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Wallace E. Baker
University of Nebraska Medical Center

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LIVER FUNCTION TESTS.

by

WALLACE E. BAKER.

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LIVER FUNCTION TESTS

INTRODUCTION.

As one scans a list of the available writings on the subject of liver function tests, one can not help but be impressed by the yearly increase in the number of articles that have been published in the last 15 years. One might well conclude from this that here is a field in which there is an ever growing interest and where the final chapter must await much further work before its "Finis" can be written. Indeed, such is the case.

Disease of the liver in mankind is as old as the word "Bilious", indeed older. Knowledge of some of its manifold functions is just as old, but knowledge of many of these functions is of comparatively recent date. It is because of this that attempts at methods of testing these functions are of still more recent times.

To cover the entire field of the various tests aimed at the function of the liver both clinically and experimentally in the field of research is obviously beyond the scope of this paper. The objective of this thesis then is to review the clinically applicable tests of the present times considering the essentials of their history, physiological basis, method and technique, and comparative value.

Before proceeding with the discussion of the tests
proper, it would seem logical to lay some groundwork with regard to the anatomy and physiology of the liver which comprehend these functions.

**PHYSIOLOGICAL ANATOMICAL DISCUSSION OF LIVER FUNCTIONS.**

That the liver combines endocrine as well as exocrine glandular activities is a well known fact that still its manifold functions are not comprehended.

Maximow and Bloom (1) in introducing their section on the histological anatomy of the liver make the following statement. "The liver plays an indispensable part in many processes in the body, particularly those concerned with its metabolism and with certain digestive processes. The removal of the liver quickly leads to the death of the animal, and it is only within the last few years that methods have been evolved which permit the study of hepatectomized dogs and rabbits for a day or so. This procedure has thrown some light on the functions of the liver, as a whole, but it has not helped in correlating the structure of the organ with its known functions."

It is not that the liver structure is so complex, but rather that its roles in body economy are so diverse.

Perhaps it would be well to review some of the essentials of its anatomical architecture.

The liver in man shows grossly a more or less distinct mosaic of regular, polygonal small areas. Each of these areas represents a small architectural unit or lobule.
Although the liver is a true gland, its lobules do not depend on a duct system; they depend upon the distribution of blood vessels. Running through the cent of the lobule, in its long axis is the central vein, while at the periphery of the lobule are the branches of the portal vein, the interlobular bile ducts, branches of the hepatic artery, and the lymphatics which form a network about the portal vein and its branches.

In order to understand that structure of the liver lobule it is necessary to know something of the arrangement and inter-relations of its two main vessels, the hepatic and portal veins. It should be born in mind that the principal afferent blood vessel of the liver is the portal vein. It receives only a small part of its blood supply from the afferent artery (hepatic artery). The latter supplies the biliary system and the connective tissue elements in the walls of the large veins, and only to a small extent does it nourish the parenchyma of the gland. The portal vein collects the blood from the digestive tract from the cardia to the anus and from the spleen, pancreas, and gallbladder and enters the liver at the porta together with the hepatic artery. The blood is drained from the liver by the two or more hepatic veins which in turn enter the inferior vena cava.
Throughout the liver the ramifications of the hepatic and portal veins everywhere avoid each other so that the terminal branches of the portal vein and the radicals of the hepatic vein are so arranged so that they are always about equal distance apart. Each radicle of the hepatic vein (central vein) is surrounded by a layer of liver tissue of uniform thickness and this mass constitutes the hepatic lobule.

Within the lobule the liver cells are arranged more or less regularly in cords which form columns extending radically from the central vein to the periphery of the lobule. Between them are broad irregular, thin walled vessels which are the sinusoids. These serve to connect the small branches of the interlobular portal veins with the intralobular central veins (radicals of the hepatic veins).

The sinusoids are lined by two types of cells. One is an undifferentiated lining cell. The other is a highly phagocytic type cell which seem to be anchored in the blood stream and are the so called Kupffer cells.

The liver cell cords usually consist of Coburun of but two adjacent cells between which a thin bile capillary runs. The latter run through the length of the liver cell cord and receive short lateral branches which
extend between the sides of adjoining liver cells. At
the periphery of the lobule certain of the hepatic cords
are directly continuous with the smallest branches of the
interlobular ducts. Here the lumen of the bile cells
give way suddenly to the small compact cells of the
duct. The finer branches of the bile ducts form an
anatomosing network about the portal vein; they increase
in diameter as they proceed toward the porta of the liver.

Thus the liver cells are in intimate contact with
the thin lining of the sinusoids on one hand and the bile
capillaries on the other.

The lobules of the liver are separated by the very
thin, translucent strands of connective tissue. It is
of small amount and barely supplies to form a framework
for the interlobular artery, vein, bile ducts, and lymphatics.

Thus we see that the liver as a functioning gland,
essentially depends upon the activity of two types of
cells, the Kupffer cells and the hepatic cells. The
Kupffer cells have been shown to be cells of the reticuloendothelial system (2) present elsewhere in the body as
well as the liver. The hepatic cells remain alone as
differentiated specialized structures. It is here that
the final answer to the question of liver function must
be found.

The hepatic cells are highly differentiated and of
and of characteristic appearance. The cytoplasm of the liver cells presents an extremely variable appearance which reflects the functional state of the cell. By appropriate methods glycogen, fat, and protein droplets and inclusions are readily demonstrable. Their actual content of any one of these constituents show great variation under normal conditions. Sometimes particularly during certain periods after digestion, the liver cells may be almost completely filled with granules which give the microchemical reactions for glycogen while at other times these may give way in part to a large number of fat droplets. The content of the liver cells in protein inclusions also shows correspondingly great variations. The relative amount of these substance demonstrable in the liver cells depend primarily on the amounts of carbohydrate, fat and protein in the diet and on the stage of digestion. Accordingly to one author (Noel 3) in the white rat, the liver lobule may be divided into three zones depending on histophysiologic differences in mitochondria, (a) zone of permanent repose, surrounding the central vein. This zone probably constitutes a region of reserve, which becomes active only when feeding is excessive or when the adjoining parenchyma is infused. (b) Zone of permanent function lies at the periphery of the lobule. Here there is always evidence of activity. (c) An intermediate zone lies between the two previous ones. This zone is at rest during
starvation and begins to function with digestion. Accordingly, mitochondrial activity proceeds from the periphery to the center of the lobule during the course of digestion.

In view of the manifold functions which the liver cells perform it is quite striking that there should be such a marked similarity in appearance in all the liver cells. From the above it would appear that all of the liver cells are equally endowed with the same functional capacities, but their active participation in these processes under normal conditions depends on the location of the cell in the lobule.

It would seem time to answer the question, "What are these functions of the liver?"

Every student of medicine knows that there are two main divisions of the activities of the liver cells. One of these is concerned with the production of bile; the other has a profound effect on the composition of the blood passing through the liver.

Ivy (4) outlines the activity of the liver as follows:

1. the liver stores food materials.
   a. Carbohydrate in the form of glycogen or animal starch. In fasting the store lasts from 12-24-hours.
   b. Protein is stored, but probably as liver protein and not as a special protein.
c. Fat. On a well balanced diet, liver fat is fairly constant. Liver fat is increased by a fatty diet, and the liver may become 50% fat. When the glycogen content of liver decreases, the fat content usually increases. Thus, on fasting the liver fat increases, if fat is present in the fat depots.

d. The liver stores the anti P. A. factor, the substance important (anti-secondary anemia factor) for building hemoglobin, and Vitamins A and D. It is rich in Vitamin B and G and stores iron and copper.

2. The liver manufactures food materials.

Glucose is the sugar of choice of the body cells. Some levulose and but little galactose can be oxidized by body cells.

a. Liver converts glucose, levulose and galactose to glycogen and then, as needed, the glycogen is changed to glucose. Galactose is not a good glycogen former.

Lactic acid is converted to glycogen in the liver. The liver maintains blood sugar level.

b. Forms glucose from certain amino acids.

c. May make fat from glucose, protein and glycerol.

d. Synthesizes certain amino acids.
e. Probably desaturates fatty acids.

f. Makes various organic acids, which may be oxidized or used for synthesis and which result from deamination of proteins.

g. Makes Vitamin A from carotene.

h. Ketone bodies.

3. Other substances manufactured by the liver.

a. Blood fibrinogen and heparin, or antiprothrombin. Red blood cells are formed in embryo.

b. Ketone bodies, glucose retards or prevents formation of.

c. Bile salts, makes and also destroys.

d. Cholesterol possibly: at least liver is concerned in cholesterol metabolism.

e. Urea, from ammonia, which arises chiefly from deamination of proteins.

f. Uric acid possibly; liver may destroy it also.

g. Next to muscle, liver is an important source of body heat.

h. Changes urobilinogen (urobilin is oxidized urobilinogen), which is produced normally in, and absorbed from the intestine, to bilirubin.

i. Forms some bile pigment or bilirubin from hemoglobin.

j. Blood albumin and globulin.

k. Glycuronic acid conjugation products.
1. Produces histamine in anaphylactic shock.
   m. Very probably produces antibodies.

4. Detoxication.

A. By chemical means:

1. Conjugation of toxic substance with:
   a. sulphuric acid, e.g. indoxyl sulphate,
   b. glucuronic acid, e.g. phenol, benzoic acid, menthol, etc., form glucuronides,
   c. glycine, e.g. benzoic acid plus glycine yields hippuric acid.

2. (a) by oxidation
   (b) by reduction
   (c) methylation
   (d) acetylation

B. By excretion in bile:
   a. heavy metals such as mercury.
   b. certain drugs.
   c. bacteria.

C. By storage in liver cells:
   a. strychnine

D. By reticul-endothelial activity:
   a. removes bacteria, foreign proteins, dyes, etc. from blood stream.

   a. Serves as a reservoir for red blood cells and fluid.
b. Tends to prevent blood dilution after drinking water.
c. "Flood chamber" to prevent over distention of right heart.
d. Some claim that the liver is important in maintaining the normal ionic equilibrium of the blood.

6. Excretory substance in bile:

<table>
<thead>
<tr>
<th>Bile pigment</th>
<th>Bile salts</th>
<th>Cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatty Acids</td>
<td>Alkali</td>
<td>Phospholipins</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Calcium</td>
</tr>
</tbody>
</table>

Certain dyes, drugs (cinchophen, salicylic acid, mercury).

From the above brief list of the many possible activities of the liver it is clear that if the liver possesses a single vital function, it is not yet known. If there were one, which is not likely (3) then a single functional test might be devised. As it is the tests of function are now directed toward ascertaining the extent of certain individual activities of the liver. Not all of these lend themselves to testing. Let us then examine further into the physiological aspects of some of those functions for which tests have been devised with sometimes more, but more often less success.

**BILE PIGMENT METABOLISM:**

The oldest known function of the liver is the excretion of bile. Among the many constituents of this
product is bile pigment or bilirubin. Since the suggestion of the relationship between hematoiden and bilirubin, much work has been done to demonstrate that bile pigments are formed from hemoglobin. The question has arisen as to whether hemoglobin represents the only sources. Whipple and Hooper (5 & 6) agreed that there were other sources. They demonstrated that diet rich in carbohydrate increased the amount of bilirubin excreted by the liver in dogs, and that an Eck-fistula because of resulting harm to the liver cells caused a decrease. Approximately 10 years later Rous, Brown, and McMaster (7) and Inlow (8) showed by collecting bile over a longer period that there was no average increase in the amount of bilirubin on finding large amounts of carbohydrate. They contend that the Eck-fistula would cause a decrease through the diversion of blood from the liver and hence a decrease in the amount of bilirubin brought to the liver for excretion. The same investigators in an earlier paper (9) concluded that their data supported the theory that bilirubin has no sources other than the hemoglobin of destroyed blood. Rich (10) demonstrated the formation of bilirubin crystals in cultures of red cells and phagocytes in vitro, as the red cells were ingested and destroyed.

The next question concerns itself with the relation of the liver to the actual manufacture of bilo pigments.
For a long time and up to as recently as 1921 (11) it was assumed by many that the liver cells manufactured bilirubin.

McNee (2) in 1913 began to establish the importance of the reticulo-endothelial system throughout the body in the manufacture of bilirubin. The final proof of the extrahepatic formation of bile pigment was presented by Mann, Ballman and McGoth (12-13). They reported that in hepectomized dogs, bile pigment appears in the blood plasma (normally absent). Furthermore it also appeared when the abdominal viscera were removed. It was also shown that the bile pigment is manufactured by the reticulo-endothelial system. Subsequent investigations (4) lead to the conclusion that the liver was the chief, although not the only site, of formation of bilirubin.

Summarizing the results of this investigation certain conclusions may be drawn. There is no evidence that the epithelial cells of the liver are concerned in the manufacture of bilirubin. The evidence that it can be made extrahepatically is conclusive. The site of this formation is a matter of much less certainty. If the reticulo-endothelial cells are actually concerned in the manufacture, then the kupfer cells of the liver, which are present in enormous numbers, must be considered to play an important
part. The function of the epithelial cells of the liver, in contrast to that of the Kupfer cells, is to excrete into the bile canaliculi the bilirubin brought to it by the blood stream (15).

From the above it may be concluded (16) that an increase in serum bilirubin is an expression of the increased activity of the reticulo-endothelial cells, an indication of the decreased excretory ability of the hepatic cells, or a manifestation of obstruction of the bile ducts. Therefore a measure of serum bilirubin may be the basis for testing the liver's function with regard to the excretion of this substance.

The bilirubin which enters the intestinal tract (17) is acted on by bacteria to form urobilinogen; this is ordinarily returned to the liver and utilized in the formation of normal body pigments. In the presence of hepatic injury this resynthesis may be halted and urobilinogen therefore may be excreted in the urine, thus providing knowledge of liver function. If there is obstruction to the bile passages, no bilirubin reaches the bowel, and consequently no urobilinogen can be formed which then may indicate liver function by its absence from the stool and urine.

**THE LIVER'S FUNCTION IN CARBOHYDRATE METABOLISM.**

The most significant result of experimental hepatectomy
perhaps is the decrease in the value for the blood sugar and the hypoglycemia reaction which continues because the normal store of glycogen in the liver is abolished. Glucose administration prolongs life up to 24-48 hours (4).

In the liver's metabolism of carbohydrates certain peculiarities with regard to two of the sugars, fructose and galactose, make them somewhat more valuable in testing this particular liver activity. According to Cori (20) Strauss at the beginning of this century introduced levulose for testing hepatic function, and several years later Bauer introduced galactose for a similar purpose. Their peculiar adaptability for this is based on the following experiment.

Cori (21) in his studies of the absorption of hexosés and pentoses from the Gastro-intestinal tract and (20) the rate of glycogen formation in the liver from the same concludes that although fructose is readily converted into glycogen by the liver, galactose is so converted with difficulty. The extent to which a particular sugar is converted into glycogen probably determines the degree of hyperglycemia that results following its ingestion.

Bodansky (22) found that in the dog galactose would produce a rather marked hyperglycemia while levulose was much less effective in doing so. Which would be expected from the above. It is this property of levulose
which is made use of as a test for liver function because in the presence of hepatic disease it produces a greater hyperglycemia.

On the other hand from the evidence presented by the investigations of Foster (23), Mann (19) and Mann and Boldman (23) we may conclude that levulose can be utilized by muscle tissue, at least to some extent. While practically no galactose is so utilized. Furthermore Roe and Schwatsmass (24); Shay, Schloss, and Bell (25) have shown by their work that insulin has practically no effect on the utilization of galactose while it may have some effect on that of levulose. However, levulose has been shown by Bodonshey (22) and others to have a comparatively high renal threshold so that loss from the blood by that route is comparatively negligible.

Calactose on the other hand has a consistently low renal threshold (26) and is not affected by an artificially produced nephritis (20). It has been found (25) & (27) that a normal person can assimilate a 40 gm. oral dose of galactose without the loss of more than 2.5 to 3 gm. of sugar in the urine in the 5 hours immediately following the administration. Because galactose is utilized almost entirely by the liver (22) if there is a glycosuria above this amount, it is indicative of some failure of this particular liver function.
CHOLESTEROL AND FAT METABOLISM.

Experimental and clinical data (28) in recent years have focused attention on the important role of the liver in the cholesterol metabolism. According to the majority of authors there is in the normal blood plasma of human beings from 140-200 mg. of total cholesterol per hundred cubic centimeters, depending upon the method of examination employed, and of this, 50 to 70 per cent is in the form of cholesterol ester (the combination of one molecule of free cholesterol with one molecule of a fatty acid). Among others Hueck, quoted by Epstein (28) expressed the belief that the greatest source of cholesterol in the body is exogenous, the enterogenous resorption depending on the amount of cholesterol in the food and on the presence of fatty acids, bile and pancreatic juice in the intestines. The main excretory path of the cholesterol is via the bile. Thannheiser, according to Epstein states that the liver is the regulator not only of the cholesterol content of the blood, but also of the relation of the free cholesterol to the cholesterol metabolism. Based on these estimations of blood cholesterol in the free and ester form are used to determine this activity of the liver.

The activity of the liver in relation to fat metabolism
is not well known. It is known (4) that the liver may make fat from glucose, protein and glycerol. It is also thought that it probably that it probably desaturates fatty acids. Some contend (29) that it combines fats with phosphorus for the purpose of transportation to other tissues and depots. At least it is known that the liver functions in some way or ways in the metabolism of fats. Therefore, the determination of the amount of phospholipid and total lipids in the blood has been advocated as giving some indication of the liver's efficiency in this activity.

METABOLISM OF BILE ACIDS.

Here is a substance that is probably produced exclusively by the liver at least the following evidence would tend toward such a conclusion.

Smyth and Whipple (30) working with Eck-fistula dogs showed that minute doses of chloroform are capable of effecting a pronounced decrease in the bile salt concentration of fistula bile. Soffer (31) quotes Jenke and Steinberg showing that although the liver excretes about 10 grams of bile acids daily the amount present in the blood did not exceed 0.025 mgm. per cent. Gregory and Poscoe (32) could find none in the circulation. The late work of Smith and Whipple (33) tends to
prove the primary part of the liver in the manufacture of these elements. It would seem then that the estimation of this primary liver product should be an excellent guide to the determination of this particular activity and the condition of the liver in general.

PROTEIN METABOLISM.

The effect of total hepatectomy on the metabolism of certain protein derivatives is of theoretical interest as Ballman et al (34) have shown. The removal of the liver is followed by a rapid fall in the concentration of the urea in the blood, urine, and tissues, which indicate that the formation of urea has ceased. According to Saffer (31) protein catabolism remains the same except for the fact that the amino acids formed as a result of protein break down are not deaminized. These compounds accumulate in the blood and tissues and are excreted as such in the urine. The concentration of amino acids in the urine has been utilized as a test for hepatic function.

There is another phase of protein metabolism which is of considerable importance. This has to do with variations in plasma proteins which have been recognized as dependent on injury to the hepatic parenchyma. While there is no direct proof that the liver is the sole site of their manufacture, experimental studies furnish some indirect evidence of their possible hepatic origin. Kerr, et al (35) demonstrated that poisoning with phosphorus and carbon tetrachloride resulted in a moderate decrease in the value for the serum proteins, after plasmapheresis occurred slowly in the presence
of hepatic injury or Eck-fistula. Recent work (36) indicates that there is a reserve of protein building material in the organism, which is stored at least in part in the liver and which probably consists of at least 50% of albumin or albumin producing substance. The opinion is that there probably is a dynamic equilibrium between tissue and plasma-protein, and the material stored in the liver may figure in this equilibrium. How this is made use of as a test of the condition of the liver will be discussed later.

THE DETOXIFICATION ACTIVITY OF THE LIVER.

Within recent years the study of the detoxifying function of the liver has occupied the interest of both the physiologist and the clinician. This is the ability of the liver to remove from the circulation various noxious substances, alter them chemically or combine them with certain common constituents of the blood in such a manner as to render them physiologically inert.

Priestly et al (37) by work on normal and hepatectomized dogs were able to show that strychnine was taken up by the liver and that it was probably destroyed there.

Lichtman (38) presented some indirect evidence that cinchophen is destroyed by the liver in his comparison of the time of appearance and concentration of the oxidized cinchophen in the urine and bile obtained by transduodenal drainage.

Early studies by Bryan (39) and more recent work by Quick (40) have served to develop another test of this liver activity based on the ability of the liver to conjugate ben-
zoic acid and amino-acetic acid to form hippuric acid which in turn is excreted in the urine. Thus more methods have been added to the testing armamentarium.

EXCRETORY FUNCTION OF THE LIVER.

Rowntree et al (41) having previously in 1909 observed that phenotetra-chlor-thalein when injected into the blood was excreted almost entirely by the liver and appeared in the bile, developed a method of measuring the liver's efficiency in this regard by determining the stool content of this dye after injection. Whipple et al (42) and Rosenthal (43) determined that if the liver were injured artificially that the rate of elimination of the dye was proportionate to the degree of hepatic injury. Rosenthal (43) further showed that the dye elimination depended on the efficiency of the liver in this activity. In later investigations, Rosenthal (44) was able to prove the efficacy of bromsulphalein in this regard and its superiority over other dyes used because it is retained almost in toto after injection and because it disappears quite slowly from the blood stream. The question then remained as to whether the dye was excreted specifically by the liver. Pratt et al (45) concluded from their work, wherein the hepatic circulation was blocked and the partial vein and inferior vena cava was anastomosed, that such is the case because the excretion of the dye under such circumstances is almost negligible. In normal dogs the dye disappears rapidly from the blood stream. Rosenthal and Lillie found
that splenectomy blockage of the reticulo-endothelial apparatus with quartz does not alter the rate or degree of removal of the bromsulphalein from the blood.

Thus it has been shown that the liver plays a definite role in removing some foreign materials, such as dyes, from the blood stream. That these substances are excreted in the bile has been proven beyond any doubt. That the liver cells, as such, are the sole removing agents, is not so well established. Nevertheless, this particular phenomena may be used for testing the activity of the liver.

There are other minor tests that purport to measure liver function whose physiological basis will be discussed as they are taken up.

In examining the above physiological activities in the light of the anatomical discussion, certain facts stand out.

1. There are only three types of cells different from the ordinary run of tissue cells elsewhere in the body. They are-- (a) Those lining the bile capillaries (b) The Kupfer cells lying along the blood channels (sinusoids) (c) The liver cells proper (Hepatic cells) which lie between and form the only thin partition separating these two canals.

2. Further, in all the above discussed activities, the primary role of the liver cell has been shown. One type of the liver cell must engage in all these varied activities.

3. The fact that there are so many tests lends toward the conclusion that no one of them has proved as satisfactory.
as desired. (Which is indeed the case). And why not, if at most, three and in the main only one type of cell is involved in these activities.

In answering this latter query we must examine liver physiology a little further. As Ivy (4) has pointed out in reviewing their own and the work of others it has been shown that only 20% of normal liver tissue is necessary to maintain its normal functions. The capacity of the liver to regenerate is remarkable. On the removal of 70% of the liver in rats and dogs, 90% regeneration will occur in a few weeks. Restriction of portal blood supply, obstruction of a bile duct or cirrhosis of the liver prevents or retards regeneration. Regeneration can occur, however, in a liver in which cirrhosis is progressing due to the administration of carbon tetrachloride. The regenerated liver cells are, under certain condition, known to be more resistant to the poison which caused the necrosis.

The tremendous factor of safety in the liver means that at least 70 or 80% of the hepatic cells must be impaired to give a positive liver function test.

It has also been found that in liver disease all of the activities are not reduced in the same proportion or at the same time. Even in extirpation of the liver there is no total failure at once, but a graded loss. The results follow somewhat in the following order. (a) Death results in 12 to 18 hours. (b) Hypoglycemia occurs, and glucose administration
prolongs life up to 24-48 hours, but the animal dies with the blood laden with glucose. (c) Rise in bilirubin in the blood occurs, because it cannot be excreted by the liver. (c) Urea falls almost to zero. (e) Amino acids increase (f) Uric acid increases. The actual cause of death is unknown. Is it due to the loss of some single vital function? Or, is it due to the loss of the multiple activities of the liver.

Thus it is (46) that for twenty years or more clinicians, physiologists and clinical pathologists have attempted to devise procedures or tests that would indicate the functional capacity of the liver, in order to determine the presence and degree of disease of the liver and to obtain information with regard to prognosis. Physiologists have repeatedly warned that no one function could be depended on to indicate the general status of the whole organ, and that the reserve function of the liver was so great that functional abnormalities could be expected to appear only when most of it had been destroyed.

What then is the excuse for liver function tests? This question is answered by White et al (47) as follows: Liver function tests may be used in various ways. 1. To detect the presence and amount of liver damage, and to follow the changes and course in an attack of jaundice. 2. For prognosis during the attack to assist in the decision concerning surgical risk, and to discover the amount of residual liver damage after the attack is over. 3. For differential diagnosis.
The objective may be an elaborate biochemical study of liver functions in different diseases, or the development of a group of simple tests for routine use. It is fair to look critically at these tests and ask if they really add anything to the general clinical picture and tell more about prognosis than the patients symptoms and appearance. Since no one test is entirely satisfactory, it is better to use several, testing several types of functions such as excretion and some type of metabolism. In testing several functions of the liver, one positive test is almost as good a sign of liver damage as if all were positive, but there is usually a correlation between the amount of injury to the liver and the number of different kinds of tests which show abnormalities.

**TESTS OF THE LIVER FUNCTIONS.**

The liver acts as an excretory organ with respect to bilirubin just as the kidney does for urea. On this basis it is possible to attribute increases of serum bilirubin to three general types of disturbances which act simply or in combination: 1. Those in which bilirubin is produced in excess of the capacity of the liver to excrete it. (hemolytic jaundice) 2. Those in which the rate of production is not increased but because of toxic or infectious injury to the liver cells and finer bile passages bilirubin accumulates in the blood stream (hepatogenous jaundice) 3. Those in which obstruction to the larger bile passages causes a
reflex of bilirubin into the blood (obstructive jaundice). As Rich (16) has argued, the presence of increased amount of bilirubin in the blood does not depend on this fact alone but depends in part on an associated disturbance in the excretory function of the liver cell. The argument can be carried further and extended to prove that pure forms of one or the other types of jaundice are probably rare. There is never gross obstruction of the extrahepatic bile passages without injury to the hepatic parenchyma; and the converse is also probably true. 3. Unfortunately, in those pathological conditions such as cirrhosis or new growths of the liver, where the process is more or less localized in the sense that not all the parenchymal cells are disturbed, an increase in serum bilirubin not infrequently fails to occur. Of 100 instances of clinically well established cases of liver disease, which does not include the acute episodes mentioned above, fully one-half showed normal blood bilirubin values. The explanation undoubtedly resides in the fact that because of the tremendous reserve power of this organ, the excretion of this pigment was adequately cared for even in the presence of extensive although not universal parenchymal damage. In the end stages of portal cirrhosis or even earlier in the so-called biliary cirrhosis, where extensive distortion of the continuity of the smaller bile channels has taken place, bilirubin begins to accumulate in the blood stream and to manifest
itself clinically. Until shortly before this stage is reached, quantitative estimation of blood bile pigments very often fails to reveal any decided increase.

Until the very beginning of the present century, the presence of an increase of bile pigment in the blood was made purely on the clinical observation of jaundiced skin and sclera. Soffer (31) credits Gilbert and associates with the first elaboration of a method for the estimation of serum bilirubin. He states, however, that it was not until Van den Bergh and Snopfer adopted Ehrlich's diazo-reaction for the estimation of blood bilirubin that a satisfactory and fairly accurate method for this purpose was evolved. Later Thannhauser and Anderson in 1921 further increased the sensitivity of the method by salting out proteins with a saturated solution of ammonium, sulphate, thus minimizing the amount of bilirubin absorbed by the proteins of the serum.

The basis of the test (48) is Ehrlich's discovery that a mixture of sulfanilic acid, hydrochloric acid, and sodium nitrite yielded a reddish violet color when added to solutions containing bilirubin. The bilirubin combines with the diazobenzolsulphochloride to form acctophenolozorubin.

Bilirubin which has passed through the liver cells (hepatic cells) but because of obstruction in the bile ducts is not excreted yields a "direct" reaction. This means that the reddish color occurs promptly on the addition of the diazo reagent to blood serum. Bilirubin which has not
passed through the hepatic cells produces a delayed or "indirect" reaction. That is, alcohol must be added to the above mixture before a color reaction takes place. A third reaction type is known as biphasic. In this the color reaction appears at once but reaches its maximum intensity sometime later. This difference in pigment reaction is in all probability due to the medium in which the pigment is contained. (48) A solution of pure bilirubin of the same p.H as that of the blood will yield a direct reaction. If it be added to normal plasma the reaction becomes indirect. Burn (48) attributes this to the absorption of the pigment by the serum proteins, which thus prevents it from reacting with the reagent promptly. He further found that when substances which lower the surface tension of plasma are introduced before the bilirubin is added, the reaction will remain "direct". It would seem that such substances are more readily absorbed by the proteins thus permitting the pigment to remain free in solution.

The pathological significance is as follows:(46) For practical purposes it may be considered that the bilirubin ordinarily present in the serum gives the "indirect" reaction and may exist in amounts from 0.1 to 2.0 mg. per 100 c.c. In hemolytic icterus and in the hemolytic anemias the excess of bilirubin is apparently "bound" in the blood stream and is not eliminated by the kidney. It gives an indirect reaction and the total quantity seldom exceeds 6-7 mg. per 100cc.
of serum. Whether this indicates an impairment of liver function is not entirely clear, although associated visible hepatic injury of significant degree is rarely demonstrable. However, if the value for indirect-reacting bilirubin reaches a level of more than 4mg % of serum, it may safely be assumed that the function of the cells of the liver has been at least functionally impaired.

If the serum shows the presence of a direct reacting bilirubin, it is practically conclusive proof of injury to the liver and rupture of the bile capillaries. In other words, the bilirubin which is taken out of the blood serum as a result of physiological or mechanical obstruction is being resorbed through lymphatic channels while a part, at least, may pass back into the circulation through the polygonal cells of the liver.

If the Van den Bergh test is carefully performed as a ring test instead of a "mixture" test, one may often detect a direct reaction even though there is only the slightest increase in the total amount of bilirubin in the serum. This is an important point which has not been generally recognized. There is no doubt that the presence of direct-reacting bilirubin has some quantitative relation to the function of the liver; that at a certain point the functional or pathological changes become so great that the cells of the liver are forced to return some of the pigment; in this
way high values for indirect reacting bilirubin tend to give way to the presence of direct reacting bilirubin. This is typically seen in jaundice due to arsphenamine.

The biphasic reaction is probably due to the simultaneous presence of bound and free bilirubin in the blood serum. (31) Snell (46) thinks it represents conditions in which there is little direct reacting bilirubin as compared with the amount of indirect reacting pigment. Pathologically it would indicate both some bile canaliculi obstruction and some parenchymal damage. The difference between the so called "prompt" and delayed biphasic reaction is in all probability an expression of the degree of obstruction of the smaller bile channels.

The various types of reacting sera and their conversion from one to another is best seen in a condition such as arsphenamine jaundice. Early there is diffuse cloudy swelling of the hepatic cells. Bile pigment is not so readily excreted and as a result some is retained in the blood. Hence, then, the blood serum will yield an indirect reaction. As the pathology increases in severity and extent, there is necrosis of some of the cells. There is disruption of the integrity of the bile canaliculi. Hence, not only is there interference with the passage of bile pigment through the cells but there is blocking of the canaliculi and resorption of bile into the blood from this source. Now the reaction is "biphasic". The
pathology may go on to the point of diffuse necrosis of all the hepatic cells and blocking of all the intrahepatic bile channels. At this stage, the direct reaction will be found.

When healing takes place the reaction will change from direct back through biphasic to indirect to become negative eventually when complete healing has occurred.

According to McGoth (46) indirect reactions are found in: pernicious anemia, familial hemolytic jaundice, acute hemolytic anemia, sickle cell anemia, paroxysmal hemoglobinemia, transfusion of the wrong type of blood, pherylhycardizine poisoning, cardiac decompensation (especially in the presence of gross pulmonary infarction) hemolytic septicemia, malaria, blackwater fever, lobar pneumonia, and icterus neonatorum. It is important to recall that these conditions as a rule are associated with a typical acholuric jaundice and that deep jaundice and high values for bilirubin are rarities unless the liver is injured.

Direct reacting bilirubin is present in the serum under the following conditions: various types of toxic or infectious jaundice chronic parenchymatous disease of the liver; mechanical obstruction to bile ducts by tumor, stone, cicatriz, infectious lesions, or extrinsic pressure, or tumors, granulomas, cysts and other lesions involving the liver substance.

The relative depth of jaundice in these various types of cases may be noted. The highest values (from 30-50 mg. %) are found in acute severe hepatogenous forms of jaundice and in neoplastic biliary obstruction. The lower grades of bili-
rubenemia (from 2 to 10 mg. %) in the presence of a direct Van den Bergh reaction) are found in subsiding acute so-called intrahepatic jaundice, in very chronic forms of hepatitis, in the various forms of portal cirrhosis and in syphilitic hepatitis, in the presence of stone in the common bile duct and in infectious forms of cholecystitis without gross biliary obstruction.

The test is of prognostic value when determinations of daily variations are made (49). Either continued estimations of plasma bilirubin may be made or the icteric index, which will be discussed subsequently, may be plotted as a curve. Falling bilirubin values signify restored potency of the bile passages or a liver that is undergoing repair. The one exception is the very chronic type of biliary obstruction. High or rising values, as a rule, signify complete obstruction, or rapidly degenerating hepatic parenchyma or a combination of the two.

B. THE ICTERUS INDEX

The technique of this test, according to Foffer (31) was first described by Meulengracht in 1920. The test is, for all practical purposes a determination of the degree of "yellow" intensity of a given serum as compared to a standard solution of potassium dichromate. It was originally thought that this color was imparted to the sera only by bilirubin. It was soon found, however, that the color of serum may be
influenced by substances other than bilirubin. For example, a diet rich in vegetables, increase the color intensity of the serum due to carotin could be represented by a mean inturus of one.

The technique as originally described (52) consists of deducting 1.0 cc of serum with 0.9 cc of normal sodium chloride solution until the color matches the standard (1:10,000) potassium dichromate. The solutions are compared in a colorimeter, and the icterus index is calculated according to the following formula:

\[
\text{Reading of Standard} \times \text{dilution} = \text{Icterus Index.}
\]

The technique has been modified by precipitating the serum with two volumes of acetone filtering and comparing this solution with the dichromate standard.

Normal indices have been established as from 1 to 10 units and more recently by Moue (52) at between 4 and 6.

The icterus index attempts to test the same thing as the qualitative Van den Bergh and has the advantage of simplicity. However, it has been found in many series of cases (31) that a discrepancy between the two tests exists not infrequently in normal instances. In those cases in which the serum bilirubin is above normal, there is no way of predicting the icterus index value. There seems to be no definite correlation ratio between the icterus index value and the bilirubin content (53).

The test is a simple and may be a valuable procedure.
if it is born in mind that it gives only a general idea of the degree of bilirubinemia. Where it is normal, it is safe to assume that there is no increase in the bilirubin content of the blood.

Snell (49) is of the opinion that the icterus index determinations from day to day and plotted as a curve will aid by giving some prognostic and diagnostic information. In complete biliary obstruction due to neoplasma, a rapidly rising curve is the rule, especially if the gallbladder has been previously removed or has been rendered nonfunctioning by local disease. If the organ is intact, the abrupt rise is converted into a slow and gradual one. If obstructive biliary cirrhosis supervenes, as is frequently the case in the presence of stricture or stone in the common bile duct, a low plateau curve is the rule; if the liver is not extensively affected, as is the rule in recent neoplastic obstructions, a high plateau curve results; later a gradual fall may occur.

C. UROBILINOGENURIA

The physiological reasons for this phenomena have been discussed.

The basis for the methods to determine the presence of unobilinogen and urobilin are dependent on: (a) fluorescence in the presence of zinc salts (b) spectroscopic absorption bands and (c) production of red color by the addition of Ehrlich's aldehyde reagent. According to Soffer (31) Schlesinger introduced the fluorescence test for urobilin. In
this, urobilin gives a green fluorescence with a saturated alcoholic solution of zinc acetate in slightly alkaline solution. The spectroscopic method for estimating the combined urobilinogen and urobilin content of urine was introduced by Wilbur and Addis (54).

The method most popular at present is that introduced by Wallace and Diamond (17). It is as follows:

The reagent is 2 grams of paradimethylomidobenzoldehyde in 100 cc. of 20% hydrochloric acid solution.

A series of dilutions of the urine are made, carried to the point where no further reaction takes place. To 1 cc of urine are added 10, 20, 30, 40, 50 etc. cc. of water. Ten cc. of each dilution are placed in test tubes and 1 cc. of the reagent is added. The characteristic pink color may appear promptly or within 5 minutes and is best seen by looking through the mouth of the tube. Warming the tube gently may hasten the appearance of the color.

Care should be taken that the specimen is fresh and that the test is performed on a 24 hour specimen as the amount of this carcinogen excreted varies during the course of the day. Watson (55) has shown that much more conclusive information with regard to urobilin and urobilinogen metabolism can be obtained by quantitative studies of the fecal and urinary excretion of these substances during definite test periods; such studies show the single examination of the urine is of doubtful significance because the amount
of bilirubin entering the intestine varies from day to day. The value of urobilinogen determinations as an index of liver functions has been and still is a much disputed point. Piersol and Rothman (56) are quite enthusiastic. They state in their conclusions: "Of all the liver function tests thus far devised, those of greatest clinical value and general usefulness are (A) Estimation of urobilinogen; (B) The determination of serum bilirubin (C) The estimation of the degree of retention of the dye bromsulphalein.

Urobilinogen is probably the most delicate single test for liver dysfunction. It is always increased even when the injury to the parenchyma is slight or when excessive blood destruction brings about an increase in bile pigment formation. In our experience, urobilinogen is constantly increased to a noteworthy degree in portal cirrhosis. Slight increases in urobilinogen have been noted from time to time in a limited number of patients in whom liver disorder was suspected but not evident clinically. In many conditions a persistent increase of urobilinogen was indicative of a residual hepatitis.

Meyer-Betz (57) studied the liver function in 100 cases. They found the urobilinogen test was positive in 10 while the bromsulphalein showed abnormal results in 21 patients and was doubtful in 3. They found that urobilinuria is found only at the beginning and at the end of the course of catarrhal jaundice. Wallace and Diamond (17) explain this by reference to the fact that during the height of this disease the parenchymal damage is extensive enough to distort
the continuity of the bile canaliculi producing an actual obstructive lesion so that no bile enters the larger bile ducts. At this stage the urobilinogen will be entirely absent from the urine. When the reparative process begins and the continuity of the bile channels is again established there will be an outpouring of bile into the gastrointestinal tract with a marked increase in the urinary excretion of urobilinogen.

Robertson (58) in 24 cases of clear cut liver disease found 7 instances in which the urobilinogen was present in the urine in amounts exceeding a 1:20 dilution.

Soffers (31) in a series of 43 definite cases of various types of liver disease found only 9 patients showing abnormal excretions of the chromogen. Three were well defined cirrhosis of the liver; 2 were biliary cirrhosis; 3 were catarrhal and arsphenamine jaundice cases; 1 was a case of acute yellow atrophy. He states that in comparing the respective merits of the urobilinogen test that it was not nearly as sensitive an index of liver function as either the bromsulphalein or the levulose tolerance test, but did yield as many positive results as the galactose tolerance test.

D. THE EXCRETION OF INTRAVENOUSLY INJECTED BILIRUBIN AS A TEST OF LIVER FUNCTION

In view of the normal liver activity in regard to this substance it is rather interesting that, although the reserve power of the liver is adequate for handling the amount of bilirubin which is normally made and brought to it for excretion, when an additional burden is thrown on the excretory
function of the cells by artificially increasing the amount of bilirubin in the blood, evidence of impaired excretory ability is often revealed.

It was on this basis that Ellbott, according to Soffer & Paulson (59), originally devised this test as a means of determining the existence of hepatic dysfunction. The procedure was introduced into this country by Harrop & Baron (60) in 1931. Since then Soffer et al (59) have modified the test to increase its practicability. They submit that here is a substance which measures the excretory function of the liver, but in contrast to the dye tests, a substance is employed for measuring this particular function which is normally manufactured in the body. The excretion of the substance is through the bile. Experimentally, the bilirubin when injected is promptly absorbed by the proteins of the blood serum and hence no excretion in the urine takes place. Neither is the injected bilirubin phagocytised by the reticulo-endothelial system except in completely obstructive ujaundice. They warn the user, however, that the use of this test is limited to those patients who show no elevation of circulatory blood bilirubin beyond 1 mg %. In the presence of hyperbilirubinemia, the retention of injected bilirubin has no significance, since, the liver cannot adequately handle the amount of bile pigment in the blood prior to performance of the test.

METHOD:

A total amount of bilirubin equal to 1 mg. per kilogram of body weight is dissolved in 1 molar solution of sodium carbonate which has previously been brought to the boiling
point and lowered to 80°C. A control sample of oxalated blood is collected in a dry syringe to prevent hemolysis, and with the needle in situ, the bilirubin is then injected intravenously. Oxalated samples of blood are obtained from the other arm within 5 minutes and again in 4 hours after injection. The concentration of the bilirubin is determined as follows: The plasma is precipitated by redistilled acetone, which is used in different concentrations, depending on the amount of bilirubin in the sample. Thus with the control and with the sample taken after 4 hours, 2 cc. of acetone and 2 cc. of plasma are used while to 1 cc. of plasma of the specimen taken after 5 minutes 4 cc. of acetone are added.

After the plasma and acetone mixtures are shaken, the samples are centrifuged, filtered, and promptly matched against standards. Except during actual readings, the acetone solution of bilirubin must be protected from the light. Final calculations are made as follows:

\[
A \text{ (Control Specimen)} \times 2 \text{ (dilution)} = A'
\]

\[
B \text{ (5 minute specimen)} \times 5 \text{ (dilution)} = B'
\]

\[
C \text{ (4 hour specimen)} \times 2 \text{ (dilution)} = C'
\]

\[
\frac{C' - A'}{B' - A'} \times 100 = \% \text{ retention of bilirubin.}
\]

**Standards:** Potassium dichromatic solutions

<table>
<thead>
<tr>
<th>Dilutions</th>
<th>1 to 2000 to 1 to 10,000</th>
</tr>
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| \[
\frac{1}{2000}
\] | color identical to that of 1 mg % of circulating bile |
| \[
\frac{1}{2222}
\] | " " " " " " 0.9% " " " |
| \[
\frac{1}{2500}
\] | " " " " " " 0.8% " " " |
\[ \frac{1}{2857} = \text{color identical to that of 0.7\% of circulating bile.} \]
\[ \frac{1}{3333} = \text{" " " " 0.6\% " " " "} \]
\[ \frac{1}{4000} = \text{" " " " 0.5\% " " " "} \]
\[ \frac{1}{5000} = \text{" " " " 0.4\% " " " "} \]
\[ \frac{1}{6666} = \text{" " " " 0.3\% " " " "} \]
\[ \frac{1}{10,000} = \text{" " " " 0.2\% " " " "} \]

If a drop of HCl is added to each standard tube, the tube may be kept indefinitely. A retention over 5\% of the injected pigment at 4 hours is considered abnormal.

In investigating the comparative merits of this method with bromsulphalein, levulose tolerance, galactose tolerance, urobilinogen, and Van den Bergh procedures, it was found that the bilirubin excretion test yielded the greatest incidence of positive results. However, for closer evaluation, a comparison was made between bilirubin and bromsulphalein since both are tests of excretory function.

In 18 cases in which there was clinically well defined, although slight, liver damage, the bilirubin test was positive in 16, while in only 3 instances was the bromsulphalein test positive. In 15 cases in which there was no clinical data to indicate hepatic involvement but where such a possibility was suspected 17 comparative studies yielded 12 positive bilirubin tests and 3 positive bromsulphalein tests. In no instance was the bilirubin test negative when the bromsulphalein test was positive. Thus in a total of 33 cases in which 35 comparative tests had been made, 28 showed ab
normal retention of injected bilirubin and 6 showed abnormal retention of injected bromsulphalein. The authors felt that this served to emphasize the fact that the bilirubin test is frequently positive in mild instances of liver damage. Hence, the bilirubin excretion test is of value where other tests usually yielded negative results.

Harrop and Baron (60) performed this test on 8 cases of various types of liver disease together with bromsulphalein and, levulose tolerance tests. The bilirubin was positive in all instances, while only one result was abnormal with each of the other tests. In 7 cases of chronic anemia where liver disease was suspected, the excretion of intravenously injected bilirubin showed the existence of impaired liver function in 6 instances, while both the bromsulphalein and levulose tolerance tests were entirely negative.

Jonkelson and Gargill (61) employing a somewhat different method, performed the test on 5 instances of hepatic cirrhosis and on 6 cases of malignancy of the liver. All showed an abnormal retention of the pigment.

Snell and McGath (46) point out two drawbacks to the bilirubin "tolerance" test. First and most important from a clinical aspect is the cost of the material for the test. Second, the determinations must be extremely accurate. For instance, under some conditions, the value for the serum bilirubin at the end of 4 hours may be only 0.5 mg% greater than the control value but may represent a retention of 7%.

Even this latter argument, however, tends to show the
the extreme sensitivity of the test.

II. TESTS BASED ON METABOLISM OF FOOD STUFFS.

A. TESTS BASED ON CARBOHYDRATE METABOLISM.

As was brought out in the physiological discussion the most significant result of experimental hepatectomy, perhaps, is the decrease in the value for the blood sugar and the hypoglycemic reaction which continues because the normal store of glycogen in the liver is abolished. If the capacity of the liver (46) to maintain the normal amount of blood sugar to be a much less sensitive index of liver function than levulose.

According to McLeon and De Wesselow (62) the test had its inception in Shirokauer's observation that the value for the blood sugar of normal persons was virtually unaffected by the oral administration of levulose, whereas it was increased in the presence of hepatic disease. He administered an oral dose of 100 gm. Spence and Brett (63) and Tallerman (64) reduced the dose to 45 grams. Kimball (65) in 480 levulose tests 100 of which were done on patients who showed neither clinical or laboratory evidence of hepatically normal patients was there an increase of more than 30 mgm per cent and in no instance was the blood sugar at the end of 2 hours more than 10 mgm % above the fasting level.

METHOD.

A fasting blood sugar is obtained early in the morning and a dose of 40 or 50 grams of levulose in 250 cc. of water is administered (40 grams if patient weighs less than 140 lbs.)
Samples of oxalated blood are collected at half hour intervals for 2 hours and their sugar values determined.

Cautions to be observed are:

1. Immediate precipitation of the blood.
2. No food or water during test period.
3. Care with regard to the purity of the levulose, according to Jaliffe (66) and Kimball (65) the following criteria of abnormality should be used:
   1. With fasting blood sugar level of 80 to 100 mgm %, an increase of 30 mgm. % or more.
   2. With fasting blood sugar level of 70 to 80 mgm. % an increase of 35 mgm % or more.
   3. With fasting blood sugar level of 60 to 70 mgm. % an increase of 40 mgm. % or more.
4. Where the height of the blood sugar curve exceeds 130 mgm. % regardless of the fasting sugar level (provided it is not above 115 mgm %) the curve is considered abnormal. Where the fasting blood sugar level is more than 130 mgm. %, the possibility that the patient is a diabetic enters and interpretation of the curve may be misleading.
5. Regardless of the height of the curve the failure of blood sugar after 2 hours to return to within 15 mgm. % of the fasting level is considered abnormal.

Kimball (65) reports on a series of 142 cases; 81 of these (57%) yielded abnormal levulose curves. The incidence
was higher in patients with severe hepatic damage.

Soffer (31) reporting on 70 tests done on patients with definite liver disease found 54 (3%) giving abnormal levulose curves. The greatest incidence of positive results was obtained in patients with diarrhea of the liver particularly when associated with ascites. Comparing the results with those obtained with urobilinogen and bromsulphalein tests they found that the levulose tolerance tests yielded a greater incidence of positive results than the urobilinogenuria determinations. While the percentage of positive results was essentially the same as that yielded by the bromsulphalein test.

Snell and McGoth (46) concluded that when the test was performed on experimental animals and all outside influences were excluded, the results were reasonably reliable, but when the test was performed on patients, many difficulties and errors were encountered. A low initial value for the fasting blood sugar, mild diabetic tendencies, and chronic pancreatic disease, all tend to vitiate the results. In general the field of usefulness of the test is limited.

3. GALACTOSE TOLERANCE TEST.

Shaye and Schloss (25) and (27) are responsible for reviving this test and bringing it to this country. It was originally introduced by Bauer in 1906.

The test is based on the observation that a normal person can assimilate a 40 gm. oral dose of galactose without the loss of more than from 2.5 to 3 gm. of sugar in the urine in the 5 hours immediately following the administration of the sugar
The test is preceded by a 12 hour fast and on the morning of the test the patient is given no breakfast. A fasting specimen of urine is tested for sugar. The patient is given 40 grams of galactose dissolved in 250-500 cc. of water. Hourly voidings of the urine are collected and tested for sugar. Positive samples are added together and the sugar determined quantitatively by the method of Benedict. Excretion of more than 3.0 grams of sugar during the 5 hour period is considered abnormal. In employing the test on diabetics (25) the interfering sugars are fermented out with yeast.

Banks, Sprague and Snell (67) tested 127 cases by this method. In intrahepatic jaundice they found 62.2% to yield positive results. Of 16 tests on portal and biliary cirrhosis only 18.7% yielded abnormal figures. In 69 instances of proven obstructive jaundice due to extrahepatic neoplasma and common duct stores and without liver damage 33% gave abnormal results. In 21 cases of liver disease without jaundice both the galactose and the bromsulphalein tests were performed. There were 15 positive instances with the dye while only 2 of the galactose tests were abnormal.

Soffer (31) and Snell and McGoth (46) make the following conclusions, in general, liver disease, in the absence of jaundice, the test is one of the least sensitive. Its greatest value lies in the differentiation in patients with jaundice between the obstructive and non-obstructive types. Although, in a certain percentage of obstructive jaundice cases, positive galactose tests may be obtained (67). In the differentiation of jaundice the galactose tolerance test has definite advantages.
over the levulose test although the incidence of positive results is about the same. In the first place, the former test is easier to perform and involves no particular hardship on the patient; the galactose test may be done in the presence of diabetes. This group frequently presents jaundice as a cardinal sign of any one of a number of causes. The fact that neither the levulose nor any of the dye tests are satisfactory in these cases renders the galactose tolerance determination particularly valuable.

B. TESTS BASED ON CHOLESTEROL METABOLISM.

The physiological basis for this test has been discussed. The method of testing is quite complicated in my humble opinion, and demands considerable laboratory equipment and experience. The method recommended by some of the latest workers (68) is Smith and Marble's (69) modification of Bloor and Knudson's (70) method.

Smith and Marble (69) state that they present a new method for the direct colorimetric determination of free cholesterol together with modifications of the Bloor and Knudson procedures for total and ester cholesterol respectively. They state further that by means of the three determinations an excellent check is provided for the accuracy of each, since obviously the calculated sum of free and ester should equal the total.

In brief the procedure is as follows: Plasma lipids are extracted with alcohol-ether (3:1) as recommended by Bloor. Total cholesterol is determined on an aliquot of filtrate; Bloor's procedure is followed closely. Ester and free cholesterol are determined on a second aliquot, the former by a
method similar to that of Bloor and Knudson except that saponification is introduced, and the latter by analysis of the cholesterol digitonide precipitate.

White et al (68) consider an ester percentage of 60%–70% as normal. They warn that the blood must not be hemolyzed for the cholesterol tests, because the red cells contain only free cholesterol which, if liberated by hemolysis, gives an abnormally high value. According to Epstein (28) in the normal blood plasma of human beings there is from 140 to 200 mg. of Total Cholesterol per hundred cubic centimeters, and of this 50 to 70% is in the form of cholesterol ester.

The results obtained by Epstein (71) in his latest tests were as follows:

In obstructive jaundice there is usually a sharp rise in the values for both the cholesterol and the cholesterol esters, which may be roughly parallel to the elevation of the value for the serum bilirubin. If biliary obstruction of long duration, cholangitis or obstructive biliary cirrhosis complicates the picture, the value for the cholesterol in the plasma may be normal or decreased. In cases in which acute parenchymatous disease of the liver is associated with jaundice the value for the cholesterol may be decreased or normal and that for the cholesterol esters may be diminished or these esters may actually be absent.

Snell and McGoth (46) remark that it has been thought that the value for cholesterol esters gives some idea of the severity of the injury to the liver and of the prognosis, but in their experience this has not been entirely substantiated.
In the ordinary types of portal cirrhosis the value for the cholesterol usually is normal except when acute degeneration of the liver supervenes.

White et al (47) and (68) in their recent study of 66 cases list the following results:

Total cholesterol in cases of obstructive jaundice showed a marked increase in 90% and showed variations between 253-278 mg. per 100 cc. It was normal or only slightly decreased in the other chronic cases, i.e. cirrhosis, and gallbladder disease without obstruction of the common duct. In a few of the cases of acute hepatitis the values were moderately low during some period of the illness.

The cholesterol ester percentage (normal 60-70%) was low in about 68% of the cases of acute hepatitis; one half of which returned to normal upon improvement of the patient. There were two deaths in this group, both of which had a low ester percentage. In the chronic cirrhosis there was a lowering of the ester percentage in 45% of the cases. In 5 out of the 8 deaths in this group, the percentage of ester was normal or had improved from low to normal. In the gallbladder cases without known obstruction, one-half were low and about 1/10 remained low. In the tumors all the ester percentages were normal. In the small group of cases of obstructive jaundice, about 50% were normal and 50% were low.

Prognostically, the total cholesterol values in the blood have given very little information of value. The cholesterol ester percentage has definite prognostic value in acute liver disease. Falling values and a very low figure are, serious
signs, and a high figure shows a mild disturbance. Progressive improvement from low to normal figures is seen in the more severe cases of acute hepatitis which recovered. The ester percentage has disclosed very little information of prognostic value in the chronic cases and has been normal in about 1/2 of the fatal cases.

In the group of cirrhosis less than half showed a low ester percent.

In the physiological discussion, the determination of phospholipids and total lipids in the blood was mentioned as a method of testing the liver's activity in fat metabolism. On reviewing the literature, Snell and McBoth (46), White et al (47) and (68) and others; it seems that these determinations show no more than the Total Cholesterol estimations. The chemistry of the tests is even more involved (72). Therefore, it was thought unwise to dwell further on this method of testing liver function.

C. TESTS BASED ON BILE ACID METABOLISM.

In view of the fact that the liver plays such an important and primary role in the manufacture of these acids, one might expect that their quantitative determination would constitute a sensitive means for determining liver function.

Soffer (31) remarks that the quantitative estimation of bile acids as a means of determining the functional status of the liver has never been widely employed because of the attending technical difficulties.

In practice there are other obstacles to the use of
this phase as a function test. Soffer (31) quotes Rosenthal and Zinner to the effect that their determinations of the concentration of bile salts in the bile of patients with advanced atrophic portal cirrhosis showed no deviation from the established normal. This would tend to show that this function was maintained up to the end stages of liver disease.

Furthermore, as Foster et al (73) have shown, the concentration of these substances in bile is markedly affected by diet.

Snell and McGoth (46) suggest that, following operation, when bile can be obtained from drainage tubes or from fistulas, the study of bile acids may give useful information. They feel that low concentrations consistently are of grave prognostic import.

D. LIVER FUNCTION TESTS BASED ON PROTEIN METABOLISM.

As was assumed in the physiological discussion, the failure of deamination of amino acids should allow for an increase in amino acids in the urine, and since this is a function of the liver one might expect to find such an increase in disease of the liver. However, as Ballman et al (34) have pointed out, the amino acids are rapidly absorbed by the muscles where hepatic function is disturbed. The demonstration of an actual increase in amino acids in the blood or urine in patients with liver disease is quite difficult except in the presence of extensive hepatic pathology.
Snell and McGoth (46) however, call attention to the test proposed by Manchke, a German investigator. In this, an oral dose of 50 grams of gelatine is administered. The urine is subsequently examined for amino acids. Normally 200 mg. of amino acid is found in the 1st four hour-period. In patients with catarrhal and arsphenamine jaundice and in patients with cirrhosis of the liver a considerable increase in the amino acid nitrogen output occurred in the urine.

Bollman, Mann and McGoth (74) found that in the dog there is a rise in uric acid content of the tissues and body fluids roughly proportional to the amount of reduction of liver tissue, and suggest that this might well be an index of hepatic function.

According to Wakeman (45) and others (31), this observation is not born out clinically.

One is inclined to conclude that as yet, liver tests based on protein metabolism have not been developed to the point where they are of much help to the clinician.

In regard to the other phase of protein metabolism discussed in the physiological section:

- Myers and Keefer (76) made estimations of plasma proteins in 16 cases of cirrhosis of the liver and 14 cases of other forms of disease of the liver. They conclude from their work that there is a decrease in plasma proteins in this disease and that this is a result of the loss of protein in ascitic fluid together with defective formation of plasma
proteins. The decrease was most marked in the albumin fraction and the albumin globulin ratio was inverted. Similar but less extensive and less consistent alterations in the plasma protein values were observed in 14 cases of other forms of disease of the liver. In the less advanced lesions the amount of albumin may be only moderately reduced and the amount of globulin may be normal or increased.

Snell and McGoth (46) commenting on this observation state:

In all but a few of a fairly large series of cases of all types of hepatic disease, the albumin globulin ratio has been disturbed considerably, but there was not much reduction in value for the total proteins. The rapidity with which changes in the amount of total protein and in the albumin-globulin ratio may take place is rather striking. Repeated determinations in the same cases indicate that these changes may not be without some prognostic significance.

It would seem that more work must be done in this regard, but it is well to bear the test in mind as a possibility.

TAKATA-ARA TEST OF LIVER FUNCTION.

It seems logical to mention this test at this time inasmuch as it is thought to be based upon some change in the serum proteins.

The test was originally introduced by Takata and Ara to differentiate between lobor and bronchopneumonia (77). They believed that the test was based on a decreased stability of
of serum proteins produced essentially by an increase in the globulin fraction. Since then there has been much debate as to the basis of the test. Magath (78) states "It becomes clear that the reaction on serum is an emperic test, a colloidal phenomenon which has yet not been explained".

In general (77) the authors are inclined to agree with the Japanese investigators who thought the reaction ascribable to changes in the relative amount of albumin and globulin in the serum with the latter in excess, so that the colloid was no longer protected. This is a phenomenon dependent on a reversal or partial reversal of the albumin globulin ratio. With this latter opinion, Crane (79) and his associates are in agreement.

The test is a precipitation of mercuric oxide when a solution of sodium carbonate, mercuric chloride and acid fuchsin is added to an affected sera.

METHOD: In each of 8 small tubes is placed 1.0 cc. of 0.9 per cent sodium chloride solution. To the first tube is added 1 cc. of the fluid to be examined. This is mixed and 1 cc. of the contents is removed and added to tube 2; then 1.0 cc. is transferred from tube 2 to tube 3 etc. One cubic centimeter of the contents of the last tube is removed and discarded. To each tube 0.25 cc. of 10% sodium carbonate and 0.3 cc. of the Takata reagent is added. The latter consists of equal parts of 0.5% mercuric chloride solution and 0.25% aqueous fuchsin. Readings are made immediately after ½ hour and 24 hour periods.
Positive reactions are indicated by the appearance of a definite precipitate in 2 of the first three tubes and in any number of the following tubes. Negative reactions may show no precipitate at all or only slightly in the last 3 or 4 tubes.

The test is thought (77) to be fairly specific for cirrhosis, but early in the disease the test is negative.

Actually (78) the test is not specific for either injury to the liver or cirrhosis, but in the presence of hepatic disease, a positive reaction is more likely to indicate cirrhosis than another disease.

Meyer et al (77) in their discussion of this test believes that it is valuable in fortifying a diagnosis of cirrhosis, differentiating surgical from medical jaundice, particularly when jaundice is painless.

It may be stated with some degree of certainty that extrahepatic obstructive processes without much liver damage do not result in a positive Tαλa Aτα Aρα and therefore aid us in differentiating an intra from extra hepatic disease unless a parenchymatous liver damage is concomitant with extra-hepatic obstruction.

It must be admitted that the correlation between positive reaction and a reversal of the A/G ratio is high and since the positivity of the test depends upon the elevation of the globulin level, the reaction is likely to be positive in any disease in which a protein shift occurs. More data needs to be collected on the latter phase (31).
TESTS BASED ON THE DETOXICATION FUNCTION OF THE LIVER.

Familiar examples of this function are (46) the conversion of indole to indoxyl sulfuric acid, the conjugation of cholic acid to form bile salts and the formation of conjugate glucuronates. Various clinical tests with such substances as thymal, menthol, camphor, salicylates, phenol, p-cresol, and guaiacol sulfuric acid have been used but have not been entirely successful. The only survivor of such tests of the detoxifying function is one based on the ability of the liver to conjugate benzoic acid and amino acetic acid to form hippuric acid.

HIPPURIC ACID.

It was known that in the dog, hippuric acid is synthesized only in the kidney. Subsequent workers tacitly assumed that in man the same condition prevailed to a great degree. Bryan (39) is the only investigator who has studied the synthesis of hippuric acid in human subjects with hepatic involvement. This result definitely indicated that liver damage did influence the conjugation of benzoic acid.

Quick (40) in introducing this test makes the following statement:

"It is quite probable that in man, as in the rabbit, the synthesis of hippuric acid also takes place in the liver. Furthermore, the writer has demonstrated that the rate of the synthesis of hippuric acid is dependent upon the speed with which the organism furnishes glycine. Irrespective of the
amount of benzoic acid administered, the quantity of hippuric acid excreted per hour (in the absence of exogenous glycine) is relatively constant, indicating a definite maximum capacity of the organism to synthesize glycine. Since it is commonly accepted that glycine is formed in the liver, it seemed probable that certain types of liver damage might produce impairment of this synthesis, which should result in a diminished output of hippuric acid. On this basis, a new test of liver function was developed.

To exclude a possible factor of delayed elimination by the kidney, Kohlstaedt, et al (80) suggested making a simultaneous study of urea clearance. If this can be done, reduction of synthesis of hippuric acid less than 2 gm. may probably be regarded as significant.

**METHOD.**

5.9 grams sodium benzoate in 30 cc. of water is administered 1 hour after a breakfast consisting of coffee and toast. The patient is then given $\frac{1}{2}$ glass of water. Immediately after taking the drug the patient voids, and then collects complete hourly specimens for 4 hours. Preserve with toluene, and the hippuric acid is determined in each specimen. In normal adults, the output of benzoic acid as hippuric acid is approximately 1 gram or more during the second and third hours, and the total for the 4 hours is from 3 to 5 grams.

**CLINICAL METHOD OF DETERMINING THE HIPPURIC ACID.**

Each hour a specimen is measured, transferred to a small
beaken and acidified with concentrated hydrochloric acid until acid to congo red; 1 cc. of the acid is usually sufficient. The solution is vigorously stirred until the precipitation of the hippuric acid is complete, and then is allowed to stand for 1 hour at room temperature. The precipitate is filtered off on a small Buchner funnel or a filter paper, washed with a small quantity of cold water, and allowed to air dry. The hippuric acid thus obtained is either weighed (to the second decimal place, which is sufficiently accurate) or titrated with 0.2N sodium hyroxide, using phanalphthalein as the indicator. To obtain the total hippuric acid, one must add to the amount thus obtained the calculated quantity remaining in solution; 100 cc. of urine will dissolve 0.33 gm. of hippuric acid. In case any specimen exceeds 125 cc. it should be slightly acidified with acetic acid and concentrated on the water both to about 50 cc. before precipitating the hippuric acid. The results are best expressed in terms of benzoic acid, one multiplies by 0.68.

Quick (81) has proposed an intravenous method for administration of the test dose of benzoic acid. In this, in the morning after a light breakfast a solution containing 1.77 grams of sodium benzoate in 20 cc of distilled water is given intravenously. Five minutes before the injection, the patient is instructed to void before the test and the urine is collected 1 hour after the injection. The urine is measured and solid ammonium sulphate added in the proportion of 5 gm. for every
10 cc. of urine. When the salt is dissolved the urine is either filtered or centrifuged. Then, proceed with the determination of the quantity of hippuric acid as before.

This modification is useful in those patients showing nausea and vomiting with the oral administration.

Quick (40) from his cases feels that here is a test of a function which is first interfered with by hepatic disease. He found a diminished excretion of hippuric acid in syphilitic cirrhosis, catarrhal jaundice and in obstructive jaundice of moderately long standing. Normal results were obtained in cholecystitis and in 2 cases of portal cirrhosis. He concludes that the test offers an approximate quantitative measure of liver disease.

He feels that the test should become useful to the surgeon in his choice of anesthesia, in his preoperative care and his preparedness for emergencies. For the internist, it should prove equally valuable since a diminished excretion of hippuric acid should be a contraindication for arsphenamine cinchophen and other drugs having a potential hepatoxic properties.

Snell and McGoth (46) make the following statement concerning this test. It has been noted that for patients who are not jaundiced, the results closely parallel bromsulphalein, while in cases of hepatogenous or obstructive jaundice the reduction in hippuric acid synthesis corresponded in a general way to the
degree of hepatic injury noted at operation or necropsy.

**BROMSULPHALEIN LIVER FUNCTION TEST.**

Bromsulphalein (31) has replaced phenol-tetrachlorthalein as a test of liver function since the former substance is much less irritating; it dissolves in water with greater ease and disappears less rapidly from the blood stream. Toxic manifestations and venous thrombosis, a comparatively frequent occurrence when phenoltetrachlorthalein is employed rarely occurs with the sulfonated dye.

As first proposed (83), a dose of 2 mg. of the dye per kilogram of body weight was injected and specimens were obtained at 5 minute, 50 minute, and 1 hour intervals thereafter. Oleary et al (82) first showed that the test could be improved by injection of 5 mg. of the dye per kilogram of body weight and Soffer (31) simplified the test by omitting the 5 minute specimen.

**METHOD.**

Five mgm per kilogram of body weight of bromsulphalein are injected. Samples of the blood are collected from the other arm at the end of 30 minutes and 1 hour. One sample of the serum is alkalinized with 3 drops of 10 per cent sodium hydroxied to bring out any change in color that may be present because of the dye. This is compared with a series of standards in a comparometer box. Another sample of serum is placed in the comparometer box directly behind the standard tube to give it the general color background of the serum. Normally 20 to 50% of the dye is present in the circulatory blood stream.
after five minutes, while all the dye has disappeared from the blood after 30 minutes. Retention of 10% or more at the end of half an hour is considered definitely abnormal and therefore indicative of liver damage.

MacDonald (84) proposes a modification to the above method and makes the following objections to the single determination:

A. It tells little or nothing if the test is normal.
B. It gives no information about actual length of time required for the liver to do a given amount of work, nor does it tell the efficiency with which it is done.

It is important to know this: two livers, both of which have 0% retention at the time limit, do not necessarily remove the dye in the same length of time and so cannot be the same in working capacity. An abnormal liver with its tremendous reserve can give the same results as a normal liver, if given time enough.

He proposes the following remedies to these objections: It was thought that if the rate at which the liver removes dye from the blood could be determined there might be produced a curve typical of the normal liver, and that if a normal could be established then early and small amounts of liver changes could be told.

To do this MacDonald estimated the remaining dye in the blood at 2 minute intervals and plotted graphs. The following facts were revealed by the graphs:
A. 18 minutes and not 30 minutes is required for complete removal in the normal liver.

B. Livers which remove 100% of the dye inside of 30 minutes do not do so by having a consistently lower reading at each successive estimation, i.e. the liver may not remove any dye at all for 2, 4, 6, 8 or 10 minutes and yet may remove it all within the previous normal time limit.

C. As a working unit, a liver represented by the curve showing a consistent and continuous disappearance from the blood is more efficient than a liver which removes only 5% in 12 minutes.

Upon further development of this method of testing, MacDonald found that 5 milligrams of dye per kilogram of body weight was such a dose as would put a "maximum load on the liver". Further that 5 minute intervals were sufficient.

He concluded from his series of cases, that by repetition of the test and the use of graphs of 5 minute interval estimations:

A. Function tests will show nothing abnormal until that function fails, but the improved technique will show liver changes before the accepted tests of function are abnormal. By this one can estimate rather accurately how much reserve there is in store before function will fail. This is of value surgically. Also, it is readily seen how this reserve increases or decreases, hence its value in diagnosis and prog-
nosis, because a prognosis cannot be good in the presence of a curve moving away from the normal, nor can treatment be considered successful under the same circumstances.

Differences in rate indicate differences in efficiency, and if changes in liver efficiency either for better or worse, can be told, the prognosis can be judged so much better.

MacDonald states that one venopuncture is sufficient for taking all the blood samples. Some space has been devoted to MacDonald's work because we can find no mention of any like observations in the literature and because his reasoning appeals to us as sufficiently logical to warrant some investigations of his claims.

McGoth (85) showed that the results, with the single determination, are extremely satisfactory, since retention of the dye occurred in 96% of cases in which there was evidence of parenchymal hepatic injury, or even moderate mechanical obstruction of the bile ducts which had not yet produced clinically demonstrable jaundice.

It should be pointed out, however, that this test should not be used in instances of jaundice where the van den Bergh reaction is either direct or biphasic. This is because the abnormal retention of the dye need not be an expression of the inability of the liver cells to excrete it, since the obstruction of the intra or extra-hepatic bile channels will cause the retention of the dye in the blood stream. In general, the test is contraindicated where the value for the serum bili-
rubin is 5 to 6 mg. %.

Soffer (31) makes the following generalizations concerning this tests: In about 55 to 65% of liver disease this test will yield definite evidence of impaired hepatic function. The percentage of positive result is considerably higher in cases where the liver damage is wide-spread and extensive as it is in portal cirrhosis with ascites, in biliary cirrhosis, and in long standing cases of chronic passive congestion. When we deal with malignancies of the liver either primary or metastatic, retention of the injected dye after one-half hour occurs less frequently, while in isolated lesions of the liver, as in hepatic gumma or cyst, the incidence of positive results obtained with the dye method is low. This test is considerably more sensitive as an index of liver function than either the galactose tolerance test on the determination of urobilinogen in the urine and yields a somewhat greater proportion of positive results in liver disease than does the levulose tolerance test.

Snell and McGoth (46) state that in their opinion the test gives results which indicate the extent of parenchymatous damage only roughly, but the determinations are sufficiently accurate to yield valuable information if the test is repeated at intervals during the course of the disease.

THE ROSE BENGAL TEST.

Delprat, Epstein and Kerr (87) originally introduced this method of testing the excretory function of the liver. The substance used is a dye which is removed from the blood
by the hepatic cells and its rate of removal is proportional to the normalcy of the liver cells.

In view of the comment by Green (86) in which he states that in a study of more than 700 cases there is very little difference in the significance of the various dyes. Phenoltetrachorophthalteine, bromsulphalein and rose bengal all show essentially the same type of changes. Further, and in view of the fact that the promsulphalein test is by far the simplest to perform, we will simply give the method for the performance of this test.

METHOD. (88)

Five to ten cubic centimeters of 1 to 2% solution of rose bengal in saline is injected intravenously. Two minutes from the start of the injection an oxalated sample of blood is collected which is designated as the "standard sample" and which contains the maximum concentration of the injected dye. It is considered to have 100% of the dye. Exactly 6 minutes later, that is 8 minutes after the dye has been introduced intravenously, the "unknown" sample is withdrawn. The plasma is separated and cleared by precipitation of the proteins with 2 volumes of acetone. This is centrifuged and to the plasma thus obtained a drop of 10% Na OH is added to clear hemolysis. The resultant dye solutions are read in a calorimeter. Normally 50% or less of the injected dye should be present in the circulatory blood after 8 minutes. In view of the photosensitizing effect of rose bengal on tissues, it is advisable to keep the collected specimens in the dark until they are to be read. Similarly, patients should be warned to avoid bright sunlight for several hours after the performance of the test.
MISCELLANEOUS TEST ASSOCIATED WITH LIVER INSUFFICIENCY.

A. Measurement of the tendency to bleed.

As Ivy et al (88) have pointed out, the surgical and medical risks of infections, and other pathological processes in and about the liver are seriously augmented by the possibility of hepatic insufficiency. One of the most striking surgical features associated with these cases of liver damages is their tendency to bleed. According to the results obtained in several clinics, 50 per cent of all post operative deaths in patients with jaundice or liver insufficiency are a result of hemorrhage.

Many procedures have been designed to measure the coagulation time of the blood accurately but none give a consistent picture of the dangers of bleeding in hepatic disease. Studies on fibrinogen, on calcium in its diffusible and nondiffusible forms, and on various other constituents of the blood have failed to solve the riddle of why a patient who has hepatic disease is so subject to hemorrhage.

Determination of coagulation time is today a most widely used procedure. The general conclusions are (88), however, that prolonged clotting time seems to bear no relation to clinical bleeding, and it is often normal in severe blood dyscrasias and when bleeding occurs.

On the other hand, Nygaard and Baldes (89) have shown that the "plasma coagulation index" as measured by their method gives a satisfactory basis for prediction of the
tendency to bleed. Repeated "coagelgrams" will often show the hemorrhagic tendency.

Ivy (88) thinks that a prolonged coagulation time in jaundice is not so much an index of bleeding tendency as liver damage.

Ivy's method of determining bleeding time is the application of a pressure cuff about the arm at about 40 millimeters of Hg. pressure then determining bleeding, time from Duke's puncture on the skin of the forearm near the elbow over the pronator muscles.

White et al (68) found that prolonged bleeding time by this method was commonly associated with poor liver function.

The methods that have been mentioned are not as yet in general use and may be too involved for practical purposes.

Snell and McGoth (46) state that high values for bilirubin in cases of icterus and high grades of retention of bromsulphalein in cases in which there is no icterus are danger signals, and furnish information as reliable as that furnished by any current method of studying the coagulation of blood.

QUANTITATIVE ESTIMATION OF SERUM PHOSPHATASE.

Phosphatase (46) which is an enzyme concerned in the metabolism of bone, is present in many tissues of the body and is found in high concentrations in bone, kidney, intestinal mucosa and liver.

Roberts (90) first noted that high value for phosphatase in the serum were a feature of some cases of obstructive
jaundice, while, in the non-obstructive types, low or normal values were the rule.

**METHOD.** It involves a determination of inorganic phosphatic liberated when the enzyme present in the blood is allowed to act on a phosphoric acid ester substrate under standard conditions. The upper normal value for adults is approximately 6 units and that for children is 15 units.

Rothman et al (91) state that in most cases of proved obstructive jaundice, the value for the phosphatase was more than 10 units but that values of 20 or more units were commonly observed; in cases of non obstructive jaundice values of less than 10 units were the rule.

Greene (92) on the other hand, expressed the opinion, that the information obtained by determinations of phosphatase is inaccurate and does not differentiate the type of jaundice in question.

It is apparent that the result of the determination of phosphatase will have to be weighed against the clinical evidence in cases in which the diagnosis is doubtful.

**THE APPEARANCE OF ERYTHROCYTIS IN DISEASE OF THE LIVER.**

This may be mentioned, not as a test of liver function, but because studies in morphology of the blood may give valuable confirmatory evidence of the presence of injury to the liver. A hypochromic macrocytic anemic is a rather uniform accompaniment of portal cirrhosis and may be noted in other types of injury to the liver. (93)
Stasney and Higgens (94) have demonstrated similar changes in the experimental animal. They attempt to explain the rapidity of appearance of macrocytis in the bloodstream on the basis of hypoproteinememia and swelling of the erythrocytes because of the altered osmotic pressure of the plasma.

Soffer (31) states that the macrocytosis was observed only in cases of long standing and very wide spread liver disease.

SUMMARY AND CONCLUSIONS.

Obviously all of the tests for investigating the activity of liver have not been dealt with in this review of the subject. However, those tests, which have been considered by most of the authorities as clinically applicable, have at least been mentioned. It is further believed that the direction which future work in this field will take has been pointed out. e.g. tests directed at the evaluation of the liver's activity in protein metabolism.

Certain conclusions as to the comparative value of the tests remain to be discussed. Of these conclusions there are some general ones that are quite obvious.

A. There is no one test that will adequately test all the liver's activities.

B. There is no one test that will show abnormalities in its reactions in all types of liver injury.

C. Indeed no one test has been shown to be 100% responsive in all cases of the same type of liver injury.
D. Some of the tests are obviously more practical than others.

E. Since no one test is entirely satisfactory, it is better to use several, testing several types of functions.

F. Lastly, but perhaps not so obvious, in testing several functions of the liver, one positive test is almost as good a sign of liver damage as if all were positive, but there is usually a correlation between the amount of injury to the liver and the number of different kinds of tests which show abnormalities.

In drawing conclusions as to the comparative value of the tests discussed, I will attempt to sum up the conclusions of three comprehensive articles on this subject. The first by L. J. Soffer (31) in 1935. The second by Snell and McGoth (46) in 1938. The third by White and his associates in November of 1939 and January of this year.

Certain general conclusions have been made in all three of these papers:

There is no correlation between the type of pathological lesion present in the liver and the function which is disturbed. Nor is there any definite index, clinically, as to which function is disturbed first. Concerning any one particular liver function test, it is an index of the degree of liver damage or progression or regression of the lesion only when performed repeatedly on the same
on the same patient.

It is utmost important to recognize when these tests should not be employed. There is no point in testing with dyes in the presence of a hyperbilirubinemia with a direct or biphasic van den Bergh reaction. Similarly the bilirubin excretion test can yield no information of any value when performed in the presence of a hyperbilirubinemia however slight. The galactose test on the other hand may be performed under all circumstances.

Evaluation of the liver function tests will be made with regard to three of the ways in which they may be used:

A. To detect the presence and amount of liver damage and to follow the changes and course of the liver injury.

B. For prognosis.

C. For differential diagnosis.

A. DETECTING PRESENCE AND AMOUNT OF LIVER DAMAGE AND TO FOLLOW CHANGES.

Van den Bergh reaction:

As a general test of hepatic function the quantitative Van den Bergh is inadequate; one sees too many instances of liver disease in which there is no increase in the amount of circulatory bilirubin.

It is an excellent index of an increase or decrease in the intensity of jaundice and of tremendous help in determining the progress of these patients. It is of value in determining progress of non-obstructive jaundice. The
test is at first indirect, and as the disease progresses it becomes biphasic and direct. Improvement is heralded by a reversal of the above.

**ICTERUS INDEX:**

Determinations have to be interpreted with caution. A normal index definitely excludes hyperbilirubinemia, while a very high icterus index almost always means an increase in the bile pigment of the blood. Between these two levels, it is probably better to resort to the Van den Bergh and be sure.

The interpretations of its reaction, with caution, are the same as the Van den Bergh.

**UROBILINOMOGEN AND UROBILIN DETERMINATIONS IN URINE:**

The test has proved to be of very little value in the general diagnosis of liver dysfunction.

**EXCRETORY TESTS:**

**Dyes:** A high incidence of positive results are found in cases of cirrhosis particularly when associated with ascites. By the plotting of a curve as suggested by Mac-Donald (84) one may be able to follow the progress of the injury.

**Bilirubin:** Soffer states "A greater incidence of positive results is obtained by this method in demonstrating hepatic dysfunction in early cases of liver disease than in any other method." Others are not in complete agreement with this.

**CARBOHYDRATE TOLERANCE TEST:**

**Levulose:** Disease of the liver must be widespread and moderately severe before an abnormal response to levulose
is manifested. Its value lies in the fact that it may be used in the presence of jaundice, obstructive or otherwise.

**Galactose:** May be used under any circumstances. It has proved to be quite valueless in the diagnosis of liver disease in the absence of jaundice. The test is usually strongly positive in severe or moderately severe acute hepatitis but in chronic cases it shows much less change.

White and associates have summed up the diagnostic value of phospho-lipids, total cholesterol, cholesterol ester per cent, and hippuric acid tests in the following chart on the results obtained in testing 66 patients. It is self explanatory.

<table>
<thead>
<tr>
<th>No. of cases</th>
<th>Phospho-lipids</th>
<th>Total cholesterol</th>
<th>Ester</th>
<th>Hippuric acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal 10-8</td>
<td>Normal 150-220</td>
<td>(60-70)</td>
<td>(2.5-3)</td>
</tr>
<tr>
<td></td>
<td>% Low Norm. %</td>
<td>% Low Norm. %</td>
<td>% Low</td>
<td>% Low Norm. %</td>
</tr>
<tr>
<td>17 acute hepatitis</td>
<td>81 19</td>
<td>38 38</td>
<td>24 28</td>
<td>36 72</td>
</tr>
<tr>
<td>21 chronic cirrhosis</td>
<td>93 7</td>
<td>45 50</td>
<td>15 55</td>
<td>25 20</td>
</tr>
<tr>
<td>10 Gall-bladder</td>
<td>50 50</td>
<td>11 67</td>
<td>55 55</td>
<td>34 11</td>
</tr>
<tr>
<td>4 Tumors</td>
<td>80 20</td>
<td>100</td>
<td>100</td>
<td>45</td>
</tr>
<tr>
<td>11 Obstructions</td>
<td>10 90</td>
<td>18 82</td>
<td>19 19</td>
<td>91 72</td>
</tr>
<tr>
<td>3 Hemolytic Jaundice</td>
<td>100</td>
<td>66 34</td>
<td>66 34</td>
<td>34</td>
</tr>
</tbody>
</table>
This should be noted about the hippuric acid excretion. It was definitely diminished in a high percentage of cases. The results paralleled the cholesterol ester figures but the test seemed to be more sensitive in all types of disease of the liver.

B. FOR PROGNOSIS.

Van den Bergh reaction:

It is of prognostic value in so far as it shows the progression of the jaundice.

Bromsulphalein:

Where this test was made after jaundice had disappeared, the results of this test closely paralleled the hippuric acid test. In one case of stricture of the common duct, the hippuric acid test seemed slightly less sensitive.

MacDonald (84) claims that this test as performed by his method gives invaluable information with regard to the prognosis of cases of liver dysfunction.

Bilirubin excretion:

Soffer (59) makes practically the same claims as the above for this test.

Galactose Tolerance:

In assessing the prognosis or in detecting the presence of liver damage the hippuric acid test was far more delicate than the galactose test.

The phospho-lipids and total cholesterol values in the blood have given very little information of prognostic value. In one fatal case of portal cirrhosis there was a
progressive change from high to low normal range over a period of 16 months, whereas a definitely bad prognosis was indicated by both hippuric acid and bromsulphalein tests.

**Cholesterol ester percentage:**

It has definite prognostic value in acute liver diseases such as acute hepatitis. Falling values and a very low figure are serious signs and a high failure shows mild disturbance.

The ester percentage has disclosed very little information of prognostic value in the chronic cases and has been normal in about half of the fatal cases.

In the group of cirrhosis less than half showed a low ester percentage while all showed a low hippuric acid excretion.

**The Hippuric Acid Excretion Test:** It has been helpful in prognosis since it invariably gave low results in all severe and fatal cases. It has proved more sensitive than the ester ratio in all classes of liver disease. This test is valuable in discovering residual liver damage after the jaundice has disappeared, or in ruling out liver damage in chronic cases. In this respect, it is almost exactly like the bromsulphalein test.

It is also of assistance in assessing surgical risk. The post operative course, is generally favorable when the hippuric acid excretion is between 2.5 to 3.6 or better. On the other hand, when the hippuric acid excretion is 1 and 1½ grams before operation a stormy convalescence may be antici...
The Ivy bleeding time:

Results agree with Ivy's opinion that a prolonged bleeding time is associated with poor liver function and is a serious prognostic sign.

C. FOR DIFFERENTIAL DIAGNOSIS.

Van den Bergh:

It's value in differentiating between obstructive and non-obstructive jaundice is minimal, as also its value in differentiating between hemolytic and non hemolytic jaundice.

Urobilinogen and Urobilin determinations in urine:

It is of some help in the differentiation between obstructive and non obstructive jaundice. The total absence of urobilinogen in the urine would suggest the former. This criteria, however, is to be used with caution.

Carbohydrate Tolerance:

The tests may have some value in the limited but important field of distinguishing acute cellular damage from the early stages of obstruction. Galactose is chiefly important in this respect.

Phospho-lipids and total cholesterol: Are consistently high in cases of external obstruction, but may also be elevated in toxic hepatitis. The latter can usually be ruled out by a history of exposure to some toxic substance.

Ester percentage:

This was also found to have little value in differentiating obstructive cases.
Hippuric acid:

This has no differential value because it is consistently positive in all types of liver disease.

In the field of differential diagnosis, the available tests are the least satisfactory. Reliance must be placed on a group picture rather than on the results of a specific test.

For practical purposes, it may be said: A. In types of disease of the liver not associated with jaundice, information gained from the study of retention of bromsulphalein is as reliable as that which can be gained in any other way, and under these conditions other tests give chiefly confirmatory evidence. B. In cases of jaundice, some information, which is not altogether reliable, as to the possible hepatogenous or obstructive nature of the jaundice in any given case can be had by studies of excretion of galactose, the values for the cholesterol and cholesterol esters in the plasma, and the value for serum phosphatase. C. The best information as to the functional activity of the liver in cases of jaundice can be gained from a consideration of the value for the serum bilirubin, its daily variations, and a knowledge of the anatomic changes which these represent. So far as indirect methods of measuring liver function in the presence of icterus are concerned, the hippuric acid gives reasonably accurate results, which should not, however, supplant the impressions gained from purely clinical study.
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