1963

Review of tests of liver function

John Charles Goldner
University of Nebraska Medical Center

A REVIEW OF TESTS OF LIVER FUNCTION

John Charles Goldner

Submitted in Partial Fulfillment for the Degree of
Doctor of Medicine

College of Medicine, University of Nebraska

December 1, 1962

Omaha, Nebraska

TABLE OF CONTENTS

INTRODUCTION

INTRODUCTORY COMMENTS

The correct use of liver function tests as an aid in the diagnosis, prognosis, and follow-up of hepatobiliary disease is a distinct challenge to the physician. The large number of tests, plus the apparent inconsistencies sometimes noted in their results, often leads the clinician to disregard the values obtained, with the result that nothing has been "gained" except a higher laboratory fee for the patient.

This unhappy state of affairs is partially due to the fact that liver function tests depend upon integrated physiologic activities which are but partly understood.[65] Furthermore, estimation of the presence or absence of hepatobiliary disease is complicated by the large functional reserve of the liver and its powers of regeneration. Under certain circumstances as much as 85 to 90 per cent of the liver may be removed before impaired function is discernible by laboratory tests.[134]

Because of the imperfections of liver function tests, any discussion of them must begin with emphasis on the fact that these tests are merely aids to the clinical examination of the patient and cannot substitute for judgment and experienced observation on the part of the physician.

INDICATIONS FOR LIVER FUNCTION TESTS

Ivy and Roth list three assumptions that are involved in the intelligent use of liver function tests (or any laboratory test). First, the physician must have in mind a specific question for which he desires an answer. Second, he must realize the limitations of the test. And third, the test must be reliable for the purpose for which it is designed or adapted.[83]

Liver function tests are indicated in two types of patients. The first group is those patients with definite signs of hepatobiliary disease, such as hepatomegaly or jaundice, or an established diagnosis such as cirrhosis. The tests are used in these cases to help establish a definite etiology for the sign or symptom, or to aid in the follow-up of the disease process in an attempt to determine the prognosis.

The second group in which liver function tests are indicated is those patients with signs or symptoms suggestive of hepatobiliary disease, such as pyrexia (hepatitis?) or dyspepsia (cirrhosis?). In these patients the tests are used primarily to detect if the hepatobiliary system is functioning improperly, and secondarily to determine the etiology of this malfunction if it exists.[119]

Almost all authors recommend the use of multiple tests in any patient when liver function tests are indicated. The only possible exception to this is in the long-term follow-up of a patient with an established diagnosis of hepatobiliary disease in which

the etiology has been determined. Popper lists three reasons for the use of multiple tests. First, the clinical entities of hepatobiliary disease are usually composed of several functional and structural pathologic features, which are not all present to the same degree. Therefore, tests indicating different features are employed.[65] Second, most structural abnormalities are associated with alterations of several functions. Therefore, several tests indicating different aspects of the same feature should be used. Third, unexplained abnormal results are found in the absence of hepatobiliary disease with almost every hepatic test. Similarly, almost no test yields abnormal results in all patients with even severe hepatic insufficiency established clinically or by other laboratory procedures. Thus, the use of multiple tests attempts to serve as a check against "false positive" or "false negative" results of individual tests.[159]

CLASSIFICATION OF JAUNDICE

Perhaps the most practical application of liver function tests is in the differential diagnosis of jaundice.[159] Essentially, this is a "therapeutic differential diagnosis" to separate "surgical" jaundice (from tumors, strictures, or stones) from "medical" jaundice (from hepatitis or cirrhosis). Hemolytic jaundice may be differentiated from the previously listed types relatively easily by other tests (e.g., the reticulocyte count).[159]

The division of jaundice into "medical" and "surgical" types is just one example of the classification of jaundice. Since many classifications have been presented, and since various types of jaundice will be mentioned in the following chapters, a discussion of some of the classifications of jaundice seems suitable at this point.

The classification system of Ducci is based mainly on the stage at which bilirubin is accumulated during the process of excretion, whether within the liver or the extrahepatic biliary tract(post-hepatic). In the former case, it differentiates those instances in which bilirubin has been converted to the prompt-reacting type (hepatic) or is still delayed or indirect-reacting (prehepatic).

Thus, Ducci divides jaundice into three groups, each with two subgroups. His first group is prehepatic jaundice which he divides into hemolytic and nonhemolytic. His second group is hepatic jaundice which he separates into hepatocellular and hepatocanalicular; and his third group is posthepatic jaundice which he divides into complete obstructive and incomplete obstructive.[32]

Cohn and Kaplan adapt the classification system of Ducci and others (Young, Rich, and Dameshek) into a much more comprehensive pattern. They define prehepatic jaundice as being that jaundice caused either by overproduction of bilirubin or by anoxia interfering with normal hepatic function. Therefore, this type of jaundice may be caused by hemolytic anemia due either to "intrinsic" mechanisms acting upon the red cell (e.g., isoimmune reactions).

Another cause of prehapatic jaundice is decreased hepatic blood flow as seen in congestive heart failure or "shock syndrome".

Cohn and Kaplan describe hepatic jaundice as that jaundice caused by functionally subnormal, abnormal, and necrotic hepatic cells which are incapable of transferring bilirubin into the bile passages at a normal rate. The etiologies for this type of jaundice include congenital abnormality (e.g., familial nonhemolytic jaundice), chemical, infectious agents (e.g., viral hepatitis), neoplastic disease of the liver, nutritional deficiencies, metabolic disease (e.g., hyperthyroidism), and anoxial states (e.g., severe anemias).

The same authors state that in posthepatic jaundice there is obstruction of the biliary passages, either intra- or extrahepatic, which prevents otherwise normal cells from transferring bilirubin into the bile canaliculi. This group includes congenital abnormalities (e.g., bile duct atresia), calculi, inflamation (e.g., cholangitis), and carcinoma (e.g.,of the head of the pancreas).[134]

Other classifications of jaundice have been suggested, such as the one of Rich who divides jaundice into two types--retention and regurgitation--; however, the Ducci classification with the modifications by Miller that have been noted here will be used generally in this thesis.

ORGANIZATION OF THESIS

The method for reviewing liver function tests in this thesis will be to first consider each test individually. Following this a review of autopsies from the University of Nebraska College of Medicine Department of Pathology will be presented in an attempt to correlate results of some liver function tests with hepatobiliary findings at autopsy. Then there will be a chapter on the value and use of liver function tests in general; and finally there will be chapters on recent and current work in the field and a summary.

ALKALINE PHOSPHATASE

INTRODUCTION

Though the source of alkaline phosphatase is uncertain and the reasons for its increase in serum in various pathological states are unknown, the serum alkaline phosphatase is often a useful liver function test. Several methods are available for the determination of this enzyme including the classical method of Bodansky and that of King and Armstrong.

METHODS

The procedure used in the University of Nebraska College of Medicine laboratory is a modification of the Bodansky method. The test consists of determining the amount of phosphorus liberated by serum alkaline phosphatase from a buffered substrate--sodium glycerophosphate-- as phosphate ion during exactly one hour incubation at 37°C. and at pH 9.3. Intermediate steps include filtration of the serum-substrate mixture and 50 per cent trichloroacetic acid through Whatman number 42 filter paper, addition of molybdate-sulfuric reagent, and the use of elon solution. Readings are made on a Coleman Junior spectrophotometer at a wavelength of 700 millimicrons, and, after correcting for the dilution factor, answers in mgm. per 100 ml. of serum for phosphatase and phosphate ion are

obtained. By substracting the latter from the former, one calculates the units of alkaline phosphatase activity in the serum--a unit of phosphatase activity being defined as equivalent to 1 mgm. of phosphorus liberated from the substrate as phosphate ion under the conditions of time, temperature, and pH described for the test. Normals are 0.5 to 4 units in adults and 5 to 14 units in children. The test takes approximately three hours to run.[213]

The classical development of this test began with two separate formulations of methods to determine serum alkaline phosphatase activity. Bodansky's method was similar to the one described above except that greater quantities of all materials were used, the pH was 8.6, and the measurements were made on a colorimeter rather than on the yet-to-be-developed spectrophotometer.[10]

King and Armstrong developed their test using disodium phenylphosphate as the substrate at a pH 9.0 with the incubation at 37.5°C. for 30 minutes. The alkaline phosphatase activity was determined by the amount of phenol liberated from the substrate. They determined their normal range to be 3.0 to 13.0 units per 100 ml.[92]

Seligman and associates saw in Bodansky's method the disadvantage of requiring two controls for each determination in order to account for the spontaneous hydrolysis of the substrate and the serum inorganic phosphate present before incubation. Therefore his group used sodium B-naphthyl phosphate as the substrate.[182] Phenolphthalein phosphate has also been recommended as the substrate.[80]

King and associates determined a micro method for infants which requires only 0.1 cc. of serum. Intermediate steps include addition of molybdate, stannous chloride, and 7 per cent trichloroacetic acid. Measurements are made with a photoelectric colorimeter. Essentially, this method is similar to the one used in Nebraska's laboratory.[91]

Recently, Schwartz and associates have suggested an automated method using sodium phenylphosphate as the substrate in place of the manual procedure utilizing sodium B-glycerophosphate. A factor, 0.30, has been established which permits the conversion of units from the automated King-Armstrong to the manual Bodansky method. The authors point out the advantages of their method are speed and greater accuracy in serum with high concentration of alkaline phosphatase.[180]

THEORIES OF PHOSPHATASE ACTIVITY

The main source of alkaline phosphatase under normal conditions appears to be osteoblastic activity.[134] It is also probably formed by intestinal mucosa and possibly by the liver.[159]

The mechanisms responsible for the increase in serum alkaline phosphatase in hepatobiliary disease are poorly understood. The "retention theory" sought to explain the increase due to a reduction in enzyme excretion because of either hepatocellular failure or biliary obstruction.[72] This theory was rejected by Cantarow and Nelson who pointed out its inconsistencies in portal cirrhosis and congenital biliary atresia.[16] Another theory is that the

apparent increase is due to the presence in serum of a catalytic agent that enhances enzyme activity.[67] A third theory proposes that the increased production of the enzyme is in bone because of the development of osteomalacia secondary to the steatorrhea and vitamin D loss that occur in obstructive jaundice.[60]

Harrison notes that the most convincing case can be made for the theory that the increase in alkaline phosphatase is due to overproduction of the enzyme by the liver in response to such diverse stimuli as hepatocellular injury, increased intraductile pressure, inflammatory disease of the ducts, and expanding lesions compressing parenchyma and ducts.[12, 160] The two most impressive works of supporting evidence, according to Harrison, are (1) that ligation of the pedicle of a single lobe of the liver raises the serum alkaline phosphatase level without producing jaundice, an effect that is prevented by excising the lobe at the time of ligation, and (2) that creation of an external biliary fistula results in an increase in alkaline phosphatase concentration not only in the serum but also in the bile.

However, none of the theories has yet explained why the enzyme level is higher in obstructive than in hepatocellular jaundice.[67] The issue remains unsettled.

USE IN DIAGNOSIS OF LIVER DISEASE

Traditionally, the chief usefulness of the serum alkaline phosphatase determination has been as an aid in the diagnosis of

extrahepatic biliary tract obstruction.[5]

Franklin and associates noted that elevation of the enzyme in serum showed no relation to morphologic changes in the liver. When they analyzed the results of their series, these workers noted that in those cases where the results were from 4 to 10 Bodansky units the etiology was generally related to liver cell damage. Elevation above 10 Bodansky units was noted primarily in "surgical", or extrahepatic obstructive, jaundice.[44]

Popper notes that "very high" values are found in conditions of cholestasis (both extrahepatic and intrahepatic), cholangiolitic ("xanthomatous") cirrhosis, and primary hepatic carcinoma. Lower, but definitely elevated, values were noted in hepatitis, cirrhosis, metastatic carcinoma, biliary fistula, and hepatic tuberculosis. The test is often normal in acute hepatitis and fatty metamorphosis of the liver.[159]

Ulevitch and associates found that the serum alkaline phosphatase concentration was a more sensitive indicator of incomplete biliary obstruction than was the serum bilirubin level.[210]

Sherlock, using the King-Armstrong method, found inconsistent rises in alkaline phosphatase in acute hepatitis and cirrhosis. He observed that in obstructive jaundice the phosphatase level usually rose progressively at an early stage and did not reflect the secondary changes occuring in the liver.[187]

Cleve and associates noted a rise in alkaline phosphatase activity in a small series of cases of hepatic tuberculosis.[21]

Rapoport observed considerable increase of phosphatase in 13 cases of children with infectious hepatitis during the early part of the disease.[168] However, the phosphatase is normally elevated in children and this may have had some bearing on his results.

The use of the phosphatase determination in the diagnosis of hepatic metastases has been extensively studied. This determination is a most important one in the decision of whether or not to subject a patient to a radical surgical procedure in an attempt to fully eradicate a malignant condition.[61] Shay and Siplet reported a high incidence of hepatic metastases in patients with an elevated alkaline phosphatase, especially if accompanied by an elevated Bromsulphalein retention and a normal serum bilirubin. Rarely, gallstone disease may produce similar laboratory findings. Granulomatous lesions of the liver produce similar results but they often alter one or more of the flocculation tests.[186]

Mendelsohn and Bodansky studied 160 nonicteric cancer patients, 99 of whom had proven liver metastases. They concluded that the serum alkaline phosphatase was the liver function test of choice in the diagnosis of metastatic liver disease at a time when the condition was not advanced, although some correlation was noted with the Bromsulphalein, serum bilirubin, and cephalin-cholesterol flocculation tests. About 90 per cent of the cases with advanced liver metastases had elevated serum alkaline phosphatase values above the highest control which was 5.4 Bodansky units.[133]

In the diagnosis of primary carcomona of the liver, Greene

and Schiff have recently presented three signs and symptoms indicative of the possibility of this condition. First is the appearance of dull, progressive right upper abdominal pain over an enlarging, firm, usually nodular liver. Second is a pre-existing postnecrotic or posthepatic cirrhosis (which of itself should tend to exclude metastatic tumor). And third is an elevation of the serum alkaline phosphatase above 7.2 Bodansky units.[58]

NON-HEPATOBILIARY CAUSES OF ABNORMAL TEST RESULTS

Among the nonhepatobiliary causes of elevated serum alkaline phosphatase are childhood, due to the increased osteoblastic activity accompanying normal growth; and bone diseases associated with abnormal increases in osteoblastic activity, including carcinoma metastases, hyperparathyroidism, and Paget's disease of bone.[159] Congestive heart failure, especially when associated with auricular fibrillation, causes a mild elevation presumably due to passive congestion of the liver.[41]

The enzyme level is also elevated following the ingestion of fat and is lowered in fasting states.[204] There is also an elevation of the enzyme in Gaucher's disease.[159] One report has stated that there is a very slight increase in the serum alkaline phosphatase with age up to the age of 60.[183]

Inconsistent test results should also lead to a careful check for the error in imperfect reagents or some alteration in the con-

ditions of time, temperature, or pH of the method.

CONCLUSION

One may conclude that the serum alkaline phosphatase is a valuable test to perform when extrahepatic biliary obstruction is being considered. The test is also useful when attempting to discover the presence or absence of liver metastases in a patient with a malignancy. The test is of much less value in determining the presence and extent of hepatocellular disease such as cirrhosis, or hepatitis.[229] The elevation of the enzyme activity in children, bone disease, and following fatty meals must always be kept in mind.

SERUM AND URINE BILIRUBIN

INTRODUCTION

The detection of increased amounts of serum bilirubin or the presence of bilirubinuria is often helpful in the diagnosis of hepatobiliary disease. However, the value of these tests---especially the partition measurements of serum bilirubin--is widely questioned by many authorities. Therefore, these tests--which seem simple and straightforward--require careful study and understanding in order for one to gain the most from the laboratory values obtained.

METHOD

The serum bilirubin determination at the University of Nebraska College of Medicine laboratory is done utilizing the Coleman Junior spectrophotometer. A diluted serum mixture is prepared by adding 9 ml. of distilled water to 1 ml. of serum. Five ml. of this mixture is used as a blank after 1 ml. of bilirubin blank solution (hydrochloric acid and water) is added. This blank is placed in the spectrophotometer and the machine is adjusted to 100 per cent transmission at a wavelength of 535 millimicrons. The other 5 ml. of the serum-distilled water mixture is combined with 1 ml. of Diazo reagent, (sulfanilic acid, hydrochloric acid, water, and sodium nitrite) mixed well, and read in the

spectrophotometer against the blank at the end of one minute. This reading gives the direct-acting bilirubin when applied to a table converting the reading to mgm. per 100 ml. Immediately after this reading is taken, 6 ml. of absolute methyl alcohol is added to both the blank and the unknown, and mixed. After standing for 15 minutes, both tubes are read as before on the spectrophotometer to give the total serum bilirubin. Both the direct and total serum bilirubin are reported in mgm. per 100 ml., with the normal direct serum bilirubin being less than 0.25 mgm. per 100 ml., and the normal total serum bilirubin being less than 1.2 mgm. per 100 ml. The test takes approximately an hour to run.[213]

The urine bilirubin is determined by employing an Ictotest® made by Ames Company, Inc. The tablet imparts a blue to purple color to the urine, which is on a cellulose-asbestos test mat, when bilirubinuria is present.[213]

Early in the second decade of this century, van den Bergh discovered that the bilirubin in some serums would react with Ehrlich's diazo reagent without the use of alcohol, and thereby originated the direct, or aqueous, diazo reaction.[38]

Malloy and Evelyn introduced the photoelectric colorimeter method for measurement of total serum bilirubin in 1937. The test is almost exactly the same as the one now in use for total serum

bilirubin at the University of Nebraska. The major variation from this work presently employed is, of course, the use of the spectrophotometer in place of the photoelectric colorimeter.[122]

The currently used method for determination of the direct-acting serum bilirubin was introduced in 1945 by Ducci and Watson.[36]

Recently, a test consisting of direct spectrophotometry without addition of chemicals using the Coleman Junior spectrophotometer has been studied in attempting to determine the total serum bilirubin in newborns. The authors of the report state that the test overcomes the problem of hemolysis which interferes with the use of van den Bergh reagent, and that the test is simpler, more rapid, and may be more accurate than the Evelyn-Malloy method.[188] However, they state the disadvantages of the direct measurement method are that serum turbidity, when present, prohibits the test from being run, and that the lower limit of sensitivity has not yet been determined. At the present time the test is specific only for total serum bilirubin in newborns.[131]

Ictotest® tablets were reported by Sobotka and associates in 1953. The tablets contain 0.2 mgm. of p-nitrobenzenediazonium p-toluenesulfonate, 100 mgm. of sulfosalicylic acid, 10-20 mgm. of sodium bicarbonate, and 15-25 mgm. of boric acid. This mixture is sufficiently acid to neutralize the most alkaline urines. The test consists of placing five drops of urine on a cellulose-asbestos

test mat which absorbs any bilirubin on its surface. A tablet is placed on the spot of urine and moistened with two drops of water to bring the diazo reagent into contact with the urine. The presence of bilirubin is recognized by a blue to purple color which is read at 30 seconds.[191]

Franke, in 1931, proposed a methylene blue test for determination of urine bilirubin.[42] However, this test was not found to be accurate by Stokes and associates.[203]

A photelometric method for determining bilirubinuria was presented in 1940.[55]

In 1946, Watson and Hawkinson described a barium-strip modification of Harrison's test for urine bilirubin. This test consisted of using a barium impregnated strip of filter paper and reacting this with urine and Fouchet's reagent (trichloroacetic acid and ferric chloride) which was used in the original Harrison test.[224] The Harrison spot test is felt to be superior to the methylene blue determination of bilirubinuria.[143]

In 1948, a spectrophotometric method for determination of urine bilirubin was introduced,[209] as was a means for quantitative determination of bilirubinuria.[51]

The presence of urine bile salts may be crudely determined by shaking the urine and observing for a greenish-yellow foam which is indicative of the presence of bile salts in the urine.[134]

MECHANISM OF BILIRUBIN EXCRETION

Bilirubin is formed from the breakdown of hemoglobin in the reticulo-endothelial system. Other products of this breakdown are iron and globin which then proceed to storage pools in the plasma.

Bilirubin, too, goes into the plasma--as a bilirubin-albumin mixture which is absorbed into the liver and converted to bilirubin glucuronide which is excreted via the bile into the intestinal tract. Here the bilirubin glucuronide is reduced by bacteria into urobilinogen which is excreted in the feces and, to a lesser degree, in the kidneys by means of the enterohepatic circulation.[134]

Bilirubin exists in the serum in three forms, as free bilirubin and as the mono- and diglucuronide compounds. All three are believed to be be loosely bound to albumin. The glucuronide esters are water soluble, react promptly with the diazo reagent in the absence of alcohol, and are believed to be the direct-acting compounds.[134] They are thought to have passed through hepatic cells or Kupffer cells and to have passed directly to the blood or to have been regurgitated into the blood from the biliary passages.[159, 178]

The indirect portion (which equals the total minus the direct bilirubin) is insoluble in water (unless bound to albumin), reacts slowly with the diazo reagent, and requires the presence of alcohol for the reaction.[134] This portion, felt to be the free form of serum bilirubin, is believed to have not been taken

up by hepatic or Kupffer cells.[159, 178]

Thus, the direct and indirect portions of serum bilirubin are thought to differ from each other both biologically and chemically.[159] The partition of the two is felt by many to be of great clinical value,[159] while others feel the total serum bilirubin is the only valuable determination in most cases.[134]

Bilirubin cannot be detected by the usual techniques in urine from normal individuals. Therefore, a positive test for bilirubinuria implies evidence of disease of the liver or the extrahepatic biliary system.[134]

USE IN THE DIAGNOSIS OF LIVER DISEASE

The total serum bilirubin level is of diagnostic and prognostic significance in the detection of latent jaundice and in the recognition of fluctuations in the intensity of the jaundice not apparent to the eye, according to Harrison.[67] However, Popper and Schaffner feel that the total serum bilirubin has little diagnostic value. They feel it does not necessarily reflect the degree of hepatic injury in some cases, noting that jaundice may be absent even in instances of severe liver damage.[159]

Levels above 1.4 mgm. per 100 ml. are considered abnormal and are usually associated with clinical or "latent" (clinically invisible) jaundice. Values above 2.5 mgm. per 100 ml.

are usually required before jaundice becomes apparent in adults. The jaundice is due to the affinity of elastic tissue for bilirubin, thus imparting a characteristic yellow color as the concentration exceeds a critical level.[134]

Cohn and Kaplan feels that serum bilirubin levels are of no value in differentiating between acute hepatitis and obstructive conditions since a steady increase in concentration may occur in both conditions. Large fluctuations in serum bilirubin levels of jaundiced patients may indicate either a ball-valve type of obstruction or one associated with processes compressing the common bile duct and subject to exacerbations and remissions.[134]

Klatskin and Drill feel that the one-minute (direct) determination of serum bilirubin is of little value. They are of the opinion that the time interval is arbitrary and is on the ascending limb of a variable diphasic curve representing the rate of azobilirubin development in the direct van den Bergh reaction of serum.[97]

Their work is disagreed with scathingly by Zieve and associates who find fault with much of the technique of the Klatskin-Drill study and conclude that the validity of the one-minute determination has not been effectively refuted by the K atskin-Drill article.[248]

Schaffner and associates feel that in parenchymal jaun-

dice, the liver cells are unable to accept all of the bilirubin, and in obstructive jaundice they are unable to excret it; in either case, this results in a return of prompt-reacting bilirubin to the blood stream and the accumulation of bilirubin in the Kupffer cells. This accumulation impairs bilirubin uptake and subsequently the indirect bilirubin increases.[178]

In 1956, Watson discussed the comparison of bilirubin ratios to total serum bilirubin determinations. He finds the lowest ratios--the ratio is direct to total bilirubin--characteristically in hemolytic disease and in constitutional hepatic dysfunction of the Gilbert type. However, he finds that obstructive and hepatocellular types of jaundice occur in the same ratio range and hence partition is of little value in differentiation of these types of hepatobiliary disease.[222]

Chapman and associates report that with the exception of diagnosis of hemolytic disease, the routine use of the quantitative fractionation of serum bilirubin does not seem justified.[19]

As stated previously, the presence of detectable bilirubinuria is an abnormal condition. Bilirubin is often found in the urine one to three days prior to the onset of jaundice in viral hepatitis.[177] Therefore, this test is quite valuable in studying individuals in whom the diagnosis of hepatitis is suspected. In contrast, later in the disease, the test for urine bilirubin

becomes negative while hyperbilirubinemia is still present.[134] Hence, the test for bilirubinuria is quite unreliable as an index of completeness of recovery.[143]

Hemolytic diseases, with or without jaundice, are not accompanied by the excretion of bilirubin in urine ("acholuric jaundice").[134]

Bilirubinuria is a common finding in hepatocellular and obstructive jaundice. Therefore, testing for urine bilirubin is of little help in differentiating hepatic from obstructive jaundice.[67]

Some workers feel that the presence of bilirubinuria is directly related to the level of prompt-acting (one-minute) serum bilirubin,[159, 221] while others feel that there is no definite relationship.[143, 248]

NON-HEPATOBILIARY CAUSES OF ABNORMAL BILIRUBIN METABOLISM

The serum bilirubin level of a full term newborn may vary from 0.2 to 5.9 mgm. per 100 ml., with the average value of 2.0 mgm. per 100 ml. This rises to an average peak value of 7 mgm. per 100 ml. by the second to the fifth day and then recedes to the adult level. In premature infants, the serum bilirubin frequently reaches a maximum concentration of 10 to 15 mgm. per 100 ml. before descending to adult levels in one to four weeks; the severity of the jaundice usually varies with the degree of prematurity.

Indications of a severe hemolytic process in a full term infant are: (1) a serum bilirubin in cord blood exceeding 5 mgm. per 100 ml., and (2) a serum bilirubin exceeding 10 mgm. per 100 ml. during the first 24 hours of life.[134]

Felder and associates reported that next to the Bromsulphalein test, the bilirubin determination was the most sensitive of the liver function tests to chronic congestive heart failure. Fifty-two per cent of 126 determinations on 76 patients showed abnormally elevated serum bilirubin values.[41] Etiology, duration of failure, and type of cardiac rhythm bore no relation to the incidence of jaundice. This hyperbilirubinemia is believed to result due to passive congestion of the liver with decreased clearance of blood bilirubin.[41]

Urine bilirubin may also be noted in cases of congestive heart failure and in pulmonary infarcts.[159]

Either test may yield abnormal results if care and accuracy are not observed in the performance of the procedures.

CONCLUSION

The determination of serum bilirubin appears to be an appropriate component of a liver function study. The use of the direct and total partition seems to be valid during the initial workup to help exclude the possibility of hemolytic disease. However, following the initial test, the serum bilirubin functions

chiefly as a serial determinant from which progress of the patient can be deduced to some degree, and the determination of only the total serum bilirubin seems indicated except in cases of hemolytic disease.

The use of the urine bilirubin test should probably be increased by the general physician. This test is ideal for office use because of its simplicity, and it can be of great value in the early diagnosis of hepatitis. The test is also of value in the early diagnosis of obstructive jaundice. However, following the diagnosis, this test loses much of its value due to its lack of reliability in predicting the completeness of recovery.

BROMSULPHALEIN

INTRODUCTION

The Bromsulphalein test (BSP) is one of the most useful screening tests for liver function. It is the only commonly used liver function study utilizing injection of a foreign substance. Though it is quite sensitive to changes in the hepatobiliary system, it is also influenced by many other factors which must be considered when abnormal results are obtained.

METHOD

The first step in the Bromsulphalein test is to weigh the patient. The dosage of the dye at the University of Nebraska College of Medicine laboratory is 5 mgm. per kg. (or 1 ml. per 22 lb.) of body weight. The dye, prepared by Hynson, Wescott, and Dunning Co., is then injected slowly, generally into an antecubital vein. Exactly 45 minutes later, 6 ml. of venous blood is withdrawn from the opposite arm, with care taken to avoid hemolysis, 1 ml. of the serum from the withdrawn blood is mixed with 9 ml. of 0.9 per cent sodium chloride and the solution is divided into two 5 ml. portions. A blank is prepared by adding 2 drops of 5 per cent hydrochloric acid to one portion of the serum mixture. The unknown is prepared by adding 2 drops of 2.5 N (10 per cent) sodium hydroxide to the other portion. The reading is made on a Coleman Junior spectrophotometer at a wavelength of 575 millimi-

crons with the blank being set at 100 per cent transmission. The results are expressed as the percentage of dye retained in the blood after 45 minutes. The normal result is less than 5 per cent retention in 45 minutes, and the test takes approximately an hour and a half to run from the time of the initial injection.[213]

The test was originally devised in 1925 by Rosenthal and White. They used a dosage of 2 mgm. per kg. (1 ml. per 55 lb.) and withdrew the blood sample at 30 minutes. They also recommended a sample at 5 minutes if early liver disease was suspected. Their blank and unknown were prepared as in the method described above and the reading was made colorimetrically.[174]

Since the original work on this test, there has been a continuing disagreement as to the most favorable dosage of the dye to use, and the time interval to withdraw it. Disagreement generally has centered around lower dye dosage and shorter time periods for withdrawal of the blood sample.[52, 53, 112, 139] In 1939, Macdonald recommended the use of the 5 mgm. per kg. dosage.[113] His work was supported by Mateer[128] and associates but there has been continuing discussion. However, at the present time the 5 mgm. per kg. dosage with withdrawal of the blood sample at 45 minutes is generally accepted.[67]

MECHANISM OF BROMSULPHALEIN CLEARANCE

After Bromsulphalein (sodium phenoltetrabromophthalein sulfonate)[159] is injected intravenously, most of the dye is excreted

into the intestinal tract by the liver. The rate of removal of the dye from the blood depends on the excretory capacity of the liver, the patency of the bile ducts, and the hepatic blood flow.[67]

It has been suggested that two separate or related mechanisms are involved in the removal of BSP from the body. The first, or rapid mechanism, which seems to be related to reticulo-endothelial activity, particularly the Kupffer cells, is the removal of the dye from the blood. The second, more gradual, process, which is excretion of the dye in the bile, seems to be a function of the polygonalcells of the liver.[235]

More recently it has been suggested that the slower excretion of the BSP into bile is due to the storage of the dye in the parenchymal cells. There is general agreement that re-circulation of the dye, if it occurs at all, is not a significant factor in the rate of dye clearance from the blood.[67]

MODIFICATIONS IN THE MECHANISM

Certain prerequisites have been established for the test. Most laboratories require a fasting state for 10 hours prior to the test[53] since it has been reported that the rate of BSP clearance is increased postprandially because of increased hepatic blood flow.[67]

The test should not be run on a patient with a fever since the degree of retention is increased in hyperpyrexic subjects.[53,74,159]

Jaundice has generally been considered a contraindication for

this test.[53] In 1945, Grebler introduced a photoelectric method of BSP determination in icteric serum utilizing filters.[49] Meteer confirmed his method stating that accurate readings could be made within 1 per cent retention using the 5 mgm. per kg. technique.[127]

Zieve calculated a nomograph which he felt allowed him to estimate BSP retention in jaundiced patients by taking their one-minute and total serum bilirubins into account.[247]

However, continued objection to the BSP test in the presence of jaundice has been noted by people who believe that increased hepatic damage might result and that the presence of regurgitation jaundice invalidates the result. Reich and Davis concluded, however, that the test was reliable in jaundiced patients and that it caused no untoward reactions.[170]

Objection has been made to the 45 minute determination on the grounds that the absorption of BSP from the intestine may be responsible for certain unpredicted higher blood levels which are occasionally noted in the 45 minute determination but not in the 20 minute withdrawal procedure.[52, 112]

Exercise and standing are two other factors which increase the retention of BSP in the serum of normal patients.[159] Consequently, the prerequisites for the test stipulate that the patient should rest prior to the test and should remain supine throughout the procedure.

USE IN THE DIAGNOSIS OF LIVER DISEASE

In the absence of jaundice and circulatory disturbances, the degree of dye retention is usually proportional to the extent of parenchymal liver damage, and therefore, is a highly valuable test of hepatocellular function. In the presence of jaundice, however, the test is difficult to interpret because it is not possible to determine whether the retention of dye is due to impairment of the excretory capacity of the parenchyma or is the result of obstruction to the outflow of bile. Furthermore, the presence of excess bilirubin in the blood impairs the excretion of BSP and introduces a small error in colorimetry. Nevertheless, the test retains some value in the presence of mild jaundice since the degree of BSP retention tends to parallel the serum bilirubin level in uncomplicated obstructive jaundice but is much greater when hepatocellular damage is present.[67]

A decrease in the capacity of the liver to remove BSP from plasma may be caused by one or more of the following abnormalities: (1) decreased hepatic blood flow as in congestive heart failure; (2) hepatocellular damage; and (3) biliary obstruction.[134]

The BSP test is very sensitive in the recognition of hepatocellular damage, especially in the absence of jaundice. It is best used in screening for hepatic disease and for the detection of cirrhosis, carcinoma metastases, or persistent hepatocellular damage during recovery from hepatitis or after relief of obstruction. It has little, if any, value in differentiating "medical"

from "surgical" jaundice.[159]

The BSP determination is of greatest value in defining the status of the liver of patients considered for surgical treatment, especially during bleeding.[246]

The use of the BSP in detecting hepatic metastases has been mentioned.[148,186] However, the value of the test is debatable and the results disappointing so that one must conclude that this test does not fill the need for a test that can accurately predict the presence or absence of metastatic liver disease.[133]

NON-HEPATOBILIARY CAUSES OF ABNORMAL BSP RETENTION

Since the main use of the BSP is as a screening procedure for liver function in the absence of jaundice, it is most important to be particularly familiar with the non-hepatic causes of an increased retention of the dye.

Among the most common causes of increased dye retention outside the hepatobiliary system are: (1) congestive heart failure;[41] (2) fever;[74] (3) exercise;[159] (4) standing;[159] (5) advanced age;[159] (6) malaria;[114] (7) shock;[159] (8) gall bladder disease;[13] (9) prematurity;[147] (10) post-operative state;[207] (11) concurrent cholecystographic studies;[189] (12) concurrent phenolsulfonphthalein determination;[181] (13) morphine therapy;[13] and (14) procedural errors.[132]

A false positive result may be obtained in patients who are receiving morphine, which increases the pressure in the biliary ducts by causing spasm of the sphincter of Oddi.[13]

Burnett noted increased BSP retention in a series of patients with acute cholecystitis, but did not note similar results in patients with gall bladder disease without acute inflammation.[13]

The retention of BSP during malaria seems to be related to the fever associated with the illness.[114]

Hicks and associates reported increased retention of BSP in hyperpyrexic patients. Each subject showed increasing dye retention with increasing temperature. Hicks noted that a temperature rise of 3°F. or more appeared especially significant although there was great individual variation. He concluded that the increased retention was probably due to impaired function of the cells of the liver rather than to changes in the hepatic circulation.[74]

Obrinsky and associates reported that the normal adult BSP results are not attained in premature infants for 3 to 6 weeks postpartum.[147]

In a study of many liver function tests, Felder and associates reported that the BSP gave the highest percentage of abnormal determinations in patients with congestive heart failure.[41]

In a study of 20 patients, Tagnon and associates reported a significant increase in the retention of BSP postoperatively as compared to the test results preoperatively. These results were noted even when the operative site was such that mechanical trauma could be ruled out.[207]

BSP retention has been reported elevated when the test pro-

ceeds cholecystographic studies utilizing bunamiodyl, a tri-iodinated compound.[189] Segal reports that BSP determination should precede gall-bladder studies or should be delayed for at least 1 week following administration of agents for cholecystography. He also notes that a delay of one day should be allowed between the BSP and the phenolsulfonphthalein test for kidney function.[181]

Mendenhall and Leevy point out that false negatives are sometimes due to errors in dosage. Low initial levels of the dye may be attributed in part to expanded extracellular fluid volume. They felt that skeletal-muscle uptake, renal excretion, and accelerated hepatic extraction did not appear to be significant factors in the false-negative results they studied.[132]

ALLERGIC REACTIONS TO BSP

Capps and Ingelfinger both mention pyrogenic reactions, sometimes associated with nausea and vomiting, 6 to 12 hours after the injection of the dye which last for 1 to 2 days. Also, they have noted occasional mild allergic reactions associated with urticaria and asthma. Chambers and Moister have reported a reaction to injection of BSP dye including dyspnea, cyanosis, loss of consciousness, venous distention, tachypnea, apnea, and clonic convulsion. The patient recovered following the reaction.[18]

Other allergic responses have been reported including one case of an anaphylactoid response involving the skin, cardiovascular, and gastrointestinal systems. The authors note that BSP may act

as a hapten antigen, and that precautions should be taken to treat anaphylactoid reactions when the test is employed serially.[138]

Stecher has reported a case in which administration of BSP was followed in 3 minutes by laryngeal edema, anaphylactic shock, and death.[195]

Extravasation of the dye into surrounding tissue during injection produces severe pain, cellulitis, and occasionally tissue necrosis and sloughing.

CONCLUSIONS

The Bromsulphalein test is an excellent screening test for liver function. The conditions of the test are precise and must be followed accurately to achieve the best possible results. The results must be evaluated in terms of the patient's complete condition since many factors can affect the test. The test is of minimal value in the differential diagnosis of jaundice, but is an excellent one for detecting latent liver disease. Allergic responses to the dye must always be kept in mind.

FLOCCULATION AND TURBIDITY TESTS

INTRODUCTION

The cephalin-cholesterol flocculation and thymol turbidity tests are probably the most extensively used liver function tests. However, use does not necessarily imply understanding; and the test results often are ignored or misinterpreted by the physician. These tests are non-specific,emperical tests of serum which often furnish clues to abnormal serum protein composition and activity, and thus, can be helpful in the diagnosis and follow-up of liver disease.

METHOD

The University of Nebraska College of Medicine laboratory uses the rapid cephalin-cholesterol flocculation determination utilizing reagent prepared by Difco, Co. The test involves running a negative control of 0.85 per cent sodium chloride and 1 ml. of cephalincholesterol reagent. The positive control consists of the same ingredients in the negative control plus positive control serum. The unknown contains the same components as the positive control except that the serum to be tested is substituted for the positive control serum.

The solutions are mixed and read at once against a water or saline blank on a Coleman Junior spectrophotometer at 630 millimicrons. Then the solutions are incubated for three hours at 37°C.,

centrifuged for ten minutes, and after the supernatant is pipetted off, the sediment is read as before on the spectrophotometer.

The results are determined by multiplying 100 times the optical density in three hours over the optical density of the initial specimen. The product of this multiplication is a percentage figure which is converted to the conventional zero to four-plus scale. Normal values are zero to two-plus. The test takes approximately four hours to run.[213]

In doing the thymol turbidity test, one first filters the thymol-barbital buffer solution just before using it. The buffer solution must not be allowed to stand exposed to air and the stock bottle must be kept closed. The pH of the buffer must be 7.55.

The buffer and serum are mixed and allowed to stand for 30 minutes. A blank is prepared with the buffer solution alone. After 30 minutes, the two solutions are read on a Coleman Junior spectrophotometer using a wavelength of 650 millimicrons, and then converted to units. The normal values are zero to five units. The test takes about an hour to run.[213]

The original cephalin-cholesterol flocculation test was developed by F. M. Hanger. The cephalin was derived from sheep brain. The flocculation was read in a test tube by the observor at 24, and again at 48, hours.[63]

Frisch and Quilligan created a modified emulsion of cephalin-

cholesterol that did not flocculate with normal sera when exposed to light, as did the original mixture of Hanger's.[47]

Krautman developed a micro cephalin-cholesterol flocculation test which he proposed could be done on capillary blood. He felt it was useful in studying liver function in infants and other people on whom venipuncture would be impossible to perform.[100]

Kirschner and Glickman felt that the one-plus reaction was of no significance in distinguishing obstructive from nonobstructive jaundice.[96]

The original thymol turbidity test was originated by N. F. Maclagan. The buffer was kept at a pH of 7.8. The turbidity was read in a comparator with black line on a white background against the standards established in 1926 by Kingsbury and associates. Maclagen felt that the test should be regarded as an indicator of disturbed liver metabolism rather than as a function test.[117]

Shank and Hoagland modified the original thymol turbidity test so that it could be read on a spectrophotometer at 650 millimicrons.[184]

Ducci also advocated the use of a spectrophotometer, but he suggested a wavelength of 660 millimicrons.[34]

Probably the greatest improvement in the thymol test was the changing of the pH of the buffer solution from 7.8 to 7.55. This change was the result of work done by Mateer and associates.[127]

De la Huerga and Popper suggested standardization of the thymol buffer reagent by using a stable alcoholic stock solution and a stable buffer with half concentration of thymol. They felt this would give a uniform thymol concentration in any batch of reagent prepared.[79]

THEORIES OF CAUSES OF POSITIVE REACTIONS

The causes of abnormal flocculation and turbidity in the tests being discussed are not conclusively known because of the inadequacies in the knowledge of serum proteins and their electrophoretic components. However, several simple--perhaps oversimplified--factors have been thought to influence the tests.

In almost all the flocculation and turbidity tests, stabilizing and precipitating factors exist. For instance, normal human serum precipitates the cephalin-cholesterol flocculation reaction if the serum is sufficiently diluted, because the stabilizing effect disappears earlier in the dilution than the precipitating effect.

In almost all of the tests, a quantitative increase in gamma-globulin facilitates precipitation. In addition, qualitative gamma-globulin changes may favor the precipitation response.

Albumin seems to have a stabilizing effect on almost all these tests; and here, too, qualitative, as well as quantitative, changes have been implicated.

However, some people feel that the alpha-one globulin, rather than albumin, is the real stabilizing factor in many reactions.

In some of the tests, primarily the thymol turbidity, beta-globulin seems to have a precipitating effect.

In many tests, flocculation or turbidity is inhibited by a depression factor which is increased in any type of cholestasis.[159]

For the sake of simplicity, the following table is prepared. It is a composite of the factors thought to influence the two tests with which this chapter primarily deals.

TEST	STABILIZING FACTORS	PRECIPITATING FACTORS
Cephalin-cholesterol flocculation	(1)albumin(both qualitative and quantitative changes)[85,137]	(1)increased gamma-globulin(perhaps qualitative change)[126,137]
	(2)alpha-one globulin[8]	(2)increased beta-one globulin[8]
		(3)alpha-globulin(?)[120]
Thymol turbidity	(1)albumin(both qualitative and quantitative)[159]	(1)lowering pH of buffer[159]
	(2)cholestasis[159]	(2)decreasing ionic strength of buffer[159]
		(3)increased gamma-globulin[104]
		(4)increased beta-globulin[22,104]
		(5)increased serum lipids[104]

Evidently, the precipitating factors are not identical in these two tests, and hence the results of one test do not necessarily correlate with the findings in the other.[226]

There are many other flocculation and turbidity tests, and they will be discussed briefly later in this chapter.

USE IN THE DIAGNOSIS OF LIVER DISEASE

Neither the cephalin-cholesterol flocculation nor the thymol turbidity tests are true tests of liver function. However, these tests are valuable in detecting hepatocellular disease.

The cephalin-cholesterol flocculation test is considered one of the most useful tests for the recognition of hepatic-cell degeneration in hepatitis or cirrhosis.[159] This test is considered more dependable in detecting cirrhosis than the thymol turbidity.[67] The prognosis in patients with portal cirrhosis showing persistently strong positive reactions is not favorable, regardless of apparent clinical improvement. A negative reaction does not exclude the diagnosis of cirrhosis.[66]

The cephalin-cholesterol flocculation test often shows a positive reaction early in the course of viral hepatitis; however, this is not always the case since as many as 20 per cent of one group of patients with viral hepatitis did not exhibit a positive flocculation.[134] The cephalin-cholesterol flocculation test becomes positive earlier in the course of infectious hepatitis than does the thymol turbidity, and it also becomes negative before the latter test. Thus it is a valuable diagnostic test, but is of little value in following the course of the disease.[69]

The cephalin-cholesterol flocculation test is usually negative in biliary obstruction of short duration, fatty metamorphosis, neoplasms, solitary abscesses, chronic passive congestion, biliary cirrhosis, and cholangitic hepatitis unless there is con-

current involvement of the hepatic parenchyma.[134]

This test does not indicate the extent of hepatic damage nor does it become positive in all forms of cellular injury.[64]

The thymol turbidity test appears particularly useful in the early diagnosis of viral hepatitis, for the recognition of protracted states of hepatitis, and for differentiation of cirrhosis following hepatitis from other types of cirrhosis--since the thymol turbidity usually is positive only in postnecrotic cirrhosis.[67,159]

The test is usually normal in uncomplicated extrahepatic and intrahepatic cholestasis. Since the results are expressed in units, the test is better quantitated than the results of the cephalin-cholesterol flocculation test.[159]

The thymol turbidity test has its greatest usefulness in following the course of acute hepatitis by serial determinations, since this test is often the last one to return to normal values.[69,134] In infectious hepatitis, one can usually predict alterations in the albumin-globulin ratio by determination of the thymol turbidity test if the latter results are strongly positive. However, a lesser degree of correlation has been found when the thymol turbidity results are less strongly positive or normal.[70] No correlation between the severity of symptoms in infectious hepatitis and the degree of aberration in the thymol turbidity

test has been found.[103]

The thymol turbidity is apparently somewhat helpful in differentiating hepatocellular from obstructive jaundice since the test is usually normal in the latter and elevated in the former.[127] The combination of a highly elevated thymol turbidity test with a mildly elevated alkaline phosphatase activity favors the diagnosis of hepatocellular disease; while a normal or weakly elevated thymol turbidity test with a highly elevated alkaline phosphatase activity suggests the diagnosis of biliary obstruction.[118]

Since a majority of infectious hepatitis carriers have normal thymol turbidity test results, the use of this test to "screen " potential blood donors for the presence of the hepatitis virus is useless, and can lead to a false sense of security on the part of those people collecting and administering the blood.[4]

For the most part, the thymol turbidity test readings usually parallel the readings of the cephalin-cholesterol flocculation test in cases of parenchymal liver dysfunction.[39]

NON-HEPATOBILIARY CAUSES OF POSITIVE REACTIONS

The cephalin-cholesterol flocculation test can become positive in a number of non-hepatobiliary diseases. The test is positive in infectious mononucleosis,[159] usually during the second and third weeks of the disease.[84] Brown and associates feel that true hepatitis occurs in a significant proportion of all cases of infectious mononucleosis.[11]

The test is also positive infrequently in a number of inflammatory diseases with elevated levels of gamma-globulin.[159] These include malaria,[62,159] kala-azar, rhematoid arthritis, sarcoid, leprosy, and lymphogranuloma venereum.[134]

The test can also give abnormal results due to laboratory error. The test is photosensitive, therefore, care must be taken to protect the reagents from bright light, either natural or artificial.[142]

The thymol turbidity results can be elevated in hyperlipemic states,[67] collagen diseases (especially rheumatoid arthritis), gastrointestinal disorders, inflammatory diseases with elevated gamma-globulin, infectious mononucleosis, kala-azar, malaria, and other tropical diseases.[159]

OTHER FLOCCULATION AND TURBIDITY TESTS

The flocculation and turbidity tests have been grouped into three main families by Maclagan and associates. The first group are those tests which may be regarded primarily as modifications of the classical salting-out procedures, such as the ammonium sulfate test. The second group are those tests in which the formation of a metallic complex is the probable fundamental mechanism, such as the colloidal gold and zinc sulfate tests. The third group are those tests in which complex formation of a more complicated nature is concerned, probably involving a protein-protein

linkage.[121]

Among the countless flocculation and turbidity tests beside the cephalin-cholesterol flocculation and thymol turbidity tests are the colloidal red,[34,35] gamma-globulin turbidity,[155] cadmium turbidity,[244] zinc sulfate turbidity,[46,64,] colloidal gold,[85,141] Takata-Ara,[120] and magnesium chloride[8] tests.

The mechanisms of these tests are no better understood than are those previously discussed. With few exceptions, most of these tests have been tried and abandoned in favor of the two tests which are the main subject of this chapter.

CONCLUSION

The flocculation and turbidity tests are perhaps the most used procedures among the liver function tests. However, as has been pointed out, these tests do little to define the status of the liver except in cases of hepatocellular disease, primarily in infectious hepatitis. Consequently, though these tests are always indicated when hepatitis is suspected, they add little information when used routinely on patients.

The cephalin-cholesterol flocculation and thymol turbidity tests yield the most profitable results when used together. In this way, more than one area of serum protein derangement is tested for, and the results of the two tests together give a somewhat more accurate picture of certain hepatocellular conditions.

These tests, especially the thymol turbidity, are of use in

following the convalescence of a patient with infectious hepatitis.

The many factors that affect serum proteins which can result in positive results in these tests must be ruled out before the positive results can be interpreted as specifically representing hepatocellular pathology.

SERUM PROTEINS

INTRODUCTION

Alterations in serum proteins often point toward diseases of the liver. However, the tests of serum proteins are non-specific and variable. The advent of electrophoresis has brought about an entirely new concept in serum proteins, namely, the fractionation of the protein into its many components, rather than the simple albumin-globulin separation previously done.

METHOD

Serum proteins may be determined by any of five different techniques. The first is the traditional method of separation of serum proteins by fractional salting out, such as with ammonium sulfate. This test separates the globulins from albumin which remains in solution after the globulins are precipitated, usually by full saturation with ammonium sulfate. Efforts to refine this technique so that the various globulins may be separated from each other have been disappointing.[59]

Another salting out method is that of Howe, which utilizes sodium sulfate as the globulin precipitant. This method has been widely used despite the fact that it serves only to give an approximation of the distribution of serum protein components. Most of the work on serum proteins in disease states has been done utilizing the Howe technique.[77,78,150]

A third salting out procedure which has had only sporadic use is the potassium phosphate method of Butler and Montgomery. This method has the advantage that the pH can be well defined and maintained, except in great dilutions, over a wide range of ionic strength.[59]

The second technique is the use of water-miscible organic precipitants at low ionic strength and low temperature. Ethanol has been used;[24] although methanol is more commonly employed.[151] The methanol technique is relatively simple and gives results for albumin and total globulin which appear to be close to true values as determined by electrophoresis.[59]

The third technique is the electrophoretic method of serum protein determination. The principal limitations of this method are: (1) separation of the protein components depends upon a single property, their mobility in an electric field; (2) difficulties in accurate estimation of the components; and (3) problems applying electrophoretic analysis to abnormal sera, such as lipemic sera, abnormal proteins, and lack of sufficient concentration of some proteins of diagnostic importance.[59] However, despite these limitations, the electrophoretic method has proved an invaluable aid in analysis and purification[206] of serum proteins.[135,155]

The fourth technique involves the use of the ultracentrifuge. This method, however, has proved disappointing in its result; and its chief use in the study of serum proteins has been

in the estimation of molecular size of purified particles and in testing the homogeneity of fractions obtained by other preparative methods.[59]

The fifth technique is the use of immunochemical methods. This technique finds its greatest use in certain diseases, particularly when the protein to be detected is present in concentrations too low to be detected by other methods.[59]

The object of electrophoresis is to demonstrate the presence of constituents which have different mobilities and to measure the relative concentrations of these constituents. The conditions of pH and salt concentration influence the results, and they have to be specified.[125]

In the boundary method of electrophoresis, there is one boundary between the protein solution and the supernatant buffer in each line of the U-tube at the beginning of the procedure. On passing an electric current, these boundaries move away from the original positions at velocities equal to the velocities of the protein ions below these moving boundaries. If several protein constituents of different mobilities are present, the original boundaries will split into several boundaries moving with different speeds.

The protein components are determined by the change of refractive index measured by a horizontal ray of light passing through the tube which is deflected downward. The relative concentrations

are calculated from the relative proportions of these areas; and, if the total concentration of protein in serum has been determined, the absolute concentrations of these fractions can be calculated.[125]

Longsworth tested a variety of buffers and found that 0.1 N diethylbarbiturate solution having a pH of 8.6 at 25°C. gave the best patterns for electrophoretic analysis.[111]

Dole and Braun determined the following normal values for electrophoresis using a sodium veronal buffer (diethyl barbituric acid and sodium diethyl barbiturate) at pH of 8.6; albumin, 4.04 gm. per 100 cc.; alpha-one globulin, 0.31 gm. per 100 cc.; alpha-two globulin, 0.48 gm. per 100 cc.; beta globulin, 0.81 gm. per 100 cc.; fibrinogen, 0.34 gm. per 100 cc.; and gamma globulin, 0.74 gm. per 100 cc. The albumin globulin ratio was 1.53.[31]

Hoch felt that the diethylbarbiturate buffer of pH about 8.5 and ionic strength 0.1 was best suited for electrophoretic analysis of human sera because of the good resolution of the alpha-one globulin from the albumin. However, this buffer contains nitrogen, and therefore, further analysis of the test serum by the Kjeldahl method would not be possible. Therefore, Hoch attempted to use phosphate buffer at a pH of 8.8 and ionic strength of 0.15 in place of the diethylbarbiturate buffer, and he found that this replacement could be accomplished with good results.[76]

Kunkel and Tiselius, in 1951, introduced the method of elec-

trophoresis on filter paper. They felt that the greatest advantage of the paper method over that in free solution lay in the fact that actual separation of the protein components was accomplished.[105,106]

Mackay and associates felt that the results with paper electrophoresis compared well with analysis of duplicate samples by the Tiselius boundary method, except with certain abnormal sera, such as those of high lipid content.[115]

Smithies presented a method of zone electrophoresis in starch gels using a borate buffer with a period of electrophoresis of six hours. As little as 0.02 ml. of sample could be used.[190]

Recently, two methods of electrophoresis using combined agar gel-paper[245] and cyanogum gel[73] have been presented.

The University of Nebraska laboratory uses the paper electrophoresis method with the Spinco Model R system. The buffer solution is veronal at a pH of 8.6. The time of electrophoresis is 16 hours and the total test takes about 24 hours. The results of the electrophoresis are measured by means of the Analytrol which converts the filter paper strips dyed with brom phenol blue in methanol to graph paper from which the area under the individual peaks can be determined.[213]

In discussing this method, Stephenson and Snoddy note that the albumin migrates fastest toward the anode, followed by the alpha-one and alpha-two globulins, and then the beta globulins.

The gamma globulins migrate toward the cathode. Their normal values are: albumin, 60 per cent (of total serum protein); alpha-one globulin, 4 per cent; alpha-two globulin, 8 per cent; beta globulin, 12 per cent; and gamma globulin, 16 per cent.[201]

The chemical method for determination of total serum protein and albumin globulin fractionation used at the University of Nebraska is a spectrophotometric modification of the biuret method. Diluted serum, biuret reagent (sodium hydroxide and copper sulfate),[90,94] ether, Aerosol, and sodium sulfate are combined in various mixtures to make a total protein unknown, an albumin unknown, a blank, and a protein standard. These tubes are mixed and allowed to stand for 30 minutes, and are then read within an hour on a Coleman Junior spectrophotometer at a wavelength of 540 millimicrons. The test takes about two hours to run. Normal values are 6 to 8 gm. per 100 ml. for the total serum protein, and 3.5 to 4 gm. per 100 ml. for the albumin.[213]

The colorimetric modification of the biuret method was introduced by G. R. Kingsley. The total serum protein and albumin are determined, and then the globulin is calculated by subtracting albumin from the total serum protein.[93,94]

Levin and Brauer have used ammonium hydroxide in place of sodium hydroxide in their biuret reagent.[110]

Various other methods have utilized sodium sulfite instead of sodium sulfate.[15,237]

The Kjeldahl method was conceived by Johan Kjeldahl in 1883, and has passed through many modifications since then.[214]

Three classes of substances are added to the sulfuric acid digest. The potassium sulfate increases the boiling point and thereby accelerates the digestion process. Oxidizing agents, such as hydrogen peroxide and potassium persulfate, assist in the digestion of organic compounds without destroying any of the ammonia formed. Metallic catalysts, such as mercury, act as accelerators to the process. The ammonia formed can be determined by macro or micro-titration, or by micro-gasometric measurement,[75] in addition to the traditional distillation or nesslerization principles.[159]

Kingsley and Machella, in studying differences between albumin and globulin determinations by the biuret and Kjeldahl methods considered three factors which might influence the variation in results between the two methods. The difference might be due to (1) a change in nitrogen concentration, (2) a change in number or reactivity of biuret linkages, (3) a change in response to salting out with sodium sulfate, or (4) a combination of the factors mentioned. They felt the most likely explanation involved the first factor noted, or perhaps a combination of the first two factors.[95]

Many other methods for measuring plasma proteins have been introduced, including the refractivity measurement of a drop of serum,[205] photometric determination of gamma globulin,[175] infrared

analysis of serum proteins,[202] quantitative immunochemical method for determining serum and cerebrospinal fluid proteins,[54] density gradient electrophoresis,[25] a modified biuret reaction,[228] and flotation of serum in copper sulfate solution.[159]

THEORIES OF SERUM PROTEIN METABOLISM

Historically, the serum proteins have been divided into two major fractions. Albumins are soluble in water, and are precipitated from solution by saturation with salts such as ammonium sulfate and sodium sulfate. The globulins are insoluble in water but soluble in dilute salt solutions, and are precipitated from solution with about half saturation of such salts as ammonium sulfate and sodium sulfate. However, these classic properties of albumin and globulin are not completely valid today because of the greater knowledge of proteins.[129]

Since serum albumin is formed only by the hepatic cells, reduction of serum albumin is an indication of deranged hepatocellular function, if the other factors such as nutrition or urinary protein loss are taken into account.[159] Albumin makes up 75 to 80 per cent of plasma total osmotic pressure, which is important in plasma volume and tissue fluid pressure. Albumin also functions in tissue nutrition, as a buffer and transport vehicle for ions, and as a carrier system for a number of substances such as bilirubin, sulfonamides, penicillin, and progesterone.[129]

Alpha and beta globulins are believed to be largely synthe-

sized by the liver.[129]

Gamma globulin may be partly synthesized by the liver, the major portion being attributed to plasma cells[129] and the reticuloendothelial system throughout the body.[86] The elevation of gamma globulin in hepatic disorders can be related to three factors: (1) stimulation of hepatic mesenchymal cells; (2) infiltration by plasma cells and reticuloendothelial cells; and (3) excess formation outside the liver caused by stimulation of extrahepatic mesenchyma, or by utilization for gamma globulin formation of amino acids, which are in excess because they are not used by the damaged liver for albumin synthesis. The last factor may explain the reciprocal behavior of serum albumin and gamma globulin.[159]

USE IN THE DIAGNOSIS OF LIVER DISEASE

In hepatocellular disease there is a tendency for the serum albumin level to fall. Usually this is because of a decrease in albumin synthesis in the liver, but malnutrition may be a factor in some instances. As a rule, the decline is slow, so that low concentrations of albumin are more common in chronic than in acute liver disease.[67]

The serum albumin level is of little help in the differential diagnosis of jaundice, since it may be equally reduced in both obstructive jaundice and hepatitis. Its main value lies in the follow-up of therapy, especially in cirrhosis, where improvement in serum albumin level is the best sign of successful medical treatment.[29,159]

Prognosis as to duration of life in patients with cirrhosis becomes increasingly grave as the level of serum albumin decreases. The level of serum albumin is significantly lower in patients with ascites than in those without ascites; and diuresis is associated with a rise in serum albumin.[161]

The serum globulin level tends to rise in both acute and chronic hepatocellular disease. Usually this is because of an increase in gamma globulin, but occasionally the alpha and beta fractions also are increased.[67]

In obstructive jaundice, the beta globulins tend to rise and may be accompanied by hypergammaglobulinemia if the obstruction is complicated by infection or hepatocellular injury.[67]

Since the reticuloendothelial system is thought to be the source of gamma globulin, it is reasonable to suppose that the hypergammaglobulinemia of liver disease is due to overactivity of the Kupffer cells related to inflammatory, degenerative, or regenerative processes.[67]

Elevation of gamma globulin is probably the most frequently encountered abnormal result obtained in any of the hepatic tests. Because of the incidence of slight elevations in apparently healthy people and greater elevations in many diseases, the diagnostic value of elevated serum gamma globulin is small.[159]

Significant increases in beta globulin have been observed in all types of liver disease, but to a considerably lesser degree

and frequency than the gamma globulin changes.[57]

Usually the total serum protein is low or normal in hepatocellular disease. However, rarely, hypergammaglobulinemia may be so marked that hyperproteinemia results. This is seen particularly in the chronic form of viral hepatitis and in posthepatic cirrhosis.[67]

The practical value of the determination of the total serum protein in diseases of the liver is in appreciating the state of nutrition. It is of particular value in preoperative management. The serum protein level is not a reliable index of the protein stores, although low levels generally indicate reduced protein depots.[159]

In a study by Popper and associates, the total serum protein level shows a wide spread in the various hepatobiliary diseases. The means hardly differ from the normal, the albumin depression being compensated for to various degrees by elevation of globulins.[153]

The importance of the albumin globulin ratio has been overemphasized according to Harrison. He notes that while it often is reversed in hepatocellular disease, one must realize that the albumin and globulin concentrations vary independently and that each has its own significance.[67]

Rafsky and associates note that in acute, as well as in some

instances of chronic, hepatitis, the protein partition and albumin globulin ratio as determined chemically yields apparently normal values, in marked contrast to the results obtained by electrophoretic analysis. The inadequacy of routinely employed chemical methods is also apparent in cirrhosis and metastatic carcinoma of the liver.[167] However, Franklin and associates advocate that the routine use of plasma electrophoresis for the study of protein alterations in hepatic diseases not be done.[43] Popper and associates note that although quantitatively differing markedly, the albumin globulin ratio as determined by electrophoresis and the chemical method of Howe do not differ much in the diagnostic evaluation of hepatobiliary diseases. In fact, the Howe method, although not measuring pure protein entities, is superior in differentiating obstructive jaundice from cirrhosis.[153]

NON-HEPATOBILIARY CAUSES OF ABNORMAL SERUM PROTEINS

Serum albumin may be increased due to hemoconcentration in dehydration states.[129]

Albumin may be decreased in malnutrition and debilitating diseases, ascites, nephritis and nephrosis, chronic gastrointestinal disorders,[159] and pregnancy.[129] The albumin does not change with age, weight, height, serum cholesterol, or serum inorganic phosphorus.[28]

Alpha globulin increases in association with trauma, burns, nephrosis, diabetes mellitus, collagen vascular diseases, occasional hyperthyroid states, malignancies, leukemia, and occasional cases of multiple myeloma.[129]

Alpha globulin is decreased in occasional cases of hypothyroidism.[129]

Beta globulin increases in states of hyperlipemia, hypothyroidism, diabetes mellitus, collagen vascular disease, some cases of multiple myeloma, and pregnancy.[129]

A decrease in beta globulin is rarely seen. Occasionally there is a decrease in cases of cholestasis, anemia, and eclampsia.[129]

Gamma globulin is increased in chronic inflammatory states, and in association with collagen diseases, tuberculosis, myeloma, sarcoidosis, kala-azar, carcinoma and Hodgkin's disease (irregularly),[159] and acute and chronic nephritis.[129]

Total serum globulin and globulin fractions increase linearly with age and decrease as weight increases.[28]

Total serum proteins are decreased due to bed rest, pregnancy after the 22 week (due to drop in albumin level alone), and infancy until the age of three.[125] Children 6 to 13 years of age have a somewhat higher total serum protein level than adults.[146]

The possibility of laboratory error must always be considered

when an unsuspected result in serum protein measurement is obtained.

CONCLUSIONS

The measurement of the serum proteins and the chemical partition of them into albumin and globulin fractions is a commonly used test. The results are non-specific for liver disease; and therefore, when the test is being run to determine liver status, one must be careful to rule out other causes of abnormal results.

The routine use of electrophoresis is both time-consuming and expensive, and generally does not aid the general physician to a greater degree than the chemical partition methods.

The traditional albumin/globulin ratio is entirely unreliable unless one knows the absolute values for the two fractions, because it is the change in both fractions that is important, not the relationship of the two to each other.

PROTHROMBIN TIME

INTRODUCTION

Theoretically, the prothrombin time should be an excellent test for liver function. Prothrombin is formed by hepatic cells, consequently, damage to these cells should result in a prolongation of the prothrombin time. However, this is not always the case.

The test itself is not specific for prothrombin as will be discussed below.

METHOD

The University of Nebraska College of Medicine laboratory method for the determination of prothrombin time involves the use of Simplastin®--a thromboplastin extract, which contains calcium and sodium chloride,--so that by merely adding distilled water one has a solution containing the optimal amounts of both thromboplastin and calcium chloride.

Both plasma and Simplastin® are incubated at 37°C., and then the plasma is added to the Simplastin® and the time for a clot to form is determined. The test is reported in seconds and is compared to a control. The average control is 12 to 15 seconds. Normally the control and the test plasma clots should be formed in equal or almost equal time. The test requires approximately 10 to 15 minutes to run.[213]

The one-stage prothrombin time was developed by Quick in 1938.

It consists of adding oxalated plasma to thromboplastin, then adding calcium chloride and determining the length of time for a clot to form. A control is always run and results reported as a ratio. He reported normal plasma should clot in 12 to 13 seconds. This test is similar to that used at the University of Nebraska laboratory except that both materials used originally by Quick have been combined as Simplastin.®[164]

Kato reported a micro-prothrombin test which requires only 10 cu.mm. of capillary blood, rather than 4.5 cc. of venous blood as in Quick's test. He reported an average normal of 20 ± 2 seconds by this method.[88]

The two-stage prothrombin test was developed by Warner and associates and consists of defibrinating oxalated plasma by the addition of purified thrombin. After incubation, the plasma is diluted serially with calcium chloride, thromboplastin, and acacia, after which fibrinogen is added and the clotting time is recorded. The results are then calculated in terms of unitary prothrombin.[217] In this method the prothrombin is first converted to thrombin and the thrombin is then titrated. Though this is a more difficult method, the results are considered more accurate.[123,232]

Another two-stage method of prothrombin determination utilizing imidazole buffer has been reported.[82]

Because of its speed and simplicity and the fact that it is an accurate reflection of plasma prothrombin when compared with the two-stage method, the one-stage prothrombin determination of Quick

is generally used.[196]

THEORY OF PROTHROMBIN PRODUCTION

Prothrombin is formed by hepatic cells[159] and its synthesis requires the presence of vitamin K. Vitamin K--being fat soluble --is dependent on the presence of bile salts to be absorbed from the gastrointestinal tract.[26,157]

The prothrombin time of serum is related to at least four coagulation factors: (1) the concentration of unconverted prothrombin in the serum; (2) the thrombin formed during and after coagulation which has not been absorbed by fibrin or neutralized by the natural antithrombin; (3) the serum "accelerator effect"; and (4) the concentration of labile factor (factor V) not activated during coagulation.[196]

Of these four factors, thrombin is neutralized by the natural antithrombin--which is believed to be closely associated with albumin[163]--if sufficient incubation time is allowed. The concentration of labile factor also can be disregarded. Thus, the prothrombin time appears most affected by the concentration of residual prothrombin and the accelerator effect of the serum.[232] With this in mind, "prothrombin activity" rather than "prothrombin concentration" is the most accurate description of the value being measured by the prothrombin time.[196]

Beside prothrombin, factor VII, Ac globulin (factor V), and factor X (Stuart-Prower factor) are formed by hepatic cells and

are reduced in hepatocellular degeneration.[159] Since the conversion of prothrombin to thrombin requires these three factors,[234] an absence of any or all of them will also result in a prolonged prothrombin time. Likewise, since the prothrombin test is measured by the formation of a clot, a depletion of fibrinogen in the plasma also results in a prolongation of the plasma prothrombin time since the formation of fibrin is inhibited.[232]

This lack of specificity in the plasma prothrombin test can be an advantage in testing for coagulation pre-operatively. Since most of the elements affecting the test results are hepatocellular in origin, the same processes interfering with prothrombin formation will interfere with the formation of the other components.[159] However, the lack of specificity of the test must always be kept in mind.

USE IN DIAGNOSIS OF LIVER DISEASE

As stated previously, the prothrombin time should be an ideal method for the evaluation of hepatocellular function. However, the results are inconsistent since any hepatobiliary disease may occur in association with a normal prothrombin time. Rarely, marked liver disease may be present in a patient with a normal prothrombin time; and conversely, prolonged test results are sometimes seen in patients with no demonstrable hepatobiliary pathology. The response of prothrombin activity to parenteral injection of vitamin K is likewise inconsistent since the initial prothrombin

time does not always reflect the state of hepatocellular activity.[157]

Theoretically, parenteral administration of vitamin K should correct an abnormally long prothrombin time when the defect is due to poor absorption of vitamin K as seen in biliary obstruction which prevents bile salts from reaching the intestine. On the contrary, vitamin K parenteral injection should cause no increased prothrombin response when hepatocellular damage is present since the defect is in the liver cell preventing prothrombin production in spite of vitamin K activity.[249]

Therefore, liver response to vitamin K, and not the original prothrombin time, is the true test of liver function.[211] A decrease of a prolonged prothrombin time of at least 15 per cent within 24 hours following vitamin K administration is considered evidence of normal or only slightly damaged liver cells and hence favors the diagnosis of extrahepatic biliary obstruction.[157]

In parenchymal disease, parenteral administration of vitamin K often results in an immediate rise toward normal of prothrombin time followed by a rapid drop to the original abnormal time. Therefore, conclusions on liver response to vitamin K should be based on at least 48 hours of observation following the injection of the vitamin.[157]

Perhaps the most valuable use of the prothrombin time determination in patients with suspected liver disease is in following the hemorrhagic tendency existing in the patient. Quick noted that no serious bleeding resulted until prothrombin levels were

prolonged more than three times the control in seconds. The value of determining bleeding tendency is important before surgical procedures, including liver biopsy, are performed on the patient, and in prognostication of hemorrhagic episodes.[164]

NON-HEPATOBILIARY CAUSES OF ABNORMAL PROTHROMBIN TIME

The primary non-hepatobiliary causes of hypoprothrombinemia are (1) lack of vitamin K in the diet (a rare circumstance);[211] (2) drug therapy such as Dicumarol or salicylates; (3) chronic gastrointestinal disorders resulting in poor vitamin K absorption; (4) icterus neonatorum; (5) idiopathic hypoprothrombinemia;[26] and (6) the use of imperfect reagents and materials in performing the test.

Hyperprothrombinemia, as determined by a reduction of the prothrombin time below the normal range, has been observed in man in cases of intravascular thrombosis such as in conditions of frost bite, gangrene, or thrombosis. This hematologic condition lasts for extended periods of time.[212]

CONCLUSION

THe prothrombin time is a valuable test of bleeding tendency, and can at times be helpful in the diagnosis of liver disease. If prolonged, it may be useful to repeat the test following parenteral administration of vitamin K in an attempt to differentiate hepatocellular from extrahepatic jaundice. However, because of its

inconsistent clinical results, the prothrombin time should not be regarded as a test of choice in the evaluation of liver function.

SERUM TRANSAMINASES

INTRODUCTION

The most recent test of liver function that has attained wide-spread use is the determination of serum transaminases--both the serum glutamic-oxalacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT). These tests are not specific for liver disease but are quite helpful in the diagnosis of some hepatobiliary conditions.

METHOD

Determination of the SGOT at the University of Nebraska College of Medicine laboratory is accomplished by using buffered substrate prepared by Dade Reagents, Inc. This substrate is incubated at 37°C., following which 0.1 ml. of serum is added and the mixture incubated for one hour at 37°C. The mixture is then removed from the incubator and DPNH color developer is added and allowed to stand for 20 minutes. Then 0.4 m. sodium hydroxide is added and a reading made 5 to 65 minutes following this addition. The reading is made on a Coleman Junior spectrophotometer at a wavelength of 505 millimicrons, and the units of the enzyme are obtained from a calibration table.

The SGPT is determined in the same manner except that the buffered substrate reagent is different from that used in the SGOT determination. Also, the buffered substrate-serum mixture

is incubated at 37°C. for only 30 minutes.

Normal is 0 to 40 units for SGOT and 0 to 45 units for SGPT.[213]

The original method of SGOT determination involved serum incubation and paper chromatography. In 1955, a spectrophotometric method was devised in which the transamination reaction is coupled to the reduction of oxalacetate to malate by reduced diphosphopyridine mucleotide (DPNH), in the presence of an excess of purified malic dehydrogenase. The oxidation of DPNH, and therefore, the transamination reation, is followed by measuring the decrease in light absorption at 340 millimicrons. The determination thus involves two reactions:

$$\text{(1) alpha-keto glutarate} + \text{aspartate} \overset{\text{(SGOT)}}{\rightleftharpoons} \text{l-glutamate} + \text{oxalacetate}$$

$$\text{(2) oxalacetate} + \text{DPNH} + \text{H} \xrightarrow{\text{(malic dehydrogenase)}} \text{l-malate} + \text{DPN}^{+}$$ [87]

The original method for SGPT determination, presented in 1956, was similar to the spectrophotometric method of SGOT. The reactions involved in this test are:

$$\text{(1) alpha-keto glutarate} + \text{l-alanine} \xrightarrow{\text{(SGPT)}} \text{glutamate} + \text{pyruvate}$$

$$\text{(2) pyruvate} + \text{DPNH} \xrightarrow{\text{(lactic dehydrogenase)}} \text{lactate} + \text{DPN}^{+}$$ [243]

Here too, the spectrophotometric reading is made at a wavelength of 340 millimicrons at room temperature.

In both tests, the activity is expressed as units per ml. of serum per minute. One unit equals a decrease in optical density of 0.001 millimicron under the conditions described.

Normal range for the SGOT was reported originally as 9 to 32 units with a mean value of 19.6 units.[87] Normal range for SGPT was 5 to 35 units with a mean of 16 units.[243]

THEORY OF INCREASED ENZYME ACTIVITY

Enzymes catalyzing different transamination reactions have been found widely distributed in animal and human tissues, and have been shown to change in activity in some tissues during disease.[87] The activity of SGPT has been found to be somewhat greater in the liver than has SGOT which is particularly concentrated in cardiac muscle.[242]

The mechanism for the increase in SGOT associated with acute cardiac muscle injury appears to be primarily one of escape of SGOT from necrotic cells due to an increase in the permeability of the injured heart muscle cells;[145] while the mechanism for increase of SGOT and SGPT during acute hepatocellular injury involves, in addition, a metabolic and/or excretory aberration.[242]

USE IN THE DIAGNOSIS OF LIVER DISEASE

Elevations of SGOT and SGPT activity are most marked in cases of infectious hepatitis. This rise may be as high as

2000 to 3000 units within a week or two after the onset of the disease, with SGPT values usually somewhat higher.[134]

Although the transaminase activity generally returns to normal before the serum bilirubin does, it has been reported that these enzyme levels remain persistently elevated up to six months in a definite percentage of cases of infectious hepatitis without clinical evidence corroborating prolonged convalescence.[140] This would seem to make the test much less valuable as a means of prolonged observation in the recovery stage.

The correlation between the degree of elevation of the enzyme activity and the clinical severity of hepatitis remains a moot point. Experimental data involving carbon tetrachloride poisoning of rats showed that their SGOT elevations were parallel to the amount of the poison each received.[238] The height and duration of increased SGOT activity was proportional to the amount of carbon tetrachloride administered as well as to the severity of liver-cell damage in another experiment on rats also.[136] A relationship has also been reported between the rise in SGOT activity in rats and (1) the size of the virus inoculum, (2) the blood virus titer, and (3) the degree of liver necrosis.[48]

However, in a recent article it was noted that though high SGOT values in rats can be correlated with marked hepatocellular damage, low or normal SGOT values cannot be used to exclude the presence of significant hepatic necrosis.[236]

Enzyme elevation in cases of infectious mononucleosis has

been noted in 90 per cent of cases. The increase is moderate (up to 350 units)[134] and transient, usually disappearing in less than 3 weeks.[109]

Mild to moderate elevations of SGOT and SGPT up to 350 units, but usually below 100 units, are noted in cases of cirrhosis, extrahepatic obstructive jaundice, and liver metastasis. The mean values of both enzyme determinations are reported lower in cirrhosis than in the other two conditions, however there is considerable overlap in the results obtained from patients with the varying conditions. The transaminase activity is therefore of no value in differentiating these three conditions.[192,241]

Baden and associates have reported that though the presence of liver metastases cannot be ruled out by a normal SGOT determination, the presence of normal levels of both SGOT and alkaline phosphatase are found in only 10 per cent of cases with liver metastases.[6]

Measurement of SGPT activity may be quite useful in following patients who are receiving hepatotoxic drugs such as chlorpromazine because it is an early indicator of hepatic damage.[134]

Infants with jaundice due to "medical" causes, such as inspissated bile syndrome or viral hepatitis, show enzyme activity up to 800 units within 1 week postpartum followed by a leveling off. Infants with "surgical" jaundice due to congenital malformation of bile ducts take 4 to 6 weeks to reach an SGOT elevation of 800 units where they also level off. Neonatal jaundice due to

hepatic cirrhosis secondary to malformation of the biliary tract showed variable results from normal to levels less than 800 units.[99]

NON-HEPATOBILIARY CAUSES OF ABNORMAL ENZYME ACTIVITY

Normal newborn infants have SGOT values up to 120 units and SGPT values up to 90 units. By 2 to 3 months of age, the enzyme values are similar to those of adults.[99]

Another report gives normal values for SGOT as follows: (1) newborns less than 1 week old--up to 70 units; (2) infants from 1 week to 1 year--up to 50 (perhaps 70) units; (3) children up to 7 years--up to 40 units. This same report gives newborn SGPT normal as up to 50 units with the upper normal for adults of 30 units.[20]

Therefore, though the figures vary in different studies, it is apparent that SGOT and SGPT values are elevated in the neonatal period and reach normal or near-normal adult values sometime in the first year of life.

SGOT levels also rise sharply following myocardial infarction.[2] The values range from 2 to 20 times normal within 24 hours and return to normal range within 3 to 6 days following the infarction without exception.[108]

A rough correlation has been reported between the height of the SGOT activity and the size of the myocardial infarct.[107] This observation has not been confirmed to date.

The SGOT is unaffected by angina pectoris, coronary insufficiency, heart failure, or digitalis in the absence of active

heart cell damage.

Unexpected results should always lead to a rechecking of the reagents used and the spectrophotometric equipment.

OTHER ENZYME TESTS USED

Lactic dehydrogenase is present in venous serum of normal human adults. The measurement of this enzyme is similar to that of SGOT and SGPT. The normal range is 260 to 850 units with a slight elevation noted in infants. This enzyme is characteristically elevated following myocardial infarction.[240]

Serum leucine aminopeptidase (LAP) and beta-glucuronidase are two other enzymes that have been shown to rise in patients with acute hepatitis, cirrhosis, liver metastases, carcinoma of the pancreas, and recurrent or residual common duct stone.[152]

The number of serum enzyme tests will undoubtedly continue to increase, probably in accordance with the law of diminishing returns.

CONCLUSIONS

The measurement of serum transaminase activity adds a further tool to the diagnosis of specific liver pathology. The primary value of this test is in the discovery of hepatitis and the differentiation of this disease from infectious mononucleosis which produces much lower elevated values. The test may also be of use in the differential diagnosis of jaundice in the newborn. The eleva-

tion of these enzymes in conditions of tissue necrosis, primarily in myocardial infarction, and in children must be kept in mind.

UROBILINOGEN

INTRODUCTION

Urobilinogen is a pigment derived from bilirubin which is excreted in both feces and urine. Quantitative measurement of the pigment in the feces or urine is a sensitive indicator of bilirubin metabolism and consequently is of great value in the diagnosis of hepatobiliary disease.

METHOD

The sample needed for a fecal urobilinogen determination generally consists of a 72-hour collection of all fecal material excreted by the patient. At the University of Nebraska College of Medicine laboratory the collected sample is weighed and a filtrate prepared from it by grinding the sample and adding 20 per cent ferrous sulfate, water, and 10 per cent sodium hydroxide to a portion of it. A qualitative test is run on the filtrate to determine the amount of filtrate to use in the quantitative test. Then the correct amount of filtrate is placed in a separatory funnel and various layers are produced by the addition of petroleum ether and glacial acetic acid. The final extract is prepared by addition of Ehrlich's reagent and saturated sodium acetate and a reading is made on a Coleman Junior spectrophotometer at a wavelength of 565 millimicrons against a blank consisting of Ehrlich s reagent and sodium acetate. The result is calculated

as mgm. of urobilinogen per 100 gm. of feces and mgm. of urobilinogen per 24 hours. The results are reported in the following manner: (1) total weight of feces; (2) mgm. of urobilinogen per 100 gm. feces; and (3) mgm. of urobilinogen per 24 hours. The normal range is 40 to 280 mgm. of urobilinogen per 24 hours. The test takes approximately an hour and a half to run plus seventy-two hours to collect the sample.

The specimen for a urine urobilinogen determination consists of a two-hour sample obtained from 2 to 4 p.m. A blank is prepared by adding saturated sodium acetate to urine, mixing, and then adding Ehrlich's reagent. The unknown is prepared by adding Ehrlich's reagent to urine, mixing, and then adding saturated sodium acetate. The order that the reagents are added is important in obtaining consistent results in this test. The reading must be made within 5 minutes after mixing on a Coleman Junior spectrophotometer at a 565 millimicron wavelength. The result is then calculated in Ehrlich units per 2 hours. The test takes less than 10 minutes to run plus the two-hour collecting period. The normal value is less than one Ehrlich unit in 2 hours.[213]

The original tests for fecal and urinary urobilinogen were developed by C. J. Watson in 1936. The methods were similar to the ones described above although colorimetry was used and the amounts of the various reagents were somewhat greater. Watson proposed urinary urobilinogen excretions should be done on a 24-hour urine specimen with a normal range of 0 to 4 mgm. per

24 hours. His normal range for fecal urobilinogen was 40 to 280 mgm. per 24 hours.[218]

Another method was presented by R. Sparkman in 1939[193] but this was found to be inferior to Watson's method in a study done by Steigmann and Dyniewicz,[197] and in other studies.[223,230]

In 1944, Watson and associates presented the simple methods for quantitative determination of urine and fecal urobilinogen that are now used at the University of Nebraska. The only difference was that they still employed the colorimeter since the spectrophotometer had not yet been invented.[227]

The use of the Waring Blendor® instead of a mortar and pestle to grind the sample in the fecal urobilinogen test has been advocated.[130]

MECHANISM FOR UROBILINOGEN EXCRETION

The urobilinogens are formed by the reduction of bilirubin in the intestinal tract by bacterial action. The urobilinogens are colorless but tend to oxidize in the intestinal tract to form urobilins which impart a brown color to the feces.[67,159]

A portion of the urobilinogen is absorbed into the general circulation from which much is reabsorbed by the liver (the enterohepatic circulation) and excreted into the intestine. However, a small amount of urobilinogen is excreted by the kidneys.[67]

Urine urobilinogen actually consists of a number of pigment precursors, especially mesobilirubinogen and stercobilinogen.

However, for practical clinical purposes these elements can be considered as a single entity since each of the components gives the same reactions with most methods used in simple urinalysis.[159]

The fecal urobilinogen test actually determines both urobilinogen and urobilin. The urobilin is reduced to its corresponding urobilinogen before the total urobilinogen is determined.

Under normal conditions, no bilirubin is found in the urine or the feces, though the fecal pigments are often called bile.[67]

USE IN THE DIAGNOSIS OF LIVER DISEASE

Early in the acute phase of infectious hepatitis, normal or near-normal amounts of bilirubin are excreted into the gastrointestinal tract and are converted to urobilinogen, much of which is absorbed and reaches the liver via the circulation. However, because of parenchymal cell dysfunction, there is interference with the transfer of the urobilinogen from blood to the bile canaliculi. Therefore, the blood level of urobilinogen increases and the kidney excretion of the pigment is enhanced. Later in the course of this disease, especially if liver necrosis or dysfunction is marked, decreased amounts of bilirubin reach the gastrointestinal tract so that the urinary excretion may even fall to normal amounts.[134] On the other hand, fecal urobilinogen is diminished in infectious hepatitis because of reduced bile flow into the intestine due to the impairment of the enterohepatic circulation.[67]

Cirrhosis and toxic hepatitis produce results similar to infectious hepatitis although in some cases of cirrhosis Watson noted an increase in fecal urobilinogen due to a hemolytic component.[220]

In cholestasis, less bilirubin than normal enters the intestine, and less urobilinogen than normal is formed, absorbed, and excreted in the urine. If renal failure can be excluded, reduced or absent urinary urobilinogen indicates cholestasis. Even with complete biliary obstruction, some urobilinogen is formed by bacterial action upon bilirubin in desquamated epithelial cells and in intestinal secretions, or by transformation of bilirubin in the biliary passages in the presence of bacterial cholangitis usually associated with stasis.[159]

Serial determinations in the presence of reduced urinary and fecal urobilinogen may help in differentiating calculous obstruction in which there is fluctuating excretion, from malignant obstruction in which there is no excretion.[198,220] However, an exception to this is sometimes found in carcinoma of the Ampulla of Vater, in which cases of intermittent urobilinogenuria are found.[198]

Since hepatocellular degeneration, cholestasis, and increased blood destruction often occur simultaneously, the urobilinogen excretion may be difficult to interpret.[159]

NON-HEPATOBILIARY CAUSES OF ABNORMAL UROBILINOGEN METABOLISM

Hemolytic disease, with its excessive pigment production,[67] results in great increase in fecal urobilinogen but little or no

increase in urinary urobilinogen.[198] However, urinary urobilinogen may also be markedly elevated.[134]

Two possible explanations for the increased urinary urobilinogen in hemolytic disease are: (1) concomitant hepatic dysfunction resulting from the accompanying anoxia interfering with the transfer of urobilinogen; or (2) the load of urobilinogen presented to the liver may exceed its maximal transfer capacity, and with the rising blood levels, urobilinogen is excreted by the kidney.[134]

Conditions in which the hemolytic process is generally active in addition to hemolytic anemia include pernicious anemia, transfusion reactions, leukemia, and Hodgkin's disease.[159,197]

Urobilinogen studies are unreliable when done on patients who are receiving antibiotic therapy which interferes with the gastrointestinal bacterial flora.[159] This alteration in flora may result in prevention of the conversion of bilirubin to urobilinogen leading to an increased excretion of bilirubin in the feces.[134,176]

Urine urobilinogen is increased following acute myocardial infarction usually for about eight days following the attack. It has been postulated that the increase is due to the impaired liver function induced by the stress reaction of the myocardial infarction.[40]

Sulfonamide medication also results in an increased fecal urobilinogen with some increase also in the urinary urobilinogen.[197]

No rise in fecal or urinary urobilinogen was noted following

the ingestion of cathartics, particularly phenolphthalein, or following the intravenous injection of pectin solution.[197]

Porphobilinogen in the urine also reacts with Ehrlich's reagent to give a color similar to urobilinogen; however, the latter colored product is chloroform-soluble and the former is not.[134]

Inanition, inactivity, or a low grade infection tend to lower the excretion of urobilinogen in the feces; fever of any considerable degree probably tends to increase the amount. However, fever alone does not cause an increase in the excretion of urobilinogen in the urine; this is evidently dependent on the type and severity of the infection.[219]

Low values were noted by Watson in all cases of hypochromic anemia investigated; and slight increases were observed in half the cases of polycythemia vera.[219]

Laboratory errors may also lead to abnormal results.[89,216] The pigments are photosensitive and hence must be protected from natural light.[98,215]

The period of maximal urobilinogen excretion has been shown to vary between individuals and in the same person at various times;[149] and hence if liver disease is suspected and two successive 2-hour urine urobilinogen tests are within normal limits, a 24-hour urine urobilinogen determination should be performed.[225]

CONCLUSION

The determination of urobilinogen excretion, either in the urine or feces, is an excellent test of early liver impairment. The test is quite sensitive to changes in hepatobiliary metabolism.

Because the fecal urobilinogen test is so time consuming, the urinary excretion of the pigment is preferred in screening and routine laboratory work. However, there are times when all other determinations yield equivocal results, and it is then that the more involved fecal urobilinogen test should be use.[67]

LIMITATIONS OF LIVER FUNCTION TESTS: A REVIEW OF 108 AUTOPSIES

PURPOSE

This chapter consists of a review of 108 autopsies performed at the University of Nebraska College of Medicine between 1955 and 1960.

The initial purpose of this study was to determine if any definite pattern of results of liver function tests could be established in cases of hepatic metastasis.

INTRODUCTION

As reported in the chapter on Transaminases, Baden and associates stated that in the presence of normal alkaline phosphatase activity, a normal serum glutamic-oxalacetic transaminase determination indicated the absence of hepatic metastases in 90 per cent of cases.[6]

Little else was discovered in the literature of positive value although one report was found stating that liver metastasis should be suspected in any case in which the serum bilirubin was normal, and the alkaline phosphatase and Bromsulphalein tests were elevated.[186]

The cases that were chosen for the study fulfilled the following requirements: (1) an adequate number of liver function tests,usually at least two, must have been performed prior to the death of the patient; (2) a gross and microscopic description

of the liver must be present; and (3) an adequate case summary must have been attached to the autopsy report.

The 108 cases are classified in the following manner with respect to hepatobiliary pathology:

(1) "Normal" liver--this group includes cases of passive congestion and fatty metamorphosis when the cause of death did not involve the hepatobiliary system.(22 cases)

(2) Hepatocellular disease--includes primarily cases of cirrhosis with a few cases of hepatitis and amyloidosis. (20 cases)

(3) Malignant neoplasms of liver--the vast majority of cases were metastatic liver disease, with one case of primary hepatic carcinoma, and a few cases of hepatomas, lymphoma, and hemangiomas. (44 cases)

(4) Malignant neoplasms without liver involvement--in this group the final diagnosis had to include a malignancy without any microscopic evidence of liver metastases. (19 cases)

(5) Extrahepatic obstructive jaundice excluding neoplasm as the cause of the obstruction. (3 cases)

The liver function tests that were recorded from the case records were:

(1) Alkaline phosphatase
(2) Total serum protein with albumin/globulin fractionation
(3) Cephalin-cholesterol flocculation
(4) Thymol turbidity
(5) Bromsulphalein

(6) Serum bilirubin, both direct and total components
(7) Urine bilirubin
(8) Prothrombin time, with response to vitamin K when done
(9) Serum glutamic-oxalacetic transaminase(SGOT)

Due to the lack of determinations, fecal and urine urobilinogen tests were not tabulated in the study.

Probably the chief disadvantage to this study is that most of the laboratory tests were performed within a month of the patient's death--although this was not uniformly the case. The chief advantage is that a histological study of the liver was done in every case at autopsy.

The other forms of liver pathology besides metastatic liver disease were included in the study for the purpose of controls. Since adequate data was collected on only four general types of liver pathology, only those four--comprising 96 cases--will be included in the data.

The four types of liver pathology to be included are: (1) "normal" livers; (2) cirrhosis; (3) metastatic disease of the liver; and (4) malignant neoplasm without liver involvement.

DATA

ALKALINE PHOSPHATASE TEST RESULTS

Liver Pathology	Total Cases	No. of determinations	No. of Patients	0-4 units	4-10	Above 10
"Normals"	22	9	9	9	0	0
Cirrhosis	17	10	9	5	3	2
Metastasis to liver	38	24	22	8	9	7
Malignancy without liver involvement	19	9	9	6	1	2

TOTAL SERUM PROTEINS

				less than 6 gm.%	6-8gm.%	greater than 8 gm.%
"Normals"	22	21	18	8	13	0
Cirrhosis	17	16	14	4	12	0
Metastasis to liver	38	37	33	22	14	1
Malignancy without liver involvement	19	18	16	7	7	4

ALBUMIN/GLOBULIN RATIO

Liver pathology	Total Cases	No. of determinations	No. of Patients	TEST RESULTS reversal	non-reversal
"Normals"	22	22	19	12	10
Cirrhosis	17	14	12	14	0
Metastasis to liver	38	33	31	19	14
Malignancy without liver involvement	19	18	16	11	7

CEPHALIN-CHOLESTEROL FLOCCULATION

				3+ or 4+	2+ or less
"Normals"	22	11	11	2	9
Cirrhosis	17	10	10	8	2
Metastasis to liver	38	21	21	8	13
Malignancy without liver involvement	19	8	8	4	4

Liver pathology	Total cases	THYMOL TURBIDITY No. of determinations	No. of Patients	TEST RESULTS Less than 5 units	Greater than 5 units
"Normals"	22	12	12	10	2
Cirrhosis	17	11	9	2	9
Metastasis to liver	38	21	21	13	8
Malignancy without liver involvement	19	9	9	5	4

		BROMSULPHALEIN			
				Less than 5% retention in 45 minutes	Greater than 5% retention in 45 minutes
"Normals"	22	3	3	0	3
Cirrhosis	17	6	6	1	5
Metastasis to liver	38	7	7	2	5
Malignancy without liver involvement	19	6	6	0	6

DIRECT SERUM BILIRUBIN (1-MINUTE) TEST RESULTS

Liver pathology	Total Cases	No. of determinations	No. of Patients	less than 0.25 mgm.%	greater than 0.25 mgm.%
"Normals"	22	5	4	3	2
Cirrhosis	17	12	9	1	11
Metastasis to liver	38	21	18	4	17
Malignancy without liver involvement	19	6	6	2	4

TOTAL SERUM BILIRUBIN

				less than 1.2 mgm.%	greater than 1.2 mgm.%
"Normals"	22	11	10	9	2
Cirrhosis	17	14	11	3	11
Metastasis to liver	38	30	24	10	20
Malignancy without liver involvement	19	10	10	5	5

PROTHROMBIN TIME

Liver pathology	Total cases	No. of determinations	No. of Patients	TEST RESULTS "Normal"	prolonged 5 seconds or more beyond control time	respond to K Injected with K
"Normals"	22	2	2	2	0	0/0
Cirrhosis	17	15	11	10	5	1/2
Metastasis to liver	38	15	13	13	2	0/1
Malignancy without liver involvement	19	6	5	5	1	0/0

URINE BILIRUBIN

				positive	negative
"Normals"	22	0	0	0	0
Cirrhosis	17	2	2	1	1
Metastasis to liver	38	6	5	3	3
Malignancy without liver involvement	19	4	4	4	0

SERUM GLUTAMIC-OXALACETIC TRANSAMINASE TEST RESULTS

Liver pathology	Total cases	No. of determinations	No. of Patients	less than 40 units	greater than 40 units
"Normals"	22	4	4	2	2
Cirrhosis	17	9	6	0	9
Metastasis to liver	38	6	6	1	5
Malignancy without liver involvement	19	1	1	0	1

METASTATIC LIVER DISEASE

Case Number	Alkaline Phosphatase	SGOT
# 75	1.2 Bodansky units	97 units
# 87	12.5 Bodansky units	127 units
# 95	27.6 Bodansky units	202 units
# 96	not done	61 units
#101	4.5 Bodansky units	33 units
#106	7.7 Bodansky units	143 units

MALIGNANCY WITHOUT LIVER INVOLVEMENT

Case Number	Alkaline Phosphatase	SGOT
#107	7.9 Bodansky units	200 units

CIRRHOSIS

Case Number	Alkaline Phosphatase	SGOT
# 74	10.4 Bodansky units	55 units
# 77	9.8 Bodansky units	209 units
# 82	"normal"	82.5units
# 93	4.1 Bodansky units	208 units
# 94	3.9 Bodansky units	186 units
#103	6.2 Bodansky units	3600 units

DISCUSSION OF DATA

Though the alkaline phosphatase was below 4 Bodansky units in all nine of the "normal" patients, the results were inconclusive in studying liver metastasis. In those patients exhibiting metastatic disease of the liver; eight had alkaline phosphatase determination below 4 Bodansky units, and 16 had results above 4 Bodansky units. Of 9 patients with malignant neoplasms not involving the liver; 6 had "normal" results, while 3 showed increased enzyme activity. Therefore, though the alkaline phosphatase activity was increased more often in patients with liver metastasis, the results were not conclusive enough to warrant the use of this test as a predictor of liver metastasis.

The results of the total serum protein determinations are listed as follows:

Liver pathology	Mean value of total serum protein	Standard deviation
"Normals"	6.2 gm.%	0.95 gm.%
Cirrhosis	6.5 gm.%	1.0 gm.%
Metastatic disease of liver	6.0 gm.%	0.91 gm.%
Malignancy without liver involvement	6.6 gm.%	1.6 gm.%

By inspection, one may observe that no significant difference is apparent in the results of the total serum protein determinations in any of the four groups of liver conditions used in this study.

The albumin-globulin ratio is notable only in the group of patients with cirrhosis. Of 14 determinations done on 12 patients with cirrhosis, all 14 albumin-globulin ratios were reversed with albumins decreased in the range of 1.0 to 2.7 gm. per cent, and globulins elevated in a range of 2.65 to 7.0 gm. per cent.

Mirroring the albumin-globulin determinations to a great degree, both the cephalin-cholesterol flocculation and the thymol turbidity tests were abnormally elevated primarily in patients with cirrhosis.

The Bromsulphalein test showed abnormally elevated retention in most of the cases in which it was used. The most likely cause of many of the elevations of this test--especially in the "normals" and those patients with malignant neoplasms without liver disease--was decreased hepatic blood flow associated with congestion of the liver.

The bilirubin determination results, both direct and total, did not lend themselves well to determination of a mean and standard deviation as did the total serum proteins. The results in the bilirubin tests were either within normal limits or highly elevated, and hence the standard deviation was greater than the mean. Both the total and direct bilirubins were elevated in a majority of cases except in the "normal" patients. However, this

result could well be due to the fact that the test was often run in the presence of jaundice, and not run when jaundice was not observed or suspected.

The prothrombin time was within normal limits in the vast majority of cases. The response to vitamin K in the three cases in which the test was run was irregular and inconclusive.

The urine bilirubin test was done too seldom and with too equivocal results to be of any value in forming any conclusions.

The transaminase (SGOT) was elevated in all 9 cases of cirrhosis and in 5 of 6 cases of metastatic disease of the liver.

The combination of alkaline phosphatase and SGOT were run together in 12 cases. The results can be condensed as follows:

Liver pathology	Alkaline phosphatase	SGOT
Metastatic liver disease:	elevated in 4 normal in 1	elevated in 4 normal in 1(not the same one normal as the alka-) line phosphatase)
Malignancy without liver involvement:	elevated in 1	elevated in 1
Cirrhosis:	elevated in 3 borderline in 2 normal in 1	elevated in all 6

These results are too few to draw any conclusions from; however, it is noteworthy that the test results would prevent any differentiation between the three groups listed. Therefore, the use of the results of these two tests in the attempt to predict the presence or absence of liver metastasis is, to say the least, of dubious value and will give questionable results.

CONCLUSIONS

At first glance, the results of this study are quite discouraging. One may conclude that in the cases presented, no test or group of tests could be found that would consistently distinguish patients with metastatic disease of the liver from those who were not so affected. The inability to diagnose metastatic disease of the liver accurately, points out the greatest weakness of the present liver function tests.

However, some positive aspects are apparent from the study. Probably the most encouraging fact is the fairly uniform abnormal results found in the albumin-globulin ratio, cephalin-cholesterol flocculation, and SGO -Transaminase tests in patients with cirrhosis. However, the elevated thymol turbidity in cirrhosis is somewhat surprising, although the elevations were not generally of great magnitude.

Very few tests were run on patients with hepatitis, simply because so few people died of this disease during the five-year period of the study. However, one could expect a high degree of

correlation with some of the tests in this condition.

Noting the fact that this study included primarily chronic hepatic disease and malignancy, one may state that the inconclusive results in malignant disease were expected as was the correlation of some of the tests with cirrhosis.

AFTERTHOUGHTS OF THIS STUDY

Studies which result in negative conclusions can be of value if the procedure is analyzed and constructive criticism is directed toward the project.

The problem in studying the ability of tests to predict liver metastasis is two-fold. First, the tests must be performed over a long period of time, serially, and this entails great expense and great difficulty in continued contact with the test subjects. Second, the need for a histological examination of the liver at autopsy should be essential to determine the presence or absence of hepatic metastasis. Liver biopsies will not do in the vast majority of cases, and are unreliable themselves as predictors of metastatic liver disease.

In doing a further study, one would be well advised to begin with a large series of live clinic patients and run the experimental group of tests on them periodically. An attempt would have to be made to maintain contact with the test subjects over a period of years. Finally, autopsies would have to be performed on

all patients included in the study regardless of the presumed cause of death. Though this method would be quite long and most difficult, it appears to be the only accurate means of determining the value of any test or group of tests as a predictor of hepatic metastasis.

The basic difficulty in attempting to devise tests which measure the status of the liver is the organ itself. It is large, not easily accessible for examination, and performs a multitude of functions, not all of which are understood. Therefore, the basic biochemical and physiological actions of the liver must first be understood before truly definitive tests of liver status can be established.

At the present time, one must still rely on clinical judgment, history, and physical examination as the main sources of information in liver disease. Though the laboratory tests are helpful in confirming clinical opinions, they have not replaced clinical judgment as the chief diagnostic source of the physician.

VALUE AND USE OF LIVER FUNCTION TESTS IN GENERAL

INTRODUCTION

No single liver function test is known whose results can describe adequately the functional or pathologic state of the hepatobiliary system. Consequently, multiple tests are required when one is attempting to determine the status of the liver. This need to use several tests results in the primary problem of the clinician who must use them; namely, which ones to use to get the most information about each specific patient.

As yet, there are no tests which distinguish definitely between the various forms of hepatocellular injury, and only a few are helpful in discerning between posthepatic and hepatocellular jaundice.[67]

The use of the tests must at all times be tempered by the fact that absolute results are rarely apparent from any of the tests, even when used in combination. The value of clinical history, physical examination, and other diagnostic aids such as radiographic examination, liver biopsy, and bile studies must again be emphasized.[17]

TYPES OF LIVER FUNCTION TESTS

Cantarow and Trumper divide tests of liver function into two categories. First are those tests of "metabolic functions" which

depend entirely upon the functional integrity of the liver cells and are uninfluenced by interference with the flow of bile, unless this produces hepatocellular damage. Examples of this type of test are the flocculation and turbidity tests, the transaminase tests, and studies of serum proteins.

The other type of test is that of "excretory function", where the results depend upon maintenance of a free flow of bile as well as upon hepatocellular function. An example of this type os test is the serum bilirubin determination.[17]

Popper and Schaffner list four types of tests of hepatobiliary function. First are the "activity and tolerance" tests which theoretically measure activity, capacity, or reserve. The tests measuring activity mirror the status of the organ, and usually require a single determination of a substance or a reaction. Tests measuring capacity entail the response to a load of exogenous material such as a dye, whereas tests measuring reserve use the response to a load of endogenous material, such as bilirubin or glucose. Capacity and reserve tests sometimes cannot be clearly separated and are coupled by these authors under the category of tolerance tests. The authors state that tolerance tests measuring dynamic response are preferable to activity tests reflecting a static picture.

The second group is the "true liver function" tests that measure a function which only the liver performs and upon which other organs exert little influence. However, few of the known

functions of the liver lend themselves well to practical clinical evaluation, the main exception being the response of the prothrombin time to the administration of vitamin K.

The third group is the "conditioned liver function" tests which measure functions that the liver shares with other organs or which are influenced by the function of other organs to a great degree. These include tests concerning such processes as serum protein formation or glycogen deposition in the liver. Despite theoretical drawbacks, much practical information about hepatic function can be obtained from these tests if the non-hepatobiliary influences upon the tests are taken into account.

The fourth, and largest, group is the "hepatic" tests which, in contrast to the other groups, concern biochemical, serologic, or hematologic data on substances not necessarily formed or acted upon by the liver. They may also concern reactions influenced by functional alterations of the liver, and are sometimes acted upon by other organs.[159]

USEFULNESS OF LIVER FUNCTION TESTS

Cantarow and Trumper list seven uses of liver function tests in clinical practice. First, the diagnosis of uncomplicated hemolytic jaundice can be established rather readily.

Second, the tests aid in the diagnosis of uncomplicated posthepatic jaundice (extrahepatic duct obstruction) to a great degree.

Third, they may reveal the presence of liver damage in pa-

tients with known obstructive lesions (e.g., stone, stricture, neoplasm).

Fourth, serial studies are of great value in following the course of acute diffuse liver disease (e.g., infectious and toxic hepatitis). In these conditions, they are particularly useful in revealing residual damage after subsidence of other clinical manifestations, and in suggesting possible progression to chronic liver disease.

Fifth, certain tests may be of quite specific diagnostic value.

Sixth, they may indicate the presence of unsuspected liver disease (e.g., cirrhosis).

And seventh, they afford the only means of demonstrating the integrity of, or evaluating the degree of impairment of, various hepatic functions in the presence or absence of evidence of morphologic change.[17]

SENSITIVITY OF VARIOUS LIVER FUNCTION TESTS

Popper lists the following six tests as those liver function tests whose results are most sensitive to changes in the hepatobiliary system. The six are (1) the presence of urine bilirubin; (2) elevation of serum bilirubin; (3) the amount of urine and fecal urobilinogen; (4) increased retention of Bromsulphalein; (5) increased thymol turbidity (though not in cirrhosis); and (6) increased serum alkaline phosphatase activity.

He lists cephalin-cholesterol flocculation activity, cholesterol/ester ratio, zinc sulfate turbidity, serum esterase, intravenous hippuric acid tolerance, galactose tolerance, prothrombin time response to vitamin K injection, and serum albumin as moderately sensitive hepatic tests.

The nonsensitive hepatic tests listed by Popper include aminoaciduria, low total cholesterol, and hypoglycemia.[159]

He does not mention transaminase determinations in his discussion, but they have been noted to be as sensitive to hepatobiliary patholgy as the alkaline phosphatase, so one may assume that transaminase activity would be listed as a very sensitive hepatic test.

COST OF LIVER FUNCTION TESTS

The cost of liver function tests in various laboratories naturally varies considerably. Often, the expense of a seldom-used test in a small laboratory is greater than in a large laboratory which runs that procedure more often; while at the same time, the test run in the smaller laboratory may be less accurate due to the relative lack of familiarity of the technician with the test.

The prices for the following tests are taken from the individual price list of a private laboratory in Omaha, Nebraska. This laboratory of private pathologists does a great deal of laboratory work for local and regional physicians, and their prices

are considered to be fairly representative of the "average" cost of most tests. The cost quoted for each test would be the price for that test if it were ordered individually. In a few cases, ordering more tests lowers the cost of some of the tests.

Cost for individual liver function tests at a private laboratory, Omaha, Nebraska, August, 1962:

Test	Cost
Alkaline Phosphatase	$ 5.00
Prothrombin Time	3.00
Cephalin-Cholesterol Flocculation	4.00
Thymol Turbidity	4.00
Fecal Urobilinogen	6.00
Urine Urobilinogen	4.00
Serum Bilirubin	5.00
Urine Bile	1.00
Urine Bilirubin (Ictotest®)	1.00
Bromsulphalein®	5.00
Total serum Protein	4.00
Total serum Protein plus Albumin/Globulin ration(Biuret reaction)	8.00
Serum Electrophoresis plus Total Serum Protein and Biuret reaction	10.00
Serum Glutamic-Oxalacetic Transaminase	5.00
Serum Glutamic-Pyruvic Transaminase	5.00

USE OF LIVER FUNCTION TESTS IN THE DIFFERENTIAL DIAGNOSIS OF JAUNDICE

As stated earlier, the differential diagnosis of jaundice is in effect a "therapeutic differential diagnosis" to separate "surgical" jaundice from "medical" jaundice. The basis of the differentiation is the finding of abnormal results of tests indicative of hepatocellular degeneration and of normal results of tests indicative of cholestasis in medical jaundice and vice versa in

surgical jaundice. Intrahepatic tumor metastases or lymphomas producing jaundice belong to the medical group, since the jaundice results from intrahepatic processes not amenable to surgery.

Popper lists certain findings which indicate hepatocellular degeneration. They are abnormal cephalin-cholesterol flocculation, increased thymol turbidity,[32] increased urobilinogenuria, low serum cholinesterase level, decreased cholesterol/ester ratio, and reduced prothrombin time despite vitamin K therapy.[154,159] Cohn and Kaplan also add elevation of the level of serum transaminases.[134] Because of the frequent occurence of biological false positive tests, abnormal results in at least two of these tests are required (although abnormal results in the cephalin-cholesterol flocculation and thymol turbidity tests should be counted as only one abnormality).[159]

Popper lists the following test results as indicative of cholestasis (posthepatic jaundice); absent urinary urobilinogen, serum alkaline phosphatase activity above 15 Bodansky units (Ducci lists 10 Bodansky units as the lower limit of posthepatic obstruction[32]), and hypercholesteremia.[159] Cohn and Kaplan also list bilirubinuria and elevation of the level of serum transaminases.[134]

There are three exceptions to the basic rules set forth by Popper. First is the instance of secondary hepatocellular degeneration in surgical jaundice which is caused by prolonged extrahepatic biliary obstruction or by secondary bacterial infections of the portal tracts. In this case, laboratory evidence of both

hepatocellular degeneration and cholestasis is found.

Second is the condition of incomplete or intermittent extrahepatic cholestasis which results in fluctuating findings indicative of cholestasis in cases of surgical jaundice. When no cholestasis, but slight liver damage, is found at the time of examination, medical jaundice is probably present.

Intrahepatic cholestasis, or "cholangiolitis", is the third exception. This disease process produces abnormal results in tests indicating cholestasis while the true pathologic process is actually a type of medical jaundice.[159]

LIVER FUNCTION TESTS TO BE USED IN ROUTINE STUDY OF NON-JAUNDICED PATIENT

Popper notes that the problem of demonstrating hepatic injury in non-jaundiced patients occurs primarily in screening for anicteric or preicteric viral hepatitis during an epidemic; for toxic damage after exposure to poisons, mainly industrial in nature; and for injury resulting from the administration of drugs for clinical or experimental purposes. Since otherwise healthy persons are examined, minor alterations of hepatic function are significant. False positive results and abnormal results in carriers of hepatitis sometimes interfere with the interpretation, and isolated observations are unconvincing.[159]

Popper and Schaffner recommend the following tests in the routine study of the non-jaundiced patient; urinary bilirubin and

and urobilinogen excretion, Bromsulphalein retention, direct-reacting serum bilirubin, cephalin-cholesterol flocculation, and thymol turbidity.[159]

Cohn and Kaplan concur in Popper's choice of tests but adds serum alkaline phosphatase activity, serum transaminase levels, and total serum bilirubin in his list of routine tests.[134]

Bernhard also adds the determination of serum albumin and globulin to the routine tests to determine if occult liver disease is present.[9]

Maclagan also recommends paper electrophoresis of serum proteins and the plasma prothrombin time as special tests to be used at times in the absence of positive signs of liver disease in a patient with suspected liver pathology.[119]

LIVER FUNCTION TESTS TO BE USED IN HEPATOCELLULAR DISEASE

Neefe and Reinhold find that in the early (preicteric) stage of infectious hepatitis, the Bromsulphalein retention, bilirubinuria, increased cephalin-cholesterol flocculation, increased one-minute serum bilirubin, and increased thymol turbidity (in roughly that order) provide the earliest evidence of the initial hepatic disturbance.

During the convalescent state, the thymol turbidity test is the most consistent indicator of persistent disturbance with the colloidal gold, cephalin-cholesterol flocculation, one-minute serum bilirubin, and Bromsulphalein tests following in order of decreasing consistency.

These authors recommend a group composed of the Bromsulphalein, total and one-minute serum bilirubin, urine bilirubin and urobilinogen, cephalin-cholesterol flocculation, and thymol turbidity procedures as the most reliable minimum group of tests for the detection of mild hepatic disturbance at any stage of the disease.[143]

Hawk and associates agree with Neefe and Reinhold's recommendation above, although they list the Bromsulphalein as the test of choice when only one test is to be used serially in the "recovery" stage of hepatitis.[71]

These authors suggest that, in following the course of the jaundiced patient suffering from parenchymatous disease, the direct and total serum bilirubin, flocculation tests, the serum albumin and globulin, and the plasma prothrombin time be use.[71]

Davis recommends the use of the Bromsulphalein retention, the serum bilirubin, total serum protein determination with electrophoretic fractionation, and serum alkaline phosphatase as routine tests to determine liver damage. He noted that in 87 patients, of whom 25 died at surgery, the liver function tests were of little, or no, value, in forecasting successful completion of surgery. However, he recommends Bromsulphalein retention, serum albumin determination, and the prothrobin time as the best laboratory indicators for operative risk that are available; although, he lists clinical evaluation and liver biopsy as being far superior to any chemical test.[30]

Manning and Delp suggest the use of Bromsulphalein retention, urine urobilinogen, cephalin-cholesterol flocculation, serum transaminase, and serum bilirubin in the detection of liver disease.[124]

Popper and Schaffner note that if severe inflammation due to bacterial infection complicates extrahepatic biliary obstruction, the laboratory picture becomes indistinguishable from that of medically treated jaundice, since the resulting elevation in the serum gamma globulin level may produce abnormal results in the flocculation tests. In this case, the clinical features of chills and fever with leukocytosis or possibly liver biopsy may clarify the problem.[158]

In following patients with known liver disease to evaluate the extent of damage, and to follow the progress of disease; Cohn and Kaplan recommend serum bilirubin, thymol turbidity, cephalin-cholesterol flocculation, and albumin and globulin determinations as being useful tests in general. They note that the urinary urobilinogen and cholesterol and its esters are valuable under special conditions.[134]

Bernhard suggests the use of the direct and total serum bilirubin, Bromsulphalein retention, serum albumin and globulin, cholesterol, serum enzymes (transaminases), urine urobilinogen, and the prothrombin time in the study of known hepatocellular disease.[9]

LIVER FUNCTION TESTS TO BE USED IN KNOWN BILIARY OBSTRUCTION

Hawk and associates state that in differentiating jaundice due to biliary disease from that due to parenchymatous disease, one should use the serum alkaline phosphatase, the cephalin-cholesterol flocculation and thymol turbidity test, the prothrombin time and response to injection of vitamin K, and the fecal urobilinogen.

They feel that in following the course of the surgical patient with disease of the biliary tract one should use the alkaline phosphatase, the plasma prothrombin time, the direct and total serum bilirubin, serum albumin and globulin, electrolytes, and the blood urea nitrogen.[71]

Bernhard proposes the use of the serum bilirubin, prothrombin time, serum albumin and globulin, flocculation tests, serum transaminase, cholesterol, and alkaline phosphatase tests in patients with known obstruction.[9]

Cohn and Kaplan recommend the use of serum bilirubin, alkaline phosphatase, and albumin and globulin fractions of serum in following patients with biliary tract disease, both in diagnosis and post-operatively.[134]

Intrahepatic cholestasis cannot be differentiated from extrahepatic cholestasis by laboratory tests, since liver biopsy demonstrates extrahepatic obstruction only in protracted cases.[158]

LIVER FUNCTION TESTS IN MISCELLANEOUS CONDITIONS

In a study of a group of people 65 years old or older, Cohen and associates find that there is no distinct increase in abnormalities in the results of liver function tests.[23]

Kumate and associates studied simultaneous changes of various liver function tests in 30 cases of biliary atresia confirmed by exploratory laporotomy or necropsy.

They note a tendency for the serum bilirubin to rise as age advances which is more evident after the fourth month. However, the degree of correlation is poor.

Flocculation tests are abnormal in over 35 per cent of cases, and are more marked as age and degree of malnutrition increase.

Transaminase activities in serum show slight increases (to about 100 units per ml.), predominantly in the glutamic-oxaloacetic type, and without relation to age, nutritional condition, or flocculation tests. It is notable that some authors have placed the upper limit of normal for serum transaminase activity as high as 120 units per ml. in the postnatal period.[20,99]

Studies of Bromsulphalein clearance show increased retention at 45 minutes in about 30 per cent of cases.[102]

Richman and associates did serial liver function tests on 175 patients with right-sided heart failure of diverse etiology. Abnormal results obtained varied from 2 per cent (thymol turbidity)

to 80 per cent (Bromsulphalein and prothrombin time) of cases.

The causes of the right-sided heart failure do not appear to influence the pattern of altered liver function as much as whether failure was acute or chronic. The liver indices reflecting parenchymal cell destruction and excretory activity are most affected during acute failure.

The majority of indices of hepatic function return to normal within one to two weeks following cardiac compensation, except for those reflecting biosynthesis by the liver, which improve more slowly, and hyperglobulinemia, which tend to persist. Repeated attacks of failure (as in rheumatic heart disease) are associated with more severe impairment in liver function.[173]

OTHER LIVER FUNCTION TESTS

Because of the scope of the subject, this thesis necessarily is limited to those liver function tests which the author feels to be of general use and importance. Other liver function tests which have found some use either presently, or in the past, are listed as follows (no attempt is made at absolute completeness):

1. Serum cholesterol and cholesterol esters[134,159]
2. Icteric index[134]
3. Galactose tolearance[116]
4. Hippuric acid synthesis
5. Serum mucoproteins[159]
6. Thymol flocculation[159]
7. Zinc sulfate turbidity
8. Takata-Ara flocculation
9. Serum colloidal gold
10. Intravenous rose bengal

11. Bilirubin tolerance
12. Plasma vitamin A level
13. Blood glucose level[134]
14. Adrenalin tolerance
15. Cholinesterase level[159]
16. Intravenous lactic acid
17. Levulose tolerance
18. Ammonium sulfate
19. Blood and urine amino acids[134]
20. Duodenal aspiration[159]

RECENT WORK IN THE DEVELOPMENT OF LIVER FUNCTION TESTS

INTRODUCTION

The primary emphasis of recent work in liver function tests seems to center in the field of radio-diagnosis. Much has been written about studies utilizing radioactivated substances such as rose bengal.

Other work is being done on the development of new substances which attempt to measure hepatic clearance (e.g., indocyanine green), and on easier methods of accurately determining liver function in smaller laboratories.

USE OF RADIOACTIVATED SUBSTANCES IN TESTING LIVER FUNCTION

Rose bengal is a chemical substance which acts in much the same manner as Bromsulphalein.[144] In fact, the rose bengal test was at one time used as a liver function test. However, this material has been "tagged" with radioactive iodine (atomic weight, 131) resulting in the formation of the I^{131} rose bengal test.

In normal humans, the radioactivated substance is injected intravenously and goes in succession to the blood, liver, bile ducts, gastrointestinal tract, and finally is excreted in the feces with a small amount being excreted in the urine. The amount excreted in the feces in 72 hours can be measured by radioactive studies of a fecal sample.[144]

In infants with biliary atresia, no substance is able to get from the liver to the gastrointestinal tract, and therefore, none is excreted in the feces and most is excreted in the urine. Thus, a decrease in fecal excretion coupled with an increase in urinary excretion in infants is diagnostic of biliary atresia.[144]

Ghadimi and Sass-Kortsak report that the 72-hour fecal excretion of I^{131} rose bengal in infants with normal livers exceeds 70 per cent, with the corresponding figures for urine not exceeding 4 per cent. In four infants with atresia of the extrahepatic bile ducts, proved by laparotomy, the fecal excretion is between 2.2 and 5 per cent of the injected dose. They also find that the 72-hour fecal excretion of the same substance in 7 infants with obstructive jaundice and patent extrahepatic ducts is 10.5 or more per cent. These authors also note that the hazards involved due to exposure to a radioactive substance are no greater, in their opinion, than exposure to a conventional fluoroscopic procedure.[50]

Taplin and associates report favorably on the value of the I^{131} rose bengal test and the radioactive-gold (Au^{198}) test as indicators of liver blood flow and cellular function. Liver blood flow is measured accurately, they state, by the disappearance of the intravenously injected substance from the blood as measured by a radioactive counter attached to the patient's thigh. The cellular function is measured by a radioactive counter which is attached to the patient above his liver.[144] These authors feel this use of I^{131} rose bengal and Au^{198} allows for recognition of

conditions in which portal hypertension is present; primarily cirrhosis, lymphatic leukemia, severe congestive heart failure, and hemorrhagic shock. They conclude that these tests are most useful in the medical and surgical management of cirrhotic patients with portal hypertension and active hepatitis.[208]

However, Richman and Jacobs feel that the I^{131} rose bengal test does not give consistent results in any specific group of liver disease and hence does not offer any great value as a test of liver function.[172]

Ackerman and associates have developed a technique for scanning the liver for space-occupying lesions, which are less radioactive than normal liver tissue, using I^{131} rose bengal. They claim this technique has empirically demonstrated lesions one inch or more in diameter.[1] The possibility of the eventual use of this test in diagnosing liver neoplasms, both primary and metastatic, cannot be overlooked.

The I^{131} iodipamide test was devised by McLaren and associates in 1959 to measure the clearance and excretion of that substance by the liver. However, Freiman and associates, after using the test, conclude that it cannot be used to separate patients with normal hepatobiliary function from those with defects in their hepatobiliary system.[45]

INDOCYANINE GREEN

In this test, indocyanine green, a dye, is injected into the median basilic vein of one arm and blood is collected at various time intervals from a vein of the other arm. Approximately 0.5 mgm. per kg. of body weight of the dye is injected. No reactions, systemic or otherwise, to the dye have been noted following injection. After withdrawal of the blood, the samples are centrifuged, and the plasma dye concentrations are read against the plasma blank on a Beckman DU spectrophotometer at a wave length of 805 millimicrons.[231]

Hunton and associates have established a minimal normal plasma disappearance rate of 18 per cent per minute. With few exceptions, slower rates have been found only in patients having clinical or pathologic evidence of liver impairment. These authors feel that the indocyanine green test may be of clinical value in patients who have spurious retention of Bromsulphalein.[81]

Stekiel and associates agree that indocyanine green has potentiality as a measurer of liver blood flow but warn of two factors which serve as poorly-defined sources of error. One is the obtaining of a representative hepatic venous sample when withdrawing blood for measurement of the dye; and the other is the determination of a true extraction ratio which is complicated because of the difficulty in knowing hepatic circulatory time.[200]

Caesar and associates feel that though the plasma indocyanine green test is comparable to the Bromsulphalein test, the

former has no advantage over the latter as a screening test of liver function.[14]

A SIMPLER LIVER FUNCTION TEST

Reddin reports that many women who are chronically ill with indigestion and constipation have a poor Decholin taste time test when no other test is abnormal. He feels this is a clinically dependable test of liver dysfunction compatible with an insufficient amount of bile secreted into the duodenum. The test is recommended when excellent laboratory facilities are not available, and when hospitalization is refused. He concludes that, in general, the quicker and stronger the taste is noted and maintained, the more normal is liver function.[166]

CONCLUSION

The primary area of research in liver function testing is currently in the field of radioactivated substances. These materials are injected and then measured by radioactivity counters, either on different areas of the patient at the same time, or in the excretory products of the patient.

The possibility that hepatic neoplasms may someday be diagnosed by radioactive liver-scanning (similar to thyroid-scanning presently done) offers hope that this diagnostic enigma may still be conquered.

The work being done on substances to measure hepatic clear-

ance do not offer much hope for improvement over those tests presently available.

Simple attempts to measure liver function, such as the Decholin taste time, while interesting, have not been thoroughly tested and are probably just ways of convincing oneself that an inconclusive laboratory test may be diagnostic. In this case, perhaps no test would be better than one giving dubious results.

SUMMARY

INTRODUCTION

Liver function tests are indicated in patients with clinical signs and symptoms of hepatobiliary disease, or in those patients in whom the presence of liver and biliary tract pathology is suspected. These tests aid in the diagnosis, prognosis, and follow-up of diseases of the hepatobiliary system.

However, one must be aware from the beginning that in using liver function tests, one is using a group of imperfect instruments which are of value only as adjuncts to clinical history, physical examination, and other methods of studying the hepatobiliary system. To help reduce the error of any one test, and to measure a variety of the liver's poorly understood functions, liver function tests are usually performed in groups, and serially. The abnormal result of only one test at only one time is flimsy evidence on which to base a diagnosis of liver disease.

A primary use of liver function tests is to aid in the differential diagnosis of jaundice. Various classifications of jaundice have been put forward, but the one mainly alluded to in this thesis is that of Ducci, with modifications by Miller. They differentiate jaundice into prehapatic, hepatic, and posthepatic types corresponding to overproduction of bilirubin or anoxia, damaged hepatic cells, and obstruction of the biliary passages, respectively.

ALKALINE PHOSPHATASE

The alkaline phosphatase test often provides valuable positive evidence in the diagnosis of posthepatic jaundice. The mechanism of the test is poorly understood; and moderate rises are noted in, but are not diagnostic of, many cases of hepatocellular disease.

The test result is commonly elevated in children, diseases of the bone, hyperparathyroidism, congestive heart failure, and following ingestion of fat. Lowered test results have been noted when the patient is in a fasting state.

The test has found favor with some in attempting to diagnose the presence of metastatic carcinoma of the liver, but conclusive evidence of its value in this diagnostic dilemma has never been produced.

SERUM AND URINE BILIRUBIN

Bilirubin is a product of the breakdown of hemoglobin in the reticulo-endothelial system. It exists in plasma as both the direct-reacting (mono- and diglucuronides) and indirect-reacting (free bilirubin) forms.

Jaundice is due to the affinity of elastic tissue for bilirubin, thus imparting a characteristic yellow color as the concentration exceeds a critical level. (Jaundice is generally believed to become clinically detectable when the total serum bilirubin exceeds 2.5 mgm. per 100 ml. of serum.)

Besides jaundiced conditions, elevated serum bilirubin is noted in subclinical liver disease, newborns, and congestive heart disease.

Measurement of direct and total serum bilirubin is valuable in the diagnosis of hemolytic jaundice, where the indirect fraction is markedly elevated in the presence of near-normal direct values. However, this partition is of little value in differentiating hepatic from posthepatic jaundice.

Urine bilirubin, an abnormal finding whenever present, is noted in many cases of hepatic and posthepatic jaundice, in congestive heart failure, and in pulmonary infarcts.

Urine bilirubin is quite helpful in the early diagnosis of infectious hepatitis, but is a poor follow-up test because its early return to normal is not associated with clinical improvement. Urine bilirubin is always absent in hemolytic jaundice.

BROMSULPHALEIN

The Bromsulphalein retention test is an excellent screening test for liver function. The conditions of the test are precise and must be followed accurately to achieve reliable results.

The rate of removal of the dye from the blood depends on the excretory capacity of the liver, the patency of the bile ducts, and the hepatic blood flow. Test results must be interpreted in view of the patient's complete condition as many non-hepatobiliary factors may affect the test.

The Bromsulphalein test offers little help in the differentiation of jaundice, but is an excellent procedure in detecting latent liver disease and in follow-up studies of hepatocellular pathology.

Allergic responses to the dye must always be guarded against.

FLOCCULATION AND TURBIDITY TESTS

The cephalin-cholesterol flocculation and thymol turbidity tests are the most commonly used tests of liver function. They are non-specific, empirical tests of serum which often furnish clues to abnormal serum protein composition and activity, thus often exposing hepatocellular disease. The causes of abnormal flocculation and turbidity are partially understood and concern stabilizing and precipitating factors in many instances.

The cephalin-cholesterol flocculation test is quite useful for the diagnosis of hepatocellular degeneration in hepatitis or cirrhosis. This test is more dependable in detecting cirrhosis than the thymol turbidity, and is often positive early in the course of viral hepatitis.

The thymol turbidity is an excellent test for the follow-up of hepatitis, since it is often the last test to return to normal. The test is also useful in the diagnosis of postnecrotic cirrhosis--the only form of cirrhosis in which this test is consistently abnormal.

Neither test gives positive results regularly in cases of post-hepatic jaundice. Both are also affected by other conditions resulting in abnormal serum protein states such as infectious mononucleosis, malaria, and rheumatoid arthritis.

The tests are most profitably used together, since they each are affected by a somewhat different area of serum protein changes.

SERUM PROTEINS

Serum proteins may be measured by salting-out methods, use of water-miscible organic precipitants, electrophoresis, ultracentrifugation, and the use of immunochemical procedures. The first three methods are most commonly used.

Changes in serum proteins are quite non-specific and variable. A decreased level of serum albumin is often seen in chronic hepatocellular disease, and is a valuable prognostic tool in certain cases.

The serum globulin level tends to rise in both acute and chronic hepatocellular disease.

The traditional determination of albumin/globulin ratio is unreliable unless one knows the absolute values for both fractions, as they may be affected by different factors.

PROTHROMBIN TIME

The prothrombin time is a rapid and valuable test of bleeding tendency, and is sometimes helpful in the diagnosis of liver disease.

Since prothrombin is formed by hepatic cells, the prothrombin time should be prolonged in hepatocellular disease. Also, since fat-soluble vitamin K is required for prothrombin synthesis, the prothrombin time should also be prolonged in extrahepatic obstruction which prevents bile salts from reaching the intestinal tract.

Therefore, if prothrombin time is prolonged, and then improves more than 15 per cent within 24 hours following injection of vitamin K, a diagnosis of posthepatic jaundice would be indicated. Whereas, the absence of improvement in the prothrombin time would indicate hepatocellular pathology.

However, the clinical results of the prothrombin time have not been as encouraging as the theoretical design of the test, and its use as a liver function test is limited because of this. Though it may at times be helpful, the prothrombin time is rarely a test of choice in the evaluation of liver function.

SERUM TRANSAMINASES

Enzymes catalyzing different transamination reactions have been found widely distributed in animal and human tissues, and have been shown to change in activity in some tissues during disease. Serum glutamic-pyruvic transaminase (SGPT) is found mainly in the liver, while serum glutamic-oxalacetic transaminase (SGOT) is particularly concentrated in cardiac muscle.

The activity of these enzymes is markedly increased in cases of hepatitis; and mildly increased in infectious mononucleosis,

cirrhosis, posthepatic jaundice, and liver metastasis. Some differentiation between "medical" and "surgical" neonatal jaundice can be made by serial determination of the SGOT and SGPT enzyme activities.

The activity is also elevated somewhat in all newborns, and following myocardial infarction.

URINE AND FECAL UROBILINOGEN

The urobilinogens are formed by the reduction of bilirubin in the intestinal tract by bacterial action. The urobilinogens are colorless but tend to oxidize in the intestinal tract to form urobilins which impart a brown color to the feces.

The determination of urobilinogen excretion, either in the urine or feces, is an excellent test of early liver impairment. The test is quite sensitive to changes in hepatobiliary metabolism, and can often be a great aid in the differential diagnosis of jaundice.

Because of the time required for a fecal urobilinogen study, urinary urobilinogen measurement is preferred generally.

Elevation of urobilinogen excretion is also noted following acute myocardial infarction, pernicious anemia, leukemia, and other conditions. The test is unreliable when done on patients who are receiving antibiotic therapy which interferes with the gastrointestinal bacterial flora.

REVIEW OF 108 AUTOPSIES

A review of 108 autopsies from the University of Nebraska College of Medicine Department of Pathology is presented with reference to the results of liver function tests and their correlation with post-mortem hepatic findings, particularly the presence or absence of hepatic metastasis.

No test was found that correlated highly as a predictor of the presence of hepatic metastasis; though certain tests did show a high degree of correlation with cases of cirrhosis.

Problems of a study in predicting liver metastasis are commented upon, and certain recommendations pertaining to the possibility of future studies are made.

VALUE AND USE OF LIVER FUNCTION TESTS IN GENERAL

Different groupings of liver function tests are presented along with seven definite uses of these tests.

A discussion of the varying sensitivity of the different tests is presented. The cost for each test examined at length in this thesis is listed, using a private laboratory in Omaha, Nebraska, as the reference for the cost.

The use of liver function tests in the differential diagnosis of jaundice, the routine study of the non-jaundiced patient, the study of hepatocellular and known biliary obstructive diseases, and miscellaneous conditions is discussed.

Other less used tests of liver function are listed.

RECENT WORK IN THE DEVELOPMENT OF LIVER FUNCTION TESTS

The primary interest in current work on liver function tests has been the attempt to develop radio-isotopic diagnostic methods. The I^{131} rose bengal test is the chief example of these efforts. The results have not been conclusive to show any advantage to the use of these tests as yet.

New chemical substances are also being experimented with, notably the dye, indocyanine green, which gives reported results comparable to those of the Bromsulphalein test.

Other less promising studies have been suggested, and are noted.

CONCLUSION

A review of commonly used tests of liver function is presented stressing technique, mechanism of action, use in hepatobiliary disease, and non-hepatobiliary causes of abnormal test results.

A correlation is attempted between these tests and autopsy material obtained from the records of the University of Nebraska College of Medicine Department of Pathology.

The coordinated use of various liver function tests in an attempt to gain the most information available from them is discussed.

The recent developments in the field of liver function tests are given, with special emphasis on radio-isotopic studies.

The limitations of the tests, along with their value, use, and cost, are considered. The tests can often be of great diagnostic aid when combined with the clinical history, physical examination, and other studies; but they have not supplanted these traditional techniques in the diagnosis of hepatobiliary disease.

ACKNOWLEDGEMENTS

The advice and encouragement of Dr. M. H. Kulesh has been of inestimable help to me in the completion of this thesis. My thanks to him for his cheerfully offered assistance and criticism, and for helping to make what could have been a chore into an enjoyable and profitable experience.

My appreciation also is extended to Mrs. Bernice M. Hetzner and her staff of librarians, whose aid in the preparation and mechanics of the thesis was most helpful.

BIBLIOGRAPHY

1. Ackerman, N.B. and others, Use of an Improved Mechanical Printer for Liver Scanning with Rose Bengal dye, J. A. M. A. 179:134, 1962.

2. Agress, C.M. and others, Serum Transaminase levels in Experimental Myocardial infarction, Circulation 11:711, 1955.

3. Allen, J.G. and others, Use of Serial Dilutions in Determination of Prothrombin by One-Stage Technic, Arch. of Surg. 41:873, 1940.

4. Alsever, J.B. and Barger, J.D., Thymol Turbidity and Thymol Flocculation Tests as a Screening Technic to Detect Blood Donors Carrying the Hepatitis Virus: II. The Results of Follow-up Studies of Six Months or more on 2,385 Transfused Patients, Transfusion 1:295, 1961.

5. Armstrong, A.R. and King, E.J., Phosphatase in Obstructive Jaundice, Canad. M.A.J., 31:14, 1934.

6. Baden, H. and others, Value of Serum Glutamic Oxalacetic Transaminase in the Diagnosis of Liver Metastasis, A.M.A. Arch. Surg. 81:608, 1960.

7. Barber-Riley, G. and others, The Transfer of Bromsulphthalein from the Plasma to the Bile in Man, Clin. Sci. 20:149, 1961.

8. Bauer, R., Mechanism of Modern Serum Tests in Relation to Their Clinical Significance; Magnesium Chloride, Serum Colloidal Gold, Cephalin-Cholesterol Flocculation, and Turbidity Tests, Rev. Gastroenterology 16:158, 1949.

9. Bernhard, W.G., Liver Function Tests, Seminar Report 5:23 (summer), 1960.

10. Bodansky, A., Phosphatase Studies; Determination of Serum Phosphatase. Facts Influencing Accuracy of Determination, J. Biol. Chem. 101:93, 1933.

11. Brown, J.W., and others, Liver Function during Infectious Mononucleosis, Am. J. Med. 6:321, 1949.

12. Burke, J.O., Serum Alkaline phosphatase in Liver Disease: Concept of its Significance, Gastroenterology 16:660,1950.

13. Burnett, W., Bromsulphthalein-Retention Test in Acute Cholecystitis and in some other Acute Intra-abdominal Conditions, Lancet 1:488, 1954.

14. Caesar, J. and others, The Use of Indocyanine Green in the Measurement of Hepatic Blood Flow and as a Test of Hepatic Function, Clin. Sci. 21:43, 1961.

15. Campbell, W.R. and Hanna, M.I. Albumin, Globulins, and Fibrinogen of Serum and Plasma, J. Biol. Chem. 119:15, 1937.

16. Cantarow, A. and Nelson, J., Serum Phosphatase in Jaundice, Arch. Int. Med. 59:1045, 1937.

17. Cantarow, A. and Trumper, M., Clinical Biochemistry, Fifth edition, Philadelphia, W.B. Saunders Co., 1955.

18. Chambers, W.N. and Moister, F.C., Bromsulfalein Reaction, Am. J. Med. 5:308, 1948.

19. Chapman, J.A. and others, An Analysis of the Clinical Application of the Van Den Bergh Reaction in Jaundice, Am. J. M. Sc. 239:11, 1960.

20. Christiansson, G. and Josephson, B., A Study of the Enzyme Pattern in Children and Newborn Infants, Acta Paediatrica, 49:626, 1960.

21. Cleve, E.A. and others, A Typical Tuberculosis of Liver with Jaundice, Ann. Int. Med. 41:251, 1954.

22. Cohen, P.P. and Thompson, F.L., Mechanism of Thymol Turbidity Test, J. Lab. and Clin. Med. 32:475, 1947.

23. Cohen, T. and others, Liver Function Studies in the Aged, Geriatrics 15:824, 1960.

24. Cohn, E.J. and others, Preparation and Properties of Serum and Plasma Proteins. IV. A System for the Separation into Fractions of the Protein and Lipoprotein Components of Biological Tissues and Fluids, J. Am. Chem. Soc. 68:459, 1946.

25. Colehour, J.K., Separation of Serum Proteins by Density Gradient Electrophoresis, Clin. Chem. 6:485, 1960.

26. Covey, J.A. and others, Idiopathic Hypoprothrombinemia, Ann. Int. Med. 33:467, 1950.

27. Daikos, G.K. and others, The Value of the Quinine Test for the Differentiation of Jaundice, Amer. J. Med. Sci. 242:351,1961.

28. Das, B.C. and Bhattacharya, S.K., Changes in Human Serum Protein Fractions with Age and Weight, Canad. J. Biochem. and Physiol. 39:569, 1961.

29. Dauphinee, J.A. and Campbell, W.R., Serum Proteins in Hepatic Disease, M. Clin. North America 32:455, 1948.

30. Davis, W.D. Jr., Liver Function in Disease and in Preoperative Evaluation, Am. J. Digest. Dis. 6:240, 1961.

31. Dole, V.P. and Braun, E., Electrophoretic Patterns of Normal Plasma, J. Clin. Investigation 23:708, 1944.

32. Ducci, H., Contribution of Laboratory to Differential Diagnosis of Jaundice, J.A.M.A. 135:694, 1947.

33. -------, Thymol Test of Maclagan; Standardization and Adaptation to Evelyn Photoelectric Colorimeter, J. Lab. and Clin. Med. 32:1266, 1947.

34. -------, Colloidal Red Test for Study of Hepatic Dysfunction, J. Lab. and Clin. Med. 32:1273, 1947.

35. -------, Clinical Experience with Colloidal Red Test, Gastroenterology 14:20, 1950.

36. -------, and Watson, C.J., Quantitative Determination of Serum Bilirubin with Special Reference to Prompt-Reacting and Chloroform-Soluble Types, J. Lab. and Clin. Med. 30:293, 1945.

37. Eberlein, W.R., Simple Solvent-Partition Method for Measurement of Free and Conjugated Bilirubin in Serum, Pediatrics 25:878, 1960.

38. Elton, N.W., Van Den Bergh Reaction, Am. J. Clin. Path. 20:901, 1950.

39. Ernst, R.G. and Dotti, L.B., Evaluation of Thymol Turbidity Test, Am. J. Med. Sci. 216:316, 1948.

40. Evans, J.M. and others, Increased Urinary Urobilinogen Following Acute Myocardial Infarction, Circulation 6:925, 1952.

41. Felder, L. and others, Liver Function Tests in Chronic Congestive Heart Failure, Circulation 2:286, 1950.

42. Franke, K., Methylenblau, ein einfaches sehr empfindliches Reagens zum Nachweis von Bilirubin, Med. Klin. (German) 27:94, 1931.

43. Franklin, M. and others, Electrophoretic Studies in Liver Disease; Comparison of Serum and Plasma Electrophoretic Patterns in Liver Disease with Special Reference to Fibrinogen and Gamma Globulin Patterns, J. Clin. Investigation 30:718, 1951.

44. -------, and others, Relation between Structural and Functional Alterations of the Liver, J. Lab. and Clin. Med. 33:435, 1948.

45. Freiman, H.D. and others, The Inadequacy of Radio-Iodinated Iodipamide as a Liver Function Test, J. Nucl. Med. 3:63, 1962.

46. Friedman, M.H. Technical Factors Affecting the Estimation of Serum Gamma Globulin by the Zinc Turbidimetric Method of Kunkel, Gastroenterology 17:57, 1951.

47. Friend, C. and others, Glutamic-Oxaloacetic Transaminase Activity of Serum in Mice with Viral Hepatitis, J. Exp. Med. 102:699, 1955.

48. Frisch, A. W. and Quilligan, J.J., Modified Emulsion in the Cephalin-Cholesterol Flocculation Test, Am. J. M. Sc. 212:143, 1946.

49. Gaebler, O. H., Determination of Bromsulphalein in Normal, Turbid, Hemolyzed, or Icteric Serums, Am. J. Clin. Path. 15:452, 1945.

50. GHadimi, H. and Sass-Kortsak, A., Evaluation of the Radioactive Rose-Bengal Test for the Differential Diagnosis of Obstructive Jaundice in Infants, New Eng. J. Med. 265:351, 1961.

51. Golden, W.R.C. and Snavely, J.G., Method for Quantitative Determination of Bilirubin in Urine, J. Lab. and Clin. Med. 33:890, 1948.

52. Goodman, R.D., Bromsulfalein Clearance: Quantitative Clinical Test of Liver Function, J. Lab. and Clin. Med. 40:531, 1952.

53. -------, and Kingsley, G.R., Sulfobromophthalein Clearance Test, J.A.M.A. 153:462, 1953.

54. Goodman, M. and Vulpe, M., A Quantitative Immunochemical Method for determining Serum and Cerebrospinal Fluid Proteins, World Neurology 2:589, 1961.

55. Goodson, W.H. and Sheard, C., Photelometric Determination of Bilirubin in Urine with Diazobenzenesulfonic Acid, J. Lab. and Clin. Med. 26:423, 1940.

56. Gornall, A.G. and others, Determination of Serum Proteins by means of the Biuret Reaction, J. Biol. Chem. 177:751, 1949.

57. Gray, S.J. and others, Electrophoretic Analyses of Serum Proteins in Diseases of Liver, J. Clin. Investigation 22:191, 1943.

58. Greene, L.S. and Schiff L., Primary Carcinoma of the Liver--A Plea for Earlier Diagnosis with Emphasis on the Serum Alkaline Phosphatase Values, Gastroenterology 40:219, 1961.

59. Gutman, A.B., The Plasma Proteins in Disease, Advances Protein Chemistry 4:155, 1948.

60. -------, and others, Increased Serum Phosphatase Activity without Hyperbilirubinemia after Ligation of Hepatic Ducts in Dogs, Proc, Soc. Exper. Biol, and Med. 44:613, 1940.

61. -------, and others, Effect of Disease of Liver and Biliary Tract Upon Phosphatase Activity of Serum, J. Clin. Investigation 19:129, 1940.

62. Guttman, S.A. and others, Significance of Cephalin-Cholesterol Flocculation Test in Malarial Fever, J. Clin. Investigation 24:296, 1945.

63. Hanger, G. M., Serological Differentiation of Obstructive from Hepatogenous Jaundice by Flocculation of Cephalin-Cholesterol Emulsions, J. Clin. Investigation 18:261, 1939.

64. -------, The Meaning of Liver Function Tests, Am. J. Med. 16:565, 1954.

65. -------, Liver Functinn Tests, Med. Clin. No. Amer. 44:681, 1960.

66. -------, and Patek, A.J., The Cephalin Flocculation Test in Cirrhosis of the Liver, Am. J.M. Sc. 202:48, 1941.

67. Harrison, T.R. and others, Principles of Internal Medicine, Third Edition, New York, McGraw-Hill Book Co., Inc., 1958, Chapter 18.

68. Havens, W.P. and Marck, R.E., Leukocytic Response in Patients with Experimentally Induced Infectious Hepatitis, Am. J. M. Sc. 212:129, 1946.

69. -------, and Marck, R.E., Comparison of Cephalin-Cholesterol Flocculation and Thymol Turbidity Tests in Patients with Experimentally Induced Infectious Hepatitis, J. Clin. Investigation 25:816, 1946.

70. Havens, W.P. Jr.and Williams, T.L., The Effect of Alterations of the Serum Proteins on the Thymol Turbidity Test, Acta. med. Scandinav. Suppl. 234:141, 1949.

71. Hawk, P.B., Oser, B.L., and Summerson, W.H., Practical Physiological Chemistry, Thirteenth Edition, New York, The Blakiston Co., Inc., 1954.

72. Herbert, F.K., Plasma Phosphatase in Various Types of Jaundice, Brit. J. Exper. Path. 16:365, 1935.

73. Hermans, P.E. and others, Electrophoretic Studies of Serum Proteins in Cyanogum Gel, Proc. Staff Meet. Mayo Clinic 35:792, 1960.

74. Hicks, M.H. and others, Effect of Spontaneous and Artificially Induced Fever on Liver Function, J. Clin. Investigation 27:580, 1948.

75. Hiller, A. and others, A Study of Conditions for Kjeldahl Determination of Nitrogen in Proteins, J.Biol. Chem. 176:1401, 1948.

76. Hoch, H., Comparison of Electrophoretic Patterns of Human Sera Obtained in Phosphate and in Diethylbarbiturate Buffer, Biochem. J. 46:539, 1950.

77. Howe, P.E., Use of Sodium Sulfate as the Globulin Precipitant in the Determination of Proteins in Blood, J. Biol. Chem. 49:93, 1921.

78. -------, Determination of Proteins in Blood: A Micro Method, J. Biol. Chem. 49:109, 1921.

79. de la Huerga, J. and Popper, H., Standardized Reagent for Thymol Turbidity Test, J.Lab. and Clin. Med. 34:877, 1949.

80. Huggins, C. and Talalay, P., Sodium Phenolphthalein Phosphate as a Substrate for Phosphatase Tests, J. Biol. Chem. 159:399, 1945.

81. Hunton, D.B. and others, Studies of Hepatic Function with Indocyanine Green, Gastroenterology 39:713, 1960.

82. Hurn, M. and Mann, F.D., Determination of Prothrombin (Two-Stage Method) and Antithrombin Using Commercially Available Reagents, Am. J. Clin. Path. 17:741, 1947.

83. Ivy, A.C. and Roth, J.A., Why Do a Liver Function Test?, Gastroenterology 1:655, 1943.

84. Jordan, W.S. and Albright, R.W., Liver Function Tests in Infectious Mononucleosis, J. Lab. and Clin. Med. 35:688, 1950.

85. Kabat, E.A. and others, The Relation of Cephalin Flocculation and Colloidal Gold Reactions to the Serum Proteins, J. Clin. Investigation 22:563, 1943.

86. Kagan, B.M., Hyperglobulinemia, Am. J. Med. Sci. 206:309, 1943.

87. Karmen, A. and others, Transaminase Activity in Human Blood. Note on Spectrophotometric Assay of Glutamic-Oxalacetic Transaminase in Human Blood Serum, J.Clin. Investigation 34:126, 1955.

88. Kato, K., Micro-Prothrombin Test with Capillary Whole Blood--A Modification of Quick's Quantitative Method, Am. J. Clin. Path. 10:147, 1940.

89. Kelly, W.D. and others, Determination of Urine Urobilinogen; Interpretation of Rate of Color Development of Ehrlich Reaction, J. Lab. and Clin. Med. 31:1045, 1946.

90. Kibrick, A.C., On Determination of Protein in Serum and in Fractions Obtained from Serum with Biuret Reagent Prepared with Sodium Hydroxide, J. Lab. and Clin. Med. 34:1171, 1949.

91. King, E.J. and others, King-Armstrong Phosphatase Estimation by Determination of Liberated Phosphate, J. Clin. Path. 4:85, 1951.

92. -------, and Armstrong, A.R., Convenient Method for Determining Serum and Bile Phosphatase Activity, Canad. M.A.J. 31:376, 1934.

93. Kingsley, G.R., The Determination of Serum Total Protein, Albumin, and Globulin by the Biuret Reaction, J. Biol. Chem. 131:197, 1939.

94. -------, The Direct Biuret Method for Determination of Serum Proteins as Applied to Photoelectric and Visual Colorimetry, J. Lab. and Clin. Med. 27:840, 1942.

95. -------, and Machella, T.E., Studies of Differences between Biuret and Kjeldahl Determinations of Serum Proteins; Liver and Other Diseases, J. Lab. and Clin. Med. 34:1183, 1949.

96. Kirschner, P.A. and Glickman, S.I., Cephalin Flocculation Test in Jaundice, J. Lab. and Clin, Med. 28:1721, 1943.

97. Klatskin, G. and Drill, V.A., Significance of One-Minute Bilirubin in Serum, J. Clin. Investigation 29:660, 1950.

98. Knowlton, M., Effect of Refrigeration on Ehrlich Reaction in Urine, J. Lab. and Clin. Med. 35:328, 1950.

99. Kove, S., Patterns of Serum Transaminase Activity--Diagnostic Aid in Neonatal Jaundice, J. Pediatrics 57:802, 1960.

100. Krautman, B., Micro Cephalin-Cholesterol Flocculation Test on Blood from Finger, Am. J. Clin. Path., Tech. Sect. 10:126, 1946.

101. Krebs, J.S. and Brauer, R.W., Modification of BSP Metabolism by Bile Stasis and Liver Injury, Am. J. Physiol. 198:774, 1960.

102. Kumate, J. and others, Liver Function Tests in Infants with Biliary Atresia; Report of 30 Cases, Pediatrics 26:630, 1960.

103. Kunkel, H.G., Value and Limitations of Thymol Turbidity Test as an Index of Liver Disease, Am. J. Med. 4:201, 1948.

104. -------, and Hoaglund, C.L., Mechanism and Significance of Thymol Turbidity Test for Liver Disease, J. Clin. Investigation 26:1060, 1947.

105. -------, and Slater, R.J., Lipoprotein Patterns of Serum Obtained by Zone Electrophoresis, J. Clin. Investigation, 31:677, 1952.

106. -------, and Tiselius, A., Electrophoresis of Proteins on Filter Paper, J. Gen. Physiol. 35:89, 1951.

107. LaDue, J.S. and Wroblewski, F., Significance of Serum Glutamic-Oxalacetic Transaminase Activity Following Acute Myocardial Infarction, Circulation 11:871, 1955.

108. -------, and others, Serum Glutamic Oxalacetic Transaminase Activity in Human Acute Transmural Myocardial Infarction, Science 120:497, 1954.

109. Laursen, T. and others, Serum Glutamic-Pyruvic Transaminase in Infectious Mononucleosis, Danish Med. Bull. 8:40, 1961.

110. Levin, R. and Brauer, R.W., Biuret Reaction for Determination of Proteins--Improved Reagent and its Application, J. Lab. and Clin. Med. 38:474, 1951.

111. Longsworth, L.G., Recent Advances in the Study of Proteins by Electrophoresis, Chem. Rev. 30:323, 1942.

112. Lorber, S.H. and Shay, H., Entero-Hepatic Circulation of Bromsulphalein, Gastroenterology 20:262, 1952.

113. Macdonald, D., Practical and Clinical Test for Liver Reserve, Surg., Gyn., and Obst. 69:70, 1939.

114. Machella, T.E., Relationship of Bromsulphalein Retention to Fever of Natural P. falciparum Malaria, Am. J.M. Sc. 213:81, 1947.

115. Mackay, I.R. and others, Paper Electrophoresis of Serum Proteins' Photometric Quantitation and Comparison with Free Electrophoresis, J. Clin. Investigation 33:855, 1954.

116. Maclagan , N.F., Galactose Tolerance as Test of Liver Function, Quart. J. Med. 9:151, 1940.

117. -------, The Thymol Turbidity Test, New Indicator of Liver Dysfunction, Brit. J. Exper. Path. 25:234, 1944.

118. -------, Liver Function Tests in the Diagnosis of Jaundice; Review of 200 Cases, Brit. Med. J. 2:197, 1947.

119. -------, Liver Function Tests, Practitioner 184:253, 1960.

120. -------, and Bunn, D., Flocculation Tests with Electrophoretically Separated Serum Proteins, Biochem. J. 41:580, 1947.

121. -------, and others, The Mechanism and Interrelationships of the Flocculation Tests, J. Clin. Path. 5:1, 1952.

122. Malloy, H.T. and Evelyn, K.A., Determination of Bilirubin with Photoelectric Colorimeter, J. Biol. Chem. 119:481, 1937.

123. Mann, F.D. and others, Prothrombin in Liver Disease; Clinical Evaluation of Two-Stage Method, Gastroenterology 11:221, 1948.

124. Manning, R.T., and Delp, M.H., Liver Function Tests: Interpretation and Use of Various Methods, J. Kansas Med. Soc. 61:129, 1960.

125. Marrack, J.R., and Hoch, H., Serum Proteins--A Review, J. Clin. Path. 2:161, 1949.

126. -------, and others, Immunological Aspects of Thymol Turbidity Reaction, Brit. J. Exper. Path. 31:36, 1950.

127. Mateer, J.G. and others, Further Advances in Liver Function Tests, and Value of Therapeutic Test in Facilitating Earlier Diagnosis and Treatment of Liver Impairment, Gastroenterolgy 8:52, 1947.

128. -------, and others, Comparative Evaluation of Newer Liver Function Tests, Am. J. Digest. Dis. 9:13, 1942.

129. McCoy, K.L., Physiologic Basis of Serum Protein Studies in Liver Function, Med. Annals Dist. Columbia 30:471, 1961.

130. McDonald, R.K. and Kelly, V.C., Rapid Determination of Urobilinogen in Feces, Am. J. Clin. Path. 18:87, 1948.

131. Meites, S. and Hogg, C.K., Direct Spectrophotometry of Total Serum Bilirubin in the Newborn, Clin. Chem. 6:421, 1960.

132. Mendenhall, C.L. and Leevy, C.M., False Negative Bromsulfalein Tests, New Eng. J. Med. 264:431, 1961.

133. Mendelsohn, M.L. and Bodansky, O., Value of Liver Function Tests in Diagnosis of Intrahepatic Metastases in Nonicteric Cancer Patient, Cancer 5:1, 1952.

134. Miller, S.E., A Textbook of Clinical Pathology, Sixth Edition, Baltimore, Williams and Wilkins Co., 1960, Chapter 8 by Clarence Cohn and Alex Kaplan.

135. Milne, J., Serum Protein Fractionation; Comparison of Sodium Sulfate Precipitation and Electrophoresis, J. Biol. Chem. 169:595, 1947.

136. Molander, D.W. and others, Serum Glutamic Oxalacetic Transaminase as Index of Hepatocellular Integrity, J. Lab. and Clin. Med. 46:831, 1955.

137. Moore, D.B. and others, Mechanism of Positive Cephalin-Cholesterol Flocculation Reaction in Hepatitis, J. Clin. Investigation 24:292, 1945.

138. Morey, G. and others, Sensitization to Bromsulphalein, Gastroenterology 13:246, 1949.

139. Moses, C. and others, Importance of Rate of Dye Removal in Bromsulfalein Test of Liver Function, J. La. and Clin. Med. 33:448, 1948.

140. Mosley, J.W. and others, Elevations of Serum Transaminase Activities Following Infectious Hepatitis, Gastroenterology 41:9, 1961.

141. Neefe, J.R. and others, Studies of Responses of Certain Hepatic Tests in Diseases of the Liver and Biliary Tract, Gastroenterology 9:656, 1947.

142. -------, and Reinhold, J.G., Photosensitivity as a Cause of Falsely Positive Cephalin-Cholesterol Flocculation Tests, Science 100:83, 1944.

143. -------, and Reinhold, J.G., Laboratory Aids in Diagnosis and Management of Infectious Hepatitis; Analysis of Results Obtained by Studies on 34 Volunteers During Early and Convalescent Stages of Induced Hepatitis, Gastroenterolgy 7:393, 1946.

144. Numerof, Paul, Radiochemistry Aspects of Liver Function Tests, delivered as a Lecture at Omaha Veteran's Administration Hospital, Omaha, Nebraska, on March 15, 1962.

145. Nydick, I. and others, Evidence for Increased Serum Glutamic Oxalacetic Transaminase Activity Following Graded Myocardial Infarcts in Dogs, Circulation 12:161, 1955.

146. Oberman, J.W. and others, Electrophoretic Analysis of Serum Proteins in Infants and Children; Normal Values from Birth to Adolescence, New Eng. J.Med. 255:743, 1956.

147. Obrinsky, W., and Others, Sulfobromophthalein Sodium Excretion Test as Measure of Liver Function in Premature Infants, Pediatrics 9:421, 1952.

148. Paulson, M and Wyler C.I., Multiple Tests of Hepatic Function in Gastroenteric Malignancy, Ann. Int. Med. 16:872, 1942.

149. Pellegrino, E. and others. Observations on Diurnal Excretion of Urobilinogen in Urine of Normal Subjects and of Patients with Laennec's Cirrhosis, J. Lab. and Clin. Med. 32:397, 1947.

150. Petermann, M.L. and others, Comparison of Howe and Electrophoretic Methods for Determination of Plasma Albumin, J. Biol. Chem. 169:379, 1947.

151. Pillemer, L. and Hutchinson, M.C., Determination of Albumin and Globulin Contents of Human Serum by Methanol Precipitation, J. Biol. Chem. 158;299, 1945.

152. Pineda, E.P. and others, Serum Enzymes in the Diagnosis of Pancreatic and Hepatic Disease in Anicteric Patients, Amer. Coll. of Surgeons Surg. Forum 10:249, 1960.

153. Popper, Hans and others, Electrophoretic Serum Protein Fractions in Hepatobiliary Disease, Gastroenterolgy 17:138, 1951.

154. -------, and Franklin, M., Diagnosis of Hepatitis by Histologic and Functional Laboratory Methods, J.A.M.A. 137:230, 1948.

155. -------, and others, Turbidimetric Gamma Globulin Determinations in Hepatobiliary Diseases, J. Lab. and Clin. Med. 35:391, 1950.

156. -------, and others, Correlation between Electrophoretic and Chemical Partitions of Serum Proteins, Am. J. Clin. Path. 20:530, 1950.

157. -------, and Schaffner, Fenton, Hepatic Tests, Advances Int. Med. 4:391, 1950.

158. -------, and Schaffner, F., Laboratory Diagnosis of Liver Disease; Coordinated Use of Histological and Biochemical Observations, J.A.M.A. 150:1367, 1952.

159. -------, and Schaffner, F., Liver: Structure and Function, New York, The Blakiston Division, McGraw-Hill Book, Co., Inc., 1957.

160. -------, and others, Quantitative Correlation on Morphologic Liver Changes and Clinical Tests, Am. J. Clin. Path. 19:710, 1949.

161. Post, J. and Patek, A.J. Jr., Serum Proteins in Cirrhosis of Liver; Relation to Prognosis and to Formation of Ascites, Arch. Int. Med. 69:67, 1942.

162. Quick, A.J., Coagulation in Experimental Hemorrhagic Disease, Am. J. Physiol. 118:260, 1937.

163. -------, Normal Antithrombin and Its Relation to Heparin, Am. J. Physiol. 123:712, 1938.

164. -------, The Nature of the Bleeding in Jaundice, J.A.M.A. 110:1658, 1938.

165. -------, and others, Study of the Coagulation Defect in Hemophilia and in Jaundice, Am. J.M. Sc. 190:501, 1935.

166. Raddin, J.B., The Decholin Taste Time Test of Liver Dysfunction. A Clinical Report on its Nonspecific Diagnostic Significance and on the Therapeutic Use of Decholin, Amer. J. Gastroenterology 34:497, 1960.

167. Rafsky, H.A. and others, Symposium on Liver Disease: Electrophoretic Studies in Liver Disease, Gastroenterology 14:29, 1950.

168. Rapoport, S., Increased Serum Phosphatase and "Hyperprothrombinemia" in Infectious Hepatitis of Children, Proc. Soc. Exper. Biol. and Med. 62:203, 1946.

169. Recant, L. and others, Comparison of Cephalin-Cholesterol Flocculation with Thymol Turbidity Test, Proc. Soc. Exper. Biol. and Med. 60:245, 1945.

170. Reich, J.S. and Davis, W.D., Jr., The Differential Value of Bromsulfalein Retention Tests in Acute Hepatitis and Obstructive Jaundice, Amer. J.Digest. Dis. 5:770, 1960.

171. Reinhold, J.G., Flocculation Tests and Their Application to the Study of Liver Disease, Adv. Clin. Chem. 3:83, 1960.

172. Richman, A. and Jacobs, W., The Radioactive (I-131) Rose Bengal Test of Liver Function, J. Mt. Sinai Hosp. (New York) 27:600, 1960.

173. Richman, S.M. and others, Alterations in Indices of Liver Function in Congestive Heart Failure with Particular Reference to Serum Enzymes, Amer. J. Med. 30:211, 1961.

174. Rosenthal, S.M. and White, E.C., Clinical Application of Bromsulphalein Test for Hepatic Function, J.A.M.A. 84:1112, 1925.

175. Saifer, A. and Zymaris, M.C., Photometric Micro-Determination of Human Gamma Globulin; Development of Quantitative Flocculation-Ninhydrin Procedure, J. Clin. Investigation 31:1, 1952.

176. Sborov, V.M. and others, Effect of Aureomycin on Urobilinogen Formation and Fecal Flora, J. Lab. and Clin. Med. 37:52, 1951.

177. --------, and Keller, T.C., Diagnosis of Hepatitis, Gastroenterology 19:424, 1951.

178. Schaffner, Fenton and others, Significance of Bilirubin Partition in Hepatobiliary Disease, Am. J.M. Sc. 219:307, 1950.

179. Schalm, L. and Schulte, M.J., Quantitative Determination of the Two Types of Bilirubin Simultaneously Present in the Blood, and its Clinical Importance, Am. J. M. Sc. 219:606, 1950.

180. Schwartz, M.K. and others, Comparison of Serum Alkaline Phosphatase Activities Determined with Sodium B-Glycerophosphate and Sodium Phenylphosphate as Substrates, Am. J. Clin. Path. 33:275, 1960.

181. Segal, E., The Bromsulphalein Test of Liver Function, Postgrad. Med. 30: 61, 1961.

182. Seligman, A.M. and others, Colorimetric Determination of Phosphatases in Human Serum, J. Biol. Chem. 190:7, 1951.

183. Sewell, S., Serum Acid and Alkaline Phosphatase Values in the Adult Male, Amer. J. Med. Sci. 240:593, 1960.

184. Shank, R.E. and Hoagland, C.L., A Modified Method for the Quantitative Determination of the Thymol Turbidity Reaction of Serum, J. Biol. Chem. 162:133, 1946.

185. Shay, H. and others, The Thymol Turbidity Test as a Measure of Liver Disease, with Special Reference to Comparison of Turbidity at 18 Hours with that at 30 Minutes, Gastroenterology 9:641, 1947.

186. -------, and Siplet, H., Value of Serum Alkaline Phosphatase Determination and Bromsulphalein Test in Diagnosis of Metastatic Cancer of Liver, J. Lab. and Clin. Med. 43:741, 1954.

187. Sherlock, S.P.V., Biochemical Investigations in Liver Disease; Some Correlations with Hepatic Histology, J. Path. and Bact. 58:523, 1946.

188. Shinowara, G.Y., Spectrophotometric Studies on Blood Serum and Plasma, Physical Determination of Hemoglobin and Bilirubin, Am. J. Clin. Path. 24:696, 1954.

189. Shotton, D. and others, Bromsulphalein Retention due to Administration of a Gall-Bladder Dye (Bunamiodyl), New Eng. J. Med. 264:550, 1961.

190. Smithies, O., Zone Electrophoresis in Starch Gels: Group Variations in Serum Proteins of Normal Adults, Biochem. J. 61:629, 1955.

191. Sobotka, H. and others, A New Test for Urine Bilirubin, Am. J. Clin. Path. 23:607, 1953.

192. Sommerville, R.L. and others, Serum Transaminase in Hepatic Disease, Gastroenterology 39:574, 1960.

193. Sparkman, R., Studies of Urobilinogen: Simple and Rapid Method for Quantitative Determination of Urobilinogen in Stool and in Urine, Arch. Int. Med. 63:858, 1939.

194. Spinco., Model R. Paper Electrophoresis System Instruction Manual.

195. Steeher, J.L., Fatal Reaction to Sulfobromophthalein, New Eng. J. Med. 261:963, 1959.

196. Stefanini, M. and Crosby, W.H., Serum Prothrombin Time, A Composite Effect--an Analysis of the Factors Involved, Am. J. Clin. Path. 20:1026, 1950.

197. Steigmann, F. and Dyniewicz, J.M., Studies of Urobilinogen; Daily Urobilinogen Excretion in Urine and Feces in Health and Disease; Evaluation of Watson's and Sparkman's Methods, Gastroenterology 1:743, 1943.

198. -------, and Dyniewicz, J.M., Studies of Urobilinogen; Quantitative Urobilinogen Determinations in Differential Diagnosis of Jaundice, Gastroenterology 1:855, 1943.

199. -------, and others, Flocculation Tests in Diagnosis of Hepatobiliary Disease, Gastroenterology 13:9, 1949.

200. Stekiel, W.J. and others, Hepatic Clearance of Indocyanine in the Dog, Amer. J. Physiol. 198:881, 1960.

201. Stephenson, J.M. and Snoddy, W.T., Protein Electrophoresis: An Aid to Clinical Diagnosis, J. Okla. Med. Ass. 53:817, 1960.

202. Stewart, R.D. and others, Infrared Analysis of Serum Proteins from 105 Healthy Adults, J.Lab. and Clin. Med. 56:391, 1960.

203. Stokes, G.D. and others, Methylene Blue Test for Bilirubinuria; Clinical and Spectrophotometric Observations, J. Lab. and Clin. Med. 31:924, 1946.

204. Sukumaran, M. and Bloom W.L., Influence of Diet on Serum Alkaline Phosphatase in Rats and Men, Proc. Soc. Exper. Biol. and Med. 84:631, 1953.

205. Sunderman, F.W., A rapid Method for Estimating Serum Proteins, J. Biol.Chem. 153:139, 1944.

206. -------, and Sunderman, F.W.Jr., Studies of the Serum Proteins. V. Causes for Discrepancies in Fractionation of of Serum Proteins by Paper Electrophoresis, Am. J. Clin. Path. 33:369, 1960.

207. Tagnon, H.J. and others, Effect of Surgical Operations on Bromsulfalein Retention Test, New Eng. J. Med. 238:556, 1948.

208. Taplin, G.V. and others, Liver Blood Flow and Cellular Function in Hepatobiliary Disease. Tracer Studies with Radiogold and Rose Bengal, J. Nucl. Med. 2:204, 1961.

209. Thoma, G.E. and Kitzberger, D.M., Spectrophotometric Method for Determination of Urinary Bilirubin, J. Lab. and Clin. Med. 33:1189, 1948.

210. Ulevitch, H. and others, Importance of Serial Determinations of Serum Alkaline Phosphatase in Incomplete Biliary Tract Obstruction, J. Lab. and Clin. Med. 38:693, 1951.

211. Unger, P.N. and Shapiro, S., Prothrombin Response to Parenteral Administration of Large Doses of Vitamin K in Subjects with Normal Liver Function and in Cases of Liver Disease; Standardized Test for Estimation of Hepatic Function, J. Clin. Investigation 27:39, 1948.

212. -------, and Shapiro, S., Hyperprothrombinemia Induced by Vitamin K in Human Subjects with Normal Liver Function, Blood 3:137, 1948.

213. University of Nebraska Laboratory Procedures from card-file of Clinical Pathology Laboratory of University of Nebraska College of Medicine.

214. Vickery, H.B., Early Years of Kjeldahl Method to Determine Nitrogen, Yale J. Biol. and Med. 18:473, 1946.

215. Voegtlin, W.L., Possible Source of Error in Quantitative Determination of Urobilinogen by Watson's Method, Am. J. Clin. Path. 18:84, 1948.

216. -------, and others, Comparison of Quantitative Urinary Urobilinogen Determination (Watson) with Urine Ehrlich Test, Gastroenterology 14:538, 1950.

217. Warner, E.D. and others, Quantitative Study on Blood Clotting; Prothrombin Fluctuations Under Experimental Conditions, Am. J. Physiol. 114:667, 1936.

218. Watson, C.J., Studies of Urobilinogen; Improved Method for Quantitative Estimation of Urobilinogen in Urine and Feces, Am. J. Clin. Path. 6:458, 1936.

219. -------, Studies of Urobilinogen; Urobilinogen in Urine and Feces of Subjects without Evidence of Disease of Liver or Biliary Tract, Arch. Int. Med. 59:196, 1937.

220. -------, Studies of Urobilinogen; Per Diem Excretion of Urobilinogen in Common Forms of Jaundice and Disease of Liver, Arch. Int. Med. 59:206, 1937.

221. -------, Some Newer Concepts of Natural Derivatives of Hemoglobin, Blood 1:99, 1946.

222. -------, Importance of Fractional Serum Bilirubin Determination in Clinical Medicine, Ann. Int. Med. 45:351, 1956.

223. -------, and Bilden, E., Determination of Urobilinogen in Feces and in Urine, Comparison of Sparkman and Watson Procedure, Arch. Int. Med. 68:740, 1941.

224. -------, and Hawkinson, V., Semiquantitative Estimation of Bilirubin in Urine by Means of Barium-Strip Modification of Harrison's Test, J. Lab. and Clin. Med. 31:914, 1946.

225. -------, and Hawkinson, V., Studies of Urobilinogen; Further Experience with Simple Quantitative Ehrlich Reaction; Corrected Calibration of Evelyn Colorimeter with Pontacyl Dye Mixture in Terms of Urobilinogen, Am. J. Clin. Path. 17:108, 1947.

226. -------, and Rappaport, E.M., Comparison of Results Obtained with Hanger and Maclagan Tests in Patients with Liver Disease, J. Lab. and Clin. Med. 30:983, 1945.

227. -------, and others, Studies of Urobilinogen; Simple Method for Quantitative Recording of Ehrlich Reaction as Carried out with Urine and Feces, Am. J. Clin. Path. 14:605, 1944.

228. Weichselbaum, T.E., Accurate and Rapid Method for Determination of Proteins in Small Amounts of Blood Serum and Plasma, Am. J. Clin. Path. Tech. Suppl. 10:40, 1946.

229. Welin, G., Needle Biopsy and Liver Function Tests in Acute Hepatitis and Cirrhosis of the Liver, Acta med. Scandinav. Suppl. 268:1, 1952.

230. White, F.W. and others, Comparison of 3 Urobilinogen Tests in Urine in Jaundice and Disease of Liver, Am. J. Digest. Dis. 8:346, 1941.

231. Wiegand, B.D. and others, The Use of Indocyanine Green for the Evaluation of Hepatic and Blood Flow in Man, Am. J. Digest. Dis. 5:427, 1960.

232. Wilson, S.J., Quantitative Prothrombin and Hippuric Acid Determinations as Sensitive Reflectors of Liver Damage in Human Subjects, J. Lab. and Clin. Med. 25:1139, 1940.

233. Winkler, K. and Gran, C., Models for Description of the Bromsulfalein Elimination Curves in Man after Single Intravenous Injections, Acta med. Scandinav. 169:263, 1961.

234. Wintrobe, M.W., Clinical Hematology, Fifth Edition, Philadelphia, Lea and Febiger, 1961.

235. Wirts, C.W. and Cantarow, A., Study of Excretion of Bromsulphalein in Bile, Am. J. Digest. Dis. 9:101, 1942.

236. Wirtschafter, Z.T. and Tsujimura, J.K., Serum Glutamic Oxalacetic Transaminase, Specificity of Values in Hepatocellular Injury, A.M.A. Arch. Environ. Health 2:16, 1961.

237. Wolfson, W.Q. and others, Studies in Serum Proteins; Rapid Procedure for Estimation of Total Protein, True Albumin, Total Globulin, Alpha Globulin, Beta Globulin, and Gamma Globulin in 1.0 ml. of Serum, Am. J. Clin. Path. 18:723, 1948.

238. Wroblewski, Felix and LaDue, J.S., Serum Glutamic Oxalacetic Transaminase Activity as Index of Liver Cell Injury: Preliminary Report, Annals of Int. Med. 43:345, 1955.

239. -------, and LaDue, J.S., Serum Glutamic Oxalacetic Transaminase Activity as Index of Liver Cell Injury from Cancer, Preliminary Report, Cancer 8:1155, 1955.

240. -------, and LaDue, J.S., Lactic Dehydrogenase Activity in Blood, Proc. Soc. Exp. Biol. and Med. 90:210, 1955.

241. -------, and LaDue, J.S., Serum Glutamic Oxalacetic Aminopherase (Transaminase) in Hepatitis, J.A.M.A. 160:1130, 1956.

242. -------, and others, The Mechanism and Significance of Alterations in Serum Glutamic Oxalacetic and Serum Glutamic Pyruvic Transaminase in Liver and Heart Disease, J. Clin. Investigation 35:746, 1956.

243. -------, and LaDue, J.S., Serum Glutamic Pyruvic Transaminase in Cardiac and Hepatic Disease, Proc. Soc. Exper. Biol. and Med. 91:569, 1956.

244. Wunderly, C. and Wuhrmann, F., The Effect of Experimental Increases in the Gamma Globulin and Albumin Content of Sera on the Response Given by Turbidity and Flocculation Tests, Brit. J. of Experimental Path. 28:286, 1947.

245. Zak, B. and others, Combined Agar Gel-Paper Electrophoresis, Am. J. Clin. Path. 33:75, 1960.

246. Zamchek, N. and others, Bromsulphalein Test in Early Diagnosis of Liver Disease in Gross Upper Gastrointestinal Hemorrhage, Gastroenterology 14:343, 1950.

247. Zieve, L. and others, Derivation of a Correction Allowing Use of Bromsulphalein in Jaundiced Patients, J. Lab. and Clin. Med. 37:40, 1951.

248. -------, and others, Normal and Abnormal Variations and Clinical Significance of One-Minute and Total Serum Bilirubin Determinations, J. Lab. and Clin. Med. 38:446, 1951.

249. Ziffren, S.E. and others, Hypoprothrombinemia and Liver Function, Surg., Gynec., and Obst. 74:463, 1942.