

1954

Serum cholinesterase activity levels as a measure of liver function

Donald W. Cherry
University of Nebraska Medical Center

This manuscript is historical in nature and may not reflect current medical research and practice. Search [PubMed](#) for current research.

Follow this and additional works at: <https://digitalcommons.unmc.edu/mdtheses>

Recommended Citation

Cherry, Donald W., "Serum cholinesterase activity levels as a measure of liver function" (1954). *MD Theses*. 1964.
<https://digitalcommons.unmc.edu/mdtheses/1964>

This Thesis is brought to you for free and open access by the Special Collections at DigitalCommons@UNMC. It has been accepted for inclusion in MD Theses by an authorized administrator of DigitalCommons@UNMC. For more information, please contact digitalcommons@unmc.edu.

SERUM CHOLINESTERASE ACTIVITY LEVELS AS A MEASURE
OF LIVER FUNCTION

Donald W. Cherry

Submitted in Partial Fulfillment for the Degree of
Doctor of Medicine

College of Medicine, University of Nebraska

March 24, 1954

Omaha, Nebraska

TABLE OF CONTENTS

	<u>Page</u>
Introduction	1
Discovery of Cholinesterase	5
Site of Formation of Cholinesterase	7
Methods of Cholinesterase Determination	12
Serum Cholinesterase as a Liver Function Test	18
Summary	29
Conclusions	31
Tables	33
Bibliography	36

INTRODUCTION

The primary concern of the physician in his evaluation of the usefulness of any specific liver function test depends on a great many factors. The main considerations are the accuracy of the test, ease with which it may be performed, expense, and its specificity for certain pathological conditions.

Liver function tests offer valuable aid in the evaluation of hepatic cell function. However, these tests should be regarded only as an aid to clinical and other laboratory findings in the diagnosis of the exact pathologic conditions. Liver function tests indicate the existing functional state of the hepatic cells, but they do not reveal the cause of the impaired function. They do not indicate whether impaired function is due to a toxin circulating in the blood stream, an acute or chronic infection, faulty diet, compression of liver cells by scar tissue or metastatic carcinoma within the liver, or to any one of several other causes. Nor do these tests indicate whether the underlying cause is an acute condition or a more serious, chronic, progressive type of hepatic disease. However, if one knows from clinical and other laboratory or x-ray studies the general nature of the pathologic condition,

then the results of the liver function tests are of unusual value. They assume real importance in pre-operative and postoperative treatment and in evaluating the degree of impairment, the effectiveness of medical treatment, and the prognosis.

In employing different liver function tests in identical cases, one is impressed by the fact that there is no constant correlation of the results obtained with different tests. For example, in one case tests A and B may both show impairment of liver function, in a second case test A may show impairment and test B no evidence of it, and in a third case only test B may show impairment. This common observation can best be explained by the hypothesis that the various functions of the liver are not injured equally under different conditions. There is a dissociation of impairment of different liver functions and a corresponding dissociation of the results of liver function tests.

Therefore, the wisdom of conducting several different types of hepatic function tests is obvious, if one expects to demonstrate evidence of impaired liver function in the greatest percentage of patients with liver damage.

It is very doubtful if it will ever be possible to

select any one test that will give absolute accuracy in the differentiation of toxic and obstructive jaundice. Cases of toxic jaundice have frequently an intrahepatic obstructive element and cases of obstructive jaundice persisting for any length of time develop some degree of liver damage. To be of value in jaundice, therefore, a test dependent on the measurement of liver function must be sensitive to the diffuse liver damage usually found in toxic jaundice and relatively insensitive to the more local type of injury found in biliary obstruction.

When patients are ill with acute hepatitis, the physician is faced with the problem of deciding when the pathologic lesions have subsided, when the illness has cleared, and when the patient is ready to return to an active life. The problem is far from academic, for it has been clearly demonstrated that the chances of developing complications, be they relapse, delayed resolution, or cirrhosis, are considerably increased by allowing a patient to return to a full and active life too soon in convalescence.

The physician's decision cannot be based purely on clinical considerations because, following the subsidence of clinical symptoms and signs of liver disease, there remains a period of smouldering activity which

can be detected only by finding disturbed tests of liver function. In the majority of patients ill with acute hepatitis, liver function tests return to normal slowly, and usually all tests do not become normal simultaneously. Moreover, it has been shown that the course of recovery is usually not smooth, but is punctuated by mild remissions, which often are subclinical in nature and which are only detectable by one or more liver function tests.

The question arises as to which of the multitude of liver function tests is most reliable and of the greatest help to the physician in deciding when his patient is fit to return to work. The answer has economic as well as clinical significance.

In this paper an attempt is made to evaluate a new liver function test, serum cholinesterase activity, from the viewpoint of accuracy in differentiation of jaundice and liver disease, practicality of usage, and comparison with other standard liver function tests.

DISCOVERY OF CHOLINESTERASE

The presence in the serum of an enzyme capable of destroying acetylcholine was first suggested by Dale, 1914, (1) but it was not until 1932 that its specificity was demonstrated by Stedman, Stedman and Esson (2) and its present name of cholinesterase suggested. The site of formation and distribution was not determined by these workers.

As the chemical transmitter of a nervous impulse, acetylcholine as such has received considerable attention from clinicians, but comparatively little interest has been shown in cholinesterase. Stedman, Stedman and White, 1933, (3) reported a cholinesterase constant of human serum which was relatively high as compared with other species, but which varied considerably with different individuals. However, the level of serum cholinesterase activity was found to be constant in each individual. Again these authors fail to make mention of the site of formation or distribution in the body.

Ginsburg, Kahn and Nicholes, 1937, (4) conducted a series of experiments in which they extirpated the spleen, pancreas, gut and liver and blocked the reticulo-endothelial system in dogs with no resulting decrease in esterase levels of the serum. These authors con-

clude that the cholinesterase level in serum shows only small variations in most normal and pathological conditions, and that the cholinesterase level of the serum is rather an immobile bulwark against too much acetylcholine.

In 1943 Mendel and Rudney (5) reported a series of experiments which showed that the cholinesterase found in blood serum is a non-specific enzyme catalyzing the hydrolysis of not only choline esters, but of non-choline esters as well. This is the only mention in the literature of this esterase being non-specific for choline esters, all other articles either specifying or assuming the fact that the esterase being tested is a specific cholinesterase.

There is general agreement in the literature that cholinesterase may be isolated from blood serum and from red blood cells, in varying amounts, but it is also agreed that these esterases are identical.

McArdle, 1940, (6) reporting on a study of 269 subjects, was the first to show an association between low cholinesterase readings and liver disease. At the same time, no clinical signs or symptoms that could be attributed to a fall in the activity of the enzyme were noted and the association remained unexplained.

SITE OF FORMATION OF SERUM CHOLINESTERASE

Stedman and Stedman, 1935, (7) were the first investigators to report the distribution of serum cholinesterase in the body. These workers found that there was considerable variation occurring in cholinesterase values among individuals of a given species, and considerable variation among species. There were found to be different amounts of cholinesterase in human red blood cells and in human serum. There was also found to be great amounts of cholinesterase in brain tissue, but it was found to be absent from cerebrospinal fluid. No attempt at determining a site of formation of the esterase was reported at this time.

It had been noted by several investigators that there was an apparent correlation between low serum cholinesterase activity levels and liver disease. However, no effort was made to establish the liver as the site of formation of cholinesterase until 1943, when Faber (8) and (9) noted that the serum cholinesterase activity levels were found to be low in a number of diseases which had in common the low values of the serum albumin, and postulated that albumin and cholinesterase were both secreted into the serum from the same cells and presumably in a fairly constant ratio. Since existing

evidence indicated that the serum albumin was formed in the liver, it was Faber's conclusion that the liver must also be the organ for the origin of the serum cholinesterase.

The first conclusive work which was done toward establishing the site of formation of cholinesterase was in 1947 by Brauer and Root (10). These investigators carried out a series of experiments on dogs and found the plasma cholinesterase activity to be independent of sex and of total plasma protein concentration. A significant degree of correlation between liver function and plasma cholinesterase activity of male dogs was found. Calculations show that the liver contained five to seven times the amount of cholinesterase as the circulating blood plasma.

These authors also carried out a series of experiments with carbon tetrachloride in dogs. The poisoning of dogs with carbon tetrachloride resulted in an increase in the plasma cholinesterase activity. They concluded that this effect was due to the liver injury produced, that it was due to an actual increase of circulating plasma cholinesterase amounting to about fifty percent above normal, and that it did not reflect a failure of destruction of excesses of the enzyme.

It was their conclusion that there was a considerable store of preformed plasma cholinesterase in the liver of the dog and that an equilibrium seemed to govern the relation between liver and plasma cholinesterase activity, at least twenty-five percent of the circulating cholinesterase activity can be rapidly replaced from the hepatic store. This was interpreted as being evidence that cholinesterase was formed in the liver, stored there and released into the blood stream as needed.

Another point in support of the fact that serum cholinesterase is formed in the liver is the experiments which have been carried out using Di-Iso-Propyl Flurophosphate (DFP) in man. This agent has the unique property of inactivating irreversibly the cholinesterase in the serum.

Wescoe, Hunt, Riker and Litt, 1947, (11) were the first to use this technique. Regeneration rates of serum cholinesterase were followed in 13 normals and 15 pathologic individuals, including 13 cirrhotics, 1 carcinoma of the head of the pancreas and 1 chronic hepatitis, following injection of 2 milligrams of DFP. Regeneration rates were followed in these individuals for two weeks following the injection of the DFP. The

patients with liver disease showed significantly lower cholinesterase activity levels than did the normal patients. The regeneration rates in those with liver disease was also significantly lower than were those in the normal individuals.

DFP is rapidly destroyed in vitro and in vivo; therefore the recovery of serum cholinesterase activity is not representative of a reversal of enzyme inhibition but is indicative of synthesis of new enzyme protein. Since the regeneration rate of serum cholinesterase in patients with liver damage is significantly depressed as contrasted to the normal, it follows that the ability of such patients to synthesize this particular enzyme protein is decreased. This constitutes good evidence for the view that the liver is the primary focus for the formation of serum cholinesterase.

The above experiments have been duplicated by Grob, Lilienthal, Harvey and Jones, 1947 (12) and Comroe, Todd and Koell, 1947, (13) with the same results.

Further work has been done to correlate the serum albumin with the serum cholinesterase by Kunkel and Ward, 1947, (14); Vorhaus, Scudamore and Kark, 1950, (15); Levine and Hoyt, 1950, (16); and Fremont-Smith, Volwiler and Wood, 1952, (17).

It has been well established that serum albumin is synthesized in the liver and all the above authors have been able to directly correlate levels of serum albumin with serum cholinesterase. All have noted the depression of serum albumin and serum cholinesterase in liver disease, and the correlation between the rise of the albumin and cholinesterase as the disease improves and therapy is instituted.

Serial determinations have been done on normal individuals and individuals with liver disease and a close parallel has been found between albumin and cholinesterase in both cases. In patients undergoing rapid changes in clinical condition, a direct correlation can be demonstrated between the two levels.

METHODS OF CHOLINESTERASE DETERMINATION

There has been considerable variation in the method of determination of serum cholinesterase activity, causing great difficulty in interpreting the value of cholinesterase activity levels as liver function tests.

The majority of methods have required the usage of specialized equipment and have required considerable time and personnel, making them impractical for clinical application. In this section, the more common methods of determination will be given, and an attempt made to select the shortest, most economical and most practical methods for use in the ordinary clinical laboratory.

The first method was described by Antopol, Schifrin and Tuchman, 1938, (18), in which cholinesterase activity was expressed in cubic millimeters of carbon dioxide liberated in a total volume of 2 cc, from 7.5 milligrams acetylcholine chloride by 0.5 cc of diluted serum (diluted 50 times), the reaction continuing over a two hour period at 30 degrees centigrade. This method has the disadvantage of being long, the materials being costly, and the test requiring constant observation.

The method which had originally been described by Jones and Tod, 1937, (19) was in 1940 improved by McArdle (6) and was used quite extensively for exper-

imental work. This depends on the measurement in a Barcroft manometer of the amount of carbon dioxide liberated when acetylcholine is added to a mixture of serum and Ringers solution. The esterases break down the acetylcholine to choline and acetic acid, which reacts with the bicarbonate of the Ringers solution to form carbon dioxide. The concentration of the enzyme is expressed by the volume of carbon dioxide in cubic millimeters liberated by 1 cc of serum in 1 minute at 37 degrees centigrade. Readings were taken every two and one-half minutes for fifteen minutes. Normally the serum was diluted one in five. The mean values were 83 units by Tod and Jones and 78 units by McArdle. The equipment was not suited to use in a clinical laboratory, and if the concentration of the enzyme were high the hydrogen ion concentration would be changed sufficiently to the acid side to cause partial inhibition of the enzyme, making this test impractical for general usage.

Butt, Comfort, Cry and Osterberg, 1942, (20) developed a method utilizing the Cameron glass electrode. In this method 20 cc of acetylcholine bromide was brought to pH value of 8.5 with 1 drop of 0.1 normal sodium hydroxide. 0.2 cc of serum was added and the mixture was stirred manually. At 5 minute intervals for 30 minutes enough 0.1 normal sodium hydroxide was added

to keep the pH at 8.5. At the same time a control was run without serum. Both samples were kept at a constant temperature of 30 degrees centigrade. The value with the control was subtracted from the value with serum and expressed as cc of 0.1 normal sodium hydroxide necessary to maintain substrate at pH of 8.5 over 30 minute period. By this method a mean of 1.90, plus or minus 1.06, or 0.85 to 3.0, was used as acceptable limits of normal variability. This method was found to be impractical because of the great range of normal values, the time involved and the equipment.

A method utilizing the Beckman pH meter was used by Alcalde, 1950, (21) and Bauer, 1945, (22). This method used 1 milliliter of substrate plus 0.2 milliliters of serum in 10 milliliters of buffer, incubated 1 hour at 37 degrees centigrade, measured in a Beckman pH meter at 25 degrees centigrade and expressed in pH units. The average found was 0.60 units, normal range 0.38 to 1.09.

Snyder, Snyder and Bunch, 1951, (23) used a continuous titration method, which they described as simple and highly reproducible. They found a mean value of 2.32 units, one unit being the milliliters of 0.01 normal sodium hydroxide needed to neutralize the acetic acid liberated by 0.5 milliliters of serum from 25 milligrams

of acetylcholine chloride in 10 milliliters of carbon dioxide free water in ten minutes at pH 7.8 and a temperature of 37 degrees centigrade.

Sleissenger, Almy, Glider and Perle, 1953, (24) make mention of a colorimetric determination, with mean value of 29.6 units, but fail to describe the technique used. Heilbronn, 1953, (25) uses finger tip blood instead of venipuncture, as do the other investigators, and keeps a sample of blood-soaked filter paper. He claims good results where blood must be transported or where serial determinations are necessary, but does not make mention either of the method used for determination or of normal values.

The method which has been the most widely used and which seems to be the most easily adapted to clinical usage is that developed by Michel, 1949, (26). This method measures the acid produced by action of cholinesterase on acetylcholine by the rate of PH change in a standard buffer solution over a definite period of time, the pH change measured with a glass electrode.

The reagents are prepared as follows:

I. Plasma Buffer:

0.006 M sodium barbital (1.2371 gm); 0.001 M KH_2PO_4 (0.1361 gm); 0.30 M NaCl (17.535 gm). For 1 liter of buffer dissolve the reagents in about 900 milli-

liters of distilled water and add 11.6 milliliters of 0.1 M HCl before diluting to volume. The pH of the buffer should be 8.00 at 25 degrees centigrade.

II. Acetylcholine substrate.

0.165 M acetylcholine chloride (3.000 gm) in 100 milliliters of distilled water.

The plasma is diluted with water so that each milliliter of solution contains 0.02 milliliters of plasma. One milliliter of diluted plasma is mixed with 1 milliliter of buffer. The solution is allowed to equilibrate in a water bath at 25 degrees centigrade for 10 minutes and then 0.2 milliliters of 0.165 M acetylcholine is added with mixing.

The initial pH (pH_1) is determined with a pH meter reading to nearest 0.01 pH unit. Then 0.2 milliliters of acetylcholine solution is added with rapid mixing and the time recorded. Enzymatic reaction goes 1-1/2 hours and then final pH (pH_2) is determined. The cholinesterase is figured in units of ΔpH per hour as follows:

$$\Delta pH/\text{hour} = \frac{(pH_1 - pH_2 - b)}{(t_2 - t_1)} f$$

pH_1 = initial pH

pH_2 = final pH

t_1 = time of mixing with acetylcholine

t_2 = time of reading pH_2

b = nonenzymatic hydrolysis correction corresponding to
pH₂

f = correction for variations in Δ pH per hour with pH₁
corresponding to pH₂

The values for b and f have been worked out in tables
which may be found in Michel's original article.

The method of Michel employs simplicity, minimum of
equipment and relative ease of operation. It has been
compared to other methods and found to be very accurate.
It is the opinion of this author that this method is
the most desirable.

SERUM CHOLINESTERASE AS A LIVER FUNCTION TEST

Because of the observation that serum cholinesterase levels are consistently low in diseases of the liver, a great many attempts have been made to evaluate the serum cholinesterase activity as a measure of liver function. To date the greatest disadvantage encountered in this evaluation has been the fact that there has been a lack of standardization of the method of determination of cholinesterase activity and of the units of activity, making it extremely difficult to interpret the results of various investigators.

In this section the author presents the work of various investigators in chronological order, giving the methods used and results obtained, and attempts to correlate the results obtained with the liver function tests in standard use.

The first investigator to run levels of cholinesterase activity on pathological cases was McGeorge, 1937, (27). This author had a series of 132 cases of various pathological conditions and came to the conclusion that although great variations occurred in the cholinesterase levels in this series no correlation with type of disease or other factors could be determined. Antopol, Tuchman and Schifrin, 1937, (28) related low

cholinesterase levels to hyperthyroidism and malnutrition, but made no correlation with liver disease. In 1938, Milhorat, (29) using the method of Butt and coworkers, found that the esterase values varied greatly with individuals but were constant for the given individual, and that in patients with liver disease or debilitation the esterase values were low and changed concomitantly with the clinical status of the patient. This is the first direct correlation with liver disease.

In the ten years between 1939 and 1949, a great deal of work was done on serum cholinesterase levels. Most of the work was parallel, and only the more significant work will be mentioned in detail. A great many men did serial cholinesterase determinations, concluding only that serum cholinesterase activity was depressed in liver disease and diseases of the biliary tract as well as in various other pathological conditions. Among these were Jones and Stadie, 1939, (30); Glick and Antopol, 1939, (31); Alles and Hawes, 1940, (32); Richter and Croft, 1942, (33); Strelitz, 1944, (34); Augustinsson, 1945, (35); Maizels, 1946, (36); and McCance, Widdowson and Hutchinson, 1948, (37).

Antopol, Schifrin and Tuchman, 1938, (18), were the first to make cholinesterase determinations with the intention of correlating values with jaundice. These

investigators reported determinations on 21 cases of jaundice and 7 other instances of liver and biliary disease, as well as 60 controls. Since fever and anemia also influence the cholinesterase activity, they tabulated the temperature at the time of drawing the blood and recorded the hemoglobin. Their results are shown in Table I. It is obvious from these figures that there is a tendency to depressed values in cases of hepatic and biliary tract disease. However, there was no attempt by these authors to distinguish between obstructive and non-obstructive jaundice.

The next investigator to indicate clearly the cholinesterase level in liver disease was McArdle, 1940, (6). This investigator ran a series of 270 determinations, including 40 normal adults and 71 patients with either cirrhosis, hepatitis, metastases in the liver, or jaundice as the result of cardiac failure or uremia. Using the method of Jones and Tod, 1937, (19) the range was from 50 to 121 units, with a mean of 78 units $\frac{1}{2}$. A value below 50 units was arbitrarily set to indicate liver damage. In 79% of 71 patients with liver disease the value was below 50 units, the range being 10 to 70 units and the mean 36 units. The initial figure for cholinesterase was 50 units or above in 21 (87.5 percent) of the 24 instances of obstructive jaundice,

while in jaundice of hepatic origin it was below 50 units in 33 (87 percent) of the 38 cases. Improvement or impairment of liver function was accompanied by a rise or fall respectively in cholinesterase. A comparison with other standard liver function tests is shown in Table II. These figures suggest that the determination of the cholinesterase in the serum might be employed usefully both as a measure of liver function and as a test in differentiation of jaundice of hepatic and obstructive origin.

In 1948 Vorhaus et. al.(38) ran controls on 42 patients and serial determinations on 26 with liver disease; 7 patients with cirrhosis had serial determinations from 70 to 240 days, 3 times per week. He noted a distinct rise in cholinesterase levels in those patients undergoing treatment with albumin. His results also suggested that serial determinations of cholinesterase activity afforded a more sensitive test to slight changes in hepatic function than any of the other tests used on these patients, namely cephalin-cholesterol flocculation, thymol turbidity, bromsulfalein retention, direct and indirect serum bilirubin, urobilinogen, prothrombin time, and total serum protein and albumin-globulin ratio.

Vorhaus, Scudamore and Kark, 1950, (39) using Michel's method of determination, ran another series

with 68 normals and 69 with hepatocellular disease. This study indicated that in approximately 80 percent of patients ill with hepatocellular disorders one may expect to find serum cholinesterase activity below normal values. Conversely, the serum cholinesterase activity in patients suffering with uncomplicated extra-hepatic obstructive jaundice is normal. Because serum cholinesterase is a sensitive index of hepatocellular function, a low level in a patient ill with obstructive jaundice probably indicates that the liver has been damaged by some complicating factor. These authors believe that this is the case even if other liver function tests are normal.

In addition to these determinations, serial determinations of serum cholinesterase and other standard liver function tests were carried out in six patients ill with chronic liver disease for a period of 70 to 240 days. An excellent correlation was obtained between changes in the clinical status of the patients and changes in the serum cholinesterase activity. Such changes were not reflected in a uniform manner by the other tests.

In a series of 211 determinations compiled by Alcalde, 1950, (21) the values in liver disease were found to be consistently low, especially in cirrhotic

patients. Normal values were obtained, however, in cases of obstructive jaundice. Serial determinations in a small number of patients with hepatitis revealed that the cholinesterase level was a very good index of hepatic function. Values were always low at the peak of the disease and appeared to be correlated closely with the patient's clinical condition. These results indicate that these determinations may be quite helpful in indicating prognosis and beginning recovery in cases of hepatitis.

In 1951 Vorhaus, Scudamore and Kark (39) conducted another investigation, with serial observations of serum cholinesterase activity on 7 patients ill with acute liver disease for 22 to 76 days. Serial tests were performed in conjunction with albumin-globulin ratio, total serum protein, serum bilirubin, direct and indirect, cephalin-cholesterol flocculation, thymol turbidity, bromsulfalein retention, prothrombin time, urine urobilinogen and serum alkaline phosphatase. In all but one of the patients studied the serum cholinesterase activity was well below the lower limit of the normal range at the onset of the illness, and in every instance there was a sharp rise in serum cholinesterase activity during recovery. When compared with the 10 other tests which were done, the serum cholinesterase

activity reflected the patient's changing condition sensitively and with greater uniformity and regularity than any other test. Four patients showed evidence of mild remissions during the course of their recovery, and in every case the serum cholinesterase activity dropped temporarily during the relapse. In each instance the relapse was reflected by some, but never by all, of the other 10 liver function tests. On the basis of this work, the serum cholinesterase activity appears to reflect the status and progress of the disease more sensitively than any of the other tests performed.

Studies by Snyder, Snyder and Bunch, 1951, (23) on 53 patients undergoing biliary tract surgery indicated that the cholinesterase and albumin seemed to parallel the clinical estimate of the patient's course more closely than did any of the other standard liver function tests. However, these authors were unable to correlate the value of cholinesterase levels with any specific liver disease.

Mann, et. al., 1952, (40) reported determinations on 115 cases of hepatobiliary disease and 168 controls, using Michel's method. They also performed serum bilirubin, thymol turbidity, cephalin-cholesterol flocculation, zinc sulfate turbidity, gamma globulin turbidity

and bromsulfalein retention tests. Their results are shown in Table III.

It is apparent that the cholinesterase values are usually decreased early in acute hepatitis, and increase as the patient convalesces. However, it does not always reflect a relapse and is usually normal in chronic viral hepatitis. Values in patients with cirrhosis tend to parallel the severity of the disease. It was the conclusion of these authors that the wide range of values in normal subjects and patients with liver disease, the occurrence of low values in obstructive jaundice and its lack of discrimination of chronic viral hepatitis all limited the usefulness of the test as a practical test of liver function.

Fremont-Smith, Volwiler and Wood, 1952, (17) ran a series on 40 normal and 157 other patients with various diseases, using Michel's method. They found the lowest values in cirrhotics, and no essential difference between values in acute viral hepatitis, obstructive jaundice, or intra-abdominal carcinoma. Their data indicates that this test is of value only in differentiating cirrhosis from the remainder of the group of hepatobiliary diseases. It was felt, however, that serial determinations of serum cholinesterase activity were of more value in assessing the prognosis of patients with

portal cirrhosis than the determination of serum albumin concentration, which has been the most valuable test for this determination. It was felt that the change in cholinesterase levels is apparent at an earlier date than is the change in albumin concentration.

Investigation by Wilson, Calvert and Geohegan, 1952, (41) on 43 patients with liver disease (15 from acute, 4 from sub-acute, 14 from chronic liver disease, 10 from extra hepatic biliary obstruction) and 144 normals, were made in conjunction with the flocculation tests and serum albumin and globulin.

In acute and sub-acute liver disease, low cholinesterase values were always found, and, as a measurement of prognosis, the serum cholinesterase activity levels returned to normal in advance of the other tests. In chronic liver disease results were less accurate, normal values sometimes being found in all tests. Normal values for serum cholinesterase activity are usually observed in patients with extra-hepatic biliary obstruction of short duration.

It was the conclusion of these authors that the serum cholinesterase level is a valuable aid in estimating the prognosis of cases of hepatitis, but is of no particular value in differentiating hepato-cellular disease from obstructive jaundice.

The above conclusions were confirmed by the work of Sleissenger and associates, 1953, (24) who found that serial determinations of cholinesterase levels faithfully reflect the course of viral hepatitis and cirrhosis, but found that in no instance did these levels contradict the clinical impression or the other laboratory evidence. It was found to be of no value in differentiation of hepatocellular and obstructive jaundice.

The serum cholinesterase activity determination has never been a clinical test but rather a tool of research, largely due to the fact that technical difficulties and expense of performing the determinations have made them impractical for the general clinical laboratory. Michel's development of a relatively simple method of determination, however, makes it a test which may prove practical for the clinical laboratory. However, certain other disadvantages must be considered. The test is empirical and as such demands closer scrutiny than would be accorded to a test having a known physiological basis. It gives a fair approximation of the degree of liver damage, but is not sufficiently sensitive to show the presence of a pathological liver in the late stages of toxic jaundice or in mild hepatitis. It is very difficult to evaluate this test because of the wide

range of values in normal subjects and in patients with liver disease. Values are found to be consistently low in cirrhotics and in patients with acute viral hepatitis, but may be low or normal in patients with obstructive jaundice. It does not appear that the test is useful in differentiating hepatocellular disease from obstructive jaundice. From the work of the various investigators, it would appear that the main value of the cholinesterase determination is in predicting and following the recovery of patients with viral hepatitis.

SUMMARY

In this paper an attempt has been made to evaluate serum cholinesterase levels in the serum as a measure of liver function. Data has been presented concerning the discovery of cholinesterase and evidence given which proposes the liver as the site of formation of the cholinesterase.

One of the great disadvantages in the evaluation of cholinesterase levels has been the lack of standardization of methods of determination. In this paper the more commonly used methods have been presented, the premise set forth that Michel's method is the most practical, and this method described in detail.

The work of various investigators has been presented in chronological order, their results in various diseases of the liver has been enumerated, and comparison has been made with other liver function tests wherever possible. It has been established that serum cholinesterase levels are generally low in diseases of the liver and biliary tract, and are especially low in cirrhosis and in viral hepatitis.

It has been further established that while values are generally low in diseases of the liver, normal values may be found in cases of chronic hepatitis and in most instances of obstructive jaundice. This re-

duces the value of the determinations as a method of differentiation of hepatocellular and obstructive jaundice. The results of several series of serial determinations of cholinesterase levels in patients with acute hepatitis and cirrhosis have been presented, such results revealing that the values closely parallel the convalescence of these patients.

CONCLUSIONS

1. Serum cholinesterase levels are generally depressed in diseases of the liver and biliary tract, but may also be depressed in cases of malnutrition and other pathologic conditions.

2. The cholinesterase in the serum closely parallels the albumin levels, being depressed with DFP and rising in direct parallelism with the albumin upon recovery of the liver. This is evidence that the liver is the site of formation of cholinesterase.

3. Cholinesterase levels show great variation between individuals in both the normal state and various pathologic conditions, but are constant for the individual.

4. Cholinesterase levels are always depressed in hepatocellular disease, but may be normal in obstructive jaundice and occasionally in chronic hepatitis. In view of this, it is felt that this test is of little value in the differentiation of jaundice.

5. Cholinesterase levels, when performed serially in patients with acute hepatitis or cirrhosis, accurately reflect the convalescence of the patient.

6. Due to the technical difficulty in determining the cholinesterase levels and the expense involved, it is not felt that this test is practical for the clinical

laboratory.

7. Because of the fact that this test does not differentiate between hepatocellular disease and obstructive jaundice, and because of its impracticality for the clinical laboratory, it is felt that this test is of very little value as a test of liver function.

8. Liver function tests in standard usage, correlated with clinical observation, give more accurate results than do serum cholinesterase levels and are simpler and more economical to determine.

TABLE I

Case	Age	Diagnosis	Cholinesterase Activity	Van den Berg	Icteric Index	Hemoglobin (percent)	Temp.
1	38	Cirrhosis	10	1/67,000	14	63	99.4
2	56	Cirrhosis with hepatitis	13	1/250,000	27	85	98.6
3	62	Toxic hepatitis	15	1/17,000	35	70	101
4	54	Toxic hepatitis	18	- -	20	98	100.2
5	54	Cirrhosis	19	1/500,000	56	96	99
6	59	Cirrhosis, cholemia	20	1/100,000	2	86	99.6
7	48	Cirrhosis, toxic hepatitis	22	1/100,000	12	46	98.6
8	13	Liver abscess, actinomycosis	24	1/50,000	14	78	99.8
9	51	Acute hemolytic icterus	29	1/100,000	30	30	99
10	23	Tertian malaria	30	1/80,000	12	75	99
11	63	Cancer of pancreas	31	1/60,000	15	70	103
12	56	Cancer of pancreas	34	1/80,000	23	85	98
13	42	Acute pancreatitis	38	1/14,000	50	--	100
14	46	Cholelithiasis	40	1/60,000	40	72	99
15	56	Chronic cholecystitis	46	1/250,000	27	78	98.6
16	50	Cholecystitis with cholelithiasis	48	1/17,000	42	85	98.6
17	71	Hepatosplenomegaly	55	1/100,000	12	72	102
18	26	Catarrhal jaundice	57	--	36	75	99
19	25	Catarrhal jaundice	63	1/80,000	13	80	98.6
20	8	Catarrhal jaundice	64	1/110,000	18	90	100
21	17	Hodgkins disease	76	1/70,000	20	90	99
22	31	Actinomycosis	11	--	--	58	103
23	66	Cirrhosis	17	1/500,000	4	11	98.6
24	71	Acute cholecystitis	25	1/500,000	5	70	99.2
25	65	Splenic vein thrombosis	29	1/500,000	6	36	98.6
26	27	Bilharzia of liver	42	1/200,000	12	50	100.4
27	45	Chronic cholecystitis	48	1/500,000	8	85	99
28	51	Chronic cholecystitis	65	1/300,000	5	83	98.6

Cases 22 to 28 without jaundice
Normal value of cholinesterase activity under 50

TABLE II

Test	Authors	Number of Cases	Number of Positive Results	Percent Positive Results
Levulose Tolerance Test	Kimball (1939)	149	81	57
Estimation of Total Blood Sugar	Steward, Scarborough (1938)	43	24	56
Levulose Tolerance Test	Stoffer (1935)	70	38	54
Estimation of Blood Levulose	Steward, Scarborough (1938)	59	45	75
Estimation of Blood Levulose	Herbert & Davison (1938)	38	31	82
Bromsulphalein Test	Stoffer (1935)	52	32	62
Bromsulphalein Test	Cornell (1929)	51	26	50
Bromsulphalein Test	O'Leary, Greene & Rowntree (1929)	67	54	80
Bilirubin Tolerance Test	Stoffer (1935)	72	62	86
Hippuric Acid Synthesis Test	Quick (1936)	37	33	89
Cholinesterase Test	McArdle (1940)	71	56	79

TABLE III

Case Number	Cholinesterase pH units/hr	Bilirubin 1 min. Mgm %	Bilirubin Total. Mgm %	Thymol turbidity Units	Cephalin Flocculation	Zinc Sulfate Turbidity Units	Gamma Globulin Turbidity Units	Bromsulphalein retention % in 45 min.
1	0.22	1.9	5.4	4.8	3	14.5	8.4	--
2	0.27	0.57	2.5	3.6	3	8.6	5.5	--
3	0.28	0.16	1.2	3.4	1	4.2	6.6	42
4	0.33	0.55	1.7	1.1	0	3.7	---	51
5	0.35	0.33	1.4	7.0	3	10.4	5.9	24
6	0.37	0.81	2.1	1.7	3	4.7	7.0	23
7	0.41	2.4	4.6	3.4	0	4.5	2.6	--
8	0.43	0.39	1.0	1.2	0	2.5	2.9	18
9	0.52	0.24	1.3	6.1	3	8.4	4.5	17
10	0.56	0.50	2.1	11.9	4	16.3	11.3	37
11	0.61	0.93	2.0	1.4	0	1.0	---	22
12	0.64	0.44	1.2	6.5	2	11.9	5.4	23
13	0.70	0.81	1.8	2.9	0	2.9	2.5	35
14	0.79	13.1	2.8	4.0	4	2.2	2.5	--
15	0.83	0.32	1.0	2.3	1	3.2	2.1	14
16	0.90	0.11	0.46	5.9	1	7.5	---	13
17	0.97	0.06	0.38	2.5	0	2.2	---	30
18	1.13	0.12	0.38	1.8	0	2.5	---	8
19	1.23	0.15	0.74	1.19	0	8.1	4.7	--
20	1.28	0.10	0.98	3.2	0	3.2	3.0	15
21	1.28	0.09	0.48	1.1	0	2.5	---	2

Values above 0.7 normal.

BIBLIOGRAPHY

1. Dale: Quoted by Stedman, E; Stedman, E.; and Esson, L. H. (2)
2. Stedman, E.; Stedman, E.; and Esson, L. H. Cholinesterase, an Enzyme Present in the Blood Serum of the Horse. *Biochem. J.* 26: 2056, 1932.
3. Stedman, E.; Stedman, E.; and White, A. C.: A comparison of the Cholinesterase Activities of the Blood Sera from Various Species. *Biochem. J.* 27: 1055, 1933.
4. Ginsburg, R.; Kahn, R.; and Necholes, H.: Studies on the Blood Esterase. *Am. J. Digest. Dis. & Nutrition.* 4: 154, 1937.
5. Mendel, B. and Rudney, H.: Studies on Cholinesterase, Cholinesterase and Pseudo-cholinesterase. *Biochem. J.* 37: 59, 1943.
6. McArdle, B.: The Serum Cholinesterase in Jaundice and Diseases of the Liver. *Quart J. Med.* 9: 107, 1940.
7. Stedman, E. and Stedman, E.: The Relative Cholinesterase Activities of Serum and Corpuscles from the Blood of Certain Species. *Biochem. J.* 29: 2107, 1935.
8. Faber, M.: Serum Cholinesterase in Disease. *Acta Med. Scandinavia.* 114: 59, 1943.
9. Faber, M.: The Relationship between Serum Cholinesterase and Serum Albumin. *Acta Med. Scandinavia.* 114: 72, 1943.
10. Brauer, R. W. and Root, M. A.: Relation between Esterase Activity of Blood Plasma and of Liver of Dog. *Am. J. Physiol.* 194: 611, 1947.
11. Wescoe, W. C.; Hunt, C. C.; Riker, W. F.; and Litt, A. C.: Regeneration Rate of Serum Cholinesterase in Normal Individuals and in Patients with Liver Damage. *Am. J. Physiol.* 149: 549, 1947.

12. Grob, D.; Lilienthal, J. L., Jr.; Harvey, A. M. and Jones, B. F.: The Administration of Di-Isopropyl Fluorophosphate (DFP) to Man. Bull. John Hopkins Hosp. 81: 217-244, Oct. 1947.
13. Comroe, J. H., Jr.; Todd, J. and Koell, G. B.: Pharmacology of DFP in Man. J. Pharmacol. & Exper. Therap. 87: 281, 1946.
14. Kunkel, H. G. and Ward, S. M.: Plasma Esterase Activity in Patients with Liver Disease and the Nephrotic Syndrome. J. Exper. Med. 86: 325, 1947.
15. Vorhaus, L. J.; Scudamore, H. H.; and Kark, R. M.: Measurement of Serum Cholinesterase Activity in the Study of Diseases of the Liver and Biliary System. Gastroenterology. 15: 304, 1950.
16. Levine, M. G. and Hoyt, R. E.: The relationship between Human Serum Cholinesterase and Serum Albumin. Science, 111: 286, 1950.
17. Fremont-Smith, K.; Volwiler, W.; and Wood, P. A.: Serum Acetylcholinesterase. The Close Correlation with Serum Albumin and its Limited Usefulness as a Test of Liver Function. J. Lab. & Clin. Med. 40:4: 692-702, Oct. 1952.
18. Antopol, W.; Schiffrin, A.; and Tuchman, L.: Decreased Cholinesterase Activity of Serum in Jaundice and in Biliary Disease. Proc. Soc. Exper. Biol. & Med. 38: 363, 1938.
19. Tod, H. and Jones, M. S.: A Study of the Cholinesterase Activity in Nervous and Mental Disorders. Quart. Journ. Med. NS, V 1, 1937.
20. Butt, H. R.; Comfort, M. W.; Dry, M. B. and Osterberg, A. E.: Values for Acetylcholinesterase in the Blood Serum of Normal Persons and Patients with Various Diseases. J. of Lab. & Clin. Med. 27: 649, 1942.
21. Alcalde, M.O.: Serum Cholinesterase Determination in the Differential Diagnosis of Jaundice. J. Lab. & Clin. Med. 36: 391, 1950.

22. Bauer, R.: Cholinesterase and its Correlation with Bowel Movements and Liver Function. Rev. Gastroenterology. 12: 185, 1945.
23. Snyder, H. E.; Snyder, C. O.; and Bunch, L. D.: Serum Cholinesterase Levels in Surgical Patients. Am. Surg. 17: 959-981, 1951.
24. Sleissenger, M. H.; Almy, T. P.; Gilder, H.; and Perle, G.: Colorimetric Determination of Serum Cholinesterase, its value in hepatic and Biliary Tract Disease. J. Clin. Invest. 32: 466-472, 1953.
25. Heilbronn, E.: An Electrometric Method for the Determination of Cholinesterase Activity. Scan. Journ. of Clin. & Lab. Invest. Vol. 1, No. 4: 308, 1953.
26. Michel, H. O.: An Electrometric Method for the Determination of Red Blood Cell and Plasma Cholinesterase Activity. J. Lab. & Clin. Med. 34: 1564, 1949.
27. McGeorge, M.: Cholinesterase Activity in Disease. Lancet, I: 69, 1937.
28. Antopol, W.; Tuchman, L.; and Schifrin, A.: Cholinesterase Activity of Human Sera with Special Reference to Hyperthyroidism. Proc. Soc. Biol. & Med. XXXVI: 46, 1937.
29. Milhorat, A. T.: The Cholinesterase Activity of Blood Serum in Disease. J. Clin. Invest. 17: 649, 1938.
30. Jones, M. S. and Stadie, W. C.: The Cholinesterase Content of the Muscle of Myasthenia Gravis and of the Serum of Four other Groups of Clinical Conditions. Quart. J. Exper. Physiol. 29: 63, 1939.
31. Glick, D. and Antopol, W.: The Inhibition of Cholinesterase by Thiamin. Journ. Pharm. & Exper. Therap. LXV: 389, 1939.
32. Alles, G. A. and Hawes, R. C.: Cholinesterase in the Blood of Man. J. Biol. Chem. 133: 375, 1940.

33. Richter, D. and Croft, P. G.: Blood Esterase. Biochem. J. 36: 746, 1942.
34. Strelitz, F.: Studies on Cholinesterase. Biochem. J. 38: 86, 1944.
35. Augustinsson, K. F.: Cholinesterase. Nature. 156: 303, 1945.
36. Maizels, M.: Empirical Tests of Liver Function. Lancet, II: 451, 1946.
37. McCance, R. A.; Widdowson, E. M. and Hutchinson, A. O.: Effect of Under Nutrition and Alterations in Diet on the Cholinesterase Activity of the Serum. Nature. 161: 56, 1948.
38. Vorhaus, L. J.; Scudamore, H. H.; Gabuzda, G. J.; Morey, G. R.; Maloney, M. A. and Kark, R. M.: Serial Serum Cholinesterase determinations in Liver Disease. Proc. Central Soc. Clin. Res. 21: 25, 1948.
39. Vorhaus, L. J.; Scudamore, H. H.; and Kark, R. M.: Measurement of Serum Cholinesterase Activity; a Useful Test in the Management of Acute Hepatitis. Am. J. Med. Sc. 221: 140, 1951.
40. Mann, J. D.; Mandel, W. I.; Eichman, P. L.; Knowlton, M. A. and Sborov, V. M.: Serum Cholinesterase Activity in Liver Disease. Journ. of Lab. & Clin. Med. 39: 543-549, 1952.
41. Wilson, A.; Calvert, R. J.; Geohegan, H.: Plasma Cholinesterase Activity in Liver Disease; its Value as a Diagnostic Test of Liver Function Compared with Flocculation Tests and Plasma Protein Determinations. Journ. of Clin. Invest. 31: 815, 1952.