$ + H_2O

The inclusion of ADP in the reaction mixture causes an acceleration of the rate of coversion and stablizes the GLDH in the indicatd pH range. The amount of NADPH oxidized during the reaction is equivalent to the amount of ammonia in the specimen and can be measured phtometrically by the resulting decrease in absorbance.

XIII LACTATE DEHYDROGENASE (LD)

Although LD (EC 1.1.1.27) is not considered to be a liver function test, elevation of serum LD occurs in various types of liver disease, especially primary or secondary malignancies. Elevation of LD may occur in alcoholic, viral or drug and chemical hepatitis while LD in cirrhosis and cholestasis is usually normal. Serum LD should not be considered a specific test of liver function since damage to other organs, such as heart, lung, pancreas, kidney, prostate, skeletal muscles, and hematopoietic and lymphoreticular system, is frequently associated with serum LD elevations, (58, 59). The biochemical pattern of elevated LD, increased unconjugated bilirubin, and normal ALP is consistent with hemolytic disease. When serum LD is elevated as a result of hepatic disease, the most likely diagnosis is hepatic malignancy. Hepatocellular injury caused by infiltrating malignant cells leads to an increase in the LD-5 isoenzyme. In hepatic disease associated with malignancy, another cause for an elevated LD, particularly the LD-2 or LD-3 isoenzymes, is the release of LD from the malignant cells. Isoenzyme LD-6 has been identified in associated with hepatic disease caused by severe ischemic disease due to congestive heart failure or shock (Fig.3) (60, 61).

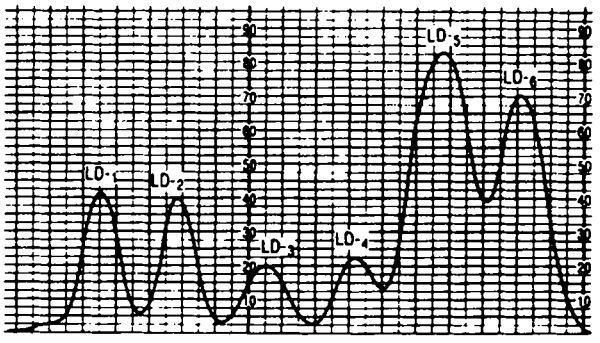

Fig. 3. Lactate dehydrogenase electrophoresis; flipped LD-1 to LD-2, increased LD-5, and presence of LD-6. Acute myocardial infarction with congestive heart failure with liver congestion with fatal outcome. Presence of LD-6 indicated a poor prognosis.

LD-6 may represent alcohol dehydrogenase derived from necrotic central lobular hepatocytes (62). The clinical chemistry procedure to determine the concentration LD is:

$$\text{L-lactate} + \text{NAD}^+ \leftarrow \text{LD} \rightarrow \text{pyruvate} + \text{NADH} + \text{H}^+$$

NAD and lactate are converted in equimolar amounts at the same rate. The rate at which NADH is formed is determined by an increase in absorbance at 340 nm and is directly proportional to enzyme activity.

XIV CERULOPLASMIN

Ceruloplasmin is a metalloprotein synthesized in the liver and released into the blood after the incorporation of copper. In Wilson's disease, an inherited metabolic disorder, tissue copper concentrations in the liver are usually markedly increased, but serum levels of ceruloplasmin and copper are decreased (63). Even when other liver function tests are normal, the most constant biochemical findings in Wilson's disease are decreases in serum ceruloplasmin and copper. Hypoceruloplasminemia can be seen in patients with nephrotc syndrome, cirrhosis, kwashiorkor, tropical sprue, and in infants with Menke's syndrome. These four conditions can be easily differentiated from Wilson's disease. Urine copper levels are increased in Wilson's disease. Ceruloplasmin is increased in serum in pregnancy, and use of oral contraceptives due to stimulated hepatic synthesis by estrogen since ceruloplasmin is an acute phase protein. Serum ceruloplasmin increases in serum with acute and chronic inflammatory illness, autoimmune diseases and malignancy (64). Ceruloplasmin is a blue protein (65). When it is elevated the color of serum becomes green (66). The blue protein combines with the yellow colored analytes to cause a green color. The clinical chemistry procedure to determine the concentration of ceruloplasmin is an immunoassay utilizing a nephelometer procedure for specific protein analysis.

XV ALPHA FETOPROTEIN (AFP)

α-Fetoprotein is a fetal globulin that appears between albumin and α_1-antitrypsin on electrophoresis. (Table 21) Normally AFP is produced by the fetal liver, gastrointestinal tract, and yolk sac. It reaches a maximum concentration in serum at approximately 12 to 15 week's gestation, then decreases to an undetectable concentration at about five weeks after birth.

Table 21. Alpha-Fetoprotein (AFP)

Glycoprotein produced in fetal liver and yolk sac
Replaced by albumin postpartum
Marker for hepatocellular carcinoma
Screening tool in Southeast Asia and Southern Africa, etc.
Increased in endodermal sinus cancers of testis, ovary

In most patients with primary carcinoma of the liver (hepatoma), the tumor cells revert to the fetal function of synthesizing and secreting AFP. Therefore, AFP is useful as a marker for hepatoma and for following the course of the disease. Markedly increased adult serum concentrations occur in hepatocellular carcinoma and endodermal sinus tumors of the testis and ovary. Moderate to slight or transient increases in AFP may be detected in other liver diseases such as alcoholic liver disease, cirrhosis,

hepatitis and tyrosinemia. The clinical chemistry procedure to determine AFP is an immunoassay utilizing specific antibodies to AFP. (67, 68).

XVI SMOOTH MUSCLE AND ANTIMITOCHONDRIAL ANTIBODIES

Patients with chronic active hepatitis may develop autoimmune antibodies that are directed against smooth muscle. These antibodies are usually of the IgG type and less frequently of the IgM type. Measuring anti-smooth muscle antibodies has become an important serologic test for the detection of chronic active hepatitis with 40-70% of the patients showing a positive test. About 50-70% of patients with primary biliary cirrhosis test positive, whereas only 28% of those with cryptogenic cirrhosis test positive. Antimitochondrial antibodies are also present in plasma of patients with primary biliary cirrhosis as well as in those with chronic active hepatitis. Immunoassys are utilized to determine serum concentrations of smooth muscle and antimitochondrial antibodies. Indirect immuno-florescent procedures are also useful.

XVII BILE ACIDS

The primary bile acids, cholic, (a trihydroxy acid) chenodeoxycholic (a trihydroxy acid), are synthesized in the liver. They are conjugated with glycine and taurine forming glycocholates and taurocholates before entering the gallbladder for storage. After a meal, the primary bile acids enter the intestinal lumen where they emulsify fats yielding a greater surface area for pancreatic lipase action. They also promote the aggregation of free fatty acids released by fat breakdown. The micelles thus formed maintain a concentration gradient of free fatty acids allowing rapid absorption. Secondary bile acids are formed by bacterial deconjugation and chemical alteration of the conjugated bile salts; deoxycholic and lithocholic acids are two prominent forms. Both the primary and secondary bile acids are reabsorbed completely from the bowel by passive and active reabsorton into the enterohepatic pathway where they are re-extracted from the blood by the hepatocytes and re-excreted into the bile. Bile salt metabolism is so well controlled that the total body content of 4 g may be cycled twice during a meal. Hepatic bile salt uptake and serum bile salt concentrations are sensitive indicators of hepatocellular dysfunction and decreased or altered blood flow in the enterohepatic pathway.

Postprandial specimens for measuring bile acids are superior to specimens drawn during fasting because of the endogenous loading of the system after eating. Exogenous loading, that is, infusing bile acids extravenously and then determining hepatic removal capability, also is used as a liver function test. Bile acid tests are not used extensively as a liver function test. The reference interval for total bile acids in serum is 3-30 mg/L (0.8 μmol/L) (about 0.8 g/dl is excreted in the feces. Total bile acids are increased in acute, chronic, and alcoholic hepatitis, cirrhosis, and cholestasis. One method used to differentiate excretory blockage from hepatocellular jaundice is to determine the trihydroxy / dihydroxy bile acid ratio. In excretory blockage, the conjugation to the trihydroxy form continues to occur, thus a great amount of the trihydroxy form is found than in hepatocellular jaundice. The clinical chemistry procedures for determining serum bile acids are GLC, HPLC and immunoassays (69, 70).

XVIII ORNITHINE CARBAMOYLTRANSFERASE (OCT)

Ornithine carbamoyltransferase catalyzes the second enzymatic reacton in the urea cycle. The enzyme is located almost exclusively in the liver mitochondria. In addition to its presence in liver, OCT is found in the intestinal mucosa and leukocytes at 1% of the activity of liver OCT. Perhaps because of initial difficulties in assaying the enzyme, OCT measurements are usually not included in the standard battery of liver function tests. Serum OCT activity, however, is both specific and sensitive for hepatocellular injury and can be quantitated enzymatically. The upper reference value in serum is about 20 μ/L. Elevated serum OCT may be found in acute viral hepatitis, liver necrosis, obstructive jaundice, cirrhosis, metastatic liver carcinoma, and congestive heart failure with ischemic liver disease. Transient elevations have been reported in women taking oral contraceptives during the sixth and ninth months after initiation of contraceptive use.

Deficiency of OCT has been reported as a primary inborn metabolic defect transmitted as a sex-linked dominant trait. The presentations of males and females with OCT deficiency differ. Typically, homozygous males have little or no hepatic OCT activity (0-2% of normal), and hyperammonemia develops within hours to days after birth, with death occurring within several weeks. A few rare cases of late onset manifestations (up to 8 years of age) of OCT deficiency have been reported in males, suggesting a pathologic mechanism secondary to hepatocellular lesion. In heterozygous females and in those rarely reported cases of late-onset males, the severity of the symptoms of OCT deficiency depends of the number of functional hepatocytes. The signs and symptoms are secondary to hyperammonemia and include vomiting, drowsiness, acute encephalopathy, failure to thrive, developmental retardation, convulsions, headache, irritability, ataxia, slurring of speech, and altered consciousness. Orotic aciduria occurs secondary to the defect in the urea cycle.

XIX NEW TESTS FOR ALCOHOL ABUSE WHICH MAY RESULT IN HEPATIC INJURY CARBOHYDRATE-DEFICIENT TRANSFERRIN

Carbohydrate-deficient transferrin (CDT) is currently a valuable biological marker available for diagnosis of sustained alcohol abuse. In well-defined populations of alcohol abusers (>60 g of alcohol dialy for 7-10 consecutive days) and sex, race and age-matched control nondrinkers, sensitivity and specificity of CDT are >80% and 90%, respectively. However, CDT tests that perform very well in a highly selected sample perform less acceptably in more heterogeneous groups such as those encountered in primary care or the general population. CDT is elevated in alcohol abuse causing disease. The damaged liver is not able to glycosylate transferrin (71-73). Various procedures are available to determine CDT. These include isoelectric focusing, immunoblotting, HPLC, turbidmetric immunoassay and laser densitometry.

XX FATTY ACID ETHYL ESTERS

The possibility exists that fatty acid ethyl esters (FAEEs), esterification products of fatty

acids and ethanol, are mediators of ethanol-induced organ damage (74,75). Individuals who died while acutely intoxicated were found to have both FAEEs and the enzyme responsible for their synthesis predominantly in the organs damaged by ethanol abuse. The highest concentrations of FAEEs and FAEE synthetase were found in the liver and the pancreas. This is in contrast to the organ distribution of oxidative products of ethanol metabolism such as acetaldehyde, which are generated primarily in the liver. In addition, FAEEs have been implicated as mediators of ethanol-induced organ damage in several *in vitro* studies. They have been shown to decrease the rate of cell growth and protein synthesis in intact Hep G2 cells, to disrupt oxidative phosphorylation in isolated mitochondria, and to increase the fragility of isolated pancreatic lysosomes.

Several biological markers of alcohol intake other than FAEEs have been identified and studies. γ-Glutamyl transferase and mean corpuscular volume are two such markers. However, the sensitivity and specificity of these tests to detect ethanol ingestion have been unsatisfactory. These values are unlikely to be abnormal in individuals ingesting small amounts of ethanol infrequently, unlike plasma or serum FAEEs and ethanol, which are detectable after a single episode of modest ethanol intake. Another biological marker for excess alcohol consumption is carbohydrate-deficient transferrin (CDT). Ingestion of more that 60 g of alcohol per day for seven to ten consecutive days produces increased concentrations, making CDT unsuitable as a short-term marker of ethanol intake. It has limited value as a longer-term marker of ethanol intake in moderate drinkers who do not ingest ethanol on a daily basis. The advantages of plasma or serum FAEEs as a biological marker in comparison to currently available indicators are high sensitivity for ethanol ingestion and utility as both a short-term and a longer-term marker for ethanol intake. FAEEs can be quantitated by GC-mass spectrometry.

XXI INTERPRETATION OF TEST PATTERNS

When liver function is evaluated, the four most important test groups re (1) the transaminase enzymes, AST and ALT, which are increased in patients whith hepatic cell necrosis, (2) ALP increased in cholestasis, (3), total, conjugated (direct), and unconjugated (indirect) bilirubin. Conjugated bilirubin is increased in defective excretory function. Increased levels of conjugated (direct) bilirubin indicate hemolytic anemia or decreased conjugating ability of hepatic cells caused by hepatic injury or inherited defects, such as Gilbert syndrome. (4) Decreased pseudocholinesterase activity, decreased levels of albumin or transferrin, and prolonged prothrombin time indicate hepatic synthetic capacity.

XXII HEPATITIS

Viral, alcoholic, drug, chemical, anoxic necrosis, congestive heart failure or shock.

Viral Hepatitis

In acute hepatitis, serum ALT is greater than AST with both frequently exceeding 1000 IU/L. (Table 22) The ALP activity is minimally increased, usually up to 200 IU/L-300 IU/L. Unconjugated bilirubin is increased owing to hepatocellular damage and conjugated bilirubin is

increased owing to intrahepatic cholestasis caused by hepatic cell swelling. Serum albumin is usually normal. In a patient with fatal acute viral hepatitis that is fulminant, AST and ALT may decline rapidly to normal or below normal because of marked hepatic necrosis and cellular exhaustion of enzymes. This type of patient has persistent or increasing jaundice. In acute hepatitis that is improving, AST, ALT and bilirubin decline concomitantly.

Table 22. Serological hepatitis markers

HA-Ab-IgM	Antibody in early disease
HA-Ab-IgG	Antibody in late disease
HBsAb	Surface antibody
HBsAg	Surface antigen
HBcAb-IgM	Core antibody early disease
HBcAb-IgG	Core antibody late disease
HBeAg	Be antigen. Only do if HBsAg(+)
HC-Ab	Antibody test
HCV	RNA copies
Delta Ab	Antibody. Only do if HBsAg(+)

In chronic persistent hepatitis, AST and ALT activity vary between 40-200 IU/L with ALP activity increased minimally. Serum bilirubin is usually below 3 mg/dl. Both conjugated and unconjugated bilirubin are increased and total albumin is minimally decreased. In chronic active hepatitis, the levels of AST and ALT are frequently above 300 IU/L with minimal increases in ALP activity. In contrast to chronic persistent hepatitis, total bilirubin is above 3 mg/dl. In chronic active hepatitis both conjugated and unconjugated bilirubin are increased. Serum albumin is often decreased and the immunoglobulins are increased.

Alcoholic Hepatitis

Ethanol causes release of M-AST from injured hepatic cell mitochondria resulting in serum AST activity greater than ALT. The total level of AST usually does not exceed 300 IU/.L but if it does, concomitant alcoholic rhabdomyolysis or alcoholic cardiomyopathy and congestive heart failure with ischemic damage of the liver should be considered. Serum ALP is minimally increased, usually up to 300 IU/L. Unconjugated bilirubin is increased owing to hepatocellular damage and conjugated bilirubin is increased owing to intrahepatic cholestasis caused by hepatic cell swelling. Serum albumin is usually normal but can be decreased due to poor nutrition.

Drug induced and chemical hepatitis

Some drugs cause hepatic cell necrosis, whereas others primarily cause cholestasis. For example, acetaminophen and isoniazid (INH) cause hepatic necrosis; in contrast, phenothiazines and oral contraceptives cause cholestasis. Drugs causing hepatic cell necrosis gives rise to abnormalities in AST and ALT that resemble those found in alcoholic hepatitis with AST exceeding ALT results. Similarly, ALT is minimally increased, and unconjugated and conjugated bilirubin elevations occur. Drugs that are associated with cholestasis cause a greater elevation of ALT and conjugated bilirubin and less elevation of AST and ALT than drugs that cause necrosis.

Anoxic necrosis, congestive heart failure or shock liver

With congestive heart failure, hepatocellular injury occurs especially in the central lobular zone of the liver. All the laboratory test

abnormalities as described under alcoholic hepatitis are present in ischemic hepatitis, but the total level of AST may exceed 1000 IU/L. The pattern includes elevation of AST and ALT greater than ALT due to release of M-AST which may be marked, elevation of ALP, normal to minimal increase in bilirubin, and decreased albumin. Elevation of LD activity occurs with the infrequent appearance of the LD-6 isoenzyme in seriously ill patients. In patients with severe cardiovascular disease and associated congestive heart failure, LD-6 is a sign of a very serious hepatic circulatory disturbance (Fig 3). Some investigators believe that LD-6 is not a true LD isoenzyme. They contend that anoxia of the hepatic lobule results in release of hepatic cell alcohol dehydrogenase into the circulation.

Cirrhosis

Cirrhosis or end-stage liver disease can be caused by alcohol, viruses, and chemicals, or ischemia or by a combination of these causative factors. The major laboratory abnormality in cirrhosis is a decrease in analytes related to hepatic cell synthesis. If the etiologic factor is persistent, other analytes, such as those of AST, ALT, ALP, unconjugated and conjugated bilirubin, become increased.

Cholestasis - obstructive disease

The two major types of cholestasis are intrahepatic and extrahepatic. Intrahepatic cholestasis can occur with or without major obstruction to bile flow. Drugs and chemicals such as the phenothiazines; viruses such as hepatitis A, B or C; alcohol; ischemia and malignant infiltrates may cause hepatic cell swelling or may directly affect the biliary canaliculi resulting in intrahepatic cholestasis. In addition, intrahepatic cholestasis affecting the portal triads include congenital bile ductule atresia, infiltration by malignant cells such as leukemia or lymphoma, and autoimmune destruction of the bile ductules in primary biliary cirrhosis. Pregnancy and Dubin-Johnson syndrome affect biliary canaliculi and may induce recurrent intrahepatic cholestasis.

Extrahepatic cholestasis may be caused by congenital atresia of the extrahepatic bile ducts, gallstones obstructing the common bile duct (choledocholithiasis), carcinoma of the common bile duct or ampulla of Vater, suppurative cholangitis and carcinoma of the head of the pancreas. With cholestasis, the main abnormalities in liver function tests are an increase in serum conjugated bilirubin and ALP. The conjugated bilirubin level is dependent on the degree of completeness of biliary obstruction. Extrahepatic biliary obstruction, if complete, may result in levels of conjugated bilirubin above 20 mg/dl while intrahepatic cholestasis may cause an increased conjugated bilirubin between 1 mg/dl to 10 mg/dl. Alkaline phosphatase is about two to three times normal in intrahepatic cholestasis, in marked contrast to extrahepatic obstruction where ALP activity is about five to ten times normal. Cholestasis causes a mild elevation of AST and ALT because of regurgitation of AST and ALT into the systemic circulation. In addition, biliary obstruction results in increased synthesis of AST and ALT by the hepatic cells. Unconjugated bilirubin and albumin are usually in normal range but may become abnormal if biliary cirrhosis occurs.

Neoplasms - Space Occupying Lesions

Malignant lesions may be focal or they may diffusely infiltrate the liver. Frequently, hepatomegaly is present without the presence of clinical or biochemical jaundice. Conjugated bilirubin may increase minimally; however, ALP, GGT and 5'-NTP are markedly elevated. Elevation of these enzymes may reach 10-15 times normal. The pattern of normal bilirubin and a moderate to markedly elevated ALP indicate neoplastic or other infiltrative lesions of the liver (76) (Table 23). With slow infiltration of the liver by malignant cells the uninvolved liver tissue is able to excrete the bilirubin into the bile ductules. Enzymes such as ALP and GGT, however are released from the hepatic cell membranes into the blood circulation. If the neoplasm obstructs a major hepatic or bile duct, the patient will become jaundiced with an elevation of conjugated bilirubin, a pattern consistent with advanced cholestasis. Elevation of ALP and a normal bilirubin suggests liver metastases, or other infiltrates, however, this pattern of dissociation of normal bilirubin and ALP elevation is also found in the hepatic disorder caused by congestive heart failure In this condition, AST and ALT are elevated and albumin is decreased. Lactate dehydrogenase is moderately to markedly elevated in liver malignancy and congestive heart failure. Release of LD occurs from injured liver cells and the malignant cells.

Table 23. Causes of increased Serum Alkaline Phosphatase and Lactate Dehydrogenase Activities with Normal Serum Bilirubin

Congestive heart failure
Myocardial infarction without congestive heart failure
Liver infiltration (from carcinoma, and myeloproliferative and lymphoproliferative conditions)
Fracture plus hematoma
Pulmonary embolism and pulmonary infarction
Chronic renal failure
Malabsorption syndrome

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