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Study of liver function tests

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Microscopically the liver is a simple organ yet its function is extremely complex; its relation and importance to general body metabolism appears to be something like the pituitary is to the endocrine glands or as the brain is to the nervous system---it is the liver which profoundly influences the complex mechanism of assimilation and utilization of our food. This utter simplicity on the one hand and the extreme complexity on the other suggests that man today is far from realizing the truths concerning the liver. Of the complexity, this author has little doubt; yet there are probably some concepts, as they are today, which will need to be altered in the future; it is this apparent simplicity which has been repeatedly referred to that the author has been unable to conceive as being the true picture. It is this extreme non-correlation of histology and functions that has stimulated particular interest in liver function.

Liver function tests are of benefit as an aid, first, in diagnosis of diseases of the liver, second, in the study of the physiology of the liver, and third, to some extent in study of extra hepatic metabolism. "Liver function test" may be construed to mean the following:

I. A laboratory procedure designed to test a specific function of the liver as is the hippuric acid test which tests the detoxification of sodium benzoate.

II. It may be a symptom as pain or a physical sign as jaundice which suggests, but does not conclusively prove there to be an obstruction.

III. It may be the clinical observation supported by pathological reports and paralled by other function tests that the liver is functioning above normal as when xanthines on a high protein diet is given to protect a liver against toxins as chloroform.

IV. It may be a complement fixation test which may indicate the presence of an organism whose disseminating presence may mislead the clinician but if and when proved to be present, may have profound influence on directing the type of therapy to be prescribed.

V. Finally it may be certain laboratory observations which someone has noticed to be consistently associated with known liver damage and such observation have been accepted as liver function test but with no or little understanding, as to how or why it work.

It is this extremely wide variety of tests which the author wishes to review, hoping to form a more decided opinion as to truth or fallacy of this simplicity of structure and complexity of function.

A knowledge of the anatomy and histology of the liver are essential for an understanding of the problem; it shall be assumed that such knowledge is common knowledge and only unusual anatomical and histological findings shall be mentioned from time to time as their importance shall arise in

this discussion.

A study of liver function tests naturally presupposes a knowledge of liver function. Table I is a list of functions ascribed to the liver; the list is by no means a complete one but has been compiled in hope of paralleling somewhat the concept that the clinician holds in regards to liver functions. It seems desirable also to attempt to classify liver function tests; a workable classification can be made roughly on the basis of the various functions of the liver that the tests are supposed to demonstrate. Table I also shows this correlation.

An ideal liver function test for the clinician should have the following characteristics; first, it should be a physiological test---that is, a test with substances employed which are normally found in the body and dealt with by the liver; second, it should by its use cause no increased pathology in the liver or organism; third, it should be agreeable to the patient; fourth, it should be easy to perform; fifth, it should be a test of a specific function; and finally, it should be accurate and denote a definite type of disturbance or lesion.

Carbohydrate Metabolism Tests

Carbohydrate metabolism tests are of greater value in throwing light on the normal physiology of the liver and the extra hepatic factors influencing carbohydrate metabolism than in detecting or denoting types of pathology in

TABLE I

Liver Functions	Tests related to said Function
I. Carbohydrate Metabolism	Glucose Tolerance Test Galactose Tolerance Test Levulose Tolerance Test
II. Bile Formation Composed of water, mucin, pigments, bile salts, fatty acids, cholesterol, lecithin, fats inorganic salts, urea, alkali and calcium.	Excretory 1. Icteric Index 2. Van den Bergh 3. Injected Bilirubin excretion 4. Urobilinogen 5. Bile Pigment of stools 6. Duodenal drainage 7. Blood Cholesterol and Cholesteral esters 8. Serum Phosphatase 9. Dye Excretion Tests 10. Blood Iodine Secretory 1. Bile-salt determination 2. Stool Examination for Fat. 3. Injected Cholic Acid
III. Protein Metabolism A. Amino Acid Synthesis 1. Glycine synthesis etc.	1. Guanidine-Blood level 2. Glycine Synthesis 3. Gelatin Excretion 4. Plasma Proteins 5. Albumin-globulin ratio 6. Tyrosinuria and Leucinuria 7. Blood Urea 8. Prothrombin
B. Fibrinogen production C. Heat production D. Purine Metabolism E. Hippuric acid synthesis F. Proteopexic function	Blood Uric acid Hippuric acid Hemoclastic crisis of Widal
IV. Fat Metabolism	
V. Detoxification A. Detoxification of 1. Toxins in disease 2. toxins in Uremia	Hippuric acid

Liver Functions	Tests related to said Function
3. Lactic acid	
4. NH_3	NH_3 concentration
5. Urea	Blood urea
6. Uric acid	Blood uric acid
7. Chloroform, strychnine, and others	
B. Detoxification by	
1. Deamination	
2. Oxidation	
3. Combination with:	
a. Glucuronic acid	
b. Sulfuric acid	
c. Amino acids	
(1) Glycine	
(2) Glutamine	
(3) Ornithine	
(4) Cysteine	Hippuric acid
(5) Clulathione	
d. Acetic acid	
e. Methol group	
4. Reticulo-endothelial activity---removal of particulate and colloidal matter	
a. Bacteria	
b. Dyes	
c. Bile pigment (only to a slight extent	
d. Foreign proteins	
5. "Excretion" in bile of	
a. Heavy metals	
b. Certain drugs	
d. Bacteria	
VI. Biochemical phenomenon(with vitamins)	
A. Utiliaation of	
1. Vitamins B,C,D,K.	
B. Formation of	Clinical observation
1. Vitamin A from carotine	
VII. Storage	
A. Carbohydrates	
B. Blood	
C. Copper and Iron	
VIII. Heparin Formation (a lipoid substance)	

Liver Functions	Tests related to said Functions
IX. Heat Formation	
X. Water Balance and Blood (volume regulation)	Fluid intake and output
XI. Regeneration	
XII. Blood formation in embryo: (hematenic principle in the adult)	Macrocytosis
XIII. Miscellaneous	1. Takata-Ara 2. Cephalin Flocculation 3. Sodium-d-lactate 4. Elimination of Na and NaCl 5. Microscopic view of sections 6. Porphyrins 7. Relief film of Esophagus 8. Wasserman 9. Cincophen Detoxification

the liver. The carbohydrates generally employed are levulose, galactose and glucose.

Galactose and levulose tolerance tests have a reliable basis but are generally reported as not satisfactory clinically. Experimentally they are accurate but there is a wide variation in the rate of dextrose utilization in normal individuals. Too many factors are involved in the metabolism of sugar in human beings to make the tests at all reliable. These two tests were originally based on the assumption that in individuals with hepatic dysfunction the rate of utilization of a predetermined amount of carbohydrate will be less than in a normal individual and that a greater amount will persist in the blood stream and will be excreted in the urine. In general this is true but it happens that the function of maintenance of normal blood sugar is conserved in spite of considerable liver damage (8). This is an interesting observation for it is the first reference to imply that all liver functions do not parallel one another.

Many modifications of this test exist but in general it is considered that a 40 gm. oral dose of galactose can be assimilated by a normal individual without the loss of more than 2.5 - 3 gm. of sugar in the urine in the five hours following its administration and that in conditions of hepatic dysfunction a degree of galactosuria above this

arbirary level is produced. In view of the fact that the kidney may have a threshold of its own, others have preferred to determine the sugar level of the blood following oral administration.

Modifications of the tests have been used, injecting the carbohydrate intravenously and evaluating the subsequent blood-sugar levels by repeated venous punctures. White (93) reported the intravenous galactose tolerance test to be of value in the differential of jaundice, especially when combined with the urobilinogen test; both remain positive for sometime after the signs of jaundice have disappeared, others (2) only further confirm that galactose tolerance maybe positive in catarrhal and toxic hepatitis and also in malignant obstruction. The test is too variable in cases of portal and biliary cirrhosis to be of reliable value. Snell and Magath call attention to the fact that the test is of no value unless the patient is visibly jaundiced. Hall (33) and others call attention to the fact that galactose clearance is impaired in infectious diseases indicating a secondary toxic damage to the liver and so suggesting that icterus in pneumonia and other infectious diseases is not one of hemolysis.

The clinical value of the levulose tolerance test has been more controversial than any other functional test of carbohydrate metabolism. Stewart, Scarborough, and David-

son (84) presented 135 cases of hepatic damage; the work of these men has been reported in table form and case reviews made of 70; a careful study of the table will reveal that approximately 60 of their patients not specifically referred to in case histories are exceptions to their generalizations and conclusions. Their work does however show the importance of taking a blood sample 30 minutes after injection rather than one hour and also shows that if the test is made for total blood sugar then diabetes mellitus may produce fallacious results; however, this objection can be overcome if the blood-levulose is determined, for it is thought that insulin does not control the first stage of levulose metabolism. Davidson, Kermack, (cited by Herbert and Davison) (38) has shown that insulin has no effect upon the first stage of levulose metabolism; these men found that when levulose and insulin were injected simultaneously into the rabbit, that the rate of removal of levulose from the blood was no greater than when levulose alsone was given. Then too Scott, Steward Scarborough and Davidson showed that administration of levulose to diabetic patients causes no abnormal rise in the blood-levulose level but it did lead to a rise in blood-glucose level. Herbert and Davison also present a case of acute pancreatitis, in which the blood-levulose curve was normal. Stewart, Scarborough and Davidson (84) also demonstrated that arteriosclerosis definitely alters the correct-

ness of their results for reason not too well understood; they also point out that the initial blood-levulose level may not be zero but they failed to show this in their table. Herbert and Davidson (38) who did consider this variable were able to set up normal values, and an abnormal level which makes the test more reliable as a test of liver functions. Best results seemed to be attained if 50 grams of levulose are administered and the blood levulose levels are estimated at 30 minute intervals after administration for a period of 2 - 3 hours. A normal curve will show a maximum rise above the initial level of not more than 15 - 20 mg. of levulose per 100 cc. of blood within an hour after injection and that at the end of the second hour the curve will show a fall to values below 10 mg. per 100 cc. Values above those stated are suggestive or indicative of hepatic dysfunction but to date the exact pathology is not to be predicted.

Snell and Magath (78) criticized the test as being invalid in mild diabetics but Snell's later work (75) on effects of thiamin chloride (B_1) would suggest that his mild diabetics are not due to hyperinsulinism but to lack of this vitamin in the liver. The invalidity of the test in chronic pancreatic disease as alleged by Snell awaits further investigation.

A study of the physiological basis of the levulose test reveals some interesting facts concerning carbohydrate

metabolism. It is a well-established fact that the liver is able to take up levulose and convert it into glycogen. Cori (15) compared the rates of formation of liver glycogen during the absorption of glucose and levulose; the rate of formation of liver glycogen during the absorption of glucose and levulose; the rate of formation of glycogen was the same for both sugars but the rate of absorption of levulose was only one-half the rate of absorption of glucose, so that the proportion of the absorbed sugar which appeared as liver glycogen was much greater in the case of levulose than in the case of glucose.

Cori and Cori (16) found that levulose is utilized far more rapidly when injected into the portal circulation than when injected into the systemic circulation, and that the rate of utilization when the sugar is given by the portal route is much the same as the normal rate of absorption from the intestine; both oxidation and glycogen formation in the liver contribute to the utilization of levulose. From the findings of Cori in 1926 and 1927, one can understand why the blood sugar rises faster with glucose than with levulose. In the case of glucose, the rate of removal is slower than the rate of absorption; and in the case of levulose, utilization keeps pace with absorption.

Bollman and Mann (7) has shown that levulose can be transformed to glucose in the absence of the liver, and

that the glucose is then available for the relief of hypoglycemia and for the formation of muscle glycogen---such a change takes place in the stomach and small intestine and no other tissue can use levulose directly other than the liver and the gastro-intestinal tract.

The glucose tolerance test is included here for completeness; it has little value as a liver function test. Glucose has the property as mentioned above of being rapidly absorbed; it is a renal threshold substance and markedly influenced by insulin.

Tests Related to Bile Formation

Of considerable importance to the clinician is the role played by the reticul-endothelial system of the liver ---that of removal of bacteria, foreign proteins, and dyes; and the role played by the parenchymal cells of excretion and secretion---secretion of bile salts and excretion of bilirubin, fatty acids, cholesterol, water, fats, lecithin, inorganic salts, bile salts, dyes, etc. The author wishes to point out that bile salts are both secreted and excreted and that bile pigments are removed by excretion and phagocytosis. An understanding of the processes of excretion and secretion will reveal that secretion of bile salts probably more nearly signifies a true metabolic process within the liver cell than does excretion of bilirubin. Furthermore the process of transporting these substances across

the cell membrane; both in and out of the cell in cases of secretion and excretion, must vary somewhat in rate. There is also the process of internal secretion and excretion of other metabolic products although at the time of this writing there seems to have been no important clinical or laboratory observations made of these phenomena. A consideration of these processes may add interest while reviewing liver function tests.

The Icteric Index Test

The icteric index test is an estimate of the amount of bile pigment in the blood not yet excreted; it denotes minor fluctuations in the intensity of the icterus which cannot be detected by clinical observation; is a simple and reasonably accurate test which demonstrates latent or sub-clinical jaundice. Snell and Magath (7) recommended curves to be plotted from the serial tests. Boyce (8) however calls attention to the fact that the test is not a true test of liver function.

Van den Bergh Test

"The Van den Bergh test is very useful to surgeons who understand what it is supposed to demonstrate and equally confusing to those who do not" (8).

Ehrlich found that when sulfanilic acid and sodium nitrite were added to a solution of bilirubin, a colored addition product, azobilirubin, was formed. This reaction

is specific and will detect bilirubin in a dilution of 1:1, 500,000. Van den Bergh precipitated the blood serum protein with alcohol, which also serves as a solvent for bilirubin, and showed the Ehrlich's reaction could be applied to this alcoholic extract. When a very small amount of bilirubin is present in the blood serum, with hemolysis as the source of th pigment, alcoholic extraction is necessary. This is the principle of the "indirect reaction". On the other hand, in obstructive jaundice with bile itself in the blood serum, alcoholic extraction is not necessary, as the characteristic color reaction appears at once on addition of the reagent. This is the "direct reaction". Either serum or plasm may be used for the test, but it must be clear and free from hemoglobin.

The underlying pathological process in cases of the direct reaction, is either an obstruction of the bile ducts or rupture of the canaliculi with necrosis of the liver cells with subsequent absorption and transfer into the blood stream of whole bile, which contains bile acids and cholesterin as well as bilirubin; these being the only two conditions which permit bile to escape from the canaliculi into the blood stream.

Snell and Magath (78) reports that one may expect a direct Van den Bergh reaction in 80% or more cases in which there is even a moderate degree of hepatic injury and in about

1% of all cases one may expect a direct reaction in which there is an absence of clinical evidence of hepatic injury. Snell and Magath believe it is better to determine the amount of both types of bilirubin in jaundiced patients rather than considering that all bilirubin was of the "direct" type.

Direct-reacting bilirubin is present in the serum under the following conditions; various types of toxic or infectious jaundice; physiological jaundice of the newborn, remaining at almost a constant level throughout the neonatal period; chronic parenchymous disease of the liver; mechanical obstruction to bile ducts by tumor, patent ductus venosus, stone, cicatrix, infectious lesions or extrinsic pressure, or tumors, granulomas, cycts and other lesions involving the liver substance.

The highest values for serum bilirubin (30 to 50 mg. per 100 cc.) are found in acute severe hepatogenous forms of jaundice and in neoplastic biliary obstruction; intermediate values (from 10 to 30 mg. per 100 cc.) are found in the milder degrees of hepatic parenchymal lesions and in conditions in which intermittent or partial biliary obstruction exists; the lower grades of bilirubinemia are found in subsiding acute "intrahepatic" jaundice, in very chronic forms of hepatitis such as those which sometimes follow biliary obstruction or prolonged intrabiliary in-

fection, and in the various forms of portal cirrhosis, in syphilitic hepatitis, and in infectious forms of cholecystitis without gross biliary obstruction.

The "indirect reaction" indicates that the bilirubin of the plasma has not been "regurgitated" from the canaliculi into the blood but represents pigment which the liver has not been able to remove from the blood stream. The underlying pathologic process may therefor be assumed to be an overproduction of bilirubin or a depressed excretory function of the liver, or a combination of these two states.

One encounters "indirect reations" in the following conditions: pernicious anemia, familial hemolytic jaundice, acute hemolyic anemia, sickle-cell anemia, paroxysmal hemoglobinemia, transfusion of the wrong type of blood, phenylhydrazine poisoning, cardiac decompensations (especially in the presence of gross pulmonary infarction), hemolytic septicemia, malaria, blackwater fever, lobar pneumonia and icterus neonatorum. It is well to notice that most of these conditions are associated with an alcholuric jaundice and that high values for bilirubin are rare unless the liver is also injured.

Snell and Magath (78) reports the Thannhauser Anderson modification of the van den Bergh's original method; the report gives the total bilirubin present and its reaction (direct or indirect). For practical purposes it may be considered

that the bilirubin ordinarily present in the serum give the "indirect reaction" and may exist in amounts varying from 0.1 to 2.0 mg. per 100 cc., according to the method of determination. In hemolytic icterus and in the hemolytic anemias, the reaction is indirect and rarely exceeds 6 - 7 mg. per 100 cc. of serum. Whether this indicates an impairment of liver function is not entirely clear although associated visible hepatic injury of significant degree is rarely demonstrated; if the value for the indirect reaction reaches a level of more than 4 mg. per 100 cc. of serum it may be safely assumed that the function of the liver cells has been at least functionally impaired.

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,--- a fact thought to be important but not 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 pathologic changes become so great that the cells of the liver are forced to return some of the pigment. In this way high values of the indirect-reacting bilirubin tend to give way to the presence of a direct-reacting bilirubin. This is typically seen in jaundice due to arsphenamine, in which the

van den Bergh is indirect at first but later is direct.

In some cases it is found that in place of the direct reaction appearing immediately as it should, it takes some little time to develop. This has the same significance as the "indirect reaction", and is given by the hemolytic type of bilirubin such as familial (sperocytic) hemolytic jaundice and sicle cell anemia (with related bone changes) (8). The biphasic van den Bergh reaction as this phenomenon is called, can occur then as the result of a high degree of pigment retention, without regurgitation, by obstruction or necrosis of the liver cells.

Rich (67) has argued that an increase of bilirubin in the blood in cases of excess production does not depend upon that factor alone but also an associated disturbance in the excretory function of the liver cells---in part this is the same as saying that a pure form of one or the other type of jaundice does not exist.

Rich has attempted to explain the direct and "indirect" reaction in a different manner. He says that bilirubin at the pH of blood gives the direct or immedicate reaction, but in normal plasm it gives the "indirect reation" because it is absorbed by the plasma proteins and as a result the pigment is prevented from reacting promptly with reagent. When substances as bile salts and cholesterin, which are present in samll quantities in blood and are increased in the blood

in various forms of regurgitant jaundice, are added to the plasm before bilirubin is introduced, or are added at the same time as the bilirubin, the Van den Bergh reaction remains direct apparently because substances which lower the surface tension seem to be absorbed by the proteins more readily than bilirubin. When the protein molecules are covered with such substances, the pigment remains free.

Rich offered a new classification of jaundice into retention and regurgitant varieties. In the retention type the bilirubin circulating in the blood stream is only partially excreted by the liver. The remainder is accumulated in the blood, where it is bound, probably to the plasma proteins, and therefore does not pass through the kidneys into the urine. This type of jaundice is associated with mild degrees of liver injury, chiefly cloudy swelling and cell atrophy, and is a secondary complication of such diseases as hemolytic anemia, congestive heart failure, pneumonia, and other conditions characterized by anoxemia and fever. In such diseases the jaundice is due partly to an increased formation of bilirubin and partly to a depressed excretory function of the liver. The Van den Bergh reaction is indirect.

In the regurgitant type of jaundice the bilirubin circulating in the blood stream is excreted by the liver cells and escapes into the tissue spaces and blood sinusoids of

the liver because of the rupture of the bile canaliculi. The larger bile ducts are obstructed, or the liver cells are necrotic, or both conditions occur in combination, and as a result both bilirubin and whole bile escape into the blood stream. The bile pigment is not bound and therefore can be excreted by the kidneys into the urine. This type of jaundice is associated with the major hepatic disorders and with the mechanical obstruction of the extrahepatic bile ducts. The Van den Bergh reaction is direct.

Among other respects in which obstructive bilirubin differs from hemolytic bilirubin is the curious fact that the kidney threshold for the two forms is entirely different (9). The former appears in the urine in any ordinary case of obstructive jaundice. In general terms it may be said that tinging of the skin and sclera occurs when the bilirubin in the blood reaches a concentration of 1 in 80,000. Bile appears in the urine when the concentration is slightly higher about 1 in 1:50,000. Hemolytic bilirubin does not pass into the urine until the concentration becomes very high. Van den Bergh is of the opinion that the bilirubin of a pure hemolytic jaundice never appears in the urine, perhaps because it is insoluble in the urine, the presence of bile in the urine always indicating that an obstructive factor has been super-added.

Theoretically this test is an excellent method of diff-

erential diagnosis of types of jaundice but actually a pure type of jaundice does not exist. It is one of the less sensative tests of liver function though Boyce, confirming the work of Snell and Magath, states that a moderate or marked degree of liver damage is present in 75 - 85 per cent of the cases in which the reaction is direct, and in a rather smaller number of cases in which the reaction is indirect.

Flint (27) in using the Van den Bergh reaction checked the results with histologic studies of biopsied sections of the liver and found it to be less accurate than the levulose tolerance test when it was considered purely as a test of liver function.

Having now determined the reaction of the patient's serum to the Van den Bergh test and the degree of bilirubinemia, one may now say more in regard to the icteric index for one still has to consider the practical problem of daily variations, since the jaundiced condition is rarely static. "This may be done by the continued use of the tests which have been mentioned or by the comparison of the color of the serum with that of a standard solution of potassium dichromate, in order to determine the so-called icteric index. The results of the last method, while not strictly comparable to the results of chemical determination of bilirubin, may be clinically useful. Plotted as a curve such determinations maybe of some diagnostic and prognostic value(78). In com-

plete biliary obstruction due to neoplasm, a rapidly rising curve is the rule, especially is the gall bladder 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 so 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 obstruction, a high plateau curve results; later, a gradual fall in the value for the bilirubin may occur. In any large group of cases of jaundice one recognizes certain other types of curves which are clinically significant. Acute "intrahepatic" forms of jaundice produce a rapid rise and an equally rapid fall in the values for the bilirubin; the longer the peak values are maintained, the slower is the decline. In chronic parenchymatous disease of the liver low irregular curves are the rule, exactly as they are in cases of long-standing biliary obstruction; in either instance, episodes of rapid degeneration of liver tissue may be marked by sustained rises in the values for the bilirubin. In general, falling curves for the values for bilirubin signify restored patency of the bile passages of 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 obstruct-

ion, a rapidly degenerating hepatic parenchyma or a combination of the two."

The Injected Bilirubin Excretion Test

The bilirubin test is an index of the excretory function of liver with respect to a substance which is normally present in the body and normally excreted by the liver; it is thus a physiological test. The test is obviously useless in frank jaundice in which an inability to handle the bilirubin already in the organisms has been demonstrated.

The test was originally described by Von Bergmann and by Eilbott in 1927 (cited by Boyce); A sterile solution of chemically pure bilirubin was injected into the blood stream in order to bring about a transient elevation of the icteric index. The ability of the liver to handle the injected bilirubin was determined by measuring the amount present in the plasma before injection and at regular intervals afterwards. The test was very satisfactory excepting that the technique itself was very complicated and too the cost of bilirubin was high. The technique was modified by Jankelson and Gargill (42) by substituting serum for plasma. The cost has been reduced but is still high if the test is run serially.

The modification by Jankelson and Gargill of the bilirubin test is as follows: The patient is prepared as for a B.M.R. and the test is done in the early morning; 10-15 cc. of blood are withdrawn from the anticubital vein to be used

as standard for the patient at that particular time. As freshly prepared bilirubin solution (1 mg. per kg. of body weight in 5% sodium bicarbonate solution) is slowly injected throught the same needle as above. Five minutes later 10 - 15 cc. of blood is withdrawn from the vein of the other arm. The patient gets neither food nor drink during the next two hours and fifty-five minutes and then another sample of blood is withdrawn. The serum of the second sample shows the highest elevation of the icteric index and the third shows the ability of the liver to eliminate bilirubin.

The bilirubin indices of the three samples are determined by colorimetry, which depends upon matching the intensity of light on two sides of a disk, or by scoptometry, which depends upon disappearance of a colored target upon the introduction of graded smoked glass; the later supplies a more definite end point and is therefore less time consuming. In the normal individual this formula gives a reading of 20-30 per cent or less; a reading above 30 per cent indicates hepatic dysfunction.

This test has for its basis at least an assumption that bilirubin will be excreted if liver function is present; it seems that no recognition is made of the accepted physiological phenomenon that secretin stimulates bile production. Because of this phenomenon, one can understand the importance of taking the test at the same time of day and under controll-

ed conditions.

It might prove interesting to use the test under controlled test meals, and one might even go so far as to perform the test with separate test meals high in carbohydrate, protein, and fat respectively.

Berman and associates (4) (5) were interested in the effect of choleresis (formation of bile as an excretory rather than a secretory process) on the rate of excretion of intravenously injected bilirubin (4). This question is of interest because sodium dehydrocholate is used clinically for such a purpose as though there were increased excretion, even though it is known that under normal conditions in the dog, the production of a choleresis by bile salts does not increase the total daily output of bile pigments in the bile; only in the anesthetized animal is there any effect and then there is merely a trend to restore the clearance rate which had been retarded by the anesthesia (23). The question is of further importance because pure bile for intravenous injection has different solubility properties from "bilirubin" in bile. Berman et. al. have cited that German workers have reported both an increase and a decrease in serum bilirubin in patients following injection of sodium dehydrocholate (Decholin-Sodium). They conclude that in the dog injection of sodium dehydrocholate or whole dog bile does not increase the rate of removal of

intravenously injected bilirubin. This same group of men along with Atkinson and Hough (5) later conclude that oxidized unconjugated bile acids, dehydrocholic acid (Decholin), ketocholanic acids (Ketochol and Kebilac) produced a marked hydrocholeresis, or an increase in the output of bile having an increased water content and a decreased content per cc. of cholates, non-volatile solids, and a decreased viscosity. Also the administration of "natural Ox-bile salts" (conjugated cholates) increases the output, but not the natural synthesis of cholates in the bile; the administration of bile acids again did not significantly affect the bile pigment.

Harrop and Barron (37), using their modification, performed the test on 10 normal individuals and found no retention of the injected pigment after four hours. In eight cases of various types of hepatic dysfunction they performed the bilirubin, bromsulphalein, and levulose tolerance test; the bilirubin test was positive in all instances, while only one result was abnormal with each of the other tests. In seven cases of chronic anemia where liver disease was suspected, the excretion of intravenously injected bilirubin showed the existence of impaired liver function in six instances, while both the bromsulphalein and levulose tolerance tests were entirely negative.

Soffer (80) reports one hundred bilirubin tests, twenty-

eight were done on normal individuals. In fifteen of these there was no retention of the injected pigment after four hours, while in eleven there was a retention of from one to three per cent and in two from five to six per cent. He concludes that retention of from five to six per cent after four hours is the upper limit of normal.. He supports the opinion of others that this is by far the most delicate of any single test that is used to detect impaired hepatic function for while other methods yield satisfactory results where damage is severe and diffuse, they are unsatisfactory in mild instances of hepatic diseases in contrast to the bilirubin excretion test which will show a high incidence of abnormal results in this type of case.

Urobilinogen Test

Wallace and Diamond (cited by Clute) (14) devised a test by which 1 cc. of Ehrlich's aldehyde is added to 10 cc. of urine and the test tube is warmed. A rose color appears if urobilinogen is present, and the amount is determined by dilution at which the reaction is still present.

Opinions as to the value of this test varies widely. The test is based on the premise that urobilin, in the intestine, is converted completely to bilirubin if liver function is normal, and incompletely if it is not normal and in the latter case is spilled over into the urine as urobilinogen.

Killian cited by Elman and McMaster (24) pointed out that

the rate of excretion of urobilinogen varies at different periods of the day, as well as from day to day, in jaundice and allied conditions, which makes it necessary to run the test over a twenty-four hour period as well as for consecutive days and that the test should be considered negative unless the reaction had persisted for five consecutive days ---which is too long a period to wait for information about a seriously ill patient.

If urobilinogen is present in dilutions greater than 1:20 it maybe assumed that liver damage is extensive. Patients with icterus due to cholelithiasis show only a slight increase in the amount of urobilinogen in the urine, except when complicating factors such as acute cholangitis, biliary cirrhosis, among others are present. The rise in the urobilinogen caused by these complications is dependent on the degree of associated biliary obstruction and the consequent amount of urobiliogen formed and reabsorbed from the bowel. Jaundice due to stone is rarely accompanied by complete biliary obstruction so that persistent values of less than 5 mg. urobilinogen in the feces per day or traces of urobilinogen in the urine are almost never found in jaundice due to cholelithiasis with obstruction and dilatation. The urobilinogen values in the stool may, also fluctuate from day to day (82).

Meyer-Betz cited by Soffer (80) pointed out in 1913 that

uribilinuria is found only at the beginning and at the end of the course of catarrhal jaundice; this is probably due to the fact that during the height of the disease the parenchymal damage is extensive enough to distort the continuity of the bile canaliculi producing an actual obstruction so that no bile enters the larger bile ducts. At this stage the van den Bergh reaction will be direct and 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 excretion of bile into the gastro-intestinal tract with a marked increase in the urinary excretion always complete by a process of obstruction and constriction so the amount of urobilinogen in the feces drops to below 5 mg./ day and the urine shows no urobilinogen or only traces of it. An exception to this may be the jaundice due to carcinoma of the Ampulla of Vater, in which cases intermittent urobilinogenuria is found.

Icterus due to hepatitis is usually associated with a marked increase of urobilinogen in the urine. The height of the increase varies, dependent on the degree of hepatic damage, although urobilinogen is not always elevated in the presence of other signs of liver disease. In some instances of severe toxic hepatitis, particularly in those due to arsenical intoxication and in cases of obstruction cirrhosis, the urobilinogen in both the urine and feces may temporarily

drop to very low levels---simulating but not actually imitating a complete obstruction on a neoplastic basis. In the simple hepatitis group, the fecal urobilinogen values are usually low except in cases where there is the added factor of increase blood destruction in which case the amount of urobilinogen in the feces is also increased.

"In hemolytic jaundice, the amount of urobilinogen in the urine is only slightly increased but in the stool it is greatly increased. With improvement, the amount of urobilinogen in the feces drops to high normal values. In some patients with hemolytic jaundice, there may be an associated hepatic dysfunction as has been demonstrated by other liver function tests." (22)

Urobilinogen is in rare instances formed in situations other than the intestinal tract and independent of bacterial action. There has been reported a case in which large quantities of urobilinogen was present in urine and a sterile ovarian cyst containing an old blood clot and a high percentage of urobilinogen was revealed by operation. When the cyst was removed the urobilinogen disappeared from the urine. In a few cases, bacteria in the bile ducts has converted bilirubin into urobilinogen (80).

Sparkman (cited by White later devised a method which he claims is more nearly a quantitative measure of urobilinogen in urine specimens. Since urinary output of urobil-

inogen may be variable throughout the day, a chance specimen of urine from a patient with hepatic dysfunction may contain normal amounts of urobilinogen and the test may therefore fail to detect an actual significant increase in daily urobilinogen which has undergone oxidation to urobilin during the period of collections is reduced back to its original form prior to estimation.

White et. al. (96) compared the three methods of estimating urobilinogen in the urine and concluded that the Watson method is superior to other more simple procedures in the evaluation of this liver function. However if serial tests are run, and such should be in cases of jaundice, the other two tests, which are easier, are satisfactory.

Lyon and Meltzer (cited by Judd) have used a method of duodenal drainage to determine bilirubin excretion but is more difficult to perform and is less accurate except in case of complete obstruction with absence of bile in which case the method may be of significance (45).

Ivy and Roth (41) however hold a different attitude for they state, "The examination of duodenal drainage, before and after the introduction of 50 cc. of a saturated solution of magnesium sulfate, for enzymes, bile pigment, bile salts, pus cells, crystals, epithelium, bacteria and parasites will yield more diagnositic information of significance than the more commonly employed gastric analysis.

However, to obtain this information considerable careful chemical and microscopic work is required and the procedure and work has to be conducted by an experienced person, one who knows more physiology and pathology than the ordinary technician. This fact, unfortunately, limits its practicality."

The presence of absence of bile pigment is of obvious diagnostic importance as is an estimation of its amount. The presence or absence of bile salts, and the amount, is important because the liver can eliminate bile pigment when it is not able to synthesize or excrete bile salts. Cholesterol and calcium bilirubinate crystals provide information regarding the existence of stones in the duct or gall bladder when the cholecystographic method fails. Blood, in the absence of a duodenal ulcer, may assist in the recognition of an ulcerating carcinoma involving the ampulla. The co-existence of cirrhosis and choleithiasis and pain in the upper abdomen is not uncommon. In cirrhosis a non-visualizing gall bladder may be of no significance because of inadequate elimination of bile, and a crystallographic study is necessary for the diagnosis of calculi. In a number of early cases of cirrhosis without calculi, but with mild jaundice and upper abdominal complaints, a flow of bile containing pigment and bile salts will prevent the patient from being subjected to an exploratory operation. The presence of an abundance of epithelial debris, pus cells and bacteria may indicate catarr-

hal inflammation of the duodenum or bile passages (cholangitis). Lightly pigmented bile and no crystals are found in the duodenal contents in acute hepatitis. A simultaneous study of the enzymes with or without the secretion test may reveal important information regarding the pancreas.

Duodenal drainage however is not without limitations for considerable skill and care is required in the technic of duodenal intubation. It is well to check the position of the tube fluoroscopically. Often the patient is too ill to be bothered with manipulations necessary in duodenal intubation. Pernicious anemia, gastric anacidity and disease of the pancreas must be considered in the interpretation of an abundance of pus cells and bacteria in the duodeno-biliary drainage.

It is generally believed that there is a substance in the intestinal mucosa (called prosecretin) which when acted upon by HCl of the stomach, or possibly by the bile, produces a substance called secretin which is absorbed by the blood stream and acts upon and stimulates the pancreas (40). Secretin has also been alleged to stimulate secretion of bile and so theoretically this factor may have some influence on the validity of the secretory and excretory tests of the liver, as has been mentioned previously.

Fat and Fat Metabolism

It has been known for many years that in the presence of

parenchymatous disease of the liver, or even following acute toxemias and infections, the amount of fat in the individual hepatic cells is greatly increased and the glycogen content is diminished at the same time. This does not necessarily imply that the liver is directly concerned in the regulation of fat metabolism, although some indirect connection of this sort cannot be excluded. In cases of poisoning due to phosphorus or chloroform, the amount of fat in the liver is much increased. Where excess food in the form of fat is given, relatively large quantities of fat concentrate in this organ. The same is true during starvation, when the fat reserves of the body are called into play. In either case, the total fatty acid content may increase from 3 to 20 per cent.

If one considers the route of entrance of fat into the body one finds that normally 60 - 70 per cent of the fat is absorbed by the lacteals and carried directly to the thoracic duct; whether this phenomenon is functioning solely for the process of assimilation of fats or whether this amount of fat is purposely shunted around the liver, at least for the "first trip" is a point which seems to be obscure in the literature. The author mentions this observation as an academic curiosity but which in the future may prove to be significant in the role of the liver in fat metabolism.

Tests for Cholesterol and Cholesterol esters

Cholesterol occurs in the plasma in two forms, the free

form as a sterol and the combined with fatty acids as esters. The normal values for blood cholesterol are usually set at 165 to 200 mg. per 100 cc. of plasma and cholesterol esters at 100 mg.

There is evidence which indicates that the liver possesses the power of removing cholesterol from the blood and storing it. Esterification of cholesterol with fatty acids is accomplished in the liver. Boyce believes that changes in the ratio are more important than changes in the concentration of total cholesterol, since the esterification of cholesterol esters from cholesterol and high fatty acids is accomplished in the liver. Green, Holz and Leahy (30) found that patients with evident hepatic damage had decreased combined cholesterol and that at times there is a decrease in total cholesterol. They state that "the ratio between cholesterol and cholesterol esters is not as diagnostic as the amount of esters". Progressive decrease in cholesterol esters in hepatic disease is a poor prognostic sign. Since it is a function of the liver to esterify cholesterol, it would seem that the statement of Boyce is more nearly correct.

Epstein and Greenspan (25) observed and studied more than 500 proved cases of hepatic and biliary tract disease and found that in obstructive jaundice a hypercholesterolemia is usually encountered, that both the free and the ester fractions were raised and that these findings paralled the degree

of hyperbilirubinemia. When the obstruction is released and the jaundice is decreased the cholesterol returns to normal.

It is important to notice if one uses this test that the usual rise does not occur in longstanding biliary stasis, superimposed infections of the biliary passage, cachexia and other complications; also in cases of acute hepatic degeneration the rise in cholesterol does not parallel the rise in bilirubin and the cholesterol values may remain normal or even subnormal. In cases of acute yellow atrophy the cholesterol value maybe greatly lowered in acute hepatic degeneration. In atrophic cirrhosis of the liver the cholesterol partition of the blood remains normal unless jaundice occurs due to acute hepatic degeneration or cholemia when the cholesterol values behave as cited above. In cases of cholecystitis and cholelithiasis, when obstruction and infection of the biliary passages are not present, the blood cholesterol values are not significantly altered. In rapidly fatal cases of severe hepatic damage the cholesterol ester values are low or zero throughout the illness whereas in mild cases the initial values are moderately depressed but return to normal with clinical improvement.

Increased cholesterol values are found in chronic and acute nephritis and such findings are significant in chronic nephritis which is accompanied by a decrease in blood urea. In diabetes, there maybe a increase of cholesterol which is

reduced with the use of insulin. In lipemia the values are high and are also elevated in pregnancy and hypothyroidism; whereas hyperthyroidism is characterized by low blood cholesterol (86).

Steiner and Turner (83) in a study of 19 patients with pneumonia showed there was a hypocholesterolemia during the febrile stage and this decrease was largely due to a decrease in the ester fraction. Hypercholesterolemia due to both fractions were noticed during days of convalescence. The number of liver function tests performed during these periods were too few to show any parallelism with decreased liver function. Epstein and Lande (25) demonstrated that blood cholesterol is high when the basal metabolic rate is low and that it is low when the basal metabolic rate is high. However Mason, Hunt, and Hurxthal (55) concluded that there was no definite correlation between cholesterol and basal metabolic rates.

Gutman et. al. (32) warns us that some cases of hepatotoxic jaundice the cholesterol values rise and may mislead one to believe there is obstruction however the values are raised at different times than in obstructive jaundice; in case of arsphenamine jaundice the rise occurs after icterus has been present for a long time and even persists after jaundice has subsided.

Serum Phosphatase Test

Roberts (69) devised a test of serum phosphatase in 1933; phosphatase activity is expressed as the number of mg. of inorganic phosphorus liberated per gram of tissue from Beta-glycerophosphate after 48 hours hydrolysis at optimum pH. (9.0) and a temperature of 38° F. Phosphatase is found greatest in ossifying cartilage, less in bone and absent from resting epiphyseal cartilage and other non-ossifying cartilage; present in teeth of young animals; milk (and floral parts of plants) (6). Bone is apparently the main if not the sole source of plasma phosphatase since there is no change when certain organs are removed.

Kay (46) has presented the theory or concept of the mechanism of the serum phosphatase test; he believes that "alkaline" serum phosphatase originates for the most part in bone-producing cells because the bone possess high phosphatase activity; the phosphatase is then taken up by the circulating blood, carried to the liver where he believes it is secreted in the bile for it is known at least that bile does contain phosphatase and is believed to have been excreted from the blood. Roberts thought the phosphatase levels was elevated above a level of 10 units in obstructive jaundice and remained below 10 units in hepatocellular jaundice. Freeman, Chen, and Ivy (28) in experimental studies on serum phosphatase concluded that it is elevated in all forms of liver in-

jury and obstruction, whether or not jaundice is present. Rothman, Meranze, and Meranze (74) found the serum phosphatase test to be satisfactory but considered it to be limited value because pure cases of obstructive and of hepatocellular jaundice do not exist---at some stage in each disease one type assumes the characteristics of the other and so they recommend that the test be run in combination with the serum bilirubin test. Cantarow and Nelson (12) found a wide overlapping of range of values in cases of obstructive and hepatocellular jaundice and so considered the test useless; however, the discrepancy probably lies in their not recognizing the overlapping of characteristics of the disease as pointed out by Rothman and his associates.

Gutman et. al. (32) has surveyed the literature and concluded after a study of a wide variety of diseases that serum phosphatase is appreciably and consistently elevated in only two pathological states: (1) diseases of the skeletal system in which there is active widespread formation of bone or cartilage; (2) diseases of the biliary system and liver in which there is impingement of the biliary tract either intrahepatic or extrahepatic. Now the presumptive cause of increase serum phosphatase in skeletal disorders is that of increased formation of the enzyme, this increase has been experimentally proved. The presumptive cause of increase phosphatase in obstructive hepatic disorders is a re-

sult of retention due to blocking of the excretory channels

It is reasoned that other factors than retention may account for increased phosphatase activity in different patients having complete and protracted obstruction of the common bile duct; in their cases of carcinoma of the head of the pancrease values ranged from 9.6 to 113.1 Bodansky units per 100 cc. One possibility for this variation might be other methods of disposing of serum phosphatase but this has not been proved. Phosphatase in bone and other solid tissues is reduced when large amounts of irradiated ergosterol are administered. This causes a withdrawal of calcium from the bone but small doses increase the phosphatase level of these tissues. Diseases dausing extensive changes in bone structure are accompanied by an increase in plasma phosphatase.

Thannhauser (85) theorizes that increased phosphatase is due not to increase concentration of this enzyme in the blood but due to the effect of an activator; the theory may be correct but his observation that ascorbic acid activates serum phosphatase (the basis of his phyothesis) has been disproved by King and Delory (47).

Still another theory as to changes of serum phosphatase in disease was proposed by Bodansky who showed that nutritional factors may influence the "alkaline" phosphatase and he raised the question as to whether or not elaboration of phosphatase by the liver itself might not influence the

phosphatase serum level.

Gutman et. al. (32) found as did Bodansky that by the Bodansky method of determination the normal range was 1-4 Bodansky units. Phosphatase activity was definitely increased in every one of their cases of obstructive jaundice; in 49 cases the values ranged from 10.9 to 113.1 Bodansky units. In the 5 cases with values less than 10.3 were found to have calculi in the common duct with incomplete obstruction; one case was typical of carcinoma of the head of the pancrease with complete obstruction and the other case was that of carcinoma of the proximal end of the common duct. They conclude that gross obstruction of the common bile duct quite regularly results in markedly increase serum phosphtase activity, the exceptions occur chiefly with incomplete or intermittent obstruction. Interposition of masses in the liver substance (tumor nodules, liver abscesses, etc.) that might block off major intrahepatic biliary passages likewise leads to elevated serum phosphatase values.

Post-operatively in cases of obstruction the serum phosphatase will return to normal if complications do not follow. Gutman et. al. related a cholecystectomy case who drained intraperitoneally and which showed for a time a normal serum phosphatase but an increased (5.2 mg%-total) bilirubin; then an external biliary fistula was produced and there developed symptoms of cholangeitis in which there was

then decreased bilirubin and increased serum phosphatase; as the febrile attacks gradually subsided, the serum phosphatase values began to fall.

In their second group, those with typical "catarrhal" jaundice, all showed increased values for phosphatase but 36 of these 41 ranged below 10 Bodansky units; the other 5 showed values to exceed 10 units---one of these was a 13 year old girl with a serum phosphatase value of 13.1 which is normal or nearly so in that age group. They conclude that "catarrhal" jaundice usually causes relatively little increase in serum phosphatase activity as compared with the degree of bilirubinemia---not a greater increase in phosphatase than might be due to varying intrahepatic obstruction of the finer biliary radicles.

The Bodansky units may exceed 10 in cases of "physiological jaundice" of the newborn but for this age group the value is not increased and then the very age of the patient would suggest the type of jaundice. Children will normally run higher serum phosphatase values than adults.

In their group of 26 patients with jaundice following exposure of hepatotoxic agents (arsenial therapy, cincophen, sulfanilamide, phosphorous, sulfapyradine, and carbon tetrachloride all showed increased serum phosphatase values with more than 50% of patients with values above 10.

It is significant to notice that Hanger (cited by

Gutman et. al.) found the cephalin-cholesterol flocculation test to be negative in patients with arsphenamine jaundice yet the serum phosphatase values were increasdd. The serum cholesterol values were also increased in this group and so misleading as is pointed out in the discussion of the test. They conclude that the varying phosphatase levels in this hepatotoxic group are indicative of the possibility of intra-hepatic biliary obstruction.

Gutman et. al., although they believe serum phosphatase test to be consistently elevated in case of biliary or obstructive cirrhosis, make no claim of consistency for the test in other types of cirrhosis. They refer to fatty degeneration and cirrhosis as "alcoholic" cirrhosis; and cirrhosis with no fatty degeneration as Laennec's. They refer to 3 cases of "cardiac" cirrhosis, 3 cases of hemochromatosis, 2 cases of "toxic" cirrhosis associated with hyperthyroidism and 1 case of schistosomiasis and 2 cases of "alcoholic" and Laennec's cirrhosis; the highest value observed was 20.3 Bodansky units and in six patients with values above 10 units there were 3 patients who failed to consistently show this elevation above 10 and that in even advanced cases of these types of cirrhosis there may be but little rise in serum phosphatase activity. When disorganization of the biliary tract, with jaundice, occurs in Laennec's cirrhosis (due to complications like infection or neoplastic degener-

ation), the serum phosphatase level rises.

Woodard and Craver (98) in a survey of 53 cases of Hodgkin's disease, 28 of lymphosarcoma, 26 leukemia and 8 miscellaneous lymphomatoid disorders found the alkaline serum phosphatase to be frequently elevated in Hodgkin's disease, but less often in lymphosarcoma and the leukemias.

Elevated serum phosphatase values occured in many patients having symptoms resulting from metastasis and referable to the bones, although no changes were demonstrable roentgenographically. Some of these patients later developed demonstrable lesions. A few cases of these diseases with no bone symptoms showed an increased phosphatase (avvalue above 5 Bodansky units).

In conclusion, then, the test maybe of value in the following cases:

1. In the relatively early detection of liver or skeletal metastasis in patients known to have malignancy.
2. In anticipating certain complications following surgery of the biliary tract.
3. In ruling out obstruction of the extra hepatic biliary tract, and improbable cause of jaundice in patients with serum phosphatase values less than 10 Bodansky units per 100 cc. When applied to the differential diagnosis of jaundice, the following sources of error should be recognized.
   (a) Unspecificity, as certain skeletal disorders increase serum phosphatase activity.
   (b) Inapplicable to jaundice in children, particularly in congenital atresia of

the bile duct.

(c) Overlapping of values in obstructive and hepatogenous groups of jaundice (about 10% or even more in either direction).

The test has a distinct and obvious advantage over the dye retention tests which are inapplicable in jaundiced patients.

For clarification it is well to mention in passing that an acid phosphatase exists which may also be found in the serum. No attempt has been made to include in this presentation a list of all the tissue where acid phosphatase is found; it is however found normally in prostatic tissue and will usually show increased "acid" phosphatase activity in cases of metastasizing prostatic carcinoma; one female with advanced Paget's disease showed values to exceed 4 units of "acid" phosphatase activity (3). Acid phosphatase has been found in the liver, spleen and kidney (Bamann and Reidel cited by Gutman and Gutman).

Dye Excretin Tests

Although a large number of dyes have been used to test the excretory function of the liver, only two, bromsulfalein and rose bengal, have come into common use.

Rowntree and his co-workers first use phenoltetrachlorphthalein intravenously but with unfavorable results; H. L. McNeil then tried to use this substance and with duodenal drainage determine the time required for the dye to appear in the bile and determine the amount which could be recovered

by duodenal drainage but the results were not too variable. Rosenthal then injected 5 mg. of dye per kilogram of body weight and finally (73) substituted bromsulfalein for phenoltetrachlorphthalein.

The bromsulfalein clearance test may be confusing to the clinician because many variations in the amount of dye has been reported in the literature (2 to 5 mg. per kilogram); then too the time intervals at which sample of blood are with drawn have varied (5,30, and 60 minute intervals, and serially every 5 minutes for half an hour). Furthermore the values for normal retention after thirty minutes have varied from zero to 10 per cent.

Magath, using 5 mgm. per kilogram of body weight and taking a single sample at the end of one hour, found that a retention of the dye occurred in 96 per cent 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. He observed that low grade retention of 4 - 12 per cent was significant; such is an aid clinically for it is at this stage that the phyicians clinical judgment is uncertain. "Occasionally retention of a low grade does occur without any evidence of hepatic disease but this is rare." (78)

Macdonald (51) has pointed out that in one patient the liver may excrete the dye in a period of five minutes be-

cause its reserve is high where as in another patient the liver may have excreted the dye completely in a period of thirty minutes but this liver may have employed its entire reserve for the full thirty minutes and so the doctor maybe misled into thinking there to be a hepatic reserve which does not exist. He proposes a plan of determining the rate at which the liver takes the dye out of the blood by examining the blood at specified shorter intervals and from these results plot a graph which can be compared with a standard based on the average determinations of a number of supposedly normal individuals.

The exact procedure of the Macdonald method is described as follows: An intravenous needle of short bevel and 18 or 20 gauge is introduced into a suitable vein, the syringe is withdrawn and a 3-4 Luer valve is attached to the needle in the vein. To this valve is connected a tube leading from a flask of saline or citrate solution which can, by the valve, be directed into the vein or out through the opening which holds the syringe. The handle in the third position allows direct suction through the needle and valve to the syringe into which blood is sucked, the syringe is detached and the fluid is first directed through the open end, which will prevent clotting in this portion, and then continued into the vein for a two minute period. This prevents clotting in the needle. When it is determined that the fluid is dripping at

the proper rate in the vacuum tube and that the valve is working properly, two mg. of bromsulphatein per kilograms of body weight is injected slowly over a period of one minute into another vein and the first specimen of blood is drawn off in exactly two minutes. The test is continued over a thirty minute period. The blood is deposited in a clean dry test tube and allowed to clot, the other specimens are centrifuged, and the clear straw-colored serum is examined to determine the percentage of dye retention in that particular specimen. The estimations should not be in numerical order, as this unconsciously tends to determine the next value. The values are then plotted on the graph and joined to produce the curve.

Ivy and Roth (41) list other limitations as the following: (a) There is evidence which indicates that the removal of bromsulfalein from the blood stream is a function of the entire reticulo-endothelial system of which the Kupfer cells of the liver are only a part. (b) The difficulty of determining accurately the degree of retention in highly icteric sera impairs the test for comparative purposes. (c) According to Snell and Magath, the bromsulfalein test is not applicable in cases in which the value of serum bilirubin is more than 5-6 mg. per cent since the element of mechanical obstruction confuses the clinical picture when the values for the serum

bilirubin are higher than this. (d) Vascular stasis will produce a false positive.

Cutler (17) reported on 75 patients who had been submitted to this test and subsequently were operated upon and more than one-third had a marked urticaria, vomiting, fever or all of these complications. Some of them had dye retention as high as 60% at the end of fifty minutes and 40% at the end of sixty minutes; there was one death due to pneumonia and nine had stormy post-operative courses. Boyce doubts the statement of Cutler that the test is of no value but points out that careful preoperative preparation might have overcome the initial dysfunction of the liver and therefore their stormy post-operative cases were probably due to liver dysfunction, a condition which the test had hinted pre-operatively.

Positive tests are the rule in chronic atrophy of the liver, cirrhosis, Banti's disease, hemochromatosis, chronic passive congestion, Pick-Concato disease, extensive fatty degeneration of the liver, amyloidosis, chronic hepatitis associated with familial hyperbilirubinemia, and in the recovery state of hepatogenous jaundice. In syphilitic cirrhosis, positive dye tests are the rule; but the degree of retention may be somewhat less than one would anticipate from the clinical evidence of hepatic injury. In these conditions, information of prognostic significance is afforded. Chapman and associates (13) cited the value of the dye test

in cases of proposed Talma-Morison omentopexy, splenectomy, and ligation of the coronary veins of the stomach, for they found that the average duration of life varied inversely with the degree of risk as indicated by this test.

The diagnostic value of dye tests in demonstrating a metastatic malignant condition must also be mentioned. Even a relatively moderate hepatic involvement will produce a significant degree of bromsulfalein retention. In many cases of abdominal and rectal malignant conditions observed, invasion of the liver has been accurately detected on the basis of the results of this test.

In another group of cases in which toxic or infectious hepatic lesions are presumed to be present the bromsulfalein test will be positive. Among the toxic conditions must be mentioned exophthalmic goiter, a condition which is known to be associated with injury to the liver, and poisonings of various types; latent hepatic injury due to cinchophen and other hepatotoxic substances also may be detected in this way. In various infectious diseases, notably undulant fever, retention of bromsulfalein may be noted during the height of the disease. Following an episode of acute cholecystitis or even biliary colic, retention of the dye may also be noted for brief periods. Finally, in the presence of biliary fistula, dye retention of a high grade is a common finding;

its significance is questionable, since in many instances the liver is anatomically normal at operation.

In testing kidney function it is to be remembered that twenty-four hours must elapse after using bromasulfalein, before a kidney function test using phenosulfanphthalein is attempted or the kidney function test will not be reliable.

Rose bengal test is done without reference to the weight or age of the patient or to the amount of dye administered. The standard is obtained from a sample of blood drawn two minutes after the dye is injected; a sample of blood drawn exactly eight minutes after injection is compared with the standard. Normally, 50 per cent or less of the injected dye (usually from 0.1 to 0.2 gm.) should be present in the eight minutes after the injection has been made. Retention of more than that amount is considered as an indication of hepatic damage. Since the dye has a photosensitizing effect, the material should be kept in the dark and the patient should be protected from direct sunlight for some hours after the use of the dye.

Test of Blood Iodine Level

De Courey (18) attempted to establish normal standards for blood iodine in the vicinity of Cincinnati; he used non-thyroid subjects of whom some were patients with cholecystitis and other biliary tract diseases. On observing that these

patients consistently had higher blood iodine levels, he considered it as a possible test of liver function to re-lace the dye test and other non-physiological methods. He first experimented with rabbits with duct legation and again found increase iodine blood levels.

He found that 3.6 gamma/100 cc. of human blood was normal for that region (Cincinnati). In 20 cases of chrinic cholecystitis with stones the average value of the blood iodine was 16.6 gamma/100 cc. of blood, in cholecystitis with common duct obstruction due to stone it was 2090 gamma, in hydrops of the gallbladder it was 13.6 gamma, and in carcinoma of the liver it was 630 gamma (18).

De Courcy considered that these results pointed to the liver as a potent factor in the regulation of blood iodine; he has suggested that the reticulo-endothelial system is particularly active in the process of removal---however this is merely his guess. He has been using the blood iodine test as an index of the safety of a proposed operation and the need of pre-operative therapy. He assumes that when the blood iodine level is below 100 micrograms/100 cc. that the operation can be performed with reasonable safety. Since the blood iodine level is not easily performed, the test is merely of academic interest now; should the test be simplified in the future, the test maybe of value---to the author it seems attractive because it is physiological.

Bile Salts

Secretory products found in bile are bile salts and a mucinous nucleo-albumin; the latter can not be considered a product of liver metabolism for this substance is secreted from the epithelium of the bile ducts and possibly from the neck of the gall bladder (56). Urea maybe found in the bile but only traces; if its presence in bile is due to a secretion, however, one might consider the bile-salt determinations to reveal considerable information as to the efficiency of liver function. A review of this physiological process will reveal some interesting facts which makes a test based on this function of limited use today.

The bile salts are sodium glycocholate and sodium tarrocholate. They are present in about equal amounts in human bile. The structural formula of cholic acid contains the tetracylic group and is therefore related to cholesterol, to the male and female sex hormones, and to cortico-sterone. There is no evidence, however, that in the body cholic acid is derived from cholesterol; feeding of cholesterol does not increase the production of bile salts. The physiological relationship between cholic acid and the sex hormones is obscure.

The precursors of cholic acid in the body are unknown, and little can be said as to the site of origin of the cholic acid; whether it is formed by the hepatic parenchyma or is merely brought preformed to the liver from other body tissue

is not known. That some is formed in the body is indicated by the fact that the bile salts continue to be discharged from a biliary fistula during long periods of starvation. That it is derived also from the food appears from the observation that increased excretion follows the ingestion of protein material. Though the supplies of glycocol and taurine within the body are apparently plentiful the supply of cholic acid is limited, for experiments in which taurine was fed alone caused no increase in the excretion of bile salts whereas cholic acid ingestion alone caused a rise in the excretion of taurocholic acid. Taurine under these circumstances was evidently supplied from body sources whereas cholic acid was not. The quantity of cholic acid available apparently determines the level of bile acid excretion.

So far as is known the liver is the only situation where the conjugation of taurine or of glycocol with cholic acid, and the production of the respective bile acid can take place. The following observations suggest that their formation is a specific function of the liver. When the function of the liver is depressed by injury, or by the establishment of an Eck fistula, the output of bile salts may be reduced by 50 per cent or more. When the common bile duct is ligated in dogs bile acids appear in the blood. On the other hand, no accumulation occurs in the blood after removal of the liver. The liver is also the site for the destruction of

bile salts, for when fed they can be recovered quantitatively from the urine of hepatectomized but not of normal animals.

After their passage into the intestine, the bile salts are reabsorbed and carried by the portal circulation back to the liver for re-excretion. Under ordinary circumstances comparatively small amounts (about 10 per cent) of bile salts are formed afresh; their concentration in the bile is maintained largely as a result of their being circulated over and over again through the portal and biliary systems. However if the bile be prevented from entering the intestine by drainage to the exterior, its concentration in bile salts does not become materially reduced. These facts indicate the existence of some mechanism controlling bile salt production; the nature of this is obscure. It is interesting to note that when cholic acid is injected intravenously it disappears from the body and is not eliminated in the excreta; the disappearance is due largely to decomposition within the cecum through the action of bacteria. The process of reabsorption and excretion however, makes the test less significant as a test of secretory function.

Determination of the amount of bile acids in duodenal contents, while entirely possible, is of little value since the material studied is a mixture of bile and secretions from the digestive tract. No known method of determining the amount of bile acids in the blood is satisfactory; this closes

a promising method of approach. The determination of the amount of bile acids in the urine is subject to too many variable factors to make it a matter of clinical importance in cases of jaundice. Following operation, when bile can be obtained by drainage tubes or from fistulas, the study of bile acids by using the Gregory-Pasoe method of determination may give useful information, as numerous investigations have shown that low concentrations consistently are of grave prognostic significance. While there is usually a period after operation when the concentration and total output of bile acids are decreased, ordinarily normal values are attained within a few days, when and if the liver regains its normal capacity to manufacture these substances. Further simplification of the method will undoubtedly make it available for more general use; even now it is clear that much may be learned from the study of bile obtained from fistulas either before or after operation.

Josephson (44) records a method of injecting cholic acid and determining subsequent blood levels. He noted that ten ml. of a 0.52 per cent solution corresponding to 0.5 gram cholic acid, was injected into a cubital vein. The solution also contained 25% glucose which prevents the pain usually caused by an intravenous injection of a pure cholate solution. One blood sample for cholate analysis was taken before the injection; subsequent samples were taken 5,30,

and 60 minutes after the injection.

The elevation of the blood cholates 5 minutes after the injection was much less than would be expected by dilution in the total volume of the blood; it is possible that this is due to adsorption of the bile acids to tissues other than the liver. The subsequent sharp decrease in cases of obstruction were thought to be due to absorption by a still functioning liver parenchyma; this concept is also held by Bollman and Mann; Chobrol, Cottel and Sallet (cited by Josephson). Following the first decrease a slight increase one hour after the injection was observed in some cases; this could be due to a partly maintained circulation of the bile salts by the aid of the lymph vessels. Doubilet (cited by Josephson) recovered bile pigments in the thoracic lymph of dogs a few minutes after applying pressure in the bile ducts. In hepatitis, the absorbing function of the liver is diminished and consequently those bile salts, which were not absorbed by other tissues, remain in the blood and the decrease is slow.

The absence of bile acids in the intestine, as in obstruction, is the cause of fatty stools; by virtue of the fact that bile acids have not united with the fats to aid in their transport across the cell membraine; fatty stools therefore serve as a rough qualitative test of the absence of the acids (45). When the bile duct is ligated, large, light colored stools are usually observed as this procedure

interferes greatly with fat absorption. Digestion is nearly complete, however, as is shown by the fact that the fat is excreted chiefly as fatty acids. Fatty stools are found also when the pancreatic ducts are tied although the absorption of fat is by no means completely prevented. When pancreatic lipase is excluded in this way the fat is excreted as glyceride rather than as fatty acids.

Protein Metablism

Protein is a basic constituent of protoplasm and consequently forms a proportion of all living tissues. Most proteins introduced directly into the blood stream or absorbed unchanged from the intestinal tract are utilized to a very limited extent or not at all, and maybe toxic. Amino acids, of which there are some 21 important to man, are absorbed from the lumen of the small intestine, pass into the portal system and thence to the liver. Some of these amino acids pass on to the tissues to form tissue protein; others are utilized for the formation of specific substances; others are de-aminized. Such generalized statements may give the impression that the clinician, and his laboratory assistants are on the brink of creating life in the test tube. Nothing could be more misleading for the process of protein metabolism is consistently dealt with in terms of generalization. Actually there is more to be learned concerning "in vitro" protein metabolism than has already been discovered; con-

sequently tests of liver function of protein metabolism are sadly wanting.

The first three liver function tests in Table I dealing with protein metabolism were included because of the frequency with which they are encountered in the literature; it seems to emphasize the "shot in-the-dark" attitude taken by various men to state that glycine is not one of the essential amino acids, that gelatine contains more than 75% of the dispensable amino acids and that guamidine is somehow remotely related to arginine and creatinine and creatin. Nevertheless Ellsworth in the Bulletin of John Hopkins' hospital reports that eight patients with liver damage as compared with eight controls showed the initial blood guanidine level to be slightly elevated and ingestion of 200 mg. of guanidine salt produced a conspicuous rise in the blood level and also mentions that renal impairment must be rigorously excluded.

Snell and Magath claim no experience with gelatin excretion but mention that the administration of 50 gm. of gelatin with subsequent quantitative examination of urine for amino acids has been tried. If the liver function is impaired, there is considerable delay in excretion, and only small amounts of amino acids are present in the first specimens of urine.

Boyce (8) did attempt to justify Hippuric acid test, a

test of glycine synthesis; he cites the work of Bruhl and Watzodse who demonstrated that in frogs, a lack of glycine brought about a cessation of glomerular circulation which makes it logical to postulate that the liver, by means of the amount of glycine released into the circulating blood stream may actually determine kidney function. If this is true, then there is justification for interpreting the progressive oliguria, passing into anuria which is an outstanding characteristic of deferred liver death (hepatorenal syndrome) as due to diminished glycine synthesis in the liver. Boyce in his studies on Quick hippuric acid test provides further support for this reasoning and reports improvement of cases of hepato-renal syndrome after administration of glucose or of Decholin which first improves the synthesis of glycine and then the formation of bile acids.

The various plasma protein tests are based on the assumption that the liver is the sole size of protein manufacture---but the evidence at hand today does not seem to be conclusive, it is also assumed that a reserve of protein building material in the organism is at least partially stored in the liver.

Changes in plasma protein may be due to alterations in nutrition rather than to variations in liver function. However there are alterations that take place too rapidly to be the result of nutritional changes and are unaffected by

feeding protein; in these cases serum protein changes correspond to variations of other liver function tests.

Serum protein tests show that in advanced hepatic lesions, particularly of the chronic type, there is a reduction in the value of serum proteins, chiefly in the albumin fraction, and a reversal of the albumin globulin ratio. In less severe lesions the changes are correspondingly less pronounced, particularly as to the albumin fraction, although the ratio is affected in much the same way. Cantarow (1938) has shown that the serum protein concentration tends to diminish immediately after paracentesis but to increase subsequently. His idea is that the primary fall must be attributed in part to a loss of protein from the blood into the rapidly re-accumulating peritoneal effusion, and the secondary increase to progressive hemoconcentration.

There is no doubt but that reduced albumin content of the blood serum of patients with hepatic damage is partially responsible for the edema and ascites; the lowering of osmotic pressure may be enough to allow transudation under certain circumstances (77). However such factors as portal venous stasis and chronic peritoneal irritation play an important part.

Leucine and tyrosine are products of protein metabolism. Their occurrence in urine is comparatively rare and when present usually appear together. Their presence in urine indicates

autolysis of tissue proteins and are seen in cases of severe fatty degeneration of the liver such as occurs in acute yellow atrophy and phosphorous poisoning. Tyrosine is also found in cases of degenerating tumors of the liver and is transitory in subacute atrophy. The crystals are deposited spontaneously only when the substances are present in large amount. Their presence is best detected by separation from urine free of albumin.

The crystals cannot be identified from their morphology alone for calcium phosphate and ammonium lecurate are similiar in appearance. Leucine crystals as they appear in urine are not pure; they are slightly yellow, oily looking spheres and many have radial and concentric striations; they may be grouped together in clusters. Tyrosine crystallizes are very fine needles, and may appear black and are usually arranged in sheaves, with a constriction at the middle. They are soluble in ammonia and hydochloric acid, but not in acetic acid. They produce a green color when Morner's reagent and sulfuric acid is added and the mixture brought to the boiling point.

Urea is formed in the liver, and uric acid, which is presumably formed in the liver, is also destroyed in that organ. Clinically, a drop in blood urea is demonstrated only toward the end in patients with acute yellow atrophy. No changes in the urea occurs in patients with the common chronic liver

disease. Wilensky and Colp (97) convey the same idea when they state, "A high blood urea content does not mean that the physiologic condition of the liver is normal or within normal limits because the smallest possible rests of liver tissue can call forth its manufacture from the simplest ammonia salts, and urea formation is possible just before death."

Wilensky and Colp mention that the destruction of uric acid (a product of purine metabolism) depends upon the liver and that increased amounts of uric acid are fairly characteristic of the failing power of the liver, and that great importance should be attached to the increase of this protein in the blood but Boyce again points out that an increase of uric acid of the blood occurs only in the terminal stages.

Mann and Bollman (54) have cited that a slight period of asphyxia will often produce a measurable accumulation of uric acid in the blood of some animals, which while it may be the result of an increase in nucleo-proteins following increased destruction of cells by the state of asphyxia, it is more easily explained on the basis of hepatic damage---that is there must be a decreased destruction of uric acid by the liver. It would seem then that studies of protein metabolism are useless when they are most needed and that such studies are of purely academic value later.

Hemoclastic Crisis Test

Widal et. al. (cited by Soffer and White) suggested a

test for the detection of proteopexic insufficiency based on the fact that the split products of protein metabolism are ordinarily stopped by the liver, broken down into harmless substances, and resorbed in the intestine. In the presence of hepatic dysfunction they may pass through the liver unaltered and give rise to mild anaphylasis (hemoclastic crisis) with resulting leukopenia, fall in blood pressure, decrease in refractometric index of the serum, and increase in the coagulation time of the blood.

Following a 5-hour fast a single control white blood cell count is made, after which 200 cc. of milk is immediately administered orally. The white blood cell counts are then repeated at 20-minute intervals for the next two hours. In normal individuals a leukocytosis occurs, but in individuals with hepatic disease a leukopenia occurs. Widal observed a hemoclastic crisis in 38 of 39 individuals similar variations in the white blood cell count have been repeatedly observed in fasting subjects and variation after food intake cannot therefor be considered a very reliable index of liver damage.

Test for Prothrombin

Experiments on laboratory animals (88) (89) and clinical observations (64) during acute hepatitis indicate that the liver is the cite of formation of prothrombin. Pohle and Stewart have suggested that in the absence of biliary ob-

struction, external biliary fistula, or an abnormal intestinal absorptive surface, the plasma prothrombin concentration is a measure of liver function. Butt, Snell and Osterberg (11) and others have shown that a decrease in prothrombin is common after operations on jaundiced individuals and so one can foresee the necessity of continued administration of vitamin K and bile salts during the post-operative period; decreased prothrombin is also common after surgery of any sort if significant liver damage is present. Lord and Andrus (cited by Hall (33) used the prothrombin to differentiate types of jaundice. They determined the effect of 2 mg. of 2 methyl 1-4 naphthoquinine, which was given in oil intramuscularly, on the prothrombin time in 24,48, and 72 hours. They concluded that if there is a rise of 10% in 24 hours the jaundice is extrahepatic or obstructive; if less than 10% in 24 hours, the jaundice is likely to be intrahepatic. It has been shown (64) that in cases of severe hepatic damage, vitamin K and bile salts fail to produce an increase in prothrombin.

The two-stage testing technic is more sensitive to hypoprothrombinemia and gives more reliable prothrombin levels, but is technical difficulties preclude its general adoption as a laboratory procedure for clinical diagnosis (in the opinion of some investigators). The one stage methods do not measure the quantity of prothrombin alone because they

are an index of many variable factors including: the amount of prothrombin, the rate of conversion of prothrombin to thrombin, and the reaction of thrombin with fibrinogen to form the fibrin clot. Although the one-step technic does not accurately determine the amount of prothrombin, it is nevertheless an excellent practical measure of the tendency to hemorrhage.

Tests Related to Detoxification

The process of detoxification of specific elements and compounds is too large a subject to be considered in a discussion of this sort but it is not beyond the scope of the problem of liver function. Table I reveals that the process of detoxification by the liver is extremely complex yet clinically one relies on but a very few related procedures as a test of detoxification, or draws conclusions from other distantly related procedures which have little known chemical basis or explanation. It seems to the author that a vast amount of knowledge might be had concerning the process of detoxification in disease and detoxification of specific substances if studies were made during the developement of various pathological states rather than relying on microscopic review of sections and vague liver function tests. Punch biopsies, which shall be spoken of in greater detail later, seem to offer a fine possibility for such studies. At present the trend is to use experimental animals, subjected to

toxins, and to observe the effect of various other substances administered for the purpose of rendering the toxin less dangerous to the animal.

Ravdin et. al. (65) conducted their experiments on rats and used suspensions of sodium xanthine or xanthine nitrate injected subcutaneously 24 hours prior to administration of the "toxin", chloroform; these substances protected the liver of the rat from injury; sodium allantoin and caffeine decrease the incidence of hepatic necrosis but not the total number of injured livers. Sodium ricinoleate solutions may confer absolute protection of the liver against the necrotizing action of chloroform. They conclude that the liver is protected by the protein split products set free from the body as a result of the increased protein metabolism incident to the inflammatory reactions, subcutaneously. High protein diets can give the same protection.

In some diseases the ammonia concentration within the body is believed to reach proportions as to be toxic to the body. An increase of blood ammonia concentration, which is present normally in the serum in a concentration of 0.02-0.04 mg. per cent as ammonium bicarbonate, is due to lack of urea synthesis (43). Beard (3) believes that in some animals at least, urea, ammonia, creatinine and uric acid can be transformed into creatine. The extra renal metabolism of ammonia is still too obscure to offer any clinical aid in

detecting hepatic disturbances. From what has already been stated concerning urea formation one can conclude that significant changes will not be observed until the terminal stage; furthermore by virtue of the fact that ammonia is eliminated by the kidney one would expect increase blood ammonia concentration to follow decrease urea formation in hepatic diseases not preceded or accompanied by renal dysfunction.

The microscopic appearance of the liver produced by various toxic substances does present some interesting findings which is not entirely consistent with the concept that the liver is a homogenous organ. It is seen that central necrosis is produced by chronic congestion and by chloroform poisoning; peripheral necrosis is associated with eclampsia; at other times the necrosis is mid-zone as in cases of extensive superficial burns and some infectious diseases; finally there are the areas of focal necrosis as seen in cases of typoid fever, pneumonia, diphtheria, and septicemia. If the liver were truly a homogenous organ then one would expect to find either focal or diffuse types and possibly peripheral necrosis the latter would theoretically occur when a limited amount of toxin were present and was insufficient in concentration to reach the central area. This does not seem to be the case and must be due to a difference in the biochemical substance present in these areas. Punch biopsies

and immediate biochemical studies of these biopsies should reveal an enormous amount of new material in regard to liver physiology; the author feels that such a study would completely abandon the concept of the liver being a homogeneous organ.

Hippuric Acid Test

Various substances as thymol, menthol, camphor, salicylates, phenol and para-cresol and guaiacol sulfuric acid have been used in the past to test the ability of the liver to remove ravious noxious substances from the circulation, alter them chemically, or combine with them in such a manner as to render them physiologically inert. The synthesis of hippuric acid is a process of detoxification brought about by the conjugation of benzoic acid which is administered orally or intravenously, with glycine which is produced in the liver. It is interesting to note that the test was originally devised as a test of renal function. Bryan (10) suggested its use as a test of liver function; it has been popularized by Quick whose name it now bears. If the value for the blood urea is normal, that is to say if renal pathology has been excluded, the rate of synthesis of hippuric acid can be correlated with the degree of hepatic injury.

The product of the conjugation in this test, is eliminated in the urine as hippuric acid except for a small fraction which is conjugated with glycuronic acid and eliminated

as glycuronic acid monobenzoate. There is no store of preformed glycine in the body; the liver has a maximum hourly synthesis of endogenous glycine, and in the absence of an exogenous supply cannot produce more than this maximum amount to combine with the ingested benzoic acid. The rate of synthesis of hippuric acid is therefor governed by the ability of the organism to produce glycine. Since the liver is the site of the synthesis of glycine, it can reasonably be assumed that this synthesis will be adversely affected in certain types of liver damage, and that the output of hippuric acid, which will be correspondingly diminished because of the lack of glycine to combine with benzoic acid, will serve as an index of this damage.

The equations may be expressed as follows:

1. Sodium benzoate ⟶ benzoic acid + glycine ⟶ hippuric acid
2. Sodium benzoate ⟶ benzoic acid + glycuronic acid ⟶ glycuronic acid monobenzoate.

One possible objection to this line of reasoning is that the reserve of the liver is so great that impairment can be demonstrated only after extensive damage has occurred. This is true in the sense that large portions of the liver can be removed surgically without lethal consequences, but proof also exists that certain mechanisms are so delicate that there is practically no margin of safety. Quick cited the experiments of Smyth and Whipple, which showed that a dose of cholorform

too small to produce changes in the hepatic epithelium can cause a marked reduction in the excretion of the bile acids, and his own similar experiments showed a marked reduction in the output of glycuronic acid even when there were no histologic changes in the liver structure. The test indicates that the mechanism of the synthesis of glycine seems to have no great margin of reserve, since the marked reduction in excretion of hippuric acid in catarrhal jaundice is out of all proportion to the characteristic structural changes in this type of pathologic change.

The second possible objection is that the test indicates only one function of the liver. This is certainly true, but the hepatic functions are "so intimately related to each other that injury to one mechanism may conceivably affect others" (8). Thus the synthesis of amino-acids such as glycine depends upon a precursor presumably derived from the metabolism of carbohydrates, while the formation of bile acids requires an ample supply of glycine and taurine. The mechanism which synthesizes glycocholic acid is probably the same as that which effects the conjugation of benzoic acid and glycine (8). Quick considered it significant that long-continued obstructive jaundice, which has been shown to bring about a decrease in the formation of bile acids, also brings about a reduction in the output of hippuric acid, neither

amount returning to normal levels until a considerable time after the relief of the obstruction.

Mann (53) has pointed out however that no conclusive proof yet exists that the liver is the sole site of the synthesis of hippuric acid. Snapper and Grunbaum (cited by Snell and Plunkett) (79) contend that there is some relation between the elimination of water and the elimination of hippuric acid. They suggest that erroneously low values may be obtained in patients who are dehydrated and secrete only small amounts of urine; this objection need further investigation but it would seem that the test would be reliable if the kidneys retain their power of concentration.

The amount of hippuric acid may be determined by precipation as described by Quick or by extraction as suggested by Kohlstaedt and Helmer (49). Equally good results seemed to be attained by the Quick method if the specimens are stirred vigorously to aid in full precipitation of hippuric acid crystals. The Quick method of determination eliminates the hourly specimens and the possible catheterization and the hourly specimen can be stored in the icebox for as long as several days without affecting the accuracy of the test.

The sodium benzoate is non-toxic, it is inexpensive, and the test appears to be even more accurate than the bilirubin test and because of its non-toxicity may be run on subsequent

days to determine the post-operative progress of the patient. If administered orally cherry syrup maybe given to over come the unpleasant test. Even in post-operative cases, with altered absorption due to the altered gastric physiology, Boyce (8) believes that the four hour period occupied by the test might eliminate that possible error. However Quick (65) reports that the intravenous method of administration yielded 85% more positive results than the oral method upon identical cases. Such a method tests the maximum amount of work the liver can perform in three hours shorter time.

A single high or normal reading may be considered as reliable if it is certain that there has been no unusual absorption of carbohydrates; a low reading should always be rechecked and possibility of vomiting should also be considered. A transfusion may also raise the correct reading. Weichselbaum and Probstein (93) have pointed out that in using the precipitation methpd of determination that low-values or zero values may result if the urine contains no more than 0.50 to 0.60 gm. of hippuric acid. However such an error could be eliminated by saturating the urine with sodium choride for at ordinary room temperatures 100 cc. of urine in which 30 gm. of Na Cl had been dissolved holds in solution only 0.110 - 0.137 gm. of hippuric acid; and it has been recommended that an average figure of 0.123 gm. be used as a correction.

Rosenberg (72) contends that excessive excretion signi-

fies early hepatic damage on the basis of "hyperirritability" of the damaged liver cells. Moser et. al. (57) suggest that an excessive excretion may be due to the excretion of sodium benzoate itself which contaminates the precipitate to yield a falsely high value.

Kohestaedt and Helmer (49) have recommended making a simultaneous study of urea clearance; however Vaccara (87) pointed out that the test is contra-indicated in nephritics with nitrogen retention and also points out that benzoic acid decidedly depresses the excretion of uric acid.

Clinical Observation with Administration of Vitamins

Today the clinician, experimentalist, and laymen are all subjected to the alleged importance of vitamins in the daily diet. It is not surprising that because the liver does profoundly influence the utilization of food that vitamins have been used in treatment of various hepatic disturbances; indeed if vitamins do play any role in food metabolism early evidence of their action would be expected to be found in the liver. A few observations are included here to demonstrate the modern trend of the use of vitamins.

Patek and Haig (63) has shown that very large doses of vitamin A will correct absolute night blindness where as a diet that contains an ordinary deficiency state will not affect the condition. "This may imply that the ability of the cirrhotic liver to utilize vitamin A is definitely less

than normal." (63)

Patek (62) reported 13 cases, treated with a high vitamin intake, in which there was alcoholic cirrhosis of the liver with ascites. Ten patients experienced remarkable improvement in respect to both clinical and laboratory findings; a few patients had spontaneous diuresis and the ascites disappeared. He used various modifications of percomorphic acid (vitamin A and D), orange juice or pure vitamin C (ascorbic acid), Valentine's liver extract orally (as a source of riboflavin) or parenteral liver extract and yeast or a yeast concentrate plus thiamine chloride to supply other portions of vitamin B complex. In order to facilitate the absorption of fat soluble vitamins, patients were given 5 - 15 gr. of animal bile salts with meals.

Fleming (unpublished data cited by Snell) (76) reported a similiar group of fifty cases. After approximately a year to a year and a half, one half of these cases were still living, one-third showed considerable improvement, of which some appeared to be making a complete recovery and others remarkable cures with disappearance of ascites. Some of the others showed temporary improvement but later succombed to hemorrhage from esophageal varices or from intercurrent infection. It is reported that the amount of progress closely paralleled the regularity with which this routine was maintained.

Patek defends the therapy as he outlined it as not being

entirely a "shot-gun" therapy because it is alleged to be a function of the liver to normally store considerable amounts of vitamin A and D; also the liver is a principal storehouse for vitamin C; and it stores part of the B complex and is essential in the utilization of vitamin K to form prothrombin.

A few attempts have been made to determine whether or not normal storage of thiamin chloride is obtained in persons who have cirrhosis; the results are not entirely conclusive but it appears from the reports of Robinson et. al. (70) that patients with advanced hepatic injury have a low urinary output of thiamin and that they respond to a test dose of the material in a manner indicative of impairment of storage. Rich and Hamilton (68) working with rabbits fed a diet essential in all its case constituents and containing adequate amounts of thiamin chloride, nicotinic acid, riboflavin and vitamin B, was capable of producting cirrhosis of liver, but when rabbits were fed yeast instead of individual components, no hepatic lesions of any consequence developed. This might indicate that there was present some substance yet unknown. The diet of the animals that developed cirrhosis was deficient in choline, whereas the animals that did not develop cirrhosis were obtaining choline from the yeast and so choline may have been responsible for the difference.

That there may be some therapeutic and biochemical basis for the use of vitamin B complex is based on the facts that

three enzyme systems depend for their functional activities upon constituents of the vitamin B complex and so in case of hepatic damage the complex storage is reduced and if the patient is on a high carbohydrate diet it would seem logical to attempt to supply these vitamins. The three enzyme systems and their action are listed below.

1. The enzymes (co-enzymes) phosphopyridine nucleotides are active in the metabolism of hexose-phosphate, each phosphopyridine nucleotide contains one moecule of nicolinic acid.
2. The co-enzyme (cocarboxylase) is the pyrophosphate of thiamin which acts in the breakdown of pyruvic acid.
3. Riboflavin enters into the composition of the Wasburg-Christian respiratory enzyme which is active in cellular oxidation reactions.

As for vitamin C, it has been shown by Snell (76) that patients with cirrhosis showed a reduction in the concentration of ascorbic acid in the plasma and urine. This might be due to a low storage level. However this is a water soluble vitamin, and it seems logical to the author that a decrease should not be noticed in a 24 hour sample of urine if the diet were sufficiently fortified with vitamin C. An hourly excretion curve would seem to be of more importance; if there were decreased storage one would expect to get increased excretion following a meal with a rapid fall in excretion 3-4 hours later.

Snell (75) claims to have demonstrated vitamin D deficiencies in cases of hepatic disease as evidenced by a form of osteoporosis. Conclusive evidence is at present lacking.

Fluid Intake and Output

Although the production of ascites is generally attributed to the loss of plasma proteins and the reversal of the albumen-globulin ratio, this may not be the entire picture. Bollman and Mann (7) were able to produce ascites spontaneously in animal with very extensive cirrhosis and following obstructive jaundice of long duration; under both of these conditions ascites could be produced and removed by dietary means. Animals with obstructive jaundice were maintained for three or four months on a diet of milk, bread, and syrup and showed no evidence of ascites; they were then fed meat for three or four days and most animals developed ascites 24 hours after the meat diet was instituted.

The active substance which produces ascites in these animals is probably not protein according to these men. Proteins of milk do not favor the production of ascites. The active principle appears to be in the water soluble extract of meat. The feeding of meat extract which is free of protein and fat produces results which are more striking than the feeding of meat. Within 4-6 hours after feeding meat extract to animals with obstructive jaundice or obstruction of long standing with extensive cirrhosis, ascites of 2-3

liters of fluid can be withdrawn; the ascites disappeared again in most instances when meat or meat extract was withdrawn.

Meat extract might produce ascites by one of three mechanisms none of which is proved: (1) because of a specific colloidal effect on the blood or vessels, which enables water to diffuse into body cavities more readily than normally; (2) a specific effect on the liver, which produces constriction of the intrahepatic portion of the portal system with subsequent sufficient increase in portal pressure to produce vascular changes in the abdominal viscera; (3) specific irritation of the peritoneal surfaces which increases the secretion of fluid from these surfaces and inhibits their absorption of fluid. In any event, extensive liver injury must be present so that it appears that a normal liver must compensate for or detoxify this active agent which will produce ascites when the liver is extensively injured. A measure of fluid intake and output in such instances would detect early changes of "hepatic" function.

Macrocytosis

The appearance of the erythrocytes in disease of the liver may be mentioned, not as a true test of liver function, but because studies on the morphology of the blood may give valuable confirmatory evidence of the presence of injury of the liver. A hypochromic macrocytic anemia is a rather uni-

form accompaniment of portal cirrhosis and may be noted in other types of hepatic injury. The rapidity with which such macrocytes appear in the blood stream has been explained by Higgins and Stasney (working on experimental animals) (39) on the basis of hypoproteinemia and swelling of erythrocyte because of the altered osmotic pressure of the plasma.

Takata-ara Test

The Takata-ara test was originally devised to differentiate between meningitis and syphilitic envolvement of the central nervous system and then soon extended to other conditions; Jezler in 1930 was the first to use it in hepatic disease. It is essentially a study of the precipiation of serum proteins of the colloid system with mercuric chloride-fuchsin solution due to their diminished stability. Takata originally thought that the reaction was accounted for by an increase in the globulin fraction and the corresponding reversal of the albumin-globulin ratio. Others do not accept this theory, and Magath (52) concludes that the test has the major defect of being an emperic phenomenon, which is not yet explained on any satisfactory scientific basis.

Kirk (48) collected from the literature 3,583 cases in which this test had been employed and concluded that it was likely to be positive in any condition, hepatic or non-hepatic, in which the globulin level was elevated, and so is definitely too insensative to be used as a liver function test.

The reported margin of error varies from 10 to 50 per cent, and although the test was earlier believed to be uniformly positive in hepatic cirrhosis, this generalization now seems to be accepted only when the diagnosis can be made clinically.

Cozzutti (cited by Boyce)(8) used Ucko's modification of the Takata-ara reaction in 433 patients without liver involvement and 83 with hepatic disease; he concluded that a positive test is more likely to indicate cirrhosis than any other disease, particularly if the test is used in combination with other liver function tests; in early cases a negative test should be interpreted as a doubtful diagnosis of cirrhosis.

Cephalin Flocculation Test

The mechanism of the cephalin flocculation test is speculative; it is believed that a strongly flocculating serum contains a nitrogen bearing constituent in the globulin fraction, which during the reaction, becomes attached to the surface of the cephalin-cholesterol particles. The film of adsorbed protein probably brings about changes in surface potential and increase the cohesive forces between the colloidal elements. The flocculation reaction depends upon the specific properties of certain cephalins. It is not obtained with cholesterol or cephalin alone or with emulsions composed of cholesterol and other lipids such as egg lecithin. The role of cholesterol in the reaction is

probably that of orientating and furnishing a vehicle for its presence during the course of certain hepatic disturbances is still speculative.

Hanger (34) reported consistent negative cephalin flocculation in 25 cases of obstructive jaundice and questionable positive, or at the most a one plus, in the 48 hour reading and suggests that this finding probably indicates mild hepatitis associated with the biliary obstructive process. Even in cases of longstanding biliary obstruction with secondary fibrosis in the liver, the test usually remains negative.

In 33 of 38 cases of jaundice due to hepatitis, catarrhal jaundice and cirrhosis there was a prompt, strong flocculation reaction; furthermore repeated tests during the course of the disease showed a close relationship between the clinical severity of the disease and the degree of flocculation.

Post-arsphenamine cases seem to respond to this test in two ways; some show a strong flocculation reaction which resembles idiopathic hepatitis; the negative reacting group is however characterized by prompt onset of disability and jaundice following arsphenamine injection, intense itching of the skin, little disturbance of the albumin globulin ratio, and an elevation serum phosphatase ---the latter being more suggestive (by interpretation of

the test) as being obstructive. The negative flocculation might be interpreted as there being a difference in the mechanism of jaundice in some post-arsphenamine cases as compared with the mechanism of idiopathic hepatitis. One must be on guard and not diagnose a case as obstructive jaundice in patients who have a negative cephalin-flocculation test. On would be wise to confirm or deny such by use of the serum phosphatase.

Too few cases have been reported of liver damage due to toxic agents, cholangeitis, liver abscess and new growths to correctly evaluate the tests; it does however seem that in single or circumscribed supparative lesions of the liver the test is usually negative while cases of multiple or disseminated lesions usually give a positive test.

Cases of hemolytic jaundice give a negative flocculation test; cases of acute or chronic infections such as pneumonia, and septicemia usually give a positive test---the latter observation would seem to indicate that icterus following infection is due to secondary changes in the liver rather than to excessive blood destruction as is the older concept as has already been concluded with the Van den Bergh test.

Rosenberg (72) emphasized that the test offers a very sensitive and reliable index of the activity of hepatic disease process. He found in 10 per cent of cases of mild to moderate disease of the liver the flocculation test disclosed

clinically significant liver disease where all other tests and combination of tests failed. Other tests of liver function were sometimes quite strongly positive in patients with mild or subclinical liver disease when the flocculation reactions were light or negative. Rosenberg interprets these as instances of impaired liver function resulting from liver disease which is either very slowly progressive or which has become quiescent.

More recently, Rosenberg (71) has observed differences in the reaction with various cephalin preparations and suggests that cephalin be exposed to air for a number of weeks to convert it to the "oxidized" form in order that false positive reactions may be avoided. If ripened cephalin is used, the test has been found to be of value in differentiating hepatogenous from obstructive jaundice (34) (58). Nadler (56) concludes that in extra-hepatic obstructive jaundice, the cephalin flocculation is predominantly negative or faintly positive. In a group of acute catarrhal jaundice cases, there were generally three-plus and four-plus reactions.

The test is also excellent for the indication of progress and prognosis. Progressive decrease and final disappearance of an initial four-plus reaction indicates an excellent prognosis in acute hepatitis; the continued per-

sistence of a four-plus reaction, irrespective of a decrease in the degree of jaundice, indicates progressive liver degeneration and often an unfavorable prognosis (35). In cases of cirrhosis the degree of flocculation parallels the severity of the process as a rule and is usually negative in those instances in which residual scarring is apparently the sole lesion(36).

Porphyrin Metabolism

Porphyrins are red pigment substances, the chemical structure of which is based on a ring formed by four pyrrole nuclei which are connected in the form of a closed system of four methine bridges. Porphyrin derivatives are an essential part of the respiratory enzymes present in all living cells, and an understanding of their nature and relations may be expected to lead to improvement in the diagnosis and treatment of many human diseases.

Considerable knowledge of the structure and chemical properties of the various porphyrins exists, but the physiologic relations are rather obscure. The identity of a particular porphyrin is the result of the type and position of radicals attached to eight substitution points along the porphyrin ring. Porphyrin compounds in the body fall into two groups---first, those having four each of two different radicals in the eight substitution points along the porphyrin ring, and second, those having three

radicals divided into four of one type and two each of the others. The first group has four possible isomers and second has fifteen.

Examples of the first group are coproporphyrin and urophophyrin. Both have four of two different types of radicals attached to the porphyrin ring. The four isomers that any porphyrin in this group may conform to are termed "types" and are numbered from 1 to 4. Only types 1 and 3 are found in nature. Types 2 and 4 do not occur naturally but have been synthesized.

Porphyrins of the second group are characterized by three different radicals substituted and fifteen isomer; in this group are included deuteroporphyrin, mesoporphyrin, hematoporphyrin and protoporphyrin. Each of the fifteen isomers conforms to one of the four types in the first group of simpler porphyrins.

Protoporphyrin, coproporphyrin, and uroporphyrin and their isomers occur in the body; mesoporphyrin and deuteroporphyrin are exogenous compounds which are believed to be produced by bacteria of the alimentary canal. Uroporphyrin and coproporphyrin are found largely in the urine and feces. Coproporphyrin is considered the only porphyrine normally present in urine. (90) (20).

It has long been recognized that small amounts of

porphyrin are excreted normally in the urine of patients suffering from a variety of diseases. Porphyrinuria has been described as accompanying many forms of hepatic disease, including cinchophin hepatitis, atrophic cirrhosis, obstructive jaundice, chronic passive congestion, lymphosarcoma of the liver, infectious icterus, melanosarcoma of the liver, hemolytic icterus and hemochromatosis. It has been suggested that porphyrinuria observed in the presence of various other diseases and toxic states is caused by associated hepatic damage of dysfunction. Much evidence has accumulated which would indicate that porphyrin arises in the body during the process of hemopoiesis, rather than during the destruction of hemoglobin, as has been supposed formerly, and that the coproporphyrin which is excreted in the urine and bile represents, first, an amount of isomeric series I porphyrin which arises as a useless by-product of the main synthesis and, second, any isomeric series III porphyrin which has not been utilized in the production of hemoglobin. The rate of excretion of coproporphyrin in the urine and bile depends on several factors, chief of which are the rate of production of the porphyrin which is to be excreted and the efficiency of the liver in disposing of the material. The liver furnishes the most important means of excretion of porphyrins; the kidneys usually excrete only a small fraction of the total porphyrins (21).

Nesbitt and Snell (60) studied the degree of porphyrinuria present or the ratio of porphyrins excreted in the urine to porphyrin excreted in the feces in cases of disease of the liver and to see if there was any relation to the extent of parenchymatous hepatic damage. They conclude that the normal range of coproporphyrin in the feces was from 300 to 400 micrograms in 24 hours. The urinary fecal ratio varied from 0.07 to 0.24. In many instances the amount of coproporphyrin present in ascitic fluid and also in the fluid obtained on thoracentesis was determined, and although an appreciable quantity was present in each instance, the amounts in these reservoirs were not sufficient to constitute factors in the loss of porphyrin from the body.

The amount of coproporphyrin excreted in the urine in each case from day to day is distributed over a wide range which usually includes some normal values dispite severe hepatic damage, so that single determinations may not be considered as informative. However, in 16 of the 17 cases most of the values for coproporphyrin excreted in the urine was well above the normal range and the mean value of any given series of determination seems to be a fair index of the degree of hepatic damage present in the particular case. In four cases representative of moderate hepatic damage, the patients excreted normal amounts of coproporphyrin in the stool, whereas in all other instances in which determinations

of coproporphyrin in feces were carried out, and which are representative of more severe damaged livers, the patients excreted reduced amounts of coproporphyrin in the feces.

They conclude that among patients suffering from various disease of the liver the degree of porphyrinuria present and particularly the urinary-fecal ratio of the excretion of coproporphyrin are fair indices of the extent of parenchymatous hepatic damage present at the time of investigation. The data on the excretion of coproporphyrin are not necessarily of prognostic values for there was an occasional recovery of patients in whom the urinary-fecal ratio of such excretion was much disturbed.

It has been claimed that in complete obstruction of the common bile duct the total excretion of coproporphyrin is not altered but that coproporphyrin practically or entirely disappears from the feces and appears in greatly increased amounts in the urine and occurs in the blood urine, which normally does not contain any coproporphyrin.

Lageder (cited by Nesbitt and Snell) (11) studied the bile pigment values along with the urinary excretion of porphyrin by rabbits and concluded that the porphyrinuria which developed after ligation of the common bile duct was not explainable simply as an overflow but that some correlation existed between the porphyrimuria and the degree of damage of the liver cells. Watson (19) also noted in three cases of

obstruction of the common duct a definite but only a moderate increase in the urinary coproporphyrin, which was much less than the porphyrin in the urine in a case of cinchophen cirrhosis. On the other hand, the urine of a patient who had hepatic insufficiency due to advanced hepar lobatum did not contain any trace of coproporphyrin. This might indicate that more than one factor operates in the excretion of coproporphyrin by the liver.

German workers present evidence in favor and against the idea that bacteria may synthesize coproporphyrin or may produce it from blood in the intestine.

Nesbitt and Snell (61) records that, subsequent to operation for obstruction of the common bile duct of varying severity, the excretion of coproporphyrin returned to normal or nearly normal or remained elevated, apparently depending on the degree of parenchymatous hepatic damage.

Van den Bergh and his assoicates described the appearance of coproporphyrin in the bile obtained by perfusion of surviving rabbit livers with blood to which protoporphyrin has been added. The results of these experiments appeared to be so definite that they were widely accepted as proving the ability of liver tissue to convert protoporphyrin to coproporphyrin, and as indicating a close relationship between the protoporphyrin of the red blood cells and the coproporphyrin of the bile and urine. Watson, Pass and Schwartz

(92), attempt to determine if crystalline protoporphyrin in dogs with bile-renal fistulae would be followed by an increased excretion of either coproporphyrin of bilirubin. The results were a slight increase of coproporphyrin which might have resulted from conversion of porphyrin isomer of Type I to isomer of Type III such a process would have involved the splitting and rotating of the porphyrin ring, a phenomenon unlikely to occur according to Fischer. The slight increase might have been due to a stimulation of erythropoiesis by the injected protoporphyrin for an increase of coproporphyrin has been shown to occur in hemolytic jaundice, pernicious anemia and after hemorrhage---but again this is questionable (as to whether or not this is the cause of increased coproporphyrin) for no increase of hemoglobin, erythrocytes, or reticulocytes was observed. Searching in dispair they finally suggest that the injected material might have been a mixture of isomer Type I and Type III.

Welcher (99) would have one discard any further consideration of the porphyrines in hepatic disease when he states, "Coproporphyrin excretion in the urine and urinary fecal ratio have been thought to be sensitive test of hepatic function, but this is not so". He refers one to the work of Dorbriner and Rhoads (22) and Nesbitt (57) for confirmation. The author feels that Welcher has make a grave mistake; Dorbriner and Rhoads (22) state, "The coproporphyrin excretion

in the urine is thought to be a sensitive indication of hepatic dysfunction, but the author feels that studies of the urine only, without estimations of the fecal coproporphyin and determination of the types excreted, do not give adequate information.....It appears that the ratio is more signigicant for the diagnosis of liver diseaase than is the total output, the highest ratios being observed in cases with the most severe liver damage, or judged by clinical evidence". Nesbitt (59) concludes, "It is possible that by the use of more quantitative procedures, such as described recently by Watson, both coproporphyrin I and coproporphyrin III might be demonstrated in the urine of all or most patients who have hepatic disease. The occurrence in the urine of these patients of coproporphyrin I, either alone or with additional varying proportions of coproporphyrin III, is in accord with the current hypothesis of synthesis and excretion of porphyrin".

Because of the misinterpretation it seems fitting to briefly review the article by Dorbriner and Rhoads who has surveyed the literature and have recorded the various conditions in which abnormal amounts of porphyrines were found in the urine and feces. Furthermore it seems striking that so many of those conditions have a close relationship to liver function. The review is presented herein Table II

Table II

Condition	Investigators	Coporphyrin in urine	Porphyrin in feces
1. Cholelithiasis and cholecystitis without icterus	Brugsch	Increased	.....
	Lageder	Increased	.....
2. Catarrhal jaundice	Brugsch	Increased	.....
	Dorbriner	Increased	.....
	Gunther	Increased	.....
3. Obstructive Jaundice	Dobriner	Increased	none or little
	Franke	Increased	none or little
4. Passive conjestion	Thiel	Moderate increase	.....
	Brugsch	Moderate increase	.....
	Dorbriner	Moderate increase	.....
5. Acute Yellow atrophy	Gunther	Increased	.....
	Brusch	Increased	.....
	Franke	Increased	.....
6. Cirrhosis	Brugsch	Increased	.....
	Lorente	Increased	.....
	Franke	Increased	.....
7. Lenticular degeneration	Tropp and Penew	Normal	.....
8. Metastatic tumors of liver	Thiel and Gunther	Increased	.....
9. Pernicious anemia--in relapses	Thiel	Normal to increased	.....
	Lageder	Normal to increased	.....
	Brugsch	Normal to increased	.....

Condition	Investigators	Coporphyrin in urine	Porphyrin in feces
10. Hemolytic jaundice	Dorbriner	Increased	.....
	most investigators	Increased	.....
11. Hemolytic jaundiced cases after splenectomy	Dobriner	Decreased	.....
	Watson	Decreased	.....
12. Aplastic anemia	Brugsch	low	.....
	Vannotti	increased	.....
13.(a)At time of Hemorrhage produced by water and chemicals	Duesberg	normal	.....
	Dobriner	.....	.....
(b) When blood regeneration was well established following hemorrhage produced by water injection and chemicals	Dobriner	Increased	.....
14. Myeloid Leukemia			
	Duesberg	Normal	.....
	Thiel	Normal	.....
	Lorente	Increased	.....
	Vannotti	Increased	.....
15(a)Hodgkin's Disease	Duesberg	Normal	.....
	Brugsch	Normal	.....
(b)Febrile Cases before death	Dorbriner	Greatly Increased	-----
16. Eczema	Goeckermann	Increased	.....
17. Sabvarsan Dermatitis	Marquardt	Increased	.....
18. Schizophrenic patient Febrile attacks	Scheid	Increased	.....
	Brugsch	Increased	.....

Conditions	Investigators	Coproporphyrin in Urine	Porphyin in feces
20(a) Administration of alcohol (moderate amount)	Franke	Double or more	.....
20(b) Chronic alcoholics	Brugsch	Greatly increased	..Low..
21. Phosphorous poisoning in animals	Lorente	Increased	.....
	Thomas	Increased	.....
22 Selenium poisoning	Halter	Increased	.....
23 Barbiturates	Gunther	Variable	.....
24 Sulfanamides			
(a) Patients with dermatitis	Brunsting	Increased	.....
(b) Patients having no reaction to Sulfanamide	Silner and Elliot	Normal	.....

Sodium-d-lactate Test

Soffer, Dantes and Sobotka (81) devised a test of liver function based on the use of sodium-d-lactate. They injected 75 mg. of this substance per kg. of body weight, in a 10-14 per cent solution, before breakfact, after taking a control sample of blood in fluoride. Another sample was taken 30 minutes later, and blood lactic acid dterminations made in duplicate. In normal individuals the value either falls below or remains at or just above the control level at the end of the test, whereas patients with hepatic disease have a distinctly delayed utilization of the injected salt. An elevation of 5 mg. or more above the control level is considered pathologic.

Sodium and Chloride Elimination

Bansi and Strecker(cited by Boyce)(8) studied the elimination of sodium and chloride and the sodium chloride index of the urine in 42 subjects, 37 of whom had hepatic disease. Some of the tests were continued over a period of weeks, An index between 0.75 and 0.50 was taken to indicate mild impairment of function, between 0.50 and 0.25 a moderate impairment, and indices below 0.25 as indicative of severe if not of fatal damage.

Cincophen Detoxification

Lichtman (50) proposed a test for the detoxifying function of the liver which requires the oral administration of a

standard dose of cinchophen and a determination by colorimetric methods of the 24 hour excretion of oxy-cinchophen in the urine. Excretion of more than 21 per cent of the amount administered indicates hepatic dysfunction, and the test serves as a quantitative index of an altered metabolism of the cells of the liver whereby they have lost their capacity to decompose this substance further. Lichtman found that when a dose of 0.45 gm. of cinchophen was used, usually less than 200 mg. was excreted over the 24 hour period in biliary obstruction due to stone and in metastatic carcinoma of the liver, whereas in diffuse parenchymatous lesions more than 200 mg. was excreted. In cirrhosis of the liver the values rose above 200 mg. only when acute or subacute hepatic degeneration was superimposed. In catarrhal jaundice the values were usually below 200 mg., and in carcinoma of the pancreas there was a persistent increase over this amount.

Wasserman Test

Hall (33) points out that the Wasserman test is indispensable in evaluating liver and gall bladder disease because syphilis may produce the clinical picture of many if not all of these conditions. It has been estimated that 90% of patients with hepatic lues give a positive Wasserman test. Interpretation is sometimes difficult because liver damage does influence the intensity or even obscures the Wasserman reaction. In such cases as the latter the therapeutic test

with the use of KI is more important; and it is to be remembered that arsenicals are contraindicated in cases of already liver damage.

Relief Film of the Oesophagus

Another method of diagnosing the presence of a portal type of cirrhosis is the demonstration of esophageal varices by relief films of the esophagus---such a test does have clinical significance. At times portal cirrhosis may be present without any history of bloody vomitus yet varices may be demonstrated ( ).

Punch Biopsy

Summaarily it may be said that there are no satisfactory and consistently reliable techniques for ascertaining the functional efficiency of the liver or modifications in its structure in patients suspected of suffering from diseases in which this organ is seriously implicated. The extensive literature devoted to liver function tests, in itself, is an indication of the deficiencies of existing clinical methods while, according to Ivy and Roth (41) liver function tests can be unreliable, even when the gland is known to be grossly pathological.

Extensive experiments in animals indicate that the liver is implicated to a greater or lesser extent in many pathological processes. It is easily possible, by badly balanced diets,

to initiate gross lesions in the liver without necessarily producing fatal results. It has been demonstrated repeatedly by experiment that not all forms of liver injury respond to the same type of treatment. This vast body of knowledge in animals cannot be applied intelligently to human subjects because it has not been established that dietary imbalances affect man in the same way as they do animals.

In view of these conditions it would appear that an entirely different approach would be more desirable. Aspiration of abscesses and cysts of the liver has been a recognized technique for over a hundred years, exploratory puncture of the solid portion of the organ for diagnostic purposes was first reported by Bingel in 1923.

By 1939, nearly 300 aspiration biopsies had been reported in the literature; of this group five died from hemorrhage and one from peritonitis. In Baron's series of 35 punch biopsies, there was only one death from hemorrhage (1).

Iversen and Roholm (cited by Gillman and Gillman) (29) described a method for performing puncture biopsies. There were no fatalies due to aspiration of these 116 cases. One patient with obstructive jaundice died nine days after the puncture and autopsy revealed the presence of one and a half liters of blood stained fluid in the peritoneal cavity; in another case there was a large clot at the site of the punct-

ure. However with the recent developement of coagulating gauze, the danger of hemorrhage should be practically nil. Gillman and Billman report the use of puncture biopsy method to ascertain the cause of hepatic enlargement, to assess the degree of involvement of the liver in malnutrition, to control therapeutic measures directed towards the improvement of the pathological processes in the liver, especially in pellagrins, and to examine the efficiency of liver function tests. The author believes it could well be used for biochemical studies when the use of coagulating gauze was used to control hemorrhage and quick freezing to reduce enzymatic action.

Conclusion

The author has reviewed a number of liver function tests which seemed to be a test of a somewhat related function. The original problem as setforth in the introduction was that of attempting to correlate simple histological appearance with the complexity of functions. The two types of cells considered in this review were the parenchymal and the Kupffer cells; the former is usually considered to be primarily concerned with the aiding of assimilation and utilization of our food. Just what the chemical equations are in such metabolic process is almost beyond speculation. The material reviewed has presented no facts or material as to what this intracellular process may be; there has been presented evidence that a number

of enzymes are influencing such a process.

Having found no explanation the author has been left to his own conjecturing. The greatest unexplainable problem to the author seems to be that of varied area of hepatic injury within the lobule when viewed by the microscope. Just why should the necrosis be central one time, mid-zone at another and peripheral at still other times? The very nature as to the rate of organic synthesis may be a factor; it is common knowledge that all reactions do not proceed at the same rate. Now assuming that the flow of substances through the liver and through the liver cells are a constant and continuous process and that the parenchymal cells are homogeneous, one might conceive that some rather seemingly harmless substances may reach the periphery of the lobule and begin slowly the process of metabolism but as the substances moves nearer the center of the lobule the metabolic process increases in speed until the process becomes too great of a load for the cells to endure and the result is central lobule injury. In other instances the substances, which may be either a toxin or a fact acting substance, reacts completely at the periphery in such a rapid fashion that the endurance of the cell is exhausted before the original substance reaches the central area. Still other possibilities of as a cause of mid-zone or central necrosis might be that the intermediate products are toxic themselves. The author repeats again that he beieves the punch biopsy with

subsequent quick freezing may in the future offer greater value as to the process of metabolism by the liver that considerable more may be learned as to the process of excretion and secretion into the blood stream and into the bile, and that following the accumulation of such knowledge specific reliable liver function tests maybe devised which are so sadly wanting today.

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