1951

Liver biopsy : and its findings correlated with the cephalin-cholesterol flocculation test of Hanger

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LIVER BIOPSY,
AND ITS FINDINGS CORRELATED
WITH THE CEPHALIN-CHOLESTEROL FLOCCULATION TEST
OF HANGER.

by

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SENIOR THESIS
SUBMITTED TO THE FACULTY OF THE
COLLEGE OF MEDICINE
UNIVERSITY OF NEBRASKA
1951

Preface

The stimulus for this paper stems from a case of ascites which the author had the privi-ledge to follow during his clerkship at the University of Nebraska Hospital. Following the routine battery of liver function tests, a diagnosis could not be made. The ascitic fluid was then carefully examined for cellular content without findings. The suggestion then was given that a liver biopsy be attempted. The liver was not palpable and there was discussion whether such a procedure would be wise. Finally one biopsy was attempted which ended in failure to get liver tissue. The question of the value and indications for a needle biopsy of the liver naturally lead into its most interesting use, that of evaluating the liver function tests.

Classification of Jaundice

Determining the etiology of a case of jaundice has been an age old problem to the physician and remains a problem even today. During the past fifty years or more a great number of laboratory procedures have been devised to test some of the liver's multitude of functions as a supplementary aid to the doctor's history and physical findings. Many times the laboratory findings seem to conflict with the history and judgment of the physician and cause a sense of mistrust on the physician's part of the value of certain procedures. One of the suggested uses of the liver biopsy is to determine the efficacy of liver function tests (9), so the purpose of this paper is to attempt to correlate cephalin-cholestrol flocculation reaction with the findings of liver biopsy, as found particularly in cases of jaundice.

It may be well to review a classification of jaundice as proposed by Rolleston and McNee:

(I) Hemolytic, Excessive production of bilirubim. Negative direct Vanden Bergh reaction

Examples: (1) Congenital hemolyti (2) Icteyus neonatorum, (3) Erythroblastosis fetalis (4) Familial jaundice, (5) Paroxysmal hemoglobinuia,

Classification of Jaundice (cont'd)

(6) Pernicious anemia, (7) Splenic anemia, (8) Cooley's anemia, (9) Marchiafava-Micheli syndrome, (10) Extensive burns, (11) Sickle cell anemia, (12) Cerebral hemorrhage, (13) Ruptured ectopic and other intra-peritoneal hemorrhage, (14) Mushroom poisoning, (15) Favism, (16) Malaria, (17) Black-water fever, (18) Hemolytic transfusion reaction, (19) Hemolysis due to infection from: Streptococcus, Staphylococcus, Pneumococcus, C. perfrengens, Bartonella bacilliformis, (20) Poisoned with: snake venom, arsenenualted hydrogen, toluenediamine, phenylhydrozine, acentanilid, sulfonamides, dintro-benzyol, aniline, benzol, and nitro compound of phenol etc.

(II) Hepatocellular- or Medical type, Injury to liver cells. Decreased bilirubim removal from blood, if severe, with escape of bilirubim into blood stream therefore direct Vanden Bergh positive after very early stage.

Examples:(1) acute and subacute hepatic necrosis, (2) infectious hepatitis (virus), (3) Infections: syphillis, pneumonia, dysentery, C. perfringens, tuberculosis, typhus, typhoid, streptococcus, staphylococcus, relapsing fever, malaria, oroya

Classification of Jaundice (cont'd)

fever, yellow fever, Weil's disease. (4) Infectious mononucleosis, (5) Hepatic cirrhosis, (6) Congestive heart failure, (7) Hyperthyroidism, (8) Diabetes mellitus, (9) Poison with: arsphenamine, sulfonamides, mercury, lead, chloroform, carbon tetrachloride, tetrachlorethane, trinetrotoluene, cincorophen, x-ray over liver.

(III) Obstructive or Surgical type, Reflux bilirubim from canaliculin in lymphatics and into blood-Direct Vanden Bergh:

Examples (1) Calculi, biliary or pancreatic, (2) Cholangitis, (3) Neoplasm of pancreas, ducts, duodenum or lymph nodes, (4) Duodenitis and diverticulum of duodenum, (5) Parasites in ducts, (6) Stricture of ducts- congestive and acquired, (7) Adhesions, (8) Pancreatitis, pancreatic cysts, (9) Cysts, cancer or abscess of liver, (10) Aneurysm of hepatic artery or renal artery.

Since hemolytic types of jaundice are not directly related to the liver, this group of causes of jaundice will not be in the scope of this paper.

Accuracy of Differential Diagnosis

Just how accurately can the etiology of jaundice be diagnosed? An interesting study of this problem

Accurary of Differential Diagnosis (cont'd) was surveyed at Buffalo General Hospital (25) between the years 1936-1940 of 413 patients whose presenting complaint was jaundice, excluding jaundice due to hemolysis. As to determining whether it was an intrahepatic or extrahepatic lesion there were 16 errors or 3.8%. Of the cases diagnosed extrahepatic there was a 1.6% error as to the cause of obstruction. 6.3% of the cases required exploration for a final diagnosis. The authors concluded that accuracy of diagnosis of jaundice can be over 90% and that the reason such accuracy can be obtained is because so many of the cases have a typical story. The figures were then broken into two time periods; one between 1936-1940, when little laboratory work was done, and this showed 7.4% error; and the 1941-1946 period, where laboratory work was done plus bedside observation, gave 5.6% error. There was a marked improvement in ability to differentiate intrahepatic from extrahepatic jaundice, in that error dropped from 6.1% to 2.4%.

When obstruction to bile flow remains for a prolonged period damage occurs in the hepatic cells due to the back pressure and often infection begins (46). If the case of jaundice is seen in this late stage it is extremely difficult to diag-

Accuracy of Differential Diagnosis(cont'd)

nose the primary cause as extrahepatic because functionally the liver has an intrahepatic lesion. Considering this fact, that the surgical type of jaundice can often lead to a medical type and that the two are often mixed, lends emphasis to the diagnostic acumen of the clinician who can by history, physical and laboratory procedures be accurate 90% of the time.

(I) Value of History and Physical:

The value of a good thorough history can not be over emphasized on any diagnosis especially that concerning liver disease. The survey which this paper makes as well as any literature concerning any mechanistic laboratory procedure to determine the liver's efficiency will bear out the point that a liver function test is only an adjunct to a good history and physical and also that one liver test or one positive finding by laboratory methods means little to nothing unless properly correlated to the history and physical and supplemented by further tests. The preceding figures bear witness to this fact in that the percentage of diagnostic error fell only 1.8% with the greater use of laboratory procedures which is a statistically significant increase in ability only because there

Accuracy of Differential Diagnosis (cont'd)

was such a small percentage of error with history and physical findings alone. It can not be in the scope of this paper to include the proper method of history and physical in diagnosing jaundice, but because it is omitted does not in any way subtract from its importance.

Ivy and Roth(20) suggest the following requirements for the intelligent use of liver function tests: (1) That one specific question to be answered. This would require a wide knowledge of physiology and biochemistry "the proper solution to the problem is to select a few tests of liver function and learn them well." (2) That the physician know the limitations of the test. (3) That the tests be reliable to determine their designated purpose. Do the tests have a high percentage of false positives?

The liver is an organ of many functions. Many of the functions of the body are only partly performed by the liver, or in other words, the liver is responsible for only a portion of the duties required in some process. Mann has emphasized the dynamic state of the liver and its variance from hour to hour. Disease processes seldom effect only one portion of the hepatic system: for example such as, obstruction, if prolonged, will cause

Accuracy of Differential Diagnosis (cont'd)

parenchymal damage giving a picture similar to primary hepatitis (46). Then there are the individual differences in livers and their reserves as well as many other problems which make evaluation of liver function tests extremely difficult.

It is generally agreed that one liver function test is of little or no value; that a battery of tests must be used; and that tests must be repeated. Even with tests of all sorts the gastroenterologist as well as the general practioner has difficulties in diagnosis (25).

History of Liver Biopsy

The fore runner of the modern methods of liver biopsy was the needle aspiration of hepatic suppuration and hydatid disease as reported by Roberts and Biett in 1833. In 1878 Hammond reported using an approach through the mid axillary line with a needle for drainage of liver abscess. Sims, a year later, reported the same method of treatment for hepatic abscess, but using an anterior axillary approach.

Lucatello in 1895 aspirated liver tissue for use as biopsy material and 12 years later Schupfer reported having performed 40 such biopsies. The material gained by their method consisted, on the

most part, of serum, blood and scattered liver cells with loss of lobular continuity.

Benbel in 1923 was one of the first to obtain solid liver tissue suitable for study. He reported 100 cases of aspiration biopsy. He had two deaths due to hemorrhage. Olivet reported 140 aspiration biopsies in 1926 some of which were cases reported by Bengel. He suffered 2 deaths by hemorrhage and one by peritonitis but did not use sufficient care in selection of cases.

In 1936 Frola published a monograph reviewing the earliest biopsies and reported 66 cases of his own. He used a very fine bore needle which provided too small a piece of tissue for adequate histological study but his amount of hemorrhage was minimal.

Iversen and Roholm brought the liver biopsy method before medical eyes by reporting 116 cases without a fatality by the use of their new method. Baron reported 48 biopsies the same year with 1 fatal hemorrhage. Kofler adopted the Iverson and Roholm method of aspiration and reported 100 cases in 1940 with 2 non-fatal hemorrhages.

Within the next ten years many workers were attracted to this method of liver study and many

cases have been reported. Mortality rates became lower and lower as more became known concerning selection of cases.

Some outstanding contributions have been made by Dible, McMichael and Sherlock, Green, Chiray, Flessinger and Roux by their interest in improving methods and selecting cases. Gillman and Gillman from South Africa introduced new equipment which sped up the process and avoided the danger of liver capsule tears.

As doctors became more and more tumor conscious during recent years, more and more instruments were devised for tumor biopsy. Tripoli and Fader popularized the use of Silverman's needle for punch biopsy of the liver.

Technique and Equipment

As mentioned, the liver biopsy began by using the aspiration method. The earliest methods stemmed from the treatment of liver abscess and hydatid cysts and were not much more than anesthizing the skin, inserting a needle into the liver and drawing back blood, serum, and scattered liver cells into the syringe. The study of such material was often diagnostic, but, on the whole,

unsatisfactory because tissue continuity was lost and often there was not enough tissue.

Iversen and Roholm introduced in 1939 an aspiration method which obtained a larger specimen for satisfactory study. Equipment consisted of a 20 cc syringe, a canula, 2 mm in diameter and 15-17 mm in length, with a serrated edge and a pointed stylet. The patient was placed in a sitting or reclining position and the skin was prepared with an iodine solution. The approach was usually in the posterior axillary line through the ninth intercostal space. The skin was anesthized with 1-2% novocain solution and then the patient instructed to take 3-4 deep breaths and hold his breath in expiration, at which time the anesthetic was injected into the pleural space and the diaphragm. A nick was then made in the skin with a surgical knife and the canula inserted through the skin. The patient was again instructed to take several deep breaths and to hold his breath in expiration while the canula and stylet were advanced through the pleural space and diaphragm. The stylet was quickly removed and the syringe placed on the canula. The canula was then inserted further with a rotorary motion to cut the liver tissue and negative pressure is

applied by pulling back on the plunger of the syringe. The cannula and liver tissue were then removed and the tissue fixed in a formulin solution. Often the negative pressure was not effective due to intake of blood and serum. In only 22.5% of the cases did the authors fail to get sufficient material for study.

Gillman and Gillman thought that the above method involved too much manuvering while the cannula was through the diaphragm and that it predisposed to tearing of the liver capsule. They introduced a beveled needle which would tend to split instead of tear the capsule. They also had the syringe attached to the needle before the procedure begins. Attached to the needle was a depth gauge, which when adjusted properly, would not allow the needle to penetrate beyond a certain depth, which usually was $2\frac{1}{2}$-$3\frac{1}{2}$ inches, or possibly as high as 7 inches if through a great deal of adipose tissue. Preparation of the patient was much the same. The approach was made between the xiphesternum and the right costal margin. The needle was quickly inserted slightly upward and to the left as the syringe plunger was quickly withdrawn. The barrel and needle were then twisted 360° and withdrawn. With this method they were able to

obtain sufficient tissue 95% of the time. The reason for failure in 5% of the attempts was accredited to the fact that nervous patients contracted their recti muscles. They had one death, in a moribund patient, out of a series of 500 biopsies.

Tripoli and Fader introduced the punch liver biopsy method in 1941. They recommended the use of the Vim-Silverman needle which is widely used today. The needle was introduced by Silverman in 1938 as a method of biopsying tumor masses. It consists of an obturator which fits inside a 16 gauge cannula and a split 18 gauge needle which is somewhat longer than the cannula. In its use to biopsy the liver, the skin is anesthesized over the selected site; the incision made with a Band-Parker blade; and the cannula and obturator inserted until they reach the liver. The obturator is removed and the split needle inserted through the cannula into the liver. Then the cannula is pushed down over the split needle for a distance of about ½ inch to clamp the prongs of the split needle about the tissue. The needle is then twisted to loosen the tissue from its base and withdrawn. This method obtains adequate tissue with the minimum amount of masceration. It is especially recommended if the liver is palpable,

in which case a subcostal approach at the lateral edge of the recti muscle could be used. The patients suffered from only a slight feeling of weakness following the biopsy and 2 weeks later it was difficult by direct examination of the liver to determine the puncture site.

The Roth-Turkel (41) needle is another needle which is also used in this country. It consists of an outer cannula, with a long bevel, and a longer inner needle, which has a serrated cutting edge and is pushed in beyond the outer cannula. A syringe is often used to provide negative suction in removing the tissue. It has the advantage of providing a larger amount of tissue.

There is a variance as to the site to make a puncture. The transthoracic approach through the pleural cavity has the danger of the patient moving and producing a tear in the liver capsule or in the sub-diaphragmatic venous plexus resulting in hemorrhage. However, this danger is not great and the chances of obtaining liver tissue is good. The sub costal approach on a palpable liver is the most certain and probably the safest if adequate measures are taken so that the bowel is not in the way or large vessels are not punctured, and the needle is

Technique and Equipment (cont'd)

not pushed on through the liver lobe to structures beyond. If a mass can be palpated along the free liver margin it should be biopsied in preference to other sites. Any such mass should be aspirated before a punch type biopsy is performed to rule out a vascular type of lesion (43). The approach between the xiphoid sternum and the right costal margin can be used on non-palpable livers without the danger of crossing the pleural cavity. There is a risk in this region of puncturing large vessels or bile canals.

Mortality and Morbidity

During the time when aspiration liver biopsy was used between Bengel in 1923 and Baron 1939 there were 300 biopsies reported with 6 accidental deaths. Five deaths were due to hemorrhage and one due to peritonitis. With refinements in technique and equipment and with better selection of cases the mortality has dropped considerably.

Mortality and Morbidity (cont'd)

MORTALITY OF ASPIRATION LIVER BIOPSY

AUTHOR	DATE	NUMBER OF BIOPSIES	DEATHS
Bingel	1933	100	2
Olivet	1926	140	3
Huard May Joyeux	1935	163	0
Baron	1939	49	1
Iverson-Roholm	1939	160	0
Tripoli-Fader	1939	14	0
Hatieganu, Sparchez , Radu, Macavee	1943	45	0
Vanbeek, Haex	1943	200	0
Hoffbauer	1945	65	0
Sherlock	1945	264	2
Total		1200	8

Gillison and Skinner recently noted that there have been 17 reported deaths in 4,900 cases of biopsy. This is a mortality of about 0.35%.

It is often not necessary to give the patient a preoperative sedation (43) because the pain involved is comparable to a lumbar puncture. There may be some distress in the right upper abdomen as long as 24 hours following the biopsy due to the unavoidable but small amount of hemorrhage causing peritoneal irritation. Pain may be referred to the right shoulder in cases of diaphragmatic irritation. Without complications a patient should be able to leave the hospital within 48 hours following a biopsy.

Precautions

Some of the most common dangers of liver biopsy are avoidable to a great extent if proper precautions are taken.

Hemorrhage seems to be one of the leading causes of difficulty and fatalities (30). Before a biopsy is attempted it is important that adequate blood studies be completed, such as hemoglobin and white count with the differential. Bleeding and clotting time should be run to rule out a hemophilia or purpuric patient. It is suggested that the prothrombin time be at least 50% of normal (48). A platlet count would be beneficial. In view of the fact that time is valuable in fighting shock due to hemorrhage, it would be wise to have the patient typed and cross matched with at least two units of blood before the biopsy is attempted, as suggested by Sherlock.

Damage to surrounding organs is another danger which can be minimized by observing a fast of at least 5 hours prior to the biopsy so that the stomach is not distended and protruding into the right side of the abdomen. Saline or soap suds enemas should be given within 12 hours of the biopsy to collapse the large bowel and make it a smaller target. A flat plate of the abdomen just prior to biopsy may rule out the inter-position of bowel between abdominal

Precautions (cont'd)

wall and liver and also provide more exact knowledge as to the location of the liver. A chest film will rule out pathology in the lower lung fields if a transthoracic approach is anticipated. It is a practical routine to have the patient take an enema before retiring and to do the biopsy before the patient has breakfast. Sips of water would be permissable.

Infection is a real danger and it is needless to say that strict aseptic technique should be observed. Antibiotics will help to keep morbidity from this cause at a lower level than reported by earlier workers.

Tumor implantation and air embolus are dangers which can be avoided only by care and discretion.

Bile peritonitis is not a common complication. Recently a case of biliary fistula resulting from biopsy has been reported (8).

In cases of jaundice it is well to give the patient vitamin K two to three days preceding the biopsy. If the jaundice is due to extrahepatic obstruction the vitamin K should be given parentally. If the jaundice is not due to obstruction the liver should be fortified with a liver extract(43)

Ascites is an added danger in biopsy because it can allow the interposition of bowel into the

Precautions (cont'd)

path of the needle and also allows the liver to float about in the abdomen. It is difficult at times to penetrate a cirrhotic liver, even when it is solidly fixed, so every advantage must be taken by the operator and the ascitic fluid drained just prior to biopsy (43).

Some routine notations must be made before biopsy. The patient's blood pressure, pulse and temperature are important in establishing a base line.

As more of a refinement rather than a requirement, a standardized diet may be prescribed for 48 hours preceding biopsy. This would tend to keep variations of fat and carbohydrate metabolism due to diet at a minimum in the study of the liver histology.

Positioning of the patient varies from sitting to suppine and is up to the discretion and conveniences of the operator. The right side should overhang the edge of the bed and may be elevated if desired to allow other abdominal organs to fall away from the liver. The patient's right arm can be placed under his head. The eyes should be covered to allay the anxiety caused by seeing the long needles.

The area should be cleansed and draped with the caution used in any abdominal operation and the operator clothed with cap, mask, gown and sterile gloves.

Precautions (cont'd)

Pre-operative sedatives are not usually necessary, but in cases of nervous patients 1/4 to 1/6 gr. of morphine, subcutaneously can be used. Barbiturates can also be used (43).

Following the biopsy the patient should be kept at absolute bed rest for 24 hours. During the first 12 hours the pulse should be taken every hour and the blood pressure every two hours. During the second 12 hour period the pulse and blood pressure may be taken every 2 hours. It is wise to give specific instructions that the physician should be notified immediately if the blood pressure falls or if the pulse is elevated above a certain point. A routine visit should be made by the physician at 8 and 12 hours following the biopsy. Temperature should be taken every 4 hours during the first 24 hour period. Special note should be made of increasing pain or apprehension, and analgesics can be given as necessary. Diet is regulated according to how the patient can tolerate food (45).

During the second 24 hours period the patient can be allowed mild exercise under continued watch. Dangers of any complications are usually manifest by this time and the mild discomfort that may have been noted following biopsy has usually abated.

Precautions (cont'd)

Discharge from the hospital is usually 48 hours following biopsy.

Advantages and Disadvantages

The value of liver biopsy lies in the fact that the actual histology of the liver disease can be studied with little distress to the patient as well as little cost and time. Liver function tests are to be compared to finger prints left by a burglar but by mean of the biopsy needle one can get in where the burglar is working and discover who he is, what he is doing and possibly how he does it. Edgar Baron expressed it well when he stated, "In the study of hepatic disease, laboratory proceedures can only reflect the alterations in function produced by the primary disturbances and are open to error in interpretation. Hepatic aspiration, on the other hand, may yield direct information about the pathologic changes in the liver. It offers a safe, rapid, inexpensive and reliable method of reaching the correct diagnosis and in some cases the only means of studying the pathologic changes associated with certain hepatic diseases, by its use many patients have been spared the ordeal of a useless laparotomy.

Equipment for the procedure is relatively

Precautions (cont'd)

simple and economical. The expense of surgical facilities may be avoided and the biopsy done in the patient's own room.

The danger to the patient is minimal. It must be realized however that one is entering the peritoneal cavity, somewhat blindly, and puncturing a highly vascular organ, which is surrounded by many vital structures.

Liver tissue in disease and health is usually uniform in character since the lobule is the functional unit. The needle biopsy is able to obtain sufficient tissue for study since the average lobule measurements are about 1 mm in breadth and 2 mm in length and the common needle is 2 mm or more in diameter (36). It is sometimes difficult to obtain liver tissue, depending somewhat upon the operator's experience and method used. Gillman and Gillman claimed 95% effeciency in obtaining tissue. Iverson and Roholm failed to get tissue 22.5% of their reported cases. Green failed in 50% of his cases to get tissue for study using the Iverson and Roholm aspiration technique.

Liver biopsy may be the only means of making a diagnosis in difficult cases. It must be mentioned that study of the biopsy histology does not always

Precautions (cont'd)

give a diagnosis especially in differentiating early biliary hepatitis from toxic hepatitis (34).

Reliability and Uses

In which disease of the liver is a biopsy of the liver of help? The diseases of the liver may be divided into three catagories in respect to biopsy findings: (1) those with specific findings, (2) those with helpful findings and (3) those in which negative findings are not helpful.

In general diseases which are characterized by diffuse liver damage have specific findings by biopsy which are pathonomonic. Such diseases as virus hepatitis (serum and infectious), toxic hepatitis, biliary hepatitis, cirrhosis, Gaucher's disease, Leishmaniasis, malaria, various blood diseases, and malnutrition are examples.

Diseases in which one can find only helpful findings by biopsy are those diseases which only secondarily effect the liver such as cardiac congestion, amyloid disease, hemochromatosis, fatty metamorphis, obstructive jaundice, infectious mononucleosis (6), hyperthyroidism, diabetes, many infectious diseases and others which are characterized by liver damage but whose liver damage would not absolutely name the

disease.

There are then the diseases in which only positive findings are pathonomonic or helpful and negative findings do not rule out the disease such as primary and secondary cancer, cysts, abscesses, tuberculosis, gumma, parasites, focal necrosis and hemangiomas. Gillman and Gillman state that two to three biopsy attempts can be made through the same aperature with safety and it appears reasonable that if any of the above diseases are suspected that two to three biopsies taken at different angles into the liver would greatly enhance the chances of making such a diagnosis (48).

The following is suggested as uses for liver biopsy by Gillman and Gillman

(1) Diagnosis of hepatic enlargement.

(2) Degree of liver damage in malnutrition and other diseases.

(3) Controlling therapy.

(4) Morphology and metabolism of liver cells in health and disease.

(5) Examining the efficacy of liver function tests.

Cephalin-cholesterol Flocculation Test

History

In recent years there has been a great impetus to study the liver due to (1) the observation of the benefit derived from high protein diet in cases of cirrhosis (33), (2) the opportunity to study hepatitis in the Armed Services during World War II and (3) the popularization of the needle biopsy. Efforts have been made by means of the biopsy needle to better correlate the histology and pathology of the liver with the liver function tests which are coming more and more into use. One of the tests used frequently as a diagnostic and prognostic tool in liver disease is the cephalin-cholesterol flocculation test of Hanger.

The first preliminary report on the cephalin flocculation test was published by Hanger in 1938. In the cases sited in this report there was a significant preponderance of positive reactions with catarrhal hepatitis and an absence of reaction with obstructive type of jaundice. He suggested that the mechanism of the test may depend upon the interaction of defective fibrinogen produced by the diseased liver upon the colloidally dispersed cephalin.

The Following year 1939, a more full report was published along with the technique of the test (13).

Cephalin-cholesterol Flocculation Test (cont'd)

In 1944 Maclagan reported the tymol turbidity test which works much along the same principle as the cephalin-flocculation test.

Since the introduction of these two tests there have been over 200 papers written concerning their use (23).

Technique

Hanger described the technique of the cephalin-cholesterol flocculation test in detail in 1939.

The first step is the preparation of the stock solution from 100 mgm of sheep brain cephalin, 300 mgm of cholesterol and 8 cc of ether. This solution can be kept for months without deterioration if it is kept tightly stoppered.

Daily a fresh supply of emulsion is made by taking 1 cc of the stock solution and stirring it slowly into 35 cc of distilled water which has been brought to 65-70 degrees C. Bring the emulsion to a boil and simmer it down to 30 cc, driving the ether off.

1 cc of the above emulsion is mixed in a centrifuge tube with 0.2 cc of the patients serum and 4 cc of normal saline. This should be shaken well, stoppered with cotton, and allowed to stand at room temperature for 48 hours.

Cephalin-cholesterol Flocculation Test (cont'd)

In reading the results, they are graded from 0 to 4 plus. Four plus has a clear supernatant liquid.

Some precautions to be followed are that the sera be fresh or kept refrigerated, that anticoagulants given to patients may give misleading results and that the glassware be carefully washed to avoid heavy metals and strong acids.

It has been recommended, since many laboratories get inconsistant results with the cephalin-flocculant test, that the cephalin preparation be aged (29)(40).

Cephalin Flocculation Theory

The flocculation tests are not liver function tests, but should be regarded as indications of disturbed liver metabolism (16) (23). The exact mechanism by which the cephalin-cholesterol test works is not known as yet. The prominent theory to date is that the patholical sera contains a nitrogen bearing constituent in the globulin factor which becomes attached to the cephalin-cholesterol particles and the absorbed protein changes the surface potential and increases the cohesion (13).

A positive cephalin flocculation reaction may result, according to Cantarow and Trumper, when: (1) there is an increase in the gamma globulin so

Cephalin Flocculation Theory (cont'd)

that the normal component of albumin fraction can't protect the reaction, (2) there is a decrease of albumin below a concentration able to protect the colloidal suspension, (3) there is decreased flocculation-inhibiting properties of the serum albumin due to a chemical modification.

Electrophoretic studies concerning pathological serum giving positive cephalin flocculation seems to indicate qualitative change in the gamma globulin fraction and not merely just a quantitative increase (23).

Since the test is preformed on the serum it can be expected that other diseases than liver disease may give positive results. Such is the case and some examples are: bacterial endocarditis, green streptococcus and sterile pneumonia, lobar pneumonia which is uncomplicated, associated with jaundice or terminal, acute and chronic nephritis, terminal nephritis, pernicious anemia, leukemia and idiopathic anemia, tuberculosis, prontylin idiosyncrosy, general infectious, toxemias, and visceral rheumatic disturbances (12).

Popper stated " the flocculation tests are so well correlated with the clinical activity because their abnormality is the result of the interplay of

Cephalin Flocculation Theory (cont'd)

reduction and alteration of albumin (due to liver damage) with gamma globulin elevation (due to mesenchymal reaction) (36)."

Value of the Cephalin Flocculation Test

The value of the cephalin-cholesterol flocculation test can best be ascertained by noting how well it correlates with liver biopsy findings. First of all it must be remembered that a positive cephalin flocculation reaction only reflects the fact that there is some abnormality in the sera and that the true nature of this abnormality is not known. It has been shown by the works of many authorities that this abnormality of the sera is accompanied by active liver damage by study of autopsy and surgical tissue but the possibility of studying the tissue of the liver on the same day as the sera is taken by such a simple and relatively anoxious method as the liver biopsy has been a great help into the study of liver disease and evaluation of liver function tests.

Popper (35) in his early study of biopsy material discovered that the histology differed in several points from tissue from the autopsy table. The criteria for the histiological diagnosis of many of the liver diseases could not be accurately applied

to biopsy findings and often criteria for differential diagnosis was at a bare minimum. He has in recent years by a thorough study of biopsy material been able to set up a precise criteria for the diagnosis of many diseases of the liver.

Difficulties still arise in differential diagnosis. In the case of obstructive jaundice it is known that after a time the bile backs up into the liver and by some mechanism refluxes into the blood stream. Due to this stagnation and back flow, infection often sets in the liver causing biliary hepatitis (46). This type of hepatitis resembles in its early stages hepatitis due to toxins such as certain chemicals, bacteria or endogenous materials. As the obstructive condition advances the hepatitis resembles more closely a medical type of hepatitis but according to Popper it becomes easier and easier to diagnose by liver biopsy methods (31) (34) (35).

The following signs have been taken as evidence of liver cell damage: (1) irregular staining of cytoplasm and nucleus (2) hazy cell outlines (3) coagulation necrosis (4) hyalinization (Councilman bodies) and (5) evidences of active phagocytosis (24). Fatty changes were not considered as evidence of active cell destruction but rather with a healing

stage (23). Lobular patterns were disrupted in cases of active liver destruction as well as healing so is not a chief factor in determining whether the cells were undergoing active degeneration (37).

Not all the authors quoted in this paper were directly interested in correlating biopsy tissue findings with the cephalin flocculation reaction, so in analyzing their case reports, the above criteria by Levy has been used.

F. W. Hoffbauer et al (16) (17) at the University of Minnesota have done several liver biopsies in the past few years reporting on its value as a diagnostic tool. No correlations were made between the histological and functional findings, but in the selected cases which he reported it was interesting to note the following correlations between histology and the cephalin flocculant test.

Case #1--The differential diagnosis was between obstruction jaundice and cirrhosis. Laboratory and clinical findings were not able to clear the picture but biopsy showed an active phase or cirrhosis. The cephalin flocculation reaction was 4 plus.

Case #2--Biopsy diagnosis was extrahepatic obstruction. Final diagnosis was cancer of the gallbladder. The cephalin flocculation test remained

Value of the Cephalin Flocculation Test (cont'd) negative during the investigation.

Case#5--Biopsy showed amyloid disease and the cephalin flocculation test remaind negative.

Case #6--This was early biliary obstruction which on surgery was shown to be metastatic cancer. The cephalin flocculation test was negative.

Case #7--Biopsy material could hardly be recognized as liver tissue due to extreme necrosis. The diagnosis was Hodgkins disease. Cephalin flocculation was 3 plus.

Case #8--The diagnosis was obstructive jaundice and the cephalin flocculation test was negative. Obstruction was due to cancer of bile duct.

It is evident in these cases, which were reported originally for another purpose than cephalin flocculation correlation, show a close correlation between liver cell damage and a positive cephalin flocculation reaction, Dependability of the test is also evidented by the fact that the cephalin flocculation test was not positive in cases which had no active cell damage. This bears out Hanger's (15) own theory as to the meaning of positive cephalin flocculation reactions, which is, that it represents active liver cell damage

De Marsh in 1947 showed by the biopsy method

Value of Cephalin Flocculation Test (cont'd)

that the jaundice associated with infectious mononucleosis was due to parenchymal changes such as in infectious hepatitis. The histological picture of virus or infectious hepatitis is one of acute liver damage. In 19 consecutive cases with jaundice which were reported there were concomitant positive cephalin flocculation tests.

Kinsell et al reported their correlations of hepatic structure to function in 1949. They were of the belief that there was no specific findings in an individual cell which could be correlated with the clinical and laboratory findings but that the liver picture as a whole must be used. He did show a close correlation between active liver cell damage and the positive cephalin flocculation reaction. He suggests using the thymolturbidity test in conjunction with the cephalin flocculation test especially during late convalescence. It is interesting to note here that Lawler and Hirst have just recently proposed the theory that cephalin flocculation is an indication of cell damage but that thymol turbidity is a sign of cellular regeneration. They base their belief on the fact that (1) in virus hepatitis thymol turbidity is the last to be abnormal and also the last to return to normal, (2) that fatty infiltration,

considered a stage of regeneration, happens to give the highest reliability to thymol turbidity, (3) thymol turbidity will be positive in the presence of local areas of regeneration and, (4) in overwhelming liver disease the thymol turbidity remains normal with an absence of regeneration.

One case reported by Kinsell had extremely poor correlation between the liver function tests and biopsy findings. No explanation could be given. "It can be said, however, that statistically all four tests (cephalin flocculation, bromosulphalein, serum bilirubim and hepatic glycogen storage) show an impressive correlation with the clinical and histologic course of acute and chronic liver disease (210."

Levy reported a case of a negro man with cirrhosis who due to personality problems could not be kept on adequate diet and away from alcohol. Several biopsies were done on this man at various times during his remissions and exacerbations. Without fail the cephalin flocculation test would be positive each time the biopsy showed an active phase of the disease and would remain negative when the liver was healing. This again supports Hanger's (14) belief that a positive cephalin flocculation test in cirrhosis which becomes negative is a good prognostic sign and that a negative cephalin flocculation test does not

Value of the Cephalin Flocculation Test (cont'd)

rule out cirrhosis but usually indicates a quiescent stage. A strong and persistant positive cephalin flocculation test in the course of cirrhosis has a very poor prognosis.

Popper and his associates have been the champions of the idea to correlate liver histology with functional tests as a help to the clinician to better understand liver disease and the functional tests. In his early work along this line he had to develop better criteria to be used in tissue study, as has been mentioned, and to establish normals.

In 1947 he reported a close correlation between biopsy findings and cephalin flocculation, thymol turbidity and bromosulphalein, other liver tests being rated below these three liver function tests.

Focal parenchymal necrosis even though it were more severe than diffuse pathology showed only slight relationship to cephalin flocculation. Regeneration of the liver cells showed significant relationship to thymol turbidity and only a questionable relationship to cephalin flocculation. Cephalin flocculation is related to lobular pattern construction and also in some degree to inflamation in periportal regions.

In 1948 Popper and Franklin listed the liver

Value of the Cephalin Flocculation Test (cont'd)

functions tests as to their ability to distinguish liver cell dysfunction or impairment of bile flow:

Dysfunction of Liver Cells	Impaired Bile Flow
Cephalin flocculation	Absence of urinary and fecal urobilinogen
Thymol turbidity	Elevated serum alkaline phosphates
Reversed A/G	Hyper cholesteromia
Increased urinary urobilinogen	
Decrease of the chol. ester	
Reduced prothrombin	
Hippuric acid test	
Reduced galactose tol.	
Reduced plasma vitamin A	
Elevated serum bilirubin	
Urinary bilirubin	
Retention of bromosulphalein	

Hepatitis was divided into the following catagories:

I Hepatitis of medical nature

(a) Infectious or virus

(b) Toxic forms due to chemical, bacterial and endagenous toxins

II Hepatitis of surgical nature

(a) Biliary. Cell damage due to "back flow"

(b) Purulent. Infection complicating biliary hepatitis

Cephalin Flocculation Reaction

	3-4 plus	2 plus	1-0 plus
Virus	80%	7%	13%
Toxic	50%	25%	25%
Biliary	7%	3%	90%
Purulent	35%	35%	30%

Value of Cephalin Flocculation Test (cont'd)

In analysis of the above figures it can be seen that the cephalin flocculation is highly reliable in distinguishing viral hepatitis from a biliary type. Its value in toxic and purulent types of hepatitis is low as can be expected if the theory is true that cephalin flocculation indicates a sizable amount of active liver damage and active liver damage varies to a great extent in these two disorders. Repeated tests are necessary especially in the toxic and purulent types of hepatitis.

Popper, Waldstein and Szanto in 1950 by means of mathematical and statistical analysis noted a high degree of correlation between the incidence of abnormal results in the presence of significant liver cell damage in the case of elevated cephalin flocculation reactions. Function tests were run usually within 48 hours of the time biopsy was taken and the longest interval between biopsy and function tests was 7 days. It was noted that regeneration could be noted by function tests before histological changes to that effect could be noted. The correlation of the flocculation tests seemed to be with diffuse liver cell damage.

Conclusions

The liver biopsy has proven itself a valuable tool in the investigation of the labyrinthical riddles of the liver. Biopsy has not only helped the clinician to differentiate, in the difficult cases, between intrahepatic jaundice and extrahepatic jaundice, but to help evaluate the common and less anoxious liver function tests. By means of the biopsy the clinician is able to obtain tissue and function tests simultainiously and thus be better able to correlate the findings.

The literature reported to date indicates that the cephalin-cholesterol flocculation test is a highly reliable test to ascertain diffuse active liver damage. It has shown that areas of focal necrosis may not be sufficient to give a positive reaction. Biopsy has shown that the cephalin flocculation may return to normal and indicate cell regeneration even before histiological evidence is present and that it has significant prognostic ability. Biopsy has also shown that in a small percentage of cases no correlation between the histological findings and the laboratory findings can be made.

Summary

(1) A review of the history, and technique of needle biopsy of the liver is reported.

(2) A review of the history and technique of the cephalin-cholesterol flocculation test is given.

(3) The cephalin-cholesterol flocculation tests is evaluated by liver biopsy findings as reported in recent literature.

BIBLIOGRAPHY

1. Baron, E. : Aspiration for Removal of Biopsy Material From the Liver. Arch. Int. Med., 63:276, 1939.

2. Bengel, A. : Uber die Parenchympunktion der Leber. Verh. Deutscheges Inn. Med., 35:210, 1923.

3. Biett : Hydatides du foie, avec Developpement Considerable de cet Organe: Ponction Explorative; Incision; Sortie dune Grande Quantite d'acephalo cystes; Querison, Gaz. de. Hop. 7:383, 1833.

4. Canatrow, A. and Trumper, M. : Clinical Biochemistry. 4th Edition. W. B. Saunders Co., Philadelphia and London. p 413, 1950.

5. Chiray, M.; Fressenger, M.; and Roux, M.: La Ponction - biopsie du foic. La Presse Med. 49 : 785-788, 1941.

6. De Marsh, Q. B., and Alt, H. L.: Hepatitis without Jaundice in Infectious Mononucleosis. J. Lab. and Clin. Med. 32:320, 1947.

7. Frola, E. : Il Puntato Epatico Nella Diagnosi Delle Malattie del Fegato. Roma, 1935

8. Gallison, D. T. Jr. and Skenner, D. Bile Peritonitis Complicating Needle Biopsy of Liver. New Eng. S of M. 243: 47, 1950.

9. Gillman, T., and Gillman, J. : Modified Liver Aspiration Biopsy Apparatus and Technique, With Special Reference to its Clinical Application as assessed by 500 Biopsies. South Africa J.M. Sc. 10:53, 1945.

10. Green, C. H. : Diseases of the Liver and Biliary Tract. Arch. Int. Med. 73:349, 1944.

11. Hammond, W. A. : Some Points in the Pathology and Treatment of Hepatic Abscesses. St. Louis M. and S.J. 35:72-76, 1878.

12. Hanger, F. M. : The Flocculation of Cephalin-Cholesterol Emulsions by Pathological Sera. Tr. A. Am. Physicians, 53:148, 1938.

Bibliography (cont'd)

13. Hanger, F. M. Serologic Differentiation of Obstructive from Hepatogenous Jaundice by Flocculation of Cephalin-Cholesterol Emulsions. J. Clin. Investigation. 18:261, 1939.

14. Hanger, F. M. and Patelo, A. J. Jr. : The Cephalin Flocculation Test in Cirrhosis of the Liver. Am. J. M. Sc., 202:43-52, 1941.

15. Hanger, F. M. : Diagnostic Problems in Jaundice Arch. Int. M. 86:169-177, 1950.

16. Hoffbauer, F. W. ; Evans, G. T., and Watson, C. J. : Cirrhosis of the Liver with Particular Reference to Correlation of Composite Liver Function Studies with Liver Biopsy. M. Clin. North America 29:363, 1945.

17. Hoffbauer, F. W.: Needle Biopsy of the Liver. J.A.M.A. 134:666-670, 1947.

18. Iversen, P., and Roholm, K.: On Aspiration Biopsy of the Liver with Remarks on its Diagnostic Significance. Acta Med. Scand. 102: 1, 1939.

19. Ivy, A. C., and Roth, J.A.: The Limitations of Liver Function Tests. Quar, Bull. N.W. Univ. Med. School 17: 179-191, 1943.

20. Ivy, A. C. and Roth, S. A. : Why do a Liver Function Test? Gastroenterology 1:655, 1943.

21. Kinsell, L. W., Weiss, H. A., Michaels, G. D., Shaver, J. S. , and Barton, H. C., Jr: The Correlation of Hepatic Structure and Function. Am. J. Med., 6:292-308, 1949

23. Lawler, A. L., and Herst, R. R. : The Hanger Cephalin-Cholesterol Flocculation Test and the Maclagan Thymol Turbidity Test, Correlation with Autopsy Findings. U.S. Armed Forces Med. J. 1:902, 1950

24. Levy, J. S., et. al. : Serial Needle Biopsy in the Study of Hepatic Disease. South M. J. 42:659, 1949

Bibliography (cont'd)

25. Lipp, W. F., Linzner, A. R., and Aaron, A. H.: The Accuracy of Diagnosis of Jaundice. J.A.M. A. 137: 236-239, 1948.

26. Lucatello, L.: Sulla Puntura del Fegato a Scopo Diagnostico. Lavori del Congressi di Medicina Interna. Roma, 527, 1895

27. Maclagan, N. F. : Thymol Turbidity Test. A new Indicator of Liver Dysfunction, Nature 154: 670-671, 1944.

28. Mann, F. C., and Magath, T. B.: Studies on Physiology of Liver: Effect of Removal of Liver on Blood Sugar Level: Arch. Int. Med. 30:73-84, 1922.

29. Mateer, J. G., Boltz, J. I., Marion, D. F., and MacMillan, J. M. : Liver Function Tests: General Evaluation of Liver Functions Tests, and Appraisal of Comparative Sensitivity and Reliability of New Tests, With Particular Emphasis on Cephalin-Cholestrol Flocculation Test, Intravenous Hippuric Acid Test and Improved Bromsulphalein Test With Normal Standard. J.A.M.A., 121: 723-728, 1943.

30. McMichael, J.: Disease of the Liver: A Review of Some Clinical and Biochemical Problems as Revealed by Systematic Biopsy Studies, J.A.M.A. 137: 234-236, 1948.

31. Nadler, S.B., and Butler, M.F.: Cephalin-Cholesterol Flocculation Test in Jaundiced Patient: Surgery 11:732-738, 1942.

32. Olivet, J.: Die Diagnostische Leberparenchym-punction, Med. Klin, 22:1440-1443, 1926.

33. Patelo, A.J. Jr.: Treatment of Alcholic Cirrhosis of the Liver With High Vitamin Therapy. Proc. Exper. Biol. and Med. 37: 329-330, 1937.
Patelo, A. J. Jr., and Post, J. : Treatment of Cirrhosis of the Liver by a Nutritious Diet and Supplements Rich in Vitamin B. Complex. J. Clin. Investigation: 20:481-505, 1941.

Bibliography (cont'd)

34. Popper, H. S., Franklin, M., Steigmann, F., and Kozoll, D. D. : Relation Between Structural and Functional Alterations of the Liver. J. Lab and Clin. Med. 32:318, 1947.

35. Popper, H. S.: Significance of Agonal Changes in the Human Liver. Arch. Path. 46:132-144,1948..

36. Popper, H. S. , and Shaffrer, F.: Hepatic Tests. Adv. Int. Med. 4:337-443, 1950.

37. Popper, H. S., Waldstein, S. S., and Szanto, P. B.: Correlation of Clinical Features of Cirrhosis of the Liver with Findings on Biopsy. Am. J. of Clin. Path. 20: 724-737, 1950.

38. Roberts: Abscess of the Liver, with Hydatids: Operation. Lancet 1:189-190, 1835.

39. Rolleston, H. D., and McNee, J.: Diseases of the Liver, Gall Bladder and Bile Ducts. 3rd edition. Macmillian and Co., London 1929.

40. Rosenberg, D. H., Cephalin-cholesterol Flocculation Test in Cases of Disease of Liver, With Special Reference to Diagnosis of Mild and Unsuspected Forms, Arch. Surg.: 42:231-248,1941.

41. Roth, A., and Turkel, H.: Technique of Prostatic Biopsy. J. Urol. 51:66, 1944.

42. Schupfer, F.: De la possibilite de faire intra vitam undiagnostic histo-pathologique precis des maladies du foi et de la rate. Semaine Med., 27:229, 1907.

43. Sherlock, S. P. V.: Aspiration Liver Biopsy, Lancet 2:397, 1945.

44. Silverman, I.: A new Biopsy Needle. Am. J. Surg., 40:671, 1928.

45. Sims, J. M.: Treatment of Hepatic Abscess by Aspiration. Tr. M. Soc. Virginia 3:106-111, 1879.

Bibliography (Cont'd)

46. Snell, A. M., and Magath, T. B.: Use and Interpretation of Tests for Liver Function, Clinical Review, J.A.M.A., 110: 167-174, 1938.

47. Tripoli, C. J., and Fader, D. E.: The Differential Diagnosis of Certain Diseases of the Liver by Means of Punch Biopsy. Am. J. Clin. Path. 11: 516, 1941.

48. Wolff, R. A., and Haythorn, S. R.: "The Liver Biopsy in the Diagnosis of Liver Disease" Pennsylvania Med. J. 53:344-348, 1950.