liver
Tests

MEDICAL GRAND ROUNDS

Southwestern Medical School

September 26, 1985

STANDARD LIVER FUNCTION TESTS

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INTRODUCTION

Liver function tests (LFTs) are obtained from enormous numbers of patients each day. They have been incorporated into screening panels of biochemical analyses that seek to define the presence or absence of disease. Interpretations of the results of these tests err commonly insofar as their significance is both over-valued and under-valued. This review is written with the hope that an understanding of the mechanisms involved in each of these tests will enhance their clinical utility.

In essence, the usual LFTs (serum bilirubin, serum alkaline phosphatase [SAP], and serum aminotransferase [SGOT/SGPT or AST/ALT]) are not (except for serum bilirubin) liver function tests at all. Recognition of this has led a number of hepatologists to embark on a rather pedantic quest to popularize other more specific terminologies to describe this constellation of analyses. Their efforts in this, an age of the diminutive, have failed and LFTs remain LFTs.

Over the years, a broad array of true liver function tests have been investigated and proposed as means by which the presence of liver disease, its severity, its progression over time and its response to therapy could be measured more specifically. Galactose elimination test, maximum urea synthesis rate, bromsulphthalein sodium (BSP), storage (S) and transport maximum (Tm), aminopyrine breath test, and bile salt clearance test all measure different aspects of liver function and truly warrant the term liver function test. They are variably complicated and cumbersome to perform, however, and none has become widely used, either as a screening test or even as a selective test once liver disease has been defined. Despite their demonstrable imperfections in terms of both sensitivity and specificity, the standard LFTs continue to be the mainstay for the recognition and evaluation of liver disease.

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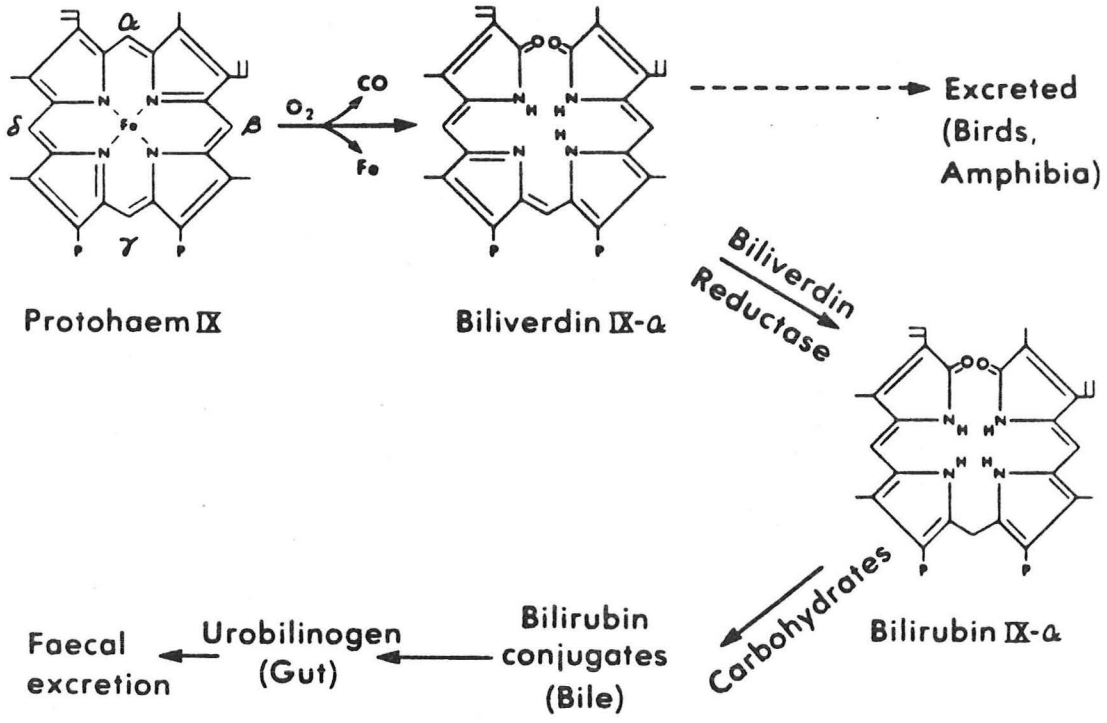
SERUM BILIRUBIN

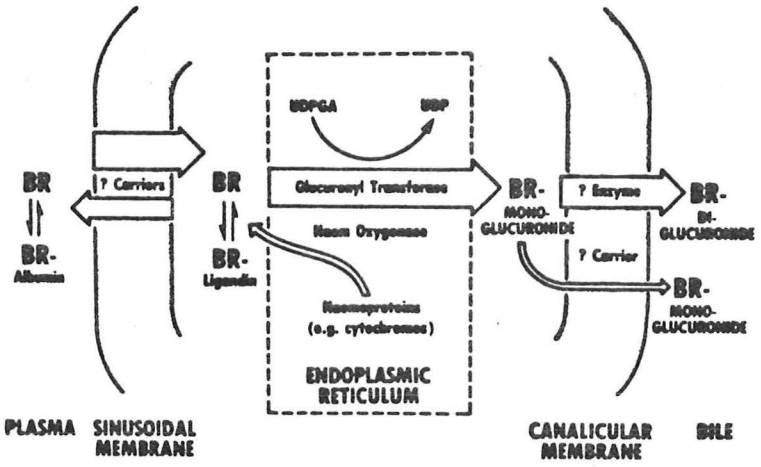
Physiology

Bilirubin is the metabolic product of heme. For the most part, heme is to be found in the hemoglobin of red blood cells. The majority of bilirubin, therefore, in a normal person derives from the reticuloendothelial degradation of heme released from senescent erythrocytes. A small portion is derived from the degradation of heme-proteins (e.g. catalase, cytochrome C) in the liver and from the metabolism of the heme in hemoglobin released in the bone marrow from abnormal red cells which are destroyed in situ. In any state of ineffective erythropoiesis this local marrow turnover of heme may increase dramatically and result in a marked over-production of bilirubin.



From Gollan and Schmid, Liver and Biliary Disease, 1979

The degradation of heme to bilirubin occurs for the most part in reticuloendothelial cells, and the pigment is transported to the liver for further metabolism and excretion. Because the molecule is essentially insoluble in water, it must be bound to protein (albumin) for transport. There is an active extraction of bilirubin from plasma into the hepatic parenchymal cells. While the mechanism of this process remains uncertain, it appears to be an active, saturable, probably energy-dependent mechanism which is not specific for bilirubin and which probably functions through some form of albumin receptor complex on the plasma membrane. The bilirubin but not the albumin is internalized. Again, the water insoluble bilirubin molecule is bound to protein (acceptor proteins Y [ligandin] and Z).



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Transport of the pigment within the cell is effected by still obscure mechanisms and the bilirubin is conjugated by the addition of 2 uridine diphosphoglucuronide (UDPG) moieties in sequence. The resultant product bilirubin diglucuronide (or conjugated bilirubin) has enhanced water solubility. It is excreted via the canalicular membrane into bile. This process is an active, competitive, partially specific (i.e. shared by some other organic anions such as bromsulphthalein sodium [BSP] but not by bile salts) process which seems to be particularly susceptible to dysfunction in the presence of parenchymal cell injury. The bilirubin reaches the intestine via the bile and is subsequently degraded to uro(sterco)bilinogen and ultimately excreted in stool and urine.

INCREASED SERUM BILIRUBIN LEVELS

The total level of serum bilirubin may be increased from elevations of the unconjugated fraction alone or from elevations of both the conjugated and unconjugated levels.

Associated with increase in serum unconjugated bilirubin only:

increased production (e.g. hemolysis, shunt hyperbilirubinemia)
impaired uptake
impaired conjugation (e.g. Gilbert's syndrome)

Associated with increase in both conjugated and unconjugated bilirubin moieties:

parenchymal cell injury
impaired canalicular excretion (e.g. Dubin Johnson syndrome)
cholestasis

Hemolysis

The capacity of the normal liver to take up, conjugate and excrete bilirubin is prodigious. It requires a very marked increase in the production of bilirubin to exceed this hepatic reserve for bilirubin disposition and to provoke an elevation of the serum level of bilirubin as long as the liver itself remains normal. Even the most extreme forms of chronic hemolysis are associated with only mild elevations of the serum bilirubin (usually <3 mg%) unless the liver itself is directly involved in the process (e.g. sickle cell disease) or is indirectly damaged (e.g. associated congestive heart failure). Blood transfusions cause a marked increase in bilirubin production from the destruction of the infused red cells. Serum bilirubin remains normal when the liver is otherwise unaffected but will rise suddenly and sometimes dramatically in the presence of underlying liver disease.

Cholestasis

When cholestasis occurs either because there is obstruction to bile flow in the ductal system or because there is diffuse disturbance of the canalicular excretory mechanism (e.g. drug-induced cholestasis), the level of conjugated bilirubin rises in the parenchymal cell as excretion ceases. A concentration gradient develops for conjugated bilirubin across the plasma membrane and the relatively water soluble pigment diffuses down this gradient into plasma from the cell. This results in an increase in the plasma level of the conjugated fraction of bilirubin. In patients with extrahepatic obstruction it is possible that some reflux of conjugated bilirubin occurs directly via canalicula to plasma reflux through the tight junctions. At the same time the acceptor proteins (Y and Z) within the cell become occupied by conjugated bilirubin. This inhibits the uptake of unconjugated bilirubin and results in an elevation of the unconjugated fraction as well. The partial water solubility of conjugated bilirubin allows for other means of excretion (e.g. in the urine and by diffusion directly into the gut). The unconjugated molecules, tightly bound to albumin, have no other avenues of elimination. The net effect of all of these factors is that in patients with jaundice from cholestasis the separation of the serum bilirubin into its component fractions usually yields approximately 50% in the conjugated fraction and 50% in the unconjugated fraction.

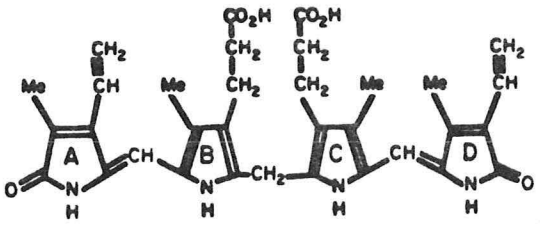
Parenchymal Disease

Jaundice associated with parenchymal cell injury (e.g. viral or alcoholic hepatitis) is the consequence of many factors. The actual destruction of parenchymal cells leads to a decrease in the mass of cells available to dispose of bilirubin. Other cells are injured but not destroyed. The canalicular membrane appears to be particularly sensitive to such partial injury. Some of these cells are able to take up and conjugate bilirubin normally but fail to excrete it. Conceivably, inflammation and its resulting edema may compress small bile ductules and aggravate the cholestatic component of these diseases. The net effect of these factors is once again to produce jaundice in which approximately half the circulating bilirubin is conjugated and approximately half is unconjugated. This is so irrespective of the cause of the hepatitis.

Fractionation of serum bilirubin is not useful, therefore, in distinguishing between parenchymal diseases and cholestatic lesions. Its value lies only in identifying those circumstances where jaundice is entirely the result of an increase in production of bilirubin (e.g. hemolysis) or the consequence of a selective defect of bilirubin uptake and/or conjugation (e.g. Gilbert' s syndrome or Crigler Najjar syndrome).

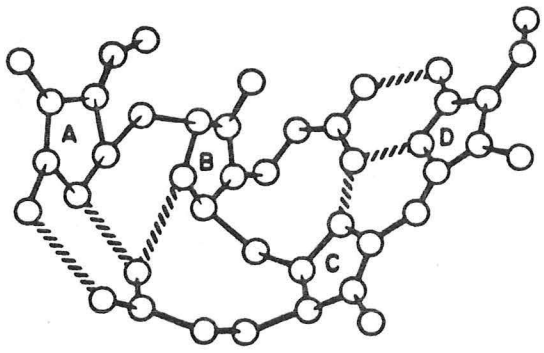
Physical Chemistry of Bilirubin

The molecular structure of bilirubin as it is commonly drawn should confer some water solubility to the pigment. Two hydrophilic anionic groups are provided by the propionic side chains.



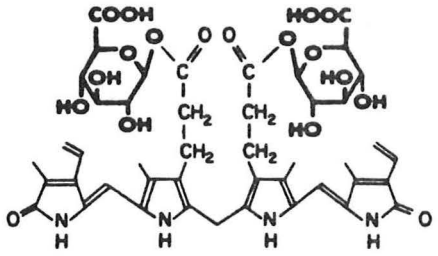
From Gollan and Schmid, Liver and Biliary Disease, 1979

In nature, however, the bilirubin molecule exists in a quite different configuration as has been shown by x-ray crystallography. Internal hydrogen bonds are formed by these propionic acid carboxyl groups and the amino groups of the outer pyrrolenone rings. These hydrogen bonds effectively remove all polar groups from the molecule and render it exceedingly water insoluble.



From Gollan and Schmid, Liver and Biliary Disease, 1979

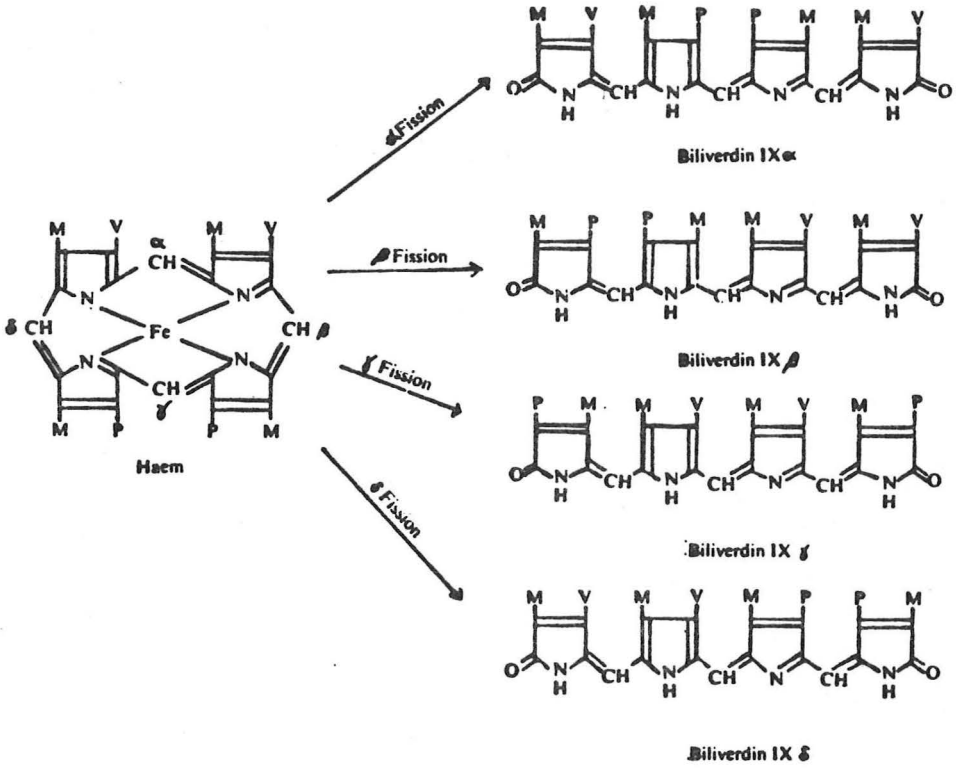
Conjugation adds one or two glucuronide residues to the propionic acid carboxyl groups. While this in fact leaves the molecule still with only two polar groups, it disrupts the internal hydrogen bonding and allows the polar groups to exert their effect on water solubility. This molecular change is enough to confer sufficient solubility to allow biliary and renal excretion.



Bilirubin IX-α diglucuronide

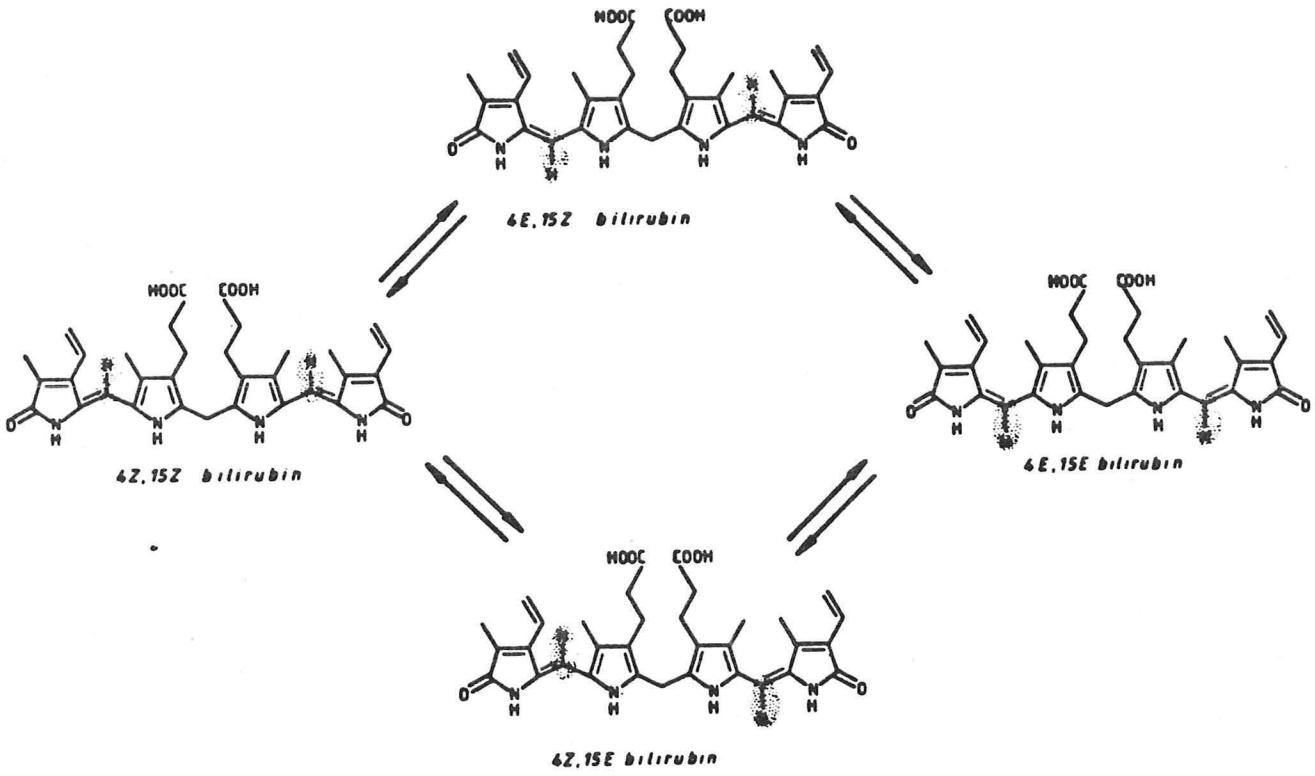
From Gollan and Schmid, Liver and Biliary Disease, 1979

This characteristic of water insolubility is critically determined by the structure of bilirubin derived from IXα hemoglobin. Other naturally occurring or synthetically produced tetrapyrroles do not demonstrate internal hydrogen bonding. The propionic side chains remain as polar groups, and these molecules therefore retain a modest water solubility.



From Gray, Nicholson and Tipton, Nature New Biology, Vol. 239, 1972

Even the isomeric structure is important. The conversion of the plane of the inter-pyrrole bridges from cis to trans is enough to disrupt the hydrogen bonds and render unconjugated bilirubin soluble and threfore excretable.



From Blanckaert and Schmid, Hepatology, A Textbook of Liver Diseases, 1982

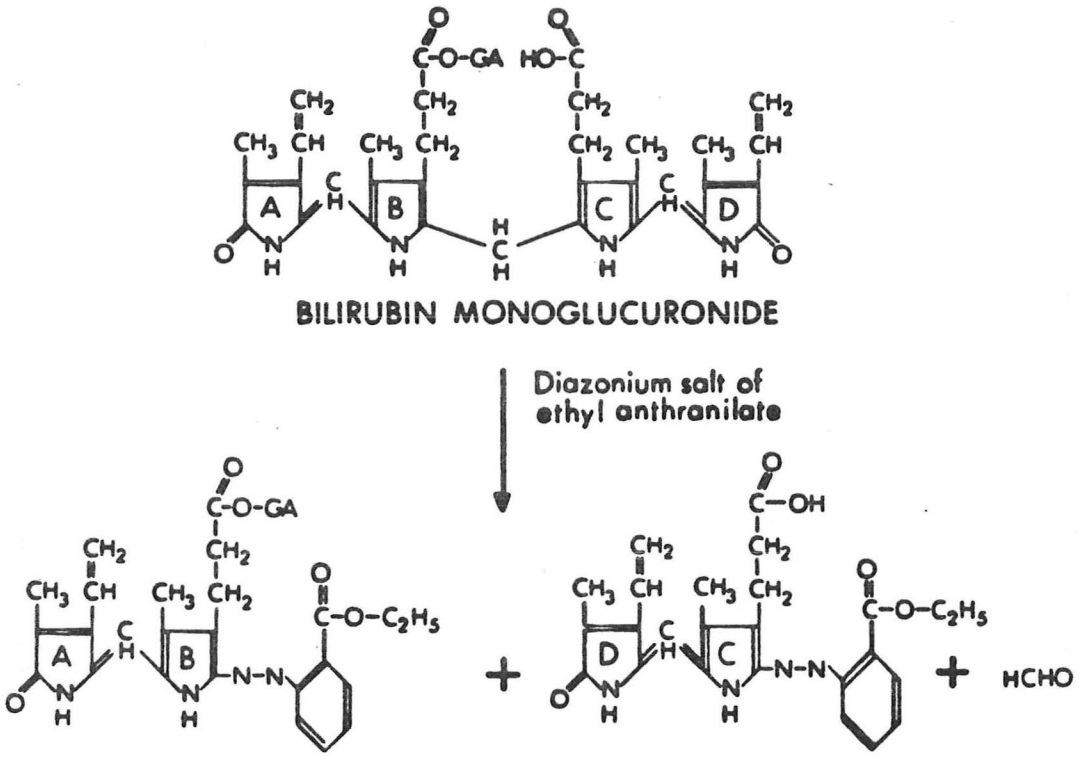
This is the basis for the efficacy of phototherapy on neonatal jaundice (secondary to under-developed glucuronidation mechanism) and the Crigler-Najjar syndrome. Phototherapy changes this geometric isomerication, thus disrupting the hydrogen bonds and promoting water solubility and excretability without the need for conjugation.

Techniques of Measuring Serum Bilirubin

There are a number of refined and sophisticated means of measuring the level of serum bilirubin and its conjugated, partially conjugated and unconjugated components. Currently the most precise appears to be the method of high pressure liquid chromatography (HPLC). The analysis used by clinical laboratories, however, remains a modification of that introduced in 1916 by van den Bergh.

Diazo Reaction of Van den Bergh

The essence of this assay is the cleaving of the bilirubin molecule at the methenyl bridge between the two pyrroles bearing the proprionic side chains. Diazotised sulfanilic acid was the original agent used by Van den Bergh, but a series of diazonium salts have subsequently been introduced.



From Chowdhury and Chowdhury, Seminars in Liver Disease, Vol. 3, 1983

This reaction results in two molecules of a diazodipyrrole which is stable and can be quantitated spectrophotometrically at 540 mμ. For this reaction to occur, the diazonium salt must have access to the appropriate methene bridge. The hydrogen bonding of unconjugated bilirubin internalizes this bridge and makes it unavailable for the reaction. Unconjugated bilirubin cannot be measured by this technique until the hydrogen bonds are disrupted. Ethanol or methanol is commonly used for this purpose. The reaction proceeds directly with conjugated bilirubin, however, because the hydrogen bonds are not present and the methene bridge is available for the reaction directly. "Indirect-reacting" bilirubin then is basically a measure of unconjugated bilirubin. "Direct-reacting" bilirubin includes conjugated bilirubin but is not synonymous with this fraction. It also includes any isomeric forms of unconjugated bilirubin which are not internally hydrogen bonded.

It has been established that normal serum contains no conjugated bilirubin. Nonetheless, a proportion (usually <30%) of the total bilirubin in normal serum reacts directly in the Van den Bergh reaction. The longer the serum and the diazonium salt are allowed to react together, the greater is the proportion of direct reacting bilirubin. At any given time, a small proportion of unconjugated bilirubin is not hydrogen bonded. One can envisage this as being a dynamic process of bonds forming and being disrupted. This small component of unconjugated bilirubin is available for the diazo-reaction to proceed. The longer the reaction time, the greater the amount of bilirubin cleaved. This phenomenon has been recognized for a long time and in the performance of the Van den Bergh reaction, a reaction time of one minute has been established as the standard time limit. Direct-reacting bilirubin has sometimes been referred to as the one-minute-bilirubin level as a consequence.

Laboratory Assay

The serum bilirubin assay is performed first of all directly on an aliquot of serum. To another aliquot alcohol is added and the assay is repeated. This second assay will measure all of the bilirubin, both conjugated and unconjugated. As long as the direct-reacting fraction is less than 30% of the total, it is probable that all of the circulating bilirubin is unconjugated. If the proportion rises above 30%, even if the total bilirubin remains normal, there must be an increase in the conjugated moiety and therefore some dysfunction of the excretory mechanism.

Serum Bilirubin as a Function Test

The capacity of a normal liver to dispose of bilirubin is very large. It follows, then, that the serum bilirubin level is not a very sensitive indicator of liver disease. A substantial amount of parenchymal injury must be present, canalicular dysfunction must be widespread, or obstruction must be almost complete before there will be a rise in the serum level. Once the level has risen because of parenchymal injury, however, the level becomes a useful prognostic indicator of severity of disease. This is so as long as there is not an increase in the production of bilirubin (e.g. hemolysis in patients with GbPD deficiency who have viral hepatitis or shunt hyperbilirubinemia in patients with alcoholic hepatitis and folate deficiency causing ineffective erythropoiesis). Under these circumstances the serum bilirubin will be high relative to the degree of injury. The co-existence of renal dysfunction will also limit the value of serum bilirubin to reflect severity. The kidney affords a real and important alternate means for excretion of conjugated bilirubin.

Patients with complete biliary obstruction who have neither an increase in bilirubin production nor a defect in renal excretion will usually stabilize their serum bilirubin betwen 20 mg% and 30 mg%. Levels higher than this in a patient indicate that there is a superimposed problem, be it increased production, impaired metabolism, or decreased alternative excretory mechanisms.

The highest serum bilirubin levels are found in patients with sickle cell disease who develop viral hepatitis. This combination of parenchymal injury coupled with marked hemolysis and ineffective erythropoiesis will produce serum levels in excess of 100 mg%. Patients tolerate these levels (half of which is unconjugated) remarkably well.

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ALKALINE PHOSPHATASE

Measurement

A group of enzymes which share the capacity to catalyze the hydrolysis of a variety of phosphate esters at an alkaline pH are known collectively as alkaline phosphatase. These enzymes are ubiquitous in nature and are present in many different tissues in humans including liver, bone, placenta, intestine, kidney, muscle and white blood cells. There have been many different assays developed to measure the serum activity of alkaline phosphatase. They have varied in the phosphate ester used as substrate, the end product measured to reflect enzyme activity (phosphate or organic alcohol), the technique used to measure the end product, and the buffer system used to maintain the pH at 9.0. The result of all these variations is that many different tests with different units of measurement and different normal ranges have developed. Because different isoenzymes have more or less affinity for different substrates, the correlation betwen serum alkaline phosphatase activity measured by different techniques is often quite poor.

Identification of Isoenzymes

Despite the demonstration of alkaline phosphatase in many different tissues and biologic secretions, diseases of liver and bone and the state of pregnancy are responsible for essentially all increased levels of serum activity. These isoenzymes are distinguishably different in many ways. When the isoenzymes are extracted from bone, liver and placenta, very clear

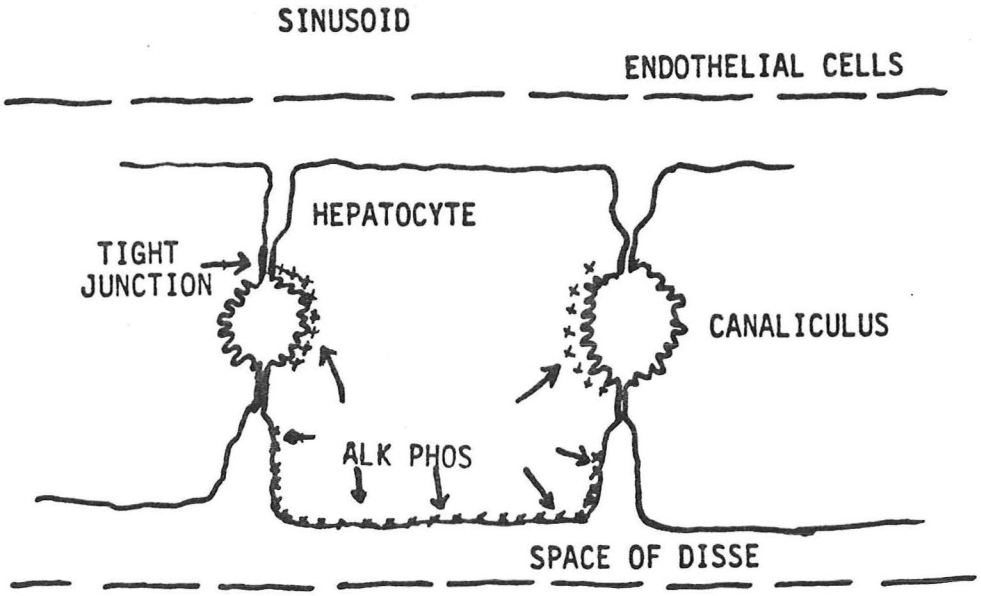
distinctions can be made between them by physico-chemical electrophoretic, and immunologic means. Thus, their relative resistance to phenylalanine and urea inhibition, their heat lability and their electrophoretic migration with and without neuraminidase digestion are distinct and different. Heat lability is the only one of these assays which has found wide clinical utility. Placental alkaline phosphatase is quite stable to heat at 56°C for 10 minutes, while bone alkaline phosphatase is largely inactivatedd by such treatment, and hepatic alkaline phosphatase is intermediate in its heat stability. Unfortunately, the precision of this separation is lost when applied to serum. Thus, when sera from patients with elevated alkaline phosphatase from pregnancy, bone disease and liver disease are evaluated for heat stability (or any of the other assays), the overlap is so great that little clinical utility results. In a patient with unexplained isolated elevation of serum alkaline phosphatase activity, discrimination as to the origin of the excess enzyme is more reliably achieved by measuring other enzymes (e.g. 5' nucleotidase, leucine aminopeptidase or gamma glutamyl transpeptidase) which are not increased by bone disease, than by trying to identify the alkaline phosphate isoenzyme. This means of identification also has its limitations, however.

Origin of Increased Serum Alkaline Phosphatase

Placental alkaline phosphatase is localized to the outermost surface of the syntrophoblast, and the serum level correlates with the mass of the placenta. Thus, the serum level begins to rise in the second trimester and progressively increases until delivery, and has usually returned to normal one month post-partum. Bone alkaline phosphatase is localized to the osteoblasts and is released by osteoblastic acctivity. Therefore, any bone disease that stimulates osteoblastic repair will result in an increase in the serum activity.

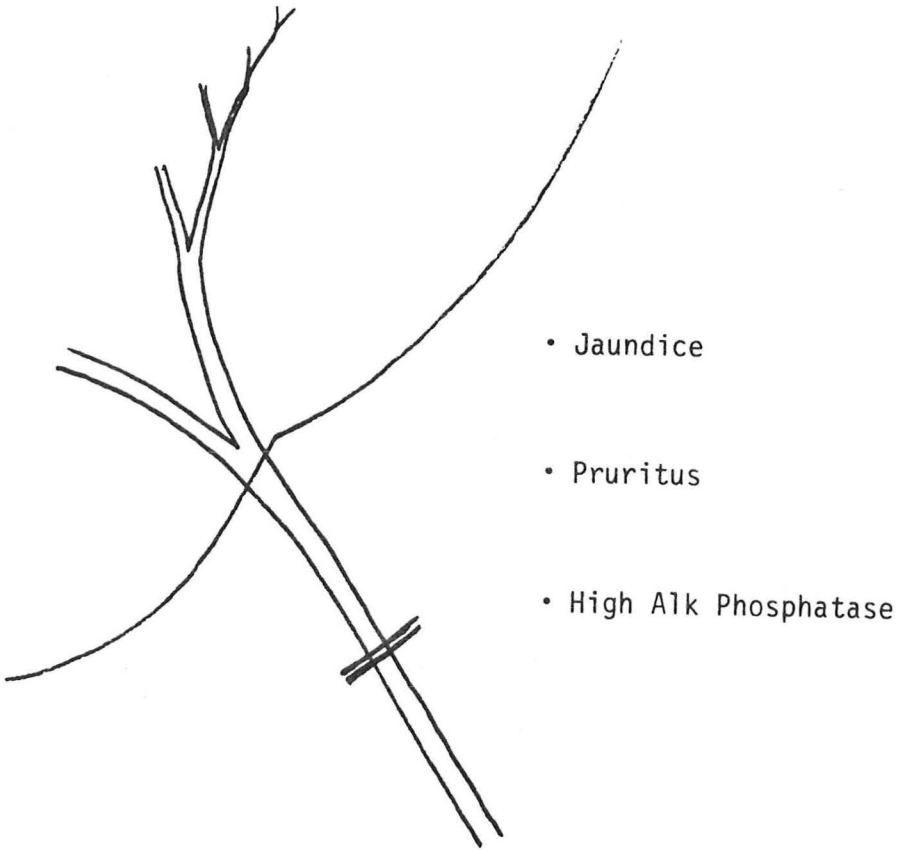
In Liver Disease

The origin of alkaline phosphatase elevations in patients with liver disease has been more controversial. An early view held that the liver was an excretory organ for the enzyme and that elevated serum levels in patients with liver disease were simply the consequence of poor clearance of enzyme derived from bone. This has been categorically disproved. Even though there is some measurable alkaline phosphatase activity in bile, the metabolic fate of this protein is similar to that of most circulating proteins, and is entirely independent of the liver. Histochemical techniques have localized some enzyme to the cuboidal cells lining bile ducts, but most of it is found in the parenchymal cells. It is particularly apparent attached to membranes bordering both the canaliculi and the sinusoids.



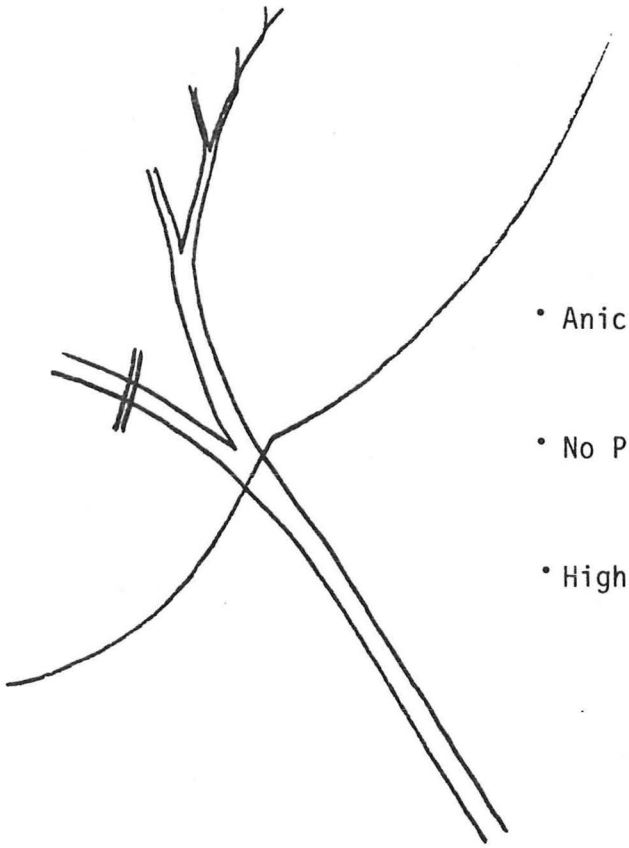
In states of cholestasis there is a marked increase in the parenchymal cell concentration of alkaline phosphatase accompanied by an increase in the serum activity. This occurs whether the cholestasis is caused by an obstruction of the common bile duct or a diffuse intrahepatic lesion involving the canalicular membrane (e.g. drug-induced cholestasis). The stimulus for this enhanced synthesis of alkaline phosphatase appears to be the intracellular concentration of bile salts. This has been convincingly demonstrated by Kaplan et al and by Hatloff and Hardison using whole animal preparations and parenchymal cell cultures. Sodium taurocholate not only stimulates the synthesis of the enzyme but also facilitates its release from cell membranes back into the plasma. Other physiologically less important bile salts stimulate synthesis but do not enhance release.

Mechanism of Increased Alkaline Phosphatase in Cholestasis



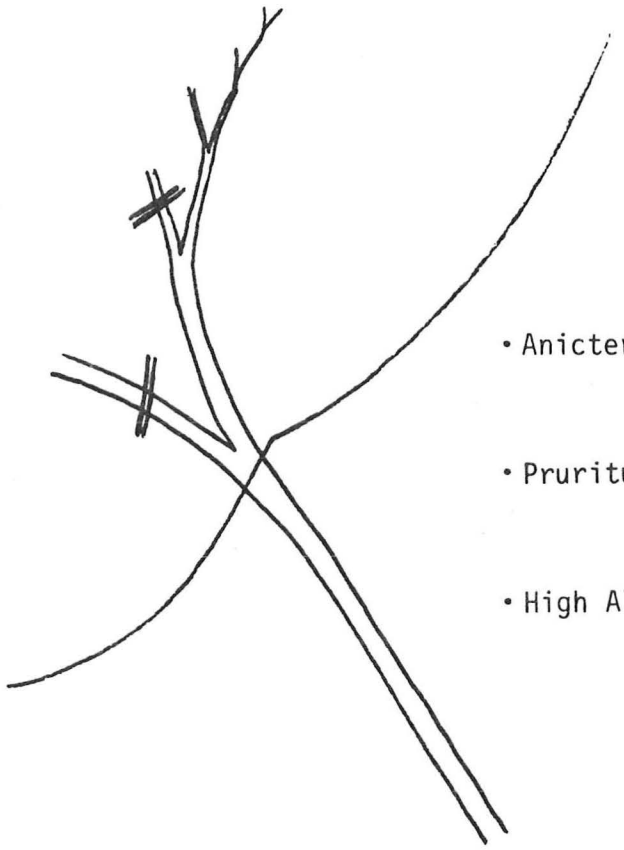
When the common bile duct is obstructed, the pressure in the extrahepatic system rises and ultimately, despite dilatation of the system, exceeds the bile secretion pressure. At this point, bile secretion across the canalicular membrane ceases. The intrahepatic concentration of bile salts rises in every cell, and the synthesis of alkaline phosphatase is stimulated. The serum activity of the enzyme rises because its release is facilitated by the retained sodium taurocholate. At the same time the excretion of bilirubin also ceases, and there is a progressive rise in the serum concentrations of both the conjugated and the unconjugated fractions. The serum bile acid levels also rise. Associated with this, there is often the development of pruritus. This symptom is caused probably by the dermal concentration of all or some specific bile salts.

The same consequences ensue when noxious stimuli (e.g. certain drugs) affect the canalicular membrane of all the parenchymal cells. There is jaundice, pruritus, high liver and serum bile salts, and a marked increase in serum alkaline phosphatase. Recent data suggests that a mechanism other than cholestasis may contribute to the high serum alkaline phosphatase in drug-induced injury. This appears to be true at least in experimental injury produced by colchicine.



- Anicteric
- No Pruritus
- High Alk Phosphatase

If obstruction is confined to either the right or left hepatic duct, the same sequence occurs to one-half of the liver. There is cholestasis with a marked rise in the synthesis and release of alkaline phosphatase from all of the obstructed cells. The serum bile salts and bilirubin level stay normal, however, because the unobstructed half of the liver is capable of disposing of the retained molecules. The net result, then, is an asymptomatic patient without jaundice, without pruritus, but with a very high alkaline phosphatase. If the degree of obstruction is increased to include some of the other lobe, a time will come when the remaining unobstructed liver is insufficient to dispose of the retained bile salts and bilirubin. The liver's capacity to deal with bile salts is not as great as is its reserve with respect to bilirubin.



One may see the situation then (e.g. a bifurcation tumor of the bile duct) where the serum bilirubin is normal but the patient has very high bile salt levels, complains of intense pruritus, and again has a very high serum alkaline phosphatase. With even more obstruction (or an increase in bilirubin production from a blood transfusion perhaps), the serum bilirubin will begin to rise as the amount of unobstructed liver becomes inadequate to deal with the bilirubin load presented to it.

Primary biliary cirrhosis provides an ideal example of such a sequence occurring in cholestasis. This is a disease whose cardinal pathologic manifestation is the slow but progressive inflammatory destruction of small bile ductules. For many years the patient (usually a woman) is quite asymptomatic and probably has entirely normal LFTs. At some point in time, enough small ducts are destroyed to cause an elevation in the serum alkaline phosphatase level. Each obstructed small duct causes cholestasis in the cells it drains. In each of these bilirubin and bile salts are retained, and consequently the synthesis and release of alkaline phosphatase is enhanced. The retained bilirubin and bile salts are readily cleared by the uninvolved liver. With time, the process becomes very widespread, and large areas of the liver become obstructed and cholestatic. The rest of the liver remains quite normal, however, and is able to maintain the patient free of symptoms and with a normal bilirubin. The serum alkaline phosphatase, however, becomes extremely high (usually >10x normal). Ultimately, the uninvolved

liver becomes inadequate to deal with bile salts, and symptoms develop. These are due either to inadequate bile salt concentrations in the gut (steatorrhea or other consequences of malabsorption) or to retention of bile salts (pruritus). The vast majority of patients with PBC are anicteric when these symptoms bring them to their physician. They usually remain so for a number of years, even though the disease is relentlessly progressive. Finally, jaundice supervenes. This has long been recognized as an ominous finding in patients with PBC. In effect, it represents end stage disease because it declares that so many bile ductules have been obliterated that there is now insufficient hepatic parenchyma left unobstructed to handle the normal daily load of bilirubin. Little wonder, then, that at this stage there is insufficient liver to synthesize proteins or detoxify toxins, and that death from liver failure is close.

Serum Alkaline Phosphatas in Infiltrative Disease of the Liver

It has been appreciated for many years that granulomatous and malignant infiltrations of the liver are characterized by a marked increase in serum alkaline phosphatase activity. Characteristically such patients have otherwise normal LFTs (although the transaminases may be increased slightly). Jaundice is very rare unless there is obstruction of the major bile ducts and otherwise only occurs in the face of massive infiltration.

The pathophysiology responsible for this is not hard to appreciate. Each focal infiltrate of malignant or granulomatous tissue causes obstruction of a small part of the biliary system. The obstructed cells are stimulated to synthesize and release alkaline phosphatase. Any retained bile salts or bilirubin are readily cleared by the otherwise uninvolved liver. Despite intense infiltration, symptoms of pruritus, malabsorption or jaundice are very unusual because only a relatively small amount of unaffected parenchyma is needed to dispose of the normal daily production of bile salts and bilirubin.

Serum Alkaline Phosphatase in Cirrhosis

It is common in cirrhotics for the serum alkaline phosphatase to remain elevated even when the cause of the liver disease has been eradicated and all of the other LFTs have returned to normal. The scar tissue in the cirrhotic liver produces the same local areas of biliary obstruction. The same sequence that is seen in infiltrative diseases is initiated, and long term elevation of serum alkaline phosphatase is predictable in patients with inactive cirrhosis.

Serum Alkaline Phosphatase in Parenchymal Disease

The serum alkaline phosphatase is usually elevated in patients with parenchymal diseases (e.g. viral and alcoholic hepatitis). This is particularly apparent in the convalescent phase of these illnesses. When parenchymal cells are destroyed, there is an immediate need for renewed parenchymal cells. Regeneration proceeds without any regard for normal architectural integrity. Thus, instead of the normal single-cell cords of parenchymal cells, rosettes of newly regenerated cells are formed.

Whenever 2 or more cells come into contact, modification of the cell membrane occurs and a canaliculus is formed. The regenerated rosettes of cells share a common canaliculus. These cells are physiologically competent. They can synthesize proteins, they can detoxify noxious agents, they can take up, metabolize and excrete organic anions. They are fully competent. The canaliculi into which they excrete their products are not connected to the biliary system, however. They are blind alleys. The cells in the rosettes then soon become "obstructed" and "cholestatic", retain bile salts and start to synthesize and release excessive quantities of alkaline phosphatase.

Alkaline Phosphatase in Dubin Johnson Syndrome

The serum activity of alkaline phosphatase is normal in patients with either the Dubin Johnson or Rotor syndromes. The canalicular defect in excretion is specific for bilirubin. Bile salt excretion is quite normal. While the parenchymal cell retains conjugated bilirubin, the cellular concentration of bile salts is normal and there is neither a stimulated synthesis nor an enhanced release of alkaline phosphatase in these patients.

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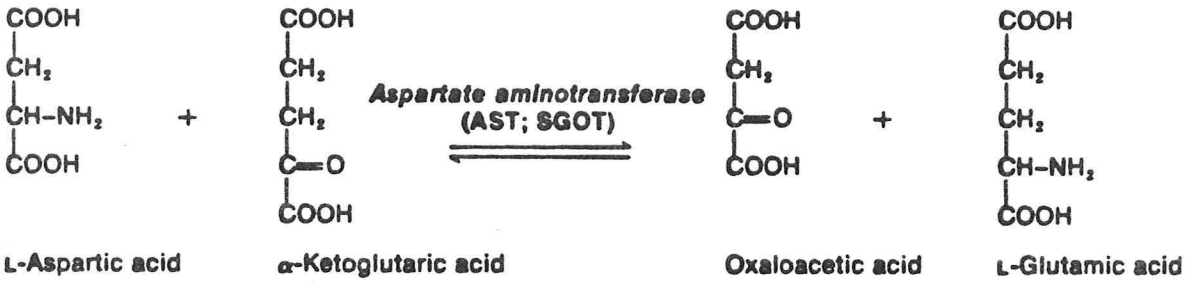
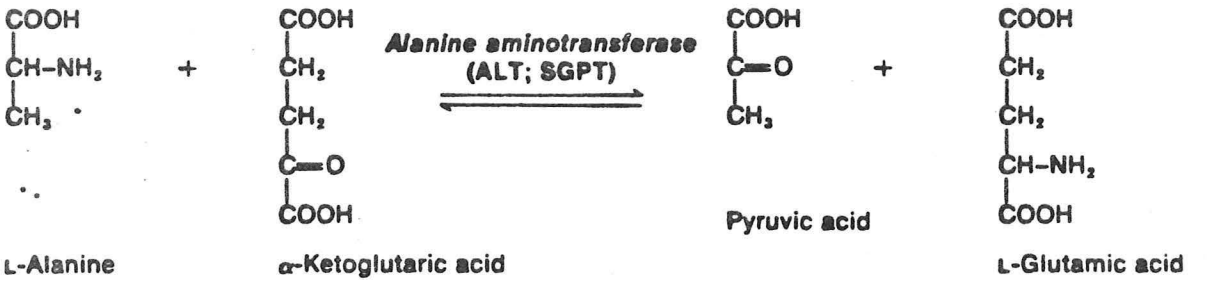
AMINOTRANSFERASES (TRANSAMINASES)

These enzymes catalyze the transfer of an amino group from an amino acid to a keto-acid, thus forming a new amino acid (and a new keto-analogue). The nomenclature of these enzymes has been changed recently (in the interests of semantic purity). The transaminases are now the aminotransferases, and serum glutaminc oxalo-acetic transaminase (SGOT) is now serum aspartate aminotransferase (AST), while serum glutamic pyruvic transaminase (SGPT) is now serum alanine aminotransferase (ALT). The name change reflects a unifying move to name enzymes by their substrate rather than their products.

The reactions catalyzed by the enzymes are:





From Kaplowitz et al, Hepatology, A Textbook of Liver Disease, 1982

The enzymes are present in tissues throughout the body but attain peak concentrations in hepatic parenchymal cells, cardiac muscle, skeletal muscle and erythrocytes. In all normal tissues except the liver, AST is present in higher concentrations than ALT.

In clinical medicine, elevations of the serum activity of the aminotransferases are seen in disorders affecting the liver, the skeletal muscle and the myocardium. Artefactual elevations may be seen in blood that has hemolyzed, although hemolytic anemias generally are associated with normal serum activity. The serum activity of AST is usually greater than the serum activity of ALT in muscle and myocardial diseases because the tissue concentration of AST is substantially higher than that of ALT. In many such patients, the serum ALT may be normal. If injury is sufficiently widespread, however, the ALT level will also rise above the normal value. An elevated serum ALT is not specific for liver disease.

In the normal liver, AST is present both in the cytosol and in the mitochondria. ALT is present only in the cytosol. In normal liver, the concentration of ALT is greater than that of AST in liver homogenates, and in most diseases of the liver, serum ALT levels are elevated earlier than, and become higher than, the serum AST level. In alcoholic patients, the cytosolic concentration of both AST and ALT are reduced. The mitochondrial concentration of AST remains unimpaired. The cause for this decrease in ALT concentration has not been definitively established, but it may be related to pyridoxine deficiency. This probably explains the well documented observation that the serum level of AST is usually higher than that of the serum ALT in patients with alcoholic hepatitis.

The serum level of these enzymes rises in patients with liver disease because the parenchymal cells that contain very high concentrations of the enzymes are either destroyed completely or are damaged in such a way that the enzymes are able to leak into the plasma through the cell membrane. The half-life of infused AST or ALT is measured in hours so that if the only mechanism for enzyme release were the complete destruction of cells, one would expect that disease would cause a very short-lived elevation of the serum level (as one sees in myocardial infarction, for example). This phenomenon is also recognized in some patients with fulminant viral hepatitis where there is massive hepatic necrosis. Serum aminotransferases which are running in the thousands of units may precipitously fall to very low levels because virtually all of the cells have been destroyed over a short period of time, and there are no cells left to synthesize more enzyme. This event is usually a portent of the patient's imminent demise.

The serum level is not a useful indicator of the severity of liver injury, however. Most patients with mild viral hepatitis will sustain serum aminotransferases of >1000 u/ml for weeks, even if there is insufficient cellular injury to cause the patient to be jaundiced. On the other hand, patients with alcoholic liver disease or chronic active lupoid hepatitis seldom have values greater than 500 u/ml, even in the most severe instances. This discrepancy has been explained by the notion of partial cellular injury prior to cell death.

In viral hepatitis, all of the parenchymal cells are affected and, irrespective of the severity of the disease, all of the cells will be destroyed and replaced during the course of the illness. It seems that the severity of the disease is a function not of the extent of cellular involvement but the time course over which this replacement of virtually all of the parenchymal cells occurs. Cell death may be protracted and for a varying period of time.

The parenchymal cells are injured in a way that allows the cell to continue to synthesize aminotransferase, but also allows the enzymes to leak into the serum, presumably through damaged cell membranes. The partial injury of almost all of the parenchymal cells accounts for the sustained high levels in otherwise mild episodes of hepatitis. The damaged cells, while leaking enzymes, are able nonetheless to function in other ways.

While the aminotransferases are not useful in judging severity of liver disease, they are useful

1. diagnostically. For the most part, the only sorts of liver disease that cause serum levels above 500 U/l are those caused by viral hepatitis, drug reactions, toxins such as carbon tetrachloride, and shock states (including acute heart failure). When the serum value is <500 U/l, it becomes entirely non-specific and of no specific diagnostic value.

2. as a screening test for the presence of liver disease. The sensitivity of the aminotransferases for the detection of liver diseases varies tremendously depending on the disease being screened. As an indicator of the presence of non-A, non-B viral hepatitis or for the occurrence of hepatotoxicity in patients receiving isoniazid, the sensitivity (particularly of ALT) seems to be very high (there is a substantial problem with specificity, however). On the other hand, the value of aminotransferase screening in patients receiving methotrexate or in patients who have received a jejuno-ileal bypass procedure is notoriously poor. It would seem that quite a large number of parenchymal cells must be partially or completely damaged before the serum level of the aminotransferases will be recognized to be abnormal.

3. prognostically. The seriousness and importance of liver diseases, by and large, depends much more on the degree of regeneration and the extent to which healing occurs by the deposition of fibrous tissue than it does on the extent of cell injury. One cannot use the height of the serum aminotransferase activity to determine the severity of chronic forms of liver disease because these enzymes reflect only on the activity of the cell injury, not on the reparative processes.

Most SMA screening panels measure serum levels of AST. Some measure both AST and ALT. It makes more sense to use the ALT level as a screening test rather than the AST because of its heightened sensitivity for liver disease and its greater specificity (insofar as muscle injury is less likely to raise the value). For no apparent reason other than that of tradition and experience, most of us continue to use the AST both in determining the presence or absence of liver disease and in declaring that point when known liver disease becomes inactive. The one exception to this has been the attempts made to screen donor blood to try to decrease the incidence of non-A, non-B viral hepatitis in the post-transfusion period. These studies used ALT as the screening enzyme.

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THE EVALUATION OF PATIENTS WITH UNEXPECTED ABNORMALITIES OF LFTs

The problem posed by the use of screening batteries of laboratory tests is that one frequently finds abnormalities. The investigative pursuit of the abnormal finding may be very expensive in financial terms and also in terms of the patient's time, comfort and even health. The dilemma faced is whether or not the cost of exploring the abnormal result is worth the benefit of knowing the cause. There is no simple answer to the question when applied to screening tests in general or when applied specifically to LFTs.

The diagnosis of a disease state is made with varying degrees of certitude depending on its importance to the patient's health and also on its therapeutic implications. An upper respiratory tract infection is frequently diagnosed in a patient with a fever without resource to any laboratory aids because the natural history of the disease is benign, short lived, and it requires only symptomatic care. Other patients who present with a fever may be subjected to an extensive laboratory investigation in an effort to identify the cause. Particular effort will be made to rule in or rule out those disorders that might be present and for which specific and effective therapy is available. The approach to an asymptomatic patient with an abnormal LFT is just as variable depending on the patient and the circumstance. I believe all abnormal LFTs need to be explained. This does not mean that the cause must always be proven, but it does mean that thought should be given each time to the patient, the probably cause and the possible implications of one's being right or wrong about the diagnosis. One should not just ignore abnormal findings even if the result is only a little above the normal range. One must remember that these tests are not useful as indicators of severity or importance of liver disease at either end of the range of abnormal values. Many very serious liver diseases may be present with only minor disturbances of LFT values.

The frequency with which one or more LFTs will be found to be abnormal in a population of patients will vary tremendously depending on which population of patients is being assessed. For example, screening tests performed on presumably healthy volunteer blood donors would be expected to result in a much lower incidence of abnormal LFTs than would a population of paid donors or an unselected population of patients attending the Outpatient Clinic at Parkland Memorial Hospital. This indeed is true. Most volunteer populations of blood donors have abnormal aminotransferases with a prevalence of 1-3%. Paid donors may show rates of abnormalities as high as 30%.

LFTs ON SMA12 FROM PMH CLINICS
(n = 405)

RESULT	No.	%
All normal	203	50.1
Increased serum bilirubin	1	0.2
Increased serum alk. phos.	135	33.3
Increased AST	24	5.9
All abnormal	11	2.7
Two abnormal	31	7.7

Over a particular 3 day period in which SMA12 assays were run on patiens attending the PMH clinic, 5.9% were found to have an isolated abnormal AST.

LFTs ON SMA12 FROM PMH WARDS
(n = 153)

RESULT	No.	%
All normal	60	39.2
Increased serum bilirubin	3	2.0
Increased serum alk. phos.	35	22.9
Increased AST	13	8.5
All abnormal	25	16.3
Two abnormal	17	11.1

8.5 Percent of patients on the Parkland Wards also had an isolated AST elevation.

LFTs ON SMA20 FROM PRIVATE LABORATORY
(n = 1300)

RESULT	No.	%
All normal	911	70.1
Increased serum bilirubin	11	0.8
Increased serum alk. phos.	205	15.8
Increased AST a/o ALT	101	7.7
All abnormal	27	2.1
Two abnormal	45	3.5

The results from a private pathology laboratory were somewhat similar. 7.7 Percent of serum specimens showed an isolated abnormal value for one or other of the aminotransferases. The frequency of isolated abnormal alkaline phosphatase (15.8%) was even more striking. With prevalence rates as high as these, it is easy to see how costly would be the definitive identification of the cause for each of these abnormal findings.

LFTs ON SMA20 FROM PRIVATE LABORATORY
(n = 1300)

RESULT	No.	%
Increased alkaline phosphatase		
<200 U	194	
>200 U	11	5.4
Increased AST		
<100 with increased ALT	42	65.6
<100 without increased ALT	15	23.4
>100	7	10.9
Increased ALT		
Without increased AST	37	43.0

Most of these abnormalities were mild. Only 5.4% of the abnormal alkaline phosphatase values were greater than 200 U while the majority of abnormal AST values were less than 100 U/l. Fifteen of the 64 samples that showed an increase in AST had a normal ALT level. Thirty-seven specimens had an isolated ALT elevation.

GENERAL CAUSES OF ABNORMAL LFTs

Laboratory error
Statistical quirk
Disease of another organ system
Artefact
Liver disease - important
- not important

Laboratory error

There are many human and mechanical reasons why such errors might occur. Repeating the assay is usually sufficient to identify and correct this cause.

Statistical Quirk

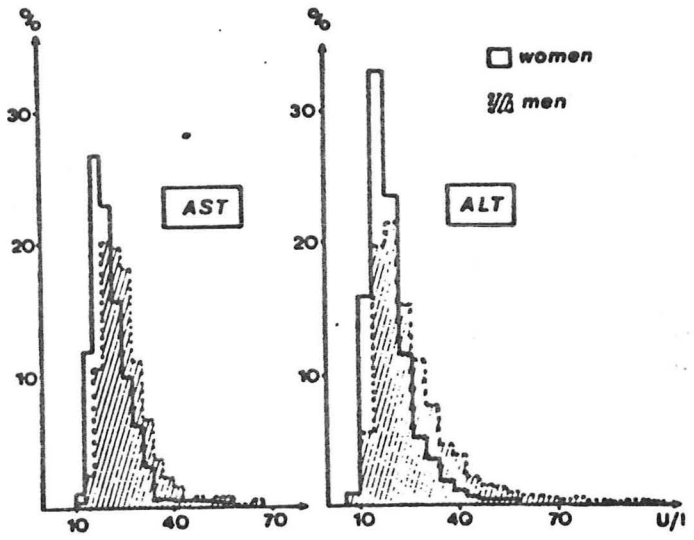
The very definition of "normal" introduces the potential for false positive results in any test. Prompted by the supposition that the values for any given laboratory test are distributed in a Gausserian (bell-shaped) curve, the definition of the normal range is often declared to be that range of values that encompasses 95% (± 2 SD from the mean) of presumed normal subjects. This defines 2 1/2 % of the normal population as being above the normal range and therefore as having abnormal results. The more tests one screens for, the greater the likelihood that one of them will be "abnormal" for this reason.

Probability of obtaining an abnormal result

Number of independent tests	Percentage of times an abnormal result is found
1	5
2	10
4	19
6	26
10	40
20	64
50	92
90	99

From Galen and Gambino, Beyond Normality; The Predictive Value and Efficiency of Medical Diagnosis, 1975

There is in fact little likelihood that a physiologic measurement will fit a Gausserian distribution, and it is common for these values to show a marked skew to the right when plotted by frequency. This magnifies the probability that a normal person will appear to have an "abnormal" result. When the assays are repeated on the same person, the result tends to move towards the mean value and fall back inside the "normal" range. The same people do not continue to provide the rightward skew when the population is studied repeatedly. Others who were initially "normal" will move temporarily into the "abnormal" range.



From Siest et al, Clinical Chemistry, 1975

LFTs are found to be abnormal because of this phenomenon rather frequently. The chance of it occurring increases with serial performances of the test in the same patient. This is the probable explanation for the reported incidence of an abnormal AST value in as many as 10% of control patients followed serially in studies designed to determine the frequency with which isoniazid causes hepatotoxicity or the frequency of post-transfusion viral hepatitis. In most instances the "false" nature of the positive test can be demonstrated by repeating the assay on a fresh sample of blood.

Disease of another organ system

In circumstances where repetition of the test confirms the abnormality of the result, the next step is to establish that the liver is the origin of the abnormality. If the AST is abnormal, this can be achieved by measuring the ALT activity in serum and the activity of creatine phosphokinase (CPK). If serum alkaline phosphatase values are high, confirmation of the hepatic origin is sought by measuring serum activities of leucine aminopeptidase (LAP), 5'nucleotidase, or gamma glutamyl transpeptidase (GGT).

Artefacts

Sometimes other substances present in serum will interfere with the reactions involved in performing LFTs and give misleading results. Rifampin, for example, is a colored compound which absorbs at 540 mμ. When present in serum in high concentrations, it will read as if it were direct-reacting bilirubin. Many different interferring substances affect the enzyme reactions depending on the techniques used. The preference of one technique over another may rest on the frequency of such artefacts (e.g. the colorimetric method of measuring AST has largely been abandoned because of the frequency of false positive reactions provoked by ketone bodies).

Liver disease

Once the abnormal test result has been established to be (a) real and (b) reflective of a hepatic disturbance, the question becomes one of cause and importance. Not all abnormal LFTs require or deserve extensive work-up, but there is no simple algorithm that allows one to predict the need for evaluation. The considerations that need to be applied to each case include:

1. The probable clinical diagnosis
2. The remotely possible diagnosis
3. The patient's general state of health
4. The response of tincture of time
5. The cost in terms of patient discomfort and risk of such testing
6. The financial situation and the cost of the further tests projected

Each particular patient needs to be assessed independently. Thus, a 65 year old obese type II diabetic who is otherwise quite well might be allowed to go unmolested with an isolated increase in her serum alkaline phosphatase of 135 U/l. On the other hand, a young man of 25 with an isolated increase in serum alkaline phosphatase might warrant a much more extensive investigation. The key is to give thoughtful consideration to the need for more extensive studies and not to dismiss abnormal findings because they are "virtually normal". Many sinister and therapeutically modifiable disorders may first declare themselves with a persistent yet minor increase in one or other of the LFTs.

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